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RÔLE DES RÉCEPTEURS 5-HT$_{1B}$ ET DE LA DOPAMINE DANS L'ACTIVITÉ DE TYPE ANTIDÉPRESSEUR DES IRSSs DANS LE TEST DE LA NAGE FORCÉE CHEZ LA SOURIS

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LISTE DES ABBREVIATIONS

5-HT : Sérotonine

5-HIAA : Acide 5-hydroxyindol-acétique

5-HT1A, 1B, … : sous types des récepteurs de la sérotonine

5,7-DHT : 5,7-dihydroxytryptamine

6-OHDA : 6-hydroxydopamine

AC : Adénylate cyclase

AMPc : Adénosine 3',5' monophosphate cyclique

ADN : Acide Désoxyribonucléique

ARN(m) : Acide Ribonucléique (messager)

BDNF : Brain Derived Neurotrophic factor

BHE : Barrière Hématoencéphalique

CF : Cortex Frontal

CPu : Caudate Putamen

DA : Dopamine

DSP-4 : N-(2-chloroéthyl)-N-éthyl-2-bromobenzylamine HCl

FST : Forced Swimming Test (test de la nage forcée ou test de Porsolt)

i.c.v. : Intracérébroventriculaire

IMAO : Inhibiteurs de la monoamine oxydase

i.p. : Intrapéritonéale

IRD : Inhibiteurs de Recapture sélectifs de la Dopamine

IRN : Inhibiteurs de Recapture sélectifs de la Noradrénaline

IRSN : Inhibiteurs de Recapture de la Sérotonine et de la Noradrénaline

IRSS : Inhibiteurs de Recapture Sélectifs de la Sérotonine

KO : Knock-out

p-CPA : Para-chlorophénylalanine

s.c. : Sous cutanée

SERT : Transporteur de la sérotonine

TCA : Antidépresseur tricycliques

TST : Tail suspension test, test de suspension caudale

1.0 INTRODUCTION

1.1 Problématique

Les deux tiers des patients traités pour une dépression ne répondent qu'après plusieurs semaines de traitement (2 à 8 semaines), alors qu'un tiers de ces patients ne répond à aucun traitement. La réponse à un traitement par antidépreseur est définie comme étant une réduction de cinquante pourcent des symptômes évalués à l'aide d'échelles permettant de déterminer l'intensité de la symptomatologie (échelle d'Hamilton ou échelle de Montgomery et Asberg). De ce fait, la réponse à ces traitements n'est pas synonyme de rémission. Compte tenu du grand nombre de patients non répondeurs, ainsi que du délai d'action nécessaire à l'apparition des effets thérapeutiques de ces molécules, il apparaît donc essentiel de trouver de nouvelles cibles d'action pour le développement de nouveaux antidépresseurs ou bien de tenter de potentialiser les traitements existants.

Le but de cette thèse est d'étudier le rôle joué par les récepteurs 5-HT_{1B} dans l'apparition des effets antidépresseurs chez la souris lors de l'administration d'IRSSs. En effet, lors de précédentes études réalisées au laboratoire (Malagie et al., 2001; Malagie et al., 2002), il a été mis en évidence que l'absence de récepteurs sérotoninergiques 1B (5-HT_{1B}) chez des souris mutantes (souris « Knock-out », K.O. 5-HT_{1B}) ou le blocage de ce récepteur par un antagoniste spécifique (GR127935) potentialisait l'augmentation de la concentration cérébrale extracellulaire de sérotonine ($[5\text{-HT}]_{EC}$) dans l'hippocampe induite par une administration intra péritonéale (i.p.) d'un IRSS par rapport à l'augmentation induite chez des souris sauvages (Wild-Type, WT). La $[5\text{-HT}]_{EC}$, a été mesurée par la technique de microdialyse intracérébrale in-vivo chez des souris éveillées après administration de paroxétine (2001) ou de fluoxétine (2002). Toutefois, lors des essais comportementaux réalisés chez des souris de même fond génétique (129/Sv), cette augmentation de la concentration extracellulaire de sérotonine n'a pas pu être corrélée à une augmentation de l'activité antidépressive. En effet, lors de la réalisation d'un test de désespoir comportemental prédictif d'une activité antidépressive, le test de la nage forcée (Forced Swimming Test, FST ou test de Porsolt) (Porsolt et al., 1977), l'administration d'un IRSS n'a pas permis de mettre en évidence une activité antidépressive chez les souris mutantes privées du récepteur 5-HT_{1B} ni chez les souris sauvages ayant reçu un antagoniste des récepteurs sérotoninergiques , alors que cet effet était présent chez les souris SAUVAGES contrôles. En parallèle, nous avons montré que l'administration i.p. d'un agoniste des récepteurs 5-HT_{1B} permet la potentialisation de l'effet anti-immobilité des antidépresseurs dans le FST (Redrobe et al., 1996 ; David et al., 2001) chez des souris de souche Swiss. Afin d'expliquer les différences entre les résultats de neurochimie

et ceux de comportement, nous avons émis l'hypothèse que l'effet antidépresseur des agonistes des récepteurs 5-HT_{1B} et des IRSSs dans le FST pourrait être lié à l'activation des hétérorécepteurs 5-HT_{1B} (situés sur des neurones non sérotoninergiques).

Ce travail a donc eu pour but :

- de vérifier que l'effet de type antidépresseur obtenu dans le FST chez la souris consécutivement à l'administration d'un agoniste des récepteurs 5-HT_{1B} (l'anpirtoline) est bien lié à l'activation des hétérorécepteurs 5-HT_{1B}.
- de déterminer les aires cérébrales impliquées dans l'apparition des effets comportementaux de l'anpirtoline
- d'étudier les effets du blocage pharmacologique de ce récepteur sur l'activité de différents antidépresseurs
- de déterminer les voies monoaminergiques impliquées dans l'apparition des effets de ces antidépresseurs.

1.2 La dépression

1.2.1 Etiologie de la dépression

La dépression est une pathologie hétérogène résultant d'un hypofonctionnement de certains systèmes de neurotransmission centraux : sérotonine (ou 5-hydroxytryptamine 5-HT), noradrénaline (NA) ou de leur métabolisme (Coppen, 1967; Duman et al., 1997). Bien que plusieurs hypothèses aient été émises, l'étiologie de la dépression est encore mal définie, plusieurs hypothèses sont à l'heure actuelle encore évoquées.

1.2.1.1 Hypothèse monoaminergique

La théorie biologique majeure de la dépression, i.e., la théorie monoaminergique, propose que cette maladie soit due en particulier à une déficience en 5-hydroxytryptamine, sérotonine (5-HT) ou en noradrénaline (NA) au niveau du cerveau (Bunney and Davis, 1965; Schildkraut, 1965; Coppen, 1967). Cette théorie repose sur le fait qu'il a été mis en évidence que le liquide céphalo-rachidien de patients déprimés contient des concentrations plus faibles de neurotransmetteurs (NA, 5-HT ainsi que de son métabolite principal, l'acide 5-hydroxy-indol acétique, 5-HIAA) que celui de patients non déprimés (Bourne et al., 1968; Beskow et al., 1976). D'autres études ont également montré le rôle prépondérant de ces monoamines dans les troubles de l'humeur, ainsi une molécule possédant la capacité d'entraîner une déplétion en monoamine (NA et 5-HT), la réserpine, permet d'induire chez les patients ainsi traités un état dépressif iatrogène (Lemieux et al., 1956); de même, une alimentation en faible teneur de tryptophane (160 mg/jour), l'acide aminé précurseur de la 5-HT, a produit une immédiate, mais réversible, rechute des symptômes dépressifs chez 67% de patients déprimés récemment guéris, mais encore sous traitement par un antidépresseur (Delgado et al., 1990).

Cette hypothèse est également en accord avec le mécanisme d'action des antidépresseurs, puisque ceux-ci permettent une augmentation de la transmission monoaminergique, et donc des concentrations extracellulaires de monoamines au niveau de la fente synaptique, soit en diminuant la dégradation des neurotransmetteurs (inhibiteurs de la monoamine oxydase : IMAO), soit en inhibant leur recapture (Inhibiteurs de Recapture Sélectifs de la Sérotonine : IRSSs, Inhibiteurs de la Recapture de la Noradrénaline : IRN ; de la Dopamine : IRD ou bien Inhibiteurs de la Recapture mixte de la Sérotonine et de la Noradrénaline : IRSN ainsi que les antidépresseurs tricycliques :

TCAs). Ces composés permettant d'améliorer l'humeur des patients souffrant de dépression, il semblait logique que l'activité de ces produits soit liée à la compensation de l'insuffisance de neurotransmission.

L'administration d'antidépresseurs permet d'augmenter les concentrations extracellulaires de monoamines au niveau de la fente synaptique, ce qui se traduit ensuite par une stimulation de l'ensemble des récepteurs postsynaptiques ; soit dans le cas des récepteurs spécifiques de la sérotonine, 14 sous-types.

Même si ceci n'a toujours pas été démontré, il semble probable que les effets thérapeutiques des antidépresseurs, ainsi que certains de leurs effets indésirables, soient liés à l'activation des récepteurs postsynaptiques. En effet, il a été prouvé qu'une déplétion en neurotransmetteurs monoaminergiques provoquerait aussi un fonctionnement anormal des récepteurs des monoamines, se traduisant soit par une modification de la sensibilité (variation d'activité intrinsèque) des récepteurs postsynaptiques, soit par une modification de leur nombre (« up-regulation » ou « down-regulation »). Ainsi, des études postmortem réalisées chez des patients déprimés ont mis en évidence une augmentation de la densité des récepteurs 5-HT_{1A} postsynaptiques et 5-HT_2 (Arora and Meltzer, 1989; Yates et al., 1990) ; traduisant bien le retentissement d'un état dépressif (diminution des concentrations de monoamine) sur l'activité des récepteurs postsynaptiques. Cependant d'autres études tendent à infirmer cette hypothèse, tout au moins pour les récepteurs 5-HT_2 (Cheetham et al., 1988; Meyer et al., 1999). Plus récemment, d'autres auteurs ont mis en évidence une diminution du nombre de récepteurs 5-HT_{2A} seulement au niveau hippocampique (aucune variation n'était observée au niveau des autre structures cérébrales) chez des sujets déprimés (Cheetham et al. 1988; Meyer et al. 1999), cette diminution était plus importante chez les sujets n'ayant pas eu de traitement antidépresseur que chez les malades traités. Ces résultats indiquent donc bien que la densité des récepteurs est fonction de leur stimulation ; dans le cas des récepteurs 5-HT_{2A}, une hypostimulation aboutit à une « down-regulation » du nombre de récepteurs.

1.2.1.2 Hypothèse moléculaire et cellulaire

De récentes études ont permis de caractériser l'influence du stress et des traitements par antidépresseurs sur d'autres substrats biologiques que le système monoaminergique et ses récepteurs (Duman et al., 1997). Une hypothèse neurodégénérative de la physiopathologie de la dépression a été proposée avec comme acteur principal un facteur

neurotrophique, le “ brain-derived neurotrophic facteur ” (BDNF) (Duman et al., 1997; Duman et al., 2001a; Duman et al., 2001b) qui augmente la survie et la croissance des neurones hippocampiques (Sklair-Tavron and Nestler, 1995) jouant ainsi un rôle dans la plasticité neuronale et peut être dans la physiopathologie de la dépression (Jacobs et al., 2000). Cette plasticité neuronale représente la capacité des neurones à se réorganiser entre eux afin d'assurer une réponse aux stimuli environnementaux. Il a été démontré que dans certaines situations, et notamment lors de l'exposition à un stress chronique (stress d'immobilisation, intrusion d'un congénère), on peut provoquer chez l'animal aussi bien une réduction de la neurogénèse cellulaire hippocampique (Smith et al., 1995; Gould et al., 1998) qu'une diminution des projections dendritiques, voire une mort neuronale (Magarinos et al., 1996) ainsi qu'une diminution des facteurs neurotrophiques, comme le BDNF, dans l'hippocampe et le cortex frontal (McEwen, 1999). Des examens postmortem ont permis de retrouver ces altérations chez des patients présentant des troubles dépressifs (MacQueen et al., 2003). Par ailleurs, un traitement chronique par électroconvulsivothérapie ou un traitement chronique par antidépresseurs (désipramine, imipramine, fluoxétine, sertraline), augmentent les concentrations de BDNF et de l'ARNm codant pour son récepteur, le TrkB, dans l'hippocampe (Nibuya et al., 1995; Nibuya et al., 1996). Dans un modèle animal de dépression, le test de résignation acquise chez le Rat, un traitement chronique par antidépresseurs ou par sismothérapie empêche la diminution de l'expression du BDNF induite par le stress (Vollmayr et al., 2001). Parallèlement, la durée d'un épisode dépressif majeur a pu être corrélée à une diminution du volume hippocampique (Sheline et al., 1999; Steffens et al., 2000) chez l'Homme.

Ces données montrent bien le potentiel que peuvent présenter des molécules permettant de stimuler la plasticité neuronale et/ou la neurogénèse dans le traitement des épisodes dépressifs majeurs.

1.2.1.3 Hypothèse endocrinienne

Une relation entre les maladies endocriniennes et la dépression a été notée il y a quelques années et de nombreuses données indiquent un mauvais rétrocontrôle négatif du système endocrinien dans la dépression. Les perturbations du système hormonal dans la dépression sont associées notamment à une hyperactivité de l'axe hypothalamo-hypophyso-adrénocorticotrope (HHA) reliant l'hypothalamus, l'hypophyse et les glandes corticosurrénales (Vetulani and Nalepa, 2000). Alors que la production de cortisol est stimulée par l'hormone adrénocorticotrope (ACTH), elle-même stimulée par deux

neurohormones, la vasopressine et la corticolibérine (CRF : Corticotropin-Releasing Factor), inversement, la production de cortisol freine par rétrocontrôle négatif la production de CRF et d'ACTH. Dans la dépression, il existe une production accrue de cortisol incapable de freiner en retour la production de CRF et d'ACTH (Nemeroff, 1998a; Sheline, 2000). Cette hypercortisolémie observée chez les patients déprimés disparaît lors de l'apparition des effets thérapeutiques des IRSSs (Holsboer, 2000). Certaines études montrent dans le LCR de patients déprimés, des concentrations élevées en CRF (Banki et al., 1987). En clinique, le test de freination à la dexaméthasone permet de se rendre compte de l'hypersécrétion de cortisol. Lors de ce test chez le sujet sain, l'administration de 1 mg de dexaméthasone, cortisone synthétique, freine le CRF, donc la sécrétion d'ACTH et diminue la production de cortisol. Chez presque la moitié des déprimés, l'administration de dexaméthasone n'entraîne pas de chute de la concentration du cortisol plasmatique, indiquant un dysfonctionnement du système de rétrocontrole endocrinien. Des recherches sur les antagonistes des récepteurs au CRF en tant qu'antidépresseur ou anxiolytique sont actuellement menées. La majorité des molécules en développement sont des antagonistes CRF_1 (Nemeroff, 1998b). Lors d'une étude clinique réalisée chez 20 patients atteints d'épisode dépressif majeur, le R121919, un des antagonistes des récepteurs CRF_1 a amélioré l'humeur des malades (Zobel et al., 2000). Des études suggèrent que ce dysfonctionnement de l'axe HHA pourrait être lié à un dysfonctionnement des récepteurs $\alpha 2$ adrénergiques (Mokrani et al., 1997). Il a également été démontré que l'administration d'une dose unique d'IRSSs induit une stimulation de l'axe HHA, alors qu'en administration chronique, ces mêmes IRSSs induisent une désensibilisation de l'axe HHA (Li et al., 1993; Jensen et al., 2001), plusieurs études ont permis de mettre en évidence que ces effets des IRSSs pourraient être transmis par l'activation des récepteurs 5-HT_{1A} et 5-HT_{2A}, car l'administration aigue d'agonistes sélectifs de ces récepteurs (respectivement le 8-OH DPAT et le DOI) induit une élévation des niveaux plasmatiques de cortisol (Pan and Gilbert, 1992; Matheson et al., 1997; Mikkelsen et al., 2004). Ces résultats ne sont pas incompatibles avec ceux suggérant l'implication des récepteurs $\alpha 2$ adrénergiques, car des études de microdialyse réalisées chez le Rat ont montré que l'activation des récepteurs 5-HT_{1A} augmente la libération de noradrénaline dans certaines aires cérébrales telles que l'hippocampe, le cortex frontal, l'aire ventrale tegmental et l'hypothalamus (Done and Sharp, 1994; Chen and Reith, 1995; Suzuki et al., 1995).

1.2.1.4 Hypothèse tachykininergique

Il existe une autre hypothèse physiopathologique de la dépression selon laquelle entre en jeu une classe de peptides neuromodulateurs, les neurokinines ou tachykinines comprenant la substance P, la neurokinine A et la neurokinine B. Celles-ci se lient respectivement aux récepteurs NK1, NK2 et NK3 mais elles présentent la particularité de se fixer et d'activer chaque sous-type de récepteurs avec des activités distinctes. Plus récemment, une quatrième tachykinine a été identifiée et clonée chez le Rat, l'hémokinine 1 (HK-1) (Zhang et al., 2000) qui se lie préférentiellement aux récepteurs NK1 (Bellucci et al., 2002). Les neurokinines sont présentes dans différentes aires cérébrales riches en monoamines, suggérant un rôle de régulation potentielle de ces neuropeptides sur les neurotransmissions monoaminergiques. Le rôle de la substance P dans l'induction de la dépression a été suggéré suite à l'observation du fait que l'administration chronique d'un antidépresseur réduit les concentrations de substance P dans le striatum, la substance noire et l'amygdale (Shirayama et al., 1996). Il a d'ailleurs été proposé que la cible thérapeutique potentielle des antagonistes des récepteurs NK_1 se situerait dans l'amygdale, une région riche en fibres tachykininergiques et fortement impliquée dans la régulation de l'humeur (Boyce et al., 2001). De plus, il a récemment été démontré chez des souris n'exprimant pas de manière constitutive le récepteur NK_1 (souris KO NK_1-/-), que le récepteur 5-HT_{1A} était désensibilisé, comme après un traitement chronique par IRSS (Froger et al., 2001; Santarelli et al., 2001). Bien que l'évaluation de l'efficacité de certains antagonistes NK_1 soit restée pendant très longtemps difficile chez les rongeurs du fait de leur faible affinité pour le récepteur de ce ligand, de nouveaux antagonistes confirment l'efficacité de ces molécules dans la dépression. Le L-733060, un antagoniste du récepteur NK-1, présente par exemple une efficacité comparable à celle des antidépresseurs sur le stress provoqué par la séparation de jeunes cobayes de leur mère (Kramer et al., 1998). Cette activité de type antidépresseur du L-733060 a été retrouvée lors d'une vaste étude préclinique utilisant différents antagonistes des récepteurs NK1 (L-742,94, CP-99,994, CP-122,721 ou MK-869) sur un modèle animal prédictif de l'efficacité clinique d'un antidépresseur, le test de suspension caudale (ou Tail Suspension Test, TST) chez la gerbille (Varty et al., 2003). L'évaluation clinique du compose MK869 chez des sujets anxieux atteints de troubles dépressifs majeurs, a révélé que le blocage chronique des récepteurs NK1 était favorable à une amélioration des troubles de l'humeur avec un délai comparable à celui des IRSS (Kramer et al., 1998; Ranga and Krishnan, 2002). Le deuxième essai clinique réalisé avec un antagoniste des récepteurs NK1, le

L759274 a également permis de mettre en évidence une amélioration de l'humeur, chez les patients traités, significativement plus importante par rapport au groupe placebo (Kramer et al., 2004). Alors que Kramer et ses collaborateurs pensaient que les antagonistes des récepteurs NK1 agissaient sans interférer sur les monoamines, différentes études d'électrophysiologie et de microdialyse ont permis de mettre en évidence d'importantes interactions entre les récepteurs NK1 / 5-HT1A d'une part (Froger et al., 2001; Santarelli et al., 2001) et NK1 / alpha-2 (Haddjeri and Blier, 2000). En effet, différentes approches génétiques et pharmacologiques révèlent que les antagonistes des récepteurs NK1, auraient un mécanisme d'action commun avec les IRSS car ils augmentent la neurotransmission 5-HT et NA en désensibilisant respectivement les autorécepteurs 5-HT1A et alpha-2. A ce jour le développement de tels composés est progressivement abandonné par les compagnies pharmaceutiques car l'amplitude de leurs effets observés lors des études cliniques n'ont pas permis de reproduire les résultats obtenus en phase II.

Toutefois, de récents travaux de microdialyse intracérébrale in-vivo réalisés chez la souris vigile ont permis de relancer l'intérêt des antagonistes des récepteurs NK1 dans le traitement de la dépression. En effet, il a été montré que les antagonistes des récepteurs permettent de potentialiser les effets neurobiochimiques des IRSSs (Guiard et al., 2004; Guiard et al., 2005). La pertinence de l'intérêt de cette coadministration reste à démontrer, puisqu'à ce jour, aucune étude n'a été réalisée pour montrer les effets comportementaux ou cliniques résultant de la coadministration IRSS (ou autre antidépresseur) et antagonistes des récepteurs NK1, et ce malgré les résultats prometteurs de microdialyse.

1.2.1.5 Relations entre ces différentes hypothèses

L'étude de la bibliographie permet de se rendre compte que chacune de ces hypothèses possède un fondement scientifique bien documenté, et qu'il existe des relations entre chacune :

Relation entre hypothèse monoaminergique et hypothèse neurodégénérative

Des études ont montré que le traitement chronique par antidépresseur permettait non seulement une augmentation des concentrations extracellulaires de monoamines chez l'animal, mais également une augmentation des concentrations de BDNF ainsi que de l'ARNm codant pour son récepteur (le TrkB) permettant ainsi de retrouver une densité de récepteurs proche de celle retrouvée chez des personnes saines.

Relation entre hypothèse monoaminergique et hypothèse endocrinienne

Il est maintenant clairement établi que l'hypercortisolémie observée chez les patients déprimés disparaît lors de l'apparition des effets thérapeutiques des IRSSs (Holsboer, 2000), et que lors d'un traitement chronique, ces mêmes IRSSs induisent une désensibilisation de l'axe HHA (Li et al., 1993; Jensen et al., 2001).

Relation entre hypothèse monoaminergique et hypothèse tachykininergique

L'administration d'une dose de paroxétine permet d'entraîner une augmentation des concentrations extracellulaires de sérotonine, et cet effet peut être potentialisé par l'utilisation d'antagonistes des récepteurs NK1, même si ceux-ci n'ont pas d'effets propres sur la libération de 5-HT (Guiard et al., 2004). Il a également été démontré que les souris dépourvues du gène codant pour le récepteur NK1 présentaient au niveau comportemental et neurobiochimique une altération du récepteur NK1 similaire à celle observée chez des souris recevant un antidépresseur de façon chronique (pour une revue de la littérature sur l'intéraction monoamine substance P voir Adell, 2004).

Relation entre hypothèse neurodégénérative et hypothèse endocrinienne

Chez l'animal, l'administration de corticostérone entraîne une diminution de la neurogénèse en diminuant les taux de BDNF (Yu et al., 2004), ce qui montre bien le rétrocontrôle effectué par l'axe HHA sur la neurogénèse.

Relation entre hypothèse neurodégénérative et hypothèse tachykininergique

Chez l'animal, le blocage constitutif (souris mutées génétiquement ne possédant plus le gène codant pour le récepteur NK1), ou pharmacologique (de façon chronique à l'aide d'un antagoniste) des récepteurs NK1, permet d'obtenir une augmentation des concentrations cérébrales de BDNF. Par contre les antidépresseurs ne permettent pas d'obtenir une augmentation du BDNF au niveau hippocampique chez des animaux dépourvues du gène codant pour le récepteur NK1, le taux de BDNF hippocampique étant déjà deux fois supérieur à celui observé chez des souris sauvages (Morcuende et al., 2003).

1.2.2 Les antidépresseurs

Il existe aujourd'hui au moins trois classifications différentes permettant de regrouper les antidépresseurs selon différents critères que sont leur structure chimique, leur activité

thérapeutique ou leur mécanisme d'action central. Cette dernière a l'avantage de regrouper les antidépresseurs en grandes classes selon les mécanismes d'action biochimique des molécules. On peut ainsi classer les médicaments antidépresseurs en différentes catégories selon la modulation de la transmission monoaminergique impliquée et leur affinité spécifique pour un des transporteurs de monoamines (Frazer, 2001). Cette classification permet en cas d'échec thérapeutique de changer de famille d'antidépresseurs bien que le mécanisme d'action des molécules ne résume pas leur pouvoir thérapeutique. La première catégorie comprend les antidépresseurs qui augmentent sélectivement la transmission sérotoninergique, on y retrouve tous les inhibiteurs du recaptage sélectifs de la sérotonine (IRSSs). La seconde catégorie regroupe les antidépresseurs qui augmentent sélectivement la transmission noradrénergique, dans laquelle on classe les inhibiteurs sélectifs du recaptage de la noradrénaline (IRN). La troisième regroupe les antidépresseurs d'action mixte qui augmentent simultanément les transmissions sérotoninergiques et noradrénergiques (IRSN). Du fait d'un mécanisme d'action différent, les IMAOs qui augmentent eux aussi la transmission monoaminergique ont été classés dans une catégorie à part entière. Enfin, des molécules comme le bupropion qui inhibe principalement la recapture de la dopamine ou la trazodone (antidépresseur dit « atypique »), ont été regroupées dans une quatrième classe (IRD).

Afin de restaurer les concentrations en monoamines au niveau de la fente synaptique dans le SNC, les antidépresseurs utilisent trois principaux mécanismes d'action basés sur l'inhibition de la MAO, l'inhibition de la recapture et enfin le blocage d'un récepteur (Artigas et al., 2002).

Après la mise sur le marché des premiers inhibiteurs de la MAO, non sélectifs d'une monoamine, la découverte des deux formes A et B de la monoamine oxydase, différant l'une de l'autre par l'affinité préférentielle de la forme A pour la NA et la 5-HT et de la forme B pour la DA, a conduit à la commercialisation d'IMAOs réversibles et sélectifs de la monoamine oxydase A ou B.

L'action de la molécule sur une cible thérapeutique autre que l'enzyme de dégradation, le transporteur monoaminergique, a permis de commercialiser de nouvelles classes innovantes de médicaments antidépresseurs. Ces molécules dont le mécanisme d'action est fondé sur l'inhibition du recaptage des monoamines qu'il s'agisse de la NA, de la 5-HT ou de la DA reste le plus fréquemment retrouvé. On a d'abord proposé l'inhibition de la recapture de la sérotonine et de la noradrénaline "induite" par les imipraminiques,

lesquels exerçaient également un effet au niveau d'autres monoamines tels que l'histamine, avec pour conséquence un effet de somnolence. Plus tard, est apparue la notion de sélectivité et des molécules "plus spécifiques", n'agissant que sur la recapture de la noradrénaline, telles que la désipramine ou la maprotiline. Puis, la génération des IRSSs a fait son apparition (fluoxétine, citalopram, paroxétine, fluvoxamine et sertraline). Il existe enfin des molécules qui agissent essentiellement sur l'inhibition du recaptage de la dopamine, telle que le bupropion, ce dernier étant commercialisé comme antidépresseur, mais seulement aux États-Unis. L'effet thérapeutique des derniers antidépresseurs développés résulte d'une action simultanée sur plusieurs grands systèmes de neurotransmission. Ainsi, sont apparues sur le marché des molécules agissant à la fois sur les voies noradrénergiques et sérotoninergiques en inhibant aussi bien la recapture de la NA que la 5-HT (milnacipran et venlafaxine). Enfin, il existe un mécanisme d'action des antidépresseurs distinct des deux précédents, fondé sur le blocage de certains sous-types de récepteurs sérotoninergiques ou noradrénergiques (i.e. antagonisme des récepteurs $\alpha 2$ présynaptiques : mianserine et mirtazapine).

Ces dernières années, les découvertes de nouveaux médicaments ont permis aux thérapeutes de disposer de médicaments induisant moins d'effets indésirables que ceux liés au traitement par les IMAOs et les tricycliques (principalement une diminution de la cardiotoxicité). Avec pour conséquence, une meilleure compliance des patients ainsi qu'une meilleure efficacité globale des traitements. Par ailleurs, des relations dose/réponse pour certains antidépresseurs commencent à être établies. L'existence de relations effet/dose a longtemps fait défaut en psychiatrie, privant les patients d'importants bénéfices potentiels. Avec les antidépresseurs tricycliques en particulier, une augmentation importante de la dose conduit à une inefficacité clinique chez l'Homme suivant une courbe effet/dose en U. Aujourd'hui, la preuve d'une relation effet/dose a été établie pour deux molécules : la paroxétine et la venlafaxine. De même que la paroxétine (Redrobe et al., 1998b), la venlafaxine est à faible dose, inhibitrice du recaptage de la sérotonine et lors de l'augmentation de la dose, inhibitrice du recaptage de la noradrénaline (Redrobe et al., 1998a; David et al., 2003). On considère ainsi qu'à partir de 150 mg/j et plus, la venlafaxine inhibe principalement le recaptage de la noradrénaline (Frazer, 2001). De même pour la paroxétine passer de 20 à 40 mg/jour augmente nettement la proportion de patients répondeurs. Cette augmentation de dose est certes limitée par la survenue d'effets indésirables, mais la relation effet/dose permet de proposer au patient une augmentation de posologie et d'éviter des changements de traitements précoces. Malgré ces avancées deux problèmes majeurs demeurent dans le

traitement des maladies dépressives : une importante proportion de patients (environ 30 %) est non répondeur et un trop long délai d'action est nécessaire pour observer une amélioration de l'humeur chez les patients traités. Ce délai entre le début du traitement et l'apparition de l'effet thérapeutique serait associé à la désensibilisation d'au moins un des deux sous-types d'autorécepteurs sérotoninergiques (Blier and de Montigny, 1994) qui peuvent avoir une localisation soit somatodendritique (5-HT_{1A}), soit sur les terminaisons neuronales (5-HT_{1B}). Une stratégie thérapeutique envisagée pour réduire ce délai d'action, est d'agir directement sur un de ces deux sous types ; ceci a notamment été mis en évidence en clinique avec l'utilisation du (-) pindolol qui agirait sur les récepteurs 5-HT_{1A} (Artigas et al., 1994; Blier and Bergeron, 1995).

1.3 Le système sérotoninergique

La 5-HT est une indolamine qui est retrouvée aussi bien au niveau périphérique que central où elle représente environ 2% de la 5-HT corporelle. La 5-HT périphérique est synthétisée par les cellules entérochromaffines de l'intestin tandis la 5-HT retrouvée au niveau du système nerveux central doit être synthétisée *in situ* car elle ne franchit pas la barrière hémato-encéphalique (BHE). La biosynthèse enzymatique de la 5-HT s'effectue à partir d'un acide aminé précurseur, le L-tryptophane apporté par l'alimentation puis véhiculé vers le SNC par un transporteur commun à d'autres acides aminés neutres à longue chaîne (Fernstrom and Wurtman, 1972; Young et al., 1977). Au niveau du SNC, le L-tryptophane est métabolisé en 5-hydroxytryptophane (5-HTP) par la tryptophane hydroxylase, enzyme dont il existe 2 isoformes : la Tph1 est retrouvée au niveau périphérique (duodénum) tandis que l'isoforme Tph2 est exprimée dans le SNC (Walther and Bader, 2003; Walther et al., 2003). Cette réaction d'hydroxylation constitue l'étape limitante de la synthèse de 5-HT. Le 5-HTP formé est ensuite décarboxylé par une enzyme pour donner la 5-HT sous sa forme finale (voir figure 1). La sérotonine est ensuite stockée dans les vésicules synaptiques où elle demeurera jusqu'à sa libération. Celle-ci fait intervenir deux modes physiologiques opposés, l'un calcium (Ca^{2+})-dépendant (exocytose) et l'autre Ca^{2+}-indépendant. L'activité de la sérotonine est exercée au niveau post synaptique par l'interaction avec ses récepteurs ; la concentration de sérotonine au niveau de la fente synaptique est fonction de deux paramètres qui sont : la recapture du neurotransmetteur via un transporteur cellulaire sélectif (SERT) qui peut recapter jusqu'à 80% de la sérotonine libérée, et la catabolisation de la sérotonine sous forme d'acide 5-Hydroxyindolacétique (5-HIAA) par la monoamine oxydase intramitochondriale qui est ensuite éliminé par voie urinaire.

De nombreuses études ont permis de cartographier les voies sérotoninergiques (voir figure 1) en utilisant différentes méthodes: histochimie de fluorescence (Dahlstrom and Fuxe, 1964), immunohistochimie (Steinbusch, 1981; Steinbusch et al., 1981). Les neurones sérotoninergiques sont restreints à des groupes de cellules autour de la ligne du pont et du cerveau moyen supérieur, numérotés de B1 à B9 (voir tableau 1) correspondant aux noyaux du raphé (Dahlstrom and Fuxe, 1964). Les groupes de cellules les plus caudales dans le raphé se projettent majoritairement dans la medulla et la moelle épinière par des voies descendantes. Les cellules les plus rostrales du raphé dorsal (B7) (NRD) et médian (B8) se projettent dans les structures limbiques comme l'hippocampe et le cortex par des voies ascendantes.

Au niveau du raphé, les voies sérotoninergiques sont sous la dépendance d'autres systèmes monoaminergiques (GABA, dopamine,...) via de nombreux hétérorécepteurs (α2, α1, $GABA_A$, …) mais également sous la dépendance d'autorécepteurs sérotoninergiques (5-HT_{1A} et 5-HT_{1B}) (voir figure 2); d'où l'importance de l'étude de ces autorécepteurs dans l'étude du mécanisme d'action des antidépresseurs puisqu'ils exercent un rétrocontrôle sur le libération de sérotonine. De plus, il existe de nombreux tissus cérébraux dans lesquels ces autorécepteurs sont colocalisés (voir figure 5).

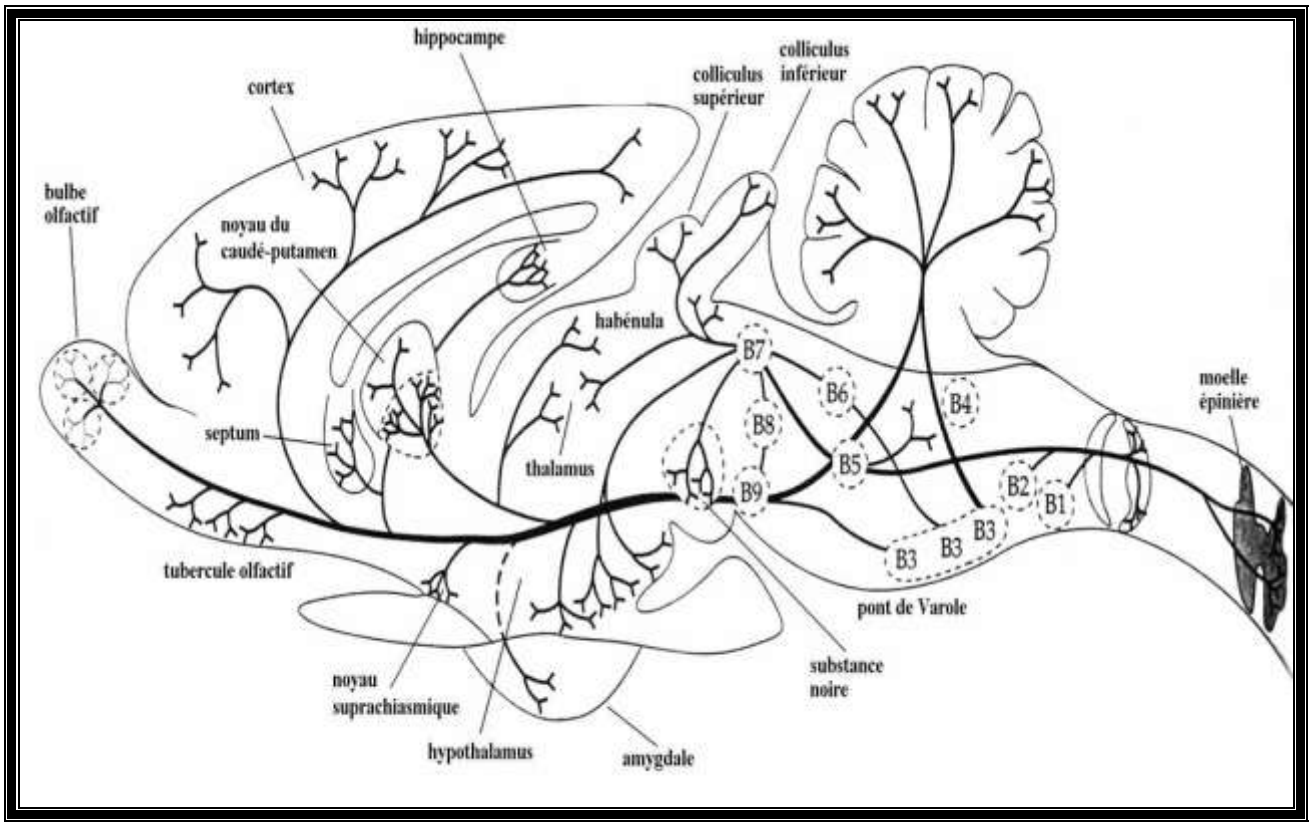

Figure 1 : Représentation schématique de l'innervation sérotoninergique du Système Nerveux Central du Rat

Les corps cellulaires des neurones sérotoninergiques sont regroupés essentiellement en deux noyaux localisés dans la région sagittale du tronc cérébral numérotés de B1 à B9. Les fibres qui en partent se projettent dans l'ensemble de l'encéphale et de la moelle épinière. (Steinbusch 1981)

Nomenclature des noyaux du raphé

	Groupe	Structure anatomique
Noyaux caudaux	B1	Raphé pallidus
	B2	Raphé obscurus
	B3	Raphé magnus
	B4	Substance grise périventriculaire
		Area postrema
Noyaux rostraux	B5	Raphé medianis caudal
		Raphé pontis
	B6	Raphé dorsalis caudal
	B7	Raphé dorsalis rostral
	B8	Raphé medianis rostra
		Noyau caudalis linearis
	B9	Raphé pontis oralis
		Région supralemiscale

Tableau 1 : Nomenclature des noyaux du raphé

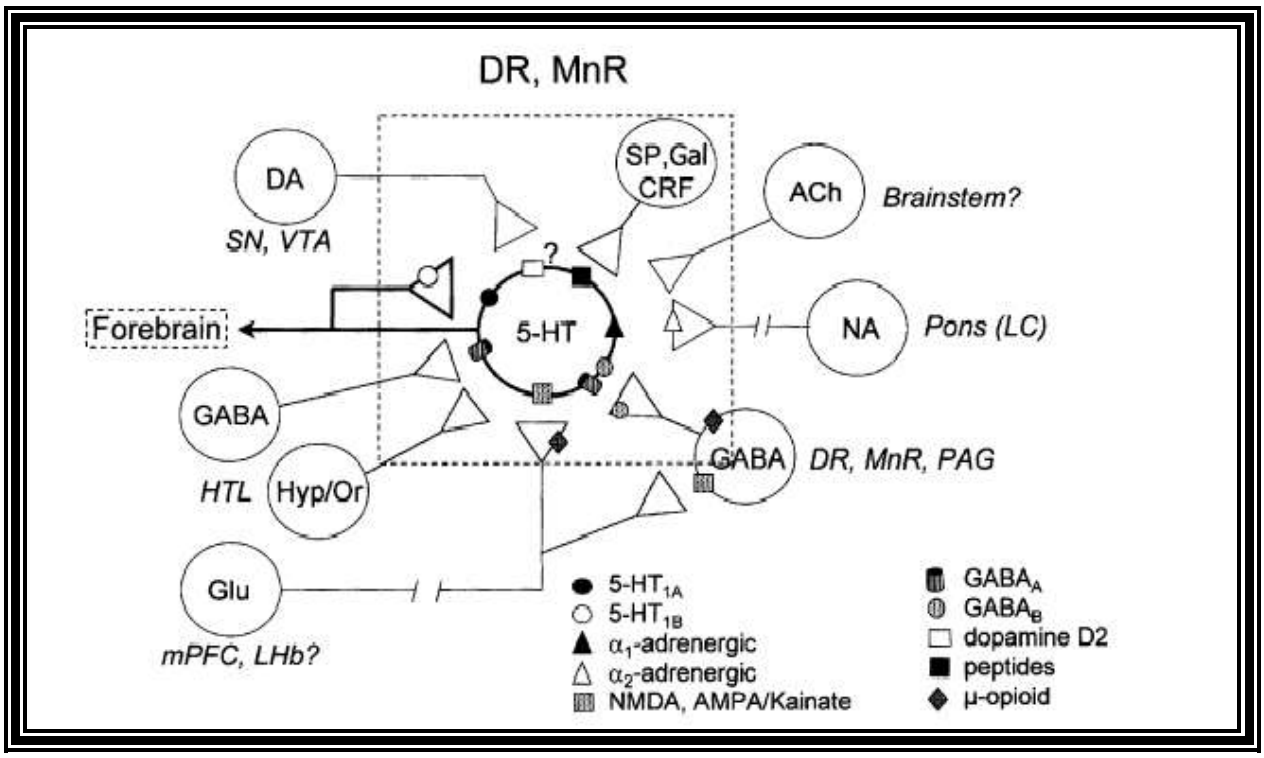

Figure 2 : Représentation schématique des principaux tissus cérébraux et des monoamines correspondantes impliqués dans la régulation des neurones sérotoninergiques au niveau du raphé.

Les aires cérébrales principales sont : le locus coeruleus (LC), le cortex prefrontal médian (mPFC), l'habenula latérale (LHb), la substance noire (SN), la matière grise périacquedutal (PAG), l'aire ventrale tegmentale (VTA) le raphé dorsal (DR) et médian (MnR) ainsi que différentes aires hypothalamiques (HTL). Les principaux neurotransmetteurs sont la noradrénaline (NA) (LC), le glutamate (Glu) (depuis le mPFC et probablement depuis la LHb), le GABA (hypothalamus, PAG), la dopamine (DA) provenant de la SN et de la VTA et probablement du DR (non montré sur le schéma) et l'acétylcholine (Ach).
Un contrôle peptidergique des neurones sérotoninergique est possible aussi bien localement (substance P (SP), galanine (GAL), corticotrophin-releasing factor (CRF)) que depuis des zones distantes (hypocretine/orexine (Hyp/Or), depuis l'HTL).

(Adell et al. 2002)

Il faut noter que du fait de sa distribution diffuse dans l'encéphale (Steinbusch 1981) et du grand nombre de ses récepteurs (Hoyer et al., 1994; Barnes and Sharp, 1999; Hoyer et al., 2002), le système sérotoninergique central est impliqué dans le contrôle de nombreuses fonctions physiologiques : respiration, régulation des comportements alimentaires, thermorégulation, vigilance, anxiété, dépression, nociception, régulation endocrinienne, agressivité, comportements sexuels, processus d'apprentissage et de mémoire, motilité intestinale et plus généralement les muscles lisses (bronches, utérus) et enfin dans la régulation de l'activité nerveuse sympathique et parasympathique des vaisseaux du cœur et enfin dans des processus pathologiques (épisodes dépressifs majeurs, anxiété, schizophrénie, troubles obsessionnels conpulsifs et certaines pathologies neurodégénératives). Ceci peut être relié à la grande diversité de types et de sous-types de récepteurs (16 identifiés à ce jour).

De ce fait, la variation de concentration extracellulaire de sérotonine (i.e. lors de l'administration d'IRSSs) s'accompagne de modifications physiologiques permettant d'expliquer les effets indésirables et thérapeutiques des antidépresseurs. Ainsi, il a été montré que l'activation des récepteurs sérotoninergiques situés dans le cortex préfrontal, l'hippocampe et l'amygdale permet une amélioration de l'humeur ; l'amélioration des troubles obsessionnels compulsifs serait quand à elle liée à l'activation des récepteurs situés au niveau des ganglions de la base. L'activité des IRSSs dans le traitement des troubles anxieux semble liée à l'activation des voies sérotoninergiques projetant du raphé vers l'hippocampe et le cortex limbique. Les phénomènes d'acathésie et d'agitation (traitement par neuroleptique ou IRSSs) seraient portés par le système sérotoninergique localisé dans les ganglions de la base alors que le contrôle central de la nausée et du vomissement s'effectue dans l'hypothalamus et tronc cérébral (via les récepteurs 5-HT3). L'augmentation de l'activité sérotoninergique au niveau du tronc cérébral (centre du sommeil) permet d'expliquer les troubles du sommeil observés lors d'un traitement par IRSS. L'activation des récepteurs 5-HT3 situés au niveau du tube permet d'expliquer les troubles gastro-intestinaux consécutifs à une augmentation des concentrations de sérotonine. L'ensemble de ces effets des IRSSs sont repris dans un article de Stephen Stahl publié en 1998 dans *Journal of Affective Disorders*.

Les récepteurs à la sérotonine sont à l'heure actuelle, classés en deux catégories distinctes :

- récepteurs ionotropiques
- récepteurs métabotropiques formés de 7 domaines transmembranaires.

Cette dernière catégorie est elle-même subdivisée en deux groupes suivant que la protéine G est couplée à une phospholipase C ou à l'AMPcyclique.

Cette nomenclature permet de classer les 16 sous-types de récepteurs de la 5-HT en sept familles: 5-HT_1 (1A, 1B/1D, 1E, 1F) ; 5-HT_2 (2A, 2B, 2C) ; 5-HT_3 (3A, 3B, 3C), 5-HT_4, 5-HT_5 (5A, 5B), 5-HT_6 et 5-HT_7 (voir figure 3). Parmi ces récepteurs, les récepteurs 5-HT_{1A} et 5-HT_{1B} peuvent avoir une localisation pré et postsynaptique, de nombreuses études ont permis de définir le rôle de chacun, tant au niveau neurobiochimique, qu'au niveau comportemental. Compte tenu de la forte colocalisation de ces récepteurs (voir figure 4), de la forte analogie structurale ainsi que du manque de ligands spécifiques, cette différenciation du rôle de chacun des autorécepteurs est récente.

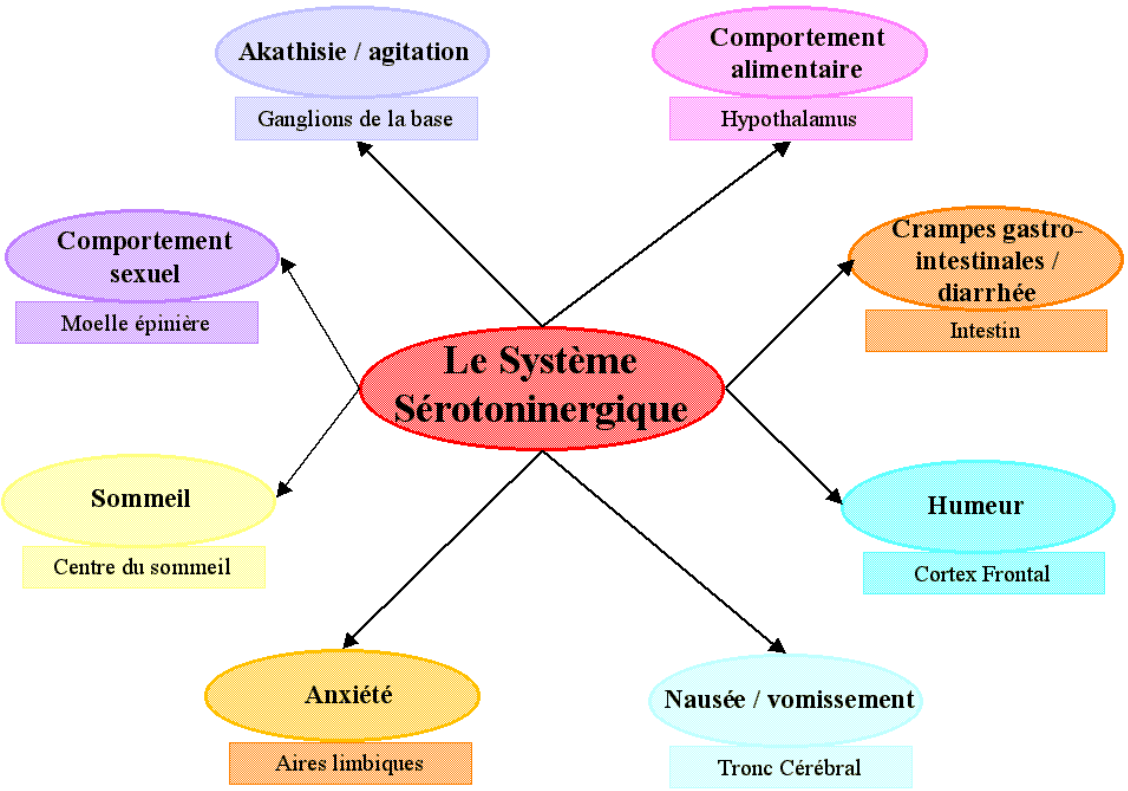

Figure 3 : Structures cérébrales impliquées dans les effets de la sérotonine
La sérotonine exerce des effets variés tant au niveau du système périphérique que central.
D'après Stahl, 2002

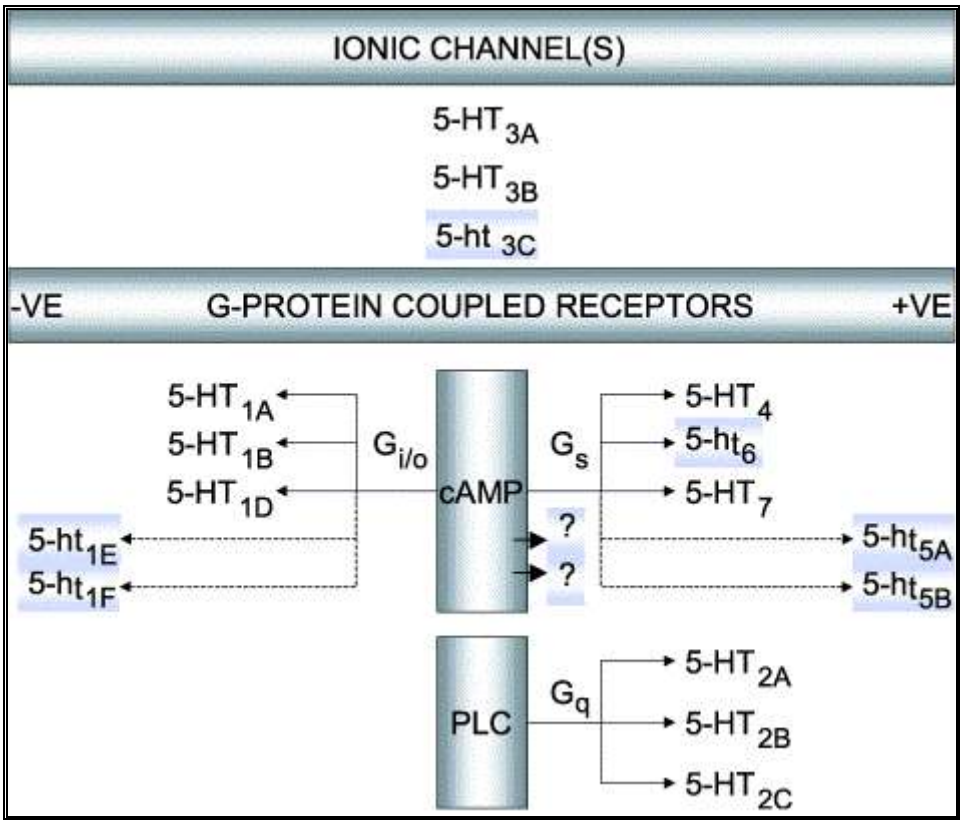

Figure 4 Représentation graphique de l'actuelle classification des récepteurs sérotoninergiques et de leur couplage.
Les récepteurs dont le nom est surligné sont ceux pour lesquels le rôle physiologique reste à définir.
(Hoyer et al. 2002)

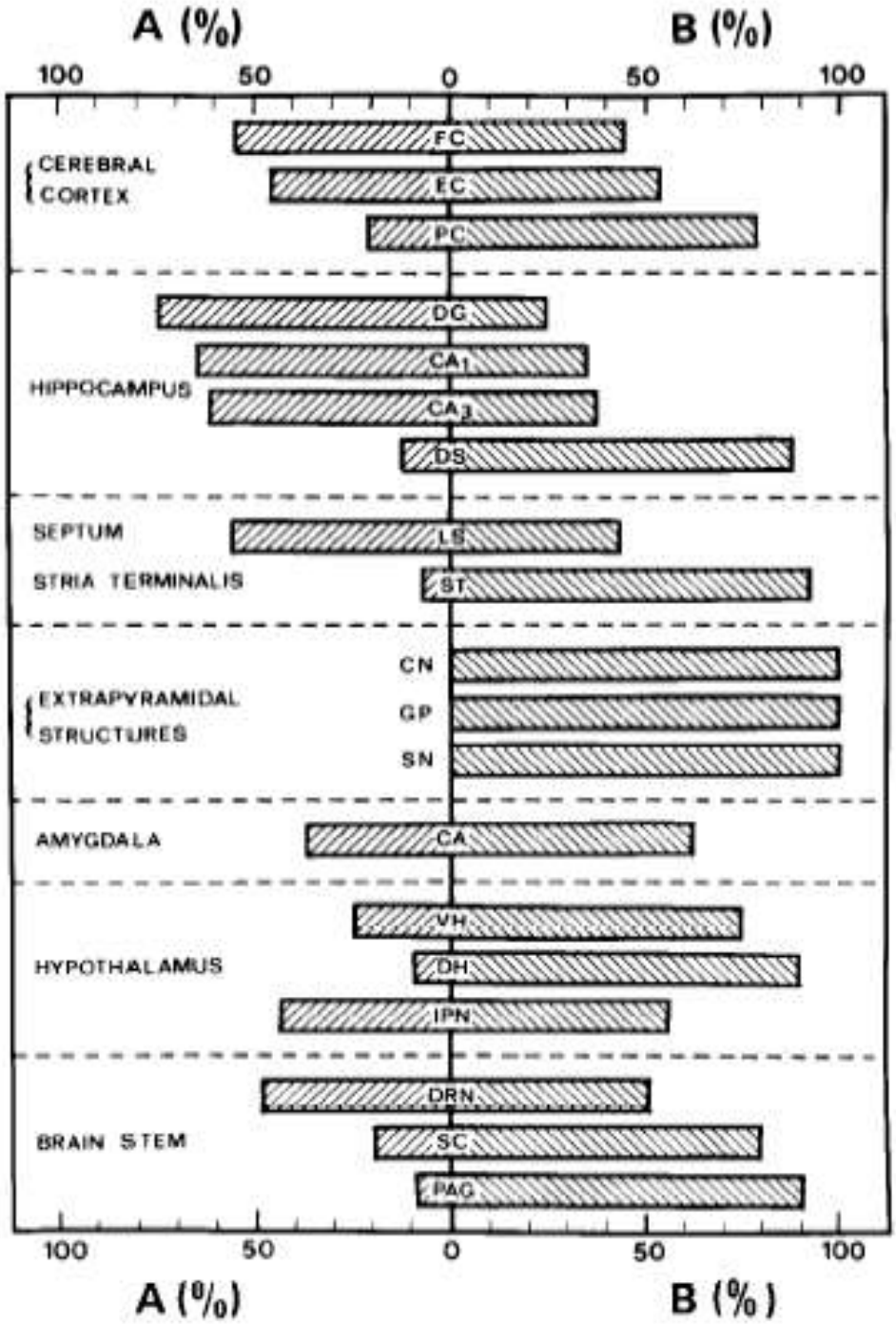

Figure 5 : Représentation schématique de la proportion de chacun des autorécepteurs 5-HT_1 (A et B) dans différentes aires cérébrales, exprimés en pourcentage de liaison total de [^{3}H]5-HT aux récepteurs 5-HT_1.

Les aires cérébrales étudiées sont le cortex frontal (FC), enthorinal (EC) et occipital (PC) ; le gyrus denté (DG), les régions CA1 et CA3, le subiculum dorsal, le noyau caudé (CN), le globus pallidus (GP), la substance noire (SN), l'hypothalamus ventral (VH) et dorsal (DH), le noyau interpedunculaire (IPN)le raphé dorsal (DRN), le colliculus supérieur (SC) et la matière grise périaqueductal (PAG).
(Verge et al. 1986)

1.4 Le récepteur 5-HT_{1B}

1.4.1 Généralités

Classification :

L'actuelle classification des récepteurs de la sérotonine distingue le récepteur 5-HT_{1B} de Rat du récepteur humain, précédemment nommé 5-$HT_{1D\beta}$ par les préfixes « r » pour Rat et « h » pour Homme (pour revue voir Hoyer et al., 2002). Le récepteur 5-HT_{1B} humain est composé de 390 acides aminés, et le gène codant pour ce récepteur est porté par le chromosome 6 (Demchyshyn et al., 1992; Hamblin et al., 1992; Jin et al., 1992; Trumpp-Kallmeyer et al., 1992; Weinshank et al., 1992) caractérisé par une homologie de plus de 90% avec le récepteur 5-HT_{1B} de Rat (Jin et al., 1992; Metcalf et al., 1992) qui est composé de 386 acides aminés (Voigt et al., 1991; Adham et al., 1992; Maroteaux et al., 1992). Seuls 32 acides aminés diffèrent entre ces récepteurs, parmi lesquels 8 sont situés dans le domaine transmembranaire (Findlay and Eliopoulos, 1990; Trumpp-Kallmeyer et al., 1992). Les différences pharmacologiques entre ces deux homologues résident principalement dans la mutation d'un seul acide aminé, l'acide aspartique (Asp^{123}) chez l'homme en arginine (Arg^{123}) chez le rongeur au niveau du $7^{ème}$ domaine transmembranaire. Cette mutation confère au récepteur de rat une affinité pour les β-bloquants tels que le pindolol (Guan et al., 1992) qui n'est pas retrouvée avec le récepteur humain. La modification génétique du récepteur humain (remplacement de l'acide aspartique par un résidu thréonine) permet de donner au récepteur humain une affinité pour les β-bloquants (Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993).

Localisation :

Les études d'autoradiographie, utilisant des molécules marquées comme la [^{3}H]-5-HT en présence de 8-OH-DPAT et du [^{125}I]-cyanopindolol et d'isoprénaline démontrent que le sous-type de récepteur sérotoninergique 5-HT_{1B} est retrouvé, chez le Rat, dans les **ganglions de la base** et particulièrement dans la **substance noire, le globus pallidus, le pallidum ventral** et les **noyaux entopédonculaires** (Pazos and Palacios, 1985; Verge et al., 1986; Bruinvels et al., 1993) ainsi qu'au niveau des **noyaux du raphé** (Pazos and Palacios, 1985; Verge et al., 1986).

Les études d'hybridation *in situ* ont permis de localiser l'ARNm codant pour les récepteurs 5-HT_{1B} dans les **noyaux du raphé médian et dorsal** (Boschert et al., 1994; Bruinvels et al., 1994; Doucet et al., 1995; Bonaventure et al., 1998), ARNm qui semble largement réduit par la lésion des fibres 5-HT par une neurotoxine, la 5,7-DHT (Doucet et al., 1995), indiquant que ces autorécepteurs situés sur les terminaisons neuronales sérotoninergiques contrôlent localement la libération de 5-HT (Engel et al., 1986; Hen, 1992; Pineyro and Blier, 1999) et que les protéines codant pour ces récepteurs sont synthétisées localement dans les cellules sérotoninergiques. De plus, les études utilisant les neurotoxines rendent indétectable la fixation de ligand radiomarqué au niveau du raphé, indiquant que les récepteurs présents dans cette structure sont des autorécepteurs. Récemment, chez l'Homme, une étude post-mortem utilisant la technique d'hybridation *in situ* a permis de retrouver de l'ARNm codant pour le récepteur 5-HT_{1B} dans le **noyau du raphé dorsal** (Bidmon et al., 2001). Certaines aires du cerveau antérieur de Rat, comme le **striatum**, contenant une grande quantité de sites de liaison exprime aussi l'ARNm du récepteur. Cependant, la distribution régionale du récepteur 5-HT_{1B} chez le Rat ne semble pas correspondre à celle de son ARNm. Des aires cérébrales comme la **substance noire, le globus pallidus ou les noyaux entopédonculaires** qui présentent elles aussi une densité importante de récepteurs, ont de faibles quantités d'ARNm. Dans l'**hippocampe**, l'ARNm du récepteur 5-HT_{1B} est localisé dans les corps cellulaires des cellules pyramidales de la région CA1, tandis que la protéine est présente dans la zone de projection des neurones CA1 pyramidaux (Boschert et al., 1994). La même étude montre que l'ARNm est exprimé dans les cellules de Purkinje dans lesquelles aucun site de liaison n'a été retrouvé. Les données anatomiques obtenues chez le Rat indiquent donc que le récepteur 5-HT_{1B} est largement exprimé dans le cerveau soit en tant qu'autorécepteurs contrôlant la libération de sérotonine, soit en tant qu'hétérorécepteurs contrôlant la libération d'autres neurotransmetteurs. Une étude d'hybridation *in situ* réalisée chez le Rat montre que la 5,7 DHT, en détruisant

l'innervation sérotoninergique présynaptique, affecte la quantité d'ARNm codant pour le récepteur 5-HT_{1B} présente dans le noyau du raphé dorsal, tandis que le signal d'hybridation reste inchangé sur les neurones postsynaptiques (Neumaier et al., 1996b). La même neurotoxine provoque une augmentation de la densité des récepteurs 5-HT_{1B} dans les ganglions de la base chez le Rat pour une déplétion en 5-HT d'au moins 95% (Compan et al., 1998). La cartographie des récepteurs 5-HT_{1B} par immunohistochimie a confirmé la présence de l'hétérorécepteur 5-HT_{1B} puisqu'il est retrouvé en densité très importante dans le globus pallidus et la substance noire (Langlois et al., 1995).

Structure	Récepteur 5-HT1B (Autoradiographie)	Récepteur 5-HT1B (Immunohistochimie)	ARNm codant pour le récepteur 5-HT1B (hybridation in situ)
Cervelet			
Stratum granulosum			-
Cellules de Purkinje	- c	- c	X b,c,g
Cortex cerebral	X d	X i	
Frontal	X d		X b
Piriform			X b
Occipital		X i	X b
Parietal		X i	X b
Ganglions de la base			
Noyau accumbens	X a,f		Xb
Bed nucleus			X
Striatum	X f	X i	X g
Caudate putamen	X a,f	X i	X b,c ,e
Substance noire	X a,c	X h,i	- b,c
Noyaux subthalamiques			X
Aire amygdalohippocampique			X
Noyau corticoamygdalien			X
Hippocampe			
Cellules pyramidales CA1	X b	X i	X b,c,e,g
CA3			-
Gyrus denté			-
Système olfactif			
Bulbe olfactif			-
Noyau olfactif antérieur			X b
Tubercule olfactif			X
Raphé			
Dorsal			X b,c,e,g
Médian			+/- b,c,e,g
Hypothalamus	X d		X b

Tableau 2 : Localisation du récepteur 5-HT_{1B} et de l'ARNm codant pour ce récepteur dans différentes aires cérébrales.

Références : a) Bonaventure et al., 1997 ; b) Bonaventure et al., 1998 ; c) Boschert et al., 1994 ; d) Bruinvels et al., 1993 ; e) Bruinvels et al., 1994 ; f) Compan et al., 1998 ; g) Doucet et al., 1995 ; h) Langlois et al., 1995 ; i) Sari et al., 1999.

Couplage moléculaire :

Les récepteurs 5-HT_{1B} sont des récepteurs à 7 hélices transmembranaires synthétisés au niveau des corps cellulaires neuronaux, migrant ensuite au niveau des terminaisons axonales; expliquant ainsi pourquoi dans certains tissus cérébraux (i.e. la substance noire) on observe la présence de récepteurs (mise en évidence par les études d'autoradiographie et d'immunohistochimie), mais pas de l'ARNm codant pour ces récepteurs. Il a été montré sur des cellules transfectées que les récepteurs 5-HT_{1B} sont couplés à une protéine G (Gi ou G0). La stimulation de ce récepteur inhibe l'adénylate cyclase aussi bien dans les études in vivo portant sur certains tissus cérébraux tels que la substance noire que dans les études in-vitro de cultures cellulaires de cellules transfectées (Bouhelal et al., 1988; Hamblin and Metcalf, 1991; Maroteaux et al., 1992; Raymond et al., 2001). Cependant, il a récemment été montré que l'activation des récepteurs 5-HT_{1B} peut également permettre l'accumulation d'AMPc ainsi que l'activation de la phospholipase C (PLC) dans des cellules transfectées, et ainsi contrôler les canaux potassiques (Ghavami et al., 1997). Ainsi, la stimulation des récepteurs 5-HT_1 augmente la conductance potassique et produit une hyperpolarisation inhibant un potentiel d'action engendré, par exemple, par la sérotonine liée aux récepteurs 5-HT3 ou par le glutamate activant les récepteurs NMDA.

Rôle Pharmacologique

L'implication du récepteur 5-HT_{1B} a été démontrée dans de nombreuses fonctions physiologiques comme le contrôle :

- De l'agressivité (Saudou et al., 1994; Geyer, 1996; Dirks et al., 2001; de Almeida and Miczek, 2002) ; lorsque l'on place un intrus dans une cage contenant des animaux mutés génétiquement, ces derniers attaquent l'intrus beaucoup plus « rapidement » et « intensément » que des animaux de type sauvages (Saudou et al., 1994), suggérant ainsi un rôle prépondérant du récepteur 5-HT_{1B} dans le comportement agressif de l'animal. Ceci est confirmé par le fait que l'administration d'anpirtoline (agoniste des récepteurs 5-HT_{1B}) chez une souris permet de diminuer son agressivité (de Almeida et al., 2001; Miczek and de Almeida, 2001; Rilke et al., 2001). Cet effet semble être lié à une activité des agonistes des récepteurs 5-HT_{1B} sur des récepteurs postsynaptiques car il est démontré que l'inhibition de l'agression est également présente chez des animaux prétraités par une neurotoxine spécifique du système sérotoninergique (la 5,7-hydroxytryptamine) qui induit une destruction de 60 à 80% du système sérotoninergique (de Almeida et al., 2001).

- Du sommeil (Boutrel et al., 1999; Monaca et al., 2003) ; le traitement par IRSS induit une diminution du sommeil paradoxal (Hendrickse et al., 1994) qui peut être reproduit par l'administration d'agonistes des récepteurs 5-HT_{1A} et/ou 5-HT_{1B} (Dzoljic et al., 1992; Boutrel et al., 1999; Boutrel et al., 2002) alors que le blocage des récepteurs 5-HT_{1A} et 5-HT_{1B} facilite le sommeil paradoxal (Boutrel et al., 1999; Boutrel et al., 2002). Il est maintenant bien établi que l'activation du récepteur 5-HT_{1B} ne joue qu'un rôle partiel dans cette inhibition du sommeil paradoxal, la majeure partie des effets étant liés au récepteur 5-HT_{1A} (Monaca et al., 2003).

- Du comportement moteur (Geyer, 1996; Skingle et al., 1996; Millan et al., 2003) ; l'administration systémique, ou locale, d'un agoniste des récepteurs 5-HT_{1B}, le RU24969 entraîne une augmentation de l'activité locomotrice dose dépendante (Green et al., 1984; De Souza et al., 1986). Cet effet sur la variation d'activité locomotrice n'est pas observé chez les animaux mutants privés du gène codant pour le récepteur sérotoninergique 1B.

- De l'appétit (Simansky, 1996; Lee and Simansky, 1997; Lucas et al., 1998; De Vry and Schreiber, 2000; Simansky and Nicklous, 2002), l'administration de différents agonistes des récepteurs 5-HT_{1B} et d'IRSSs (Nonogaki et al., 2006)permet de diminuer la prise alimentaire de façon dose-dépendante, cette hypophagie induite par des agonistes des récepteurs 5-HT_{1B} peut être bloquée par des antagonistes spécifiques (Simansky and Nicklous, 2002).

- De l'anxiété (Frances et al., 1990a; Frances et al., 1990b; Lin and Parsons, 2002) ; il a été démontré une interaction entre les benzodiazépines et les substances modulant l'activité du récepteur 5-HT_{1B} (Frances et al., 1990a) suggérant que ce récepteur joue un rôle dans le mécanisme d'action de certains anxiolytiques. De plus l'exposition d'un rat à un stress antagonise la capacité qu'ont les agonistes des récepteurs 5-HT_{1B} à diminuer la libération de sérotonine, montrant bien une désensibilisation de ce récepteur dans les états anxieux (Bolanos-Jimenez et al., 1995).

- De la pharmacodépendance (Przegalinski et al., 2003) ; dans ce domaine, les effets du récepteur 5-HT_{1B} portent à controverse puisque les effets comportementaux résultants d'un blocage aigu du récepteur (administration d'une dose unique d'antagoniste spécifique) et ceux résultants d'un blocage constitutif sont diamétralement opposés. Ainsi,

l'administration de cocaïne entraîne une diminution d'activité locomotrice chez les animaux traités par un antagoniste, et une augmentation d'activité locomotrice chez les animaux dépourvus du récepteur 5-HT_{1B}. Il a également été démontré que le prétraitement de rats par des substances agissant sur les récepteurs 5-HT_{1B} peuvent potentialiser certains des effets de produits créant une dépendance : ainsi certains agonistes des récepteurs 5-HT_{1B} (CGS-12066B, RU 24969, CP 94,253, CP 93,129) diminuent l'autoadministration d'inhibiteurs de recapture de la dopamine et de cocaïne chez ces animaux, probablement du fait d'un déplacement de la courbe effet dose vers la gauche (Parsons et al., 1996; Parsons et al., 1998).

- Du comportement sexuel (Hillegaart and Ahlenius, 1998) ; l'activation du récepteur 5-HT_{1B} par un agoniste spécifique permet d'antagoniser la « motivation sexuelle » chez le Rat (Popova and Amstislavskaya, 2002) ; les études menées chez la souris semblent confirmer que le rôle inhibiteur de la sérotonine sur le comportement sexuel soit porté, entre autre, par le récepteur 5-HT_{1B} (Rodriguez-Manzo et al., 2002). Ce dysfonctionnement sexuel est également retrouvé lors de l'injection locale intracérébrale d'agoniste 5-HT_{1B} ; ce qui a permis de mettre en évidence deux aires cérébrales impliquées dans le contrôle du comportement sexuel chez la souris : le noyau accumbens, et le noyau du raphé dorsal (Fernandez-Guasti et al., 1992).

- Ainsi que dans la régulation thermique (Hagan et al., 1997) ; l'administration d'une dose aigue de citalopram induit chez le Rat une hypothermie qui peut être reproduite par l'administration d'anpirtoline (agoniste des récepteurs 5-HT_{1B}) et antagonisée par l'administration de NAS-181 (antagoniste des récepteurs 5-HT_{1B}) (Oerther and Ahlenius, 2001).

Des études comparatives portant sur le comportement de souris privées de ce récepteur versus le comportement de souris sauvages ont permis de retrouver l'ensemble de ces implications chez ces animaux (Crabbe et al., 1996; Ramboz et al., 1996a; Ramboz et al., 1996b; Rocha et al., 1998).

Au niveau neurobiochimique, le récepteur 5-HT_{1B} peut avoir deux localisations : soit une localisation présynaptique, soit une localisation postsynaptique ; dans le cas d'une localisation présynaptique, le récepteur est alors considéré comme étant un autorécepteur (situé sur des neurones sérotoninergiques), alors qu'en cas de localisation postsynaptique, il s'agit d'un hétérorécepteur.

1.4.2 Récepteur présynaptique

Le récepteur présynaptique est impliqué dans le contrôle de la modulation du relargage de la sérotonine (d'où la dénomination d'autorécepteur) au niveau de la fente synaptique. La stimulation de ce récepteur permet un rétrocontrôle négatif de la libération de sérotonine limitant ainsi les variations de concentrations extracellulaires de sérotonine. Contrairement à l'autorécepteur de type 5-HT_{1A} qui exerce son action en diminuant l'activité électrique neuronale (diminution du « firing »), le récepteur 5-HT_{1B} agit en diminuant la libération de neurotransmetteurs. Ceci est en accord avec la localisation de ces deux sous-types de récepteurs, le récepteur 5-HT_{1A} étant situé au niveau des corps cellulaires des neurones, alors que le récepteur 5-HT_{1B} est lui situé au niveau des terminaisons axonales. De nombreuses études ont mis en évidence que le délai nécessaire à l'apparition des effets thérapeutiques des IRSSs correspond au délai nécessaire pour obtenir la désensibilisation d'un (ou des 2) sous-types de récepteur 5-HT_1 (A/B) ; l'augmentation de la neurotransmission sérotoninergique induite par les antidépresseurs étant limitée par l'activation de ces récepteurs. Cette idée est basée sur le fait que les études d'électrophysiologie chez le Rat ont montré qu'un traitement aigu par antidépresseur diminue l'activité électrique des neurones, celle-ci revenant à la normale après un traitement chronique (Chaput et al., 1986; Blier et al., 1987).

Les autorécepteurs 5-HT_{1B} sont retrouvés dans la fente synaptique des neurones situés dans le cortex, le raphé, l'hippocampe et le striatum chez l'homme, le cobaye et le rat (Hoyer and Middlemiss, 1989; Limberger et al., 1991; Davidson and Stamford, 1995; Buhlen et al., 1996; Price et al., 1997; Schlicker et al., 1997).

1.4.2.1 Autorécepteurs et antidépresseurs

Lors d'un traitement prolongé par IRSSs (i.e. la paroxétine pendant 21 jours), il a été démontré une désensibilisation des autorécepteurs 5-HT_{1B} situés dans le raphé médian chez le Rat, indiquant que les récepteurs contrôlant négativement le relargage de la

sérotonine au niveau somatodendritique sont moins sensibles à l'activation par le neurotransmetteur endogène (Pineyro and Blier, 1996); les autorécepteurs 5-HT_{1B} situés au niveau terminal dans différentes zones de projections telles que l'hippocampe, l'hypothalamus et le cortex frontal sont également désensibilisés par un traitement chronique par IRSSs (Blier et al., 1984; Moret and Briley, 1990; O'Connor and Kruk, 1994; el Mansari et al., 1995; Pineyro and Blier, 1999; Dremencov et al., 2000; Newman et al., 2000). Il est également clairement démontré qu'un traitement chronique avec un antidépresseur (fluoxetine ou sertraline) entraîne une diminution de la quantité d'ARN messager codant pour le récepteur 5-HT_{1B} au niveau du noyau du raphé dorsal de rat, qui revient à la normale après arrêt du traitement. Les concentrations d'ARNm dans les aires de projections (i.e. hippocampe, striatum et cortex frontal) ne sont quant à elles pas affectées par ce traitement (Neumaier et al., 1996a; Anthony et al., 2000). Suggérant ainsi que seuls les autorécepteurs 5-HT_{1B} sont désensibilisés lors d'un traitement chronique par un IRSS.

Il existe cependant quelques travaux qui n'ont pas permis de mettre en évidence de désensibilisation de ces autorécepteurs (Auerbach and Hjorth, 1995; Bosker et al., 1995a; Bosker et al., 1995b; Cremers et al., 2000b) lors d'un traitement chronique par un IRSS, puisque dans ces études soit les antagonistes des récepteurs 5-HT_{1B} conservent leur capacité à potentialiser les effets des antidépresseurs (augmentation des concentrations extracellulaires de sérotonine) ; soit les agonistes peuvent encore diminuer le relargage de sérotonine ; suggérant ainsi que les autorécepteurs 5-HT_{1B} ne sont pas désensibilisés, mais restent fonctionnels. Cette hétérogénéité dans les résultats peut s'expliquer par une grande diversité des protocoles utilisés : dose d'IRSS administrée, rythme d'administration : 1 à 2 fois par jour ou minipompes Alzet, voie d'administration, période de « wash-out » ou non.

1.4.2.2 Autorécepteurs et antagonistes des récepteurs 5-HT_{1B}

L'utilisation d'antagonistes des récepteurs 5-HT_{1B} n'a pas permis de faire varier les concentrations extracellulaires de sérotonine mesurées dans différentes aires cérébrales chez différents animaux (Souris et Rat) telles que le cortex et l'hippocampe (Liao et al., 2000; Adell et al., 2001; Gardier et al., 2001; Roberts et al., 2001); suggérant que la sérotonine endogène n'exerce pas d'effet sur les autorécepteurs sérotoninergiques dans les conditions basales.

Seules quelques études ont montré une augmentation des concentrations extracellulaires de sérotonine mesurées par microdialyse intracérébrale chez le cobaye dans le gyrus denté après administration systémique d'antagonistes des récepteurs 5-HT_{1B} (GR 127935 et SB-224289) (Roberts et al., 1998; Hughes and Dawson, 2004), cette augmentation n'a toutefois pas été corrélée à une variation des concentrations extracellulaires de sérotonine dans le cortex frontal de cobaye (Hughes and Dawson, 2004). Dans cette dernière étude, les auteurs ont également étudié les effets de ces antagonistes chez le Rat et ont montré qu'ils étaient dépourvus d'effets ; suggérant un effet « espèce ». De même ; il a été montré que le SB 236057, un agoniste inverse des récepteurs 5-HT_{1B}, permet d'augmenter le relargage de sérotonine dans le gyrus denté (à faible dose : 0,75 mg/kg po), ainsi que dans le cortex frontal (à forte dose : 2,5 mg/kg ; $p<0,05$) chez le cobaye (Roberts et al., 2000).

Il a donc été postulé que les effets des antagonistes des autorécepteurs sérotoninergiques quant aux concentrations extracellulaires de sérotonine chez le Rat et la Souris ne sont visibles que lorsque la neurotransmission sérotoninergique est augmentée (i.e. lors du traitement par un IRSS) (Hjorth, 1993).

1.4.2.3 Autorécepteurs et association antidépresseurs + antagonistes des récepteurs 5-HT_{1B}

Afin de valider cette hypothèse, de nombreuses études complémentaires ont été réalisées en utilisant une coadministration d'IRSSs et d'antagonistes des récepteurs 5-HT_{1B}. Les IRSSs sont utilisés dans le but d'augmenter la neurotransmission sérotoninergique, permettant ainsi l'activation des autorécepteurs 5-HT_{1B} (ceux-ci étant inactifs dans les conditions basales); les antagonistes des récepteurs 5-HT_{1B} coadministrés devraient théoriquement antagoniser l'inhibition de relargage de 5-HT et ainsi augmenter la $[5\text{-HT}]_{EC}$. Il a ainsi été démontré que les antagonistes des récepteurs 5-HT_{1B} peuvent potentialiser l'augmentation des concentrations extracellulaires de 5-HT induite par les antidépresseurs dans le cortex frontal de rat (Gobert et al., 1997; Sharp et al., 1997; Dawson and Nguyen, 2000; De Groote et al., 2003b) ; dans l'hippocampe de rat (Liao et al., 2000), dans l'hippocampe et le cortex frontal de cobaye (Roberts et al., 1996) ainsi que dans l'hypothalamus de cobaye (Roberts et al., 1996) et dans l'hippocampe de souris (Malagie et al., 1995; Trillat et al., 1998; Gardier et al., 2001; Malagie et al., 2001; Gardier et al., 2003). Toutefois, malgré les résultats obtenus chez le Rat et le Cobaye, aucune différence de $[5\text{-HT}]_{EC}$ n'est observée au niveau du cortex frontal de Souris

(Malagie et al., 1995; Trillat et al., 1998; Gardier et al., 2001; Malagie et al., 2001; Gardier et al., 2003). Considérant les voies d'innervation sérotoninergiques, de récentes études ont eu pour objectif de démontrer que les antagonistes 5-HT_{1B} exercent un effet significativement plus important au niveau du raphé médian qu'au niveau du raphé dorsal (Adell et al., 2001); ce dernier semblant plus sous la dépendance des récepteurs de type 5-HT_{1D} (Pompeiano et al., 1992; Starkey and Skingle, 1994; Davidson and Stamford, 1995; Pineyro et al., 1995; Hertel et al., 2001). Cette théorie permettrait d'expliquer les résultats obtenus chez la Souris, puisque les voies sérotoninergiques provenant du raphé médian innervent l'hippocampe, alors que celles provenant du raphé dorsal vont innerver le cortex. Cependant, cette théorie porte à controverse du fait du manque de sélectivité des produits employés ; d'autant plus que le BRL 15572, un antagoniste des récepteurs 5-HT_{1D}, permet de potentialiser le relargage de la sérotonine induit par stimulation électrique aussi bien au niveau du raphé dorsal que du raphé médian (Hopwood and Stamford, 2001; Roberts et al., 2001). De la même façon, les agonistes des récepteurs 5-HT_{1B} permettent de diminuer le relargage de sérotonine aussi bien dans les noyaux du raphé dorsal que médian et cet effet est antagonisé par l'administration d'antagonistes des récepteurs 5-HT_{1B} (Davidson and Stamford, 1995; Adell et al., 2001; Hertel et al., 2001; Hopwood and Stamford, 2001).

IRSS	Dose, voie d'administration	Antagoniste 5-HT_{1B}	Dose, voie d'administration	Potentialisation	Référence
Citalopram	1 µmol/kg ; sc	GR 127935	1 µmol/kg ; sc	Hippocampe	(Cremers et al., 2000a)
	3 µmol/kg ; sc	GR 127935	1 µmol/kg ; sc		(Cremers et al., 2000a)
	10 µmol/kg ; sc	GR 127935	1 µmol/kg ; sc		(Cremers et al., 2000a)
	5 mg/kg ; sc	Penbutolol	8 mg/kg ; sc		(Hjorth, 1993)
	5 mg/kg ; sc	AR-A000002	9 mg/kg ; sc	Cortex	(Stenfors et al., 2004)
Fluoxétine	10 mg/kg ; ip	SB 224289	4 mg/kg ; ip		(Hervas et al., 2000)
	20 mg/kg ; sc	SB 224289	4 mg/kg ; ip	Cortex	(Hervas et al., 2000)
	20 mg/kg ; sc	GR 127935	3 mg/kg ; sc		(Dawson and Nguyen, 2000)
	10 mg/kg ; sc	SB 224289	2,5 mg/kg ; sc		(Gobert and Millan, 1999)
	10 mg/kg ; sc	GR 127935	2,5 mg/kg ; sc		(Gobert et al., 1997)
Paroxétine	5 mg/kg ; sc	GR 127935	4 mg/kg ; sc	Hippocampe	(Malagie et al., 2001)
	5 mg/kg ; sc	GR 127935	4 mg/kg ; sc		(Gardier et al., 2001)
	1 mg/kg/jour ; ip	GR 127935	4 mg/kg ; sc		(Gardier et al., 2003)
	10 µmol ; cortex	SB 224289	4 mg/kg ; sc		(Roberts et al., 1999)
Fluvoxamine	1 µmol ; cortex	NAS-181	1 µmol ; cortex	Cortex	(De Groote et al., 2002b)

Tableau 3 : Potentialisation de l'augmentation de sérotonine extracellulaire induite par l'administration d'IRSSs lors de la coadministration avec un antagoniste des récepteurs 5-HT_{1B}.

1.4.2.4 Autorécepteurs et souris ne présentant pas le gène codant pour les récepteurs 5-HT_{1B}

La mise au point récente par recombinaison homologue de souris homozygotes « knockout » mutantes privées des récepteurs 5-HT_{1B} (souris KO 5-HT_{1B} -/-) constitue un moyen d'étude supplémentaire, car permettant d'observer les effets pharmacologiques consécutifs à un blocage constitutif, et non plus pharmacologique, du récepteur 5-HT_{1B}. Ainsi, les concentrations basales de sérotonine extracellulaire mesurées par la technique de microdialyse intracérébrale in-vivo chez les animaux éveillés mutés génétiquement et ne présentant pas de gène codant pour le récepteur 5-HT_{1B} étant équivalentes à celles mesurées chez des animaux non mutés dans le cortex, l'hippocampe et le striatum (De Groote et al., 2002b; Malagie et al., 2002; De Groote et al., 2003c), ceci confirme que dans les conditions basales l'autorécepteur 5-HT_{1B} est inactif ; l'absence de différence dans les concentrations basales de sérotonine ne semblant pas être liée à la mise en place de mécanismes de compensation chez l'animal muté puisque l'administration d'une dose unique d'antagoniste sélectif du récepteur permet d'obtenir les mêmes résultats.

Effets neurobiochimiques des antidépresseurs chez les animaux privés de récepteur 5-HT_{1B} :

De nombreuses études ont permis de mettre en évidence une potentialisation des effets neurobiochimiques des IRSSs chez des animaux mutés génétiquement et ne présentant pas le gène codant pour le récepteur 5-HT_{1B}. Ainsi l'administration de paroxétine par voie générale (traitement aigue ou chronique) aboutit à une augmentation des concentrations extracellulaires de sérotonine statistiquement plus importante dans l'hippocampe de souris mutantes 5-HT_{1B} (Knobelman et al., 2001) comparée à des souris sauvages ; mais cette potentialisation n'est pas retrouvée au niveau du cortex médian préfrontal (Gardier et al., 2001; Malagie et al., 2001; Gardier et al., 2003). Cette différence cortex/hippocampe (potentialisation dans l'hippocampe mais pas dans le cortex) est également retrouvée lors d'un traitement par différentes doses de fluoxétine (5 et 10 mg/kg) (Malagie et al., 2002). La fluvoxamine (0,1 ; 1 et 10 µM) administrée localement dans le striatum de souris privées des récepteurs 5-HT_{1B} induit une augmentation de $[5\text{-}HT]_{EC}$ qui n'est pas statistiquement plus importante que celle induite chez les animaux sauvages quelque soit la dose testée (De Groote et al., 2003c). A l'inverse, le même auteur a démontré que la perfusion locale de fluvoxamine (1µM) dans l'hippocampe et le

cortex préfrontal de souris induit une augmentation de $[5\text{-HT}]_{EC}$ significativement plus élevée chez les animaux mutants comparés aux animaux sauvages. Ces résultats sont en accord avec ceux montrant que l'augmentation de $[5\text{-HT}]_{EC}$ au niveau striatal consécutive à l'administration systémique de fluoxétine n'est pas potentialisée chez les souris ne possédant pas le récepteur 5-HT_{1B}, contrairement à ce qui est obtenu dans l'hippocampe chez ces mêmes animaux (Knobelman et al., 2001).

1.4.2.5 Autorécepteurs et agonistes des récepteurs 5-HT_{1B}

La perfusion locale de CP 93129 (0,5 et 1 µM respectivement), un agoniste des récepteurs 5-HT_{1B}, au niveau du striatum ou du cortex préfrontal chez la Souris permet une diminution des concentrations extracellulaires de sérotonine de plus de 50% comparé au groupe ne recevant que le véhicule; ceci n'étant pas retrouvé chez les animaux dépourvus du récepteur 5-HT_{1B}, cet effet est bien lié à une activité sélective du produit sur les récepteurs 5-HT_{1B} (De Groote et al., 2002b; De Groote et al., 2003c). Ces effets sont également retrouvés lors de la perfusion locale de CP 93129 (1µM) via la sonde de microdialyse directement dans l'hippocampe (De Groote et al., 2002a). L'administration du même agoniste (1 µM) dans la substance noire chez le Rat permet également une diminution des concentrations extracellulaires de sérotonine (-25%) (Thorre et al., 1998). Une gamme de dose montre que l'effet du CP 93129 sur les concentrations corticales de sérotonine extracellulaire chez le Rat est dose dépendant (De Groote et al., 2003a). L'administration de RU 24969 par voie générale permet elle aussi de diminuer les concentrations de sérotonine dans l'hippocampe de rats anesthésiés (mesuré par microdialyse intracérébrale) (Martin et al., 1992). Une diminution similaire des dialysats de sérotonine est également obtenue dans le cortex frontal de rats anesthésiés (Sleight et al., 1989) ainsi que dans le diencéphale de rats éveillés (Auerbach et al., 1991). De plus, l'administration locale de RU 24969 via la sonde de microdialyse est responsable d'une diminution de la concentration extracellulaire de 5-HT aussi bien au niveau de l'hippocampe de rats anesthésiés (Hjorth and Tao, 1991; Bosker et al., 1995b), que du diencéphale de rats vigiles (Auerbach et al., 1991). Les effets de l'administration locale de RU 24969 sont antagonisés par l'injection simultanée de methiothepine, un antagoniste des récepteurs 5-HT_{1B}, directement dans l'hippocampe (Martin et al., 1992). La methiothepine permet également d'antagoniser la diminution des concentrations extracellulaires de sérotonine induite par le CP 93129 (Hjorth and Tao, 1991). Compte tenu du fait que le récepteur de cobaye présente une analogie structurale avec le

récepteur humain plus forte que les récepteurs de rats et murins, de nombreuses études ont été réalisées chez cet animal. L'administration locale de 5-CT chez le Cobaye éveillé diminue les concentrations extracellulaires de sérotonine obtenues dans le cortex frontal (Lawrence and Marsden, 1992) tout comme le sumatriptan chez les animaux anesthésiés (Sleight et al., 1990; Roberts et al., 1997). Cet effet du sumatriptan est antagonisé par l'administration d'un antagoniste spécifique, le SB-224289 (Gaster et al., 1998) et n'est pas présent lors de l'administration du produit par voie générale, montrant bien que le sumatriptan ne passe pas la barrière hémato-encéphalique (Sleight et al., 1990).

Ces résultats obtenus par microdialyse sont conformes à ceux publiés auparavant et utilisant une approche in vitro (Engel et al., 1986; Maura et al., 1986).

Agoniste	Injection	Animal	[5-HT]	Structure	Auteur
CP 93129	Locale	Souris	↘	Striatum	(De Groote et al., 2003c)
	Locale	Souris	↘	Hippocampe	(De Groote et al., 2002a)
	Locale	Souris	↘	Cortex préfrontal	(De Groote et al., 2002b)
	Locale	Rat	↘	Substance noire	(Thorre et al., 1998)
	Locale	Rats	↘	Hippocampe	(Hjorth and Tao, 1991)
	Locale	Rats	↘	Cortex	(De Groote et al., 2003a)
RU 24969	Systémique	Rats	↘	Hippocampe	(Martin et al., 1992)
	Systémique	Rats	↘	Cortex frontal	(Sleight et al., 1989)
	Systémique	Rats	↘	Diencéphale	(Auerbach et al., 1991)
	Locale	Rats	↘	Hippocampe	(Hjorth and Tao, 1991; Bosker et al., 1995b)
	Locale	Rats	↘	Diencéphale	(Auerbach et al., 1991)
5-CT	Locale et systémique	Cobayes	↘	Cortex frontal	(Lawrence and Marsden, 1992)
Sumatriptan	Locale	Cobayes	↘	Cortex frontal	(Sleight et al., 1990)
	Systémique		0		

Tableau 4 : Effets de différents agonistes des récepteurs 5-HT_{1B} sur les concentrations extracellulaires de sérotonine mesurées dans différentes aires cérébrales par la technique de microdialyse intra-cérébrale in-vivo.

Au vu de l'ensemble des résultats portant sur les études neurobiochimiques, il a été démontré que le blocage constitutif (souris mutantes privées du gène codant pour le récepteur 5-HT_{1B}) ou pharmacologique à l'aide d'un antagoniste sélectif des récepteurs 5-HT_{1B} (GR 127935, NAS-181, Penbutolol, SB 224289 ou AR-A000002) permet de potentialiser l'augmentation des concentrations extracellulaires de sérotonine consécutive à l'administration aigu par voie systémique ou locale de différents IRSSs (paroxétine, fluoxétine, citalopram ou fluvoxamine) dans différentes aires cérébrales (Gobert et al., 1997; Malagie et al., 2001; De Groote et al., 2002b; De Groote et al., 2002a; Malagie et al., 2002). A l'inverse, l'administration d'agonistes des récepteurs 5-HT_{1B} permet de diminuer les concentrations extracellulaires de 5-HT aussi bien dans les conditions basales, que lors de la coadministration avec des IRSSs. Il semble donc que les antagonistes des récepteurs 5-HT_{1B}, en bloquant les autorécepteurs, potentialisent les effets neurobiochimiques des IRSSs, alors que les agonistes, en diminuant les concentrations extracellulaires de sérotonine, semblent limiter ces effets.

1.4.3 Récepteur postsynaptique

Les récepteurs 5-HT_{1B} post synaptiques permettent de moduler la libération d'autres neurotransmetteurs, d'où le nom d'hétérorécepteurs. Parmi les neuromédiateurs dont la libération est contrôlée par le récepteur 5-HT_{1B}, on trouve la dopamine (Sarhan et al., 1999; Sarhan et al., 2000), le glutamate (Bobker and Williams, 1989), l'acétylcholine; (Maura and Raiteri, 1986; Cassel et al., 1995) ainsi que le GABA (Feuerstein et al., 1996b).

1.4.3.1 Récepteur postsynaptique et acétylcholine

En 1986, il a été établi lors d'études in vitro que l'administration de doses croissantes de sérotonine exogène permet de diminuer le relargage de [^{3}H]acétylcholine à partir de neurones d'hippocampe de rat. Dans cette série d'expérimentation les effets de la sérotonine ne sont pas antagonisés par l'administration d'antagoniste des récepteurs 5-HT_2 (kétansérine) et 5-HT_{1C} ; par contre le propranolol (antagoniste mixte des récepteurs 5-HT_{1A} et 5-HT_{1B}) permet d'antagoniser cet effet. De plus, compte tenu du fait que seuls les agonistes des récepteurs 5-HT_{1B} permettent de mimer les effets de la sérotonine, il apparaît donc clairement que l'activation du récepteur 5-HT_{1B} permet un rétrocontrôle négatif du relargage de l'acétylcholine (Ach) dans l'hippocampe de rats (Maura and

Raiteri, 1986). Toutefois, des résultats opposés ont été trouvés lors de la réalisation de microdialyse dans le cortex frontal de rats ; ces études montrent que lors d'un traitement par un IRSS, l'augmentation de la neurotransmission sérotoninergique s'accompagne d'une augmentation de la libération d'Ach et que cette augmentation est limitée par la coadministration d'un antagoniste mixte des récepteurs 5-HT_{1A} et 5-HT_{1B}, le pindolol (Consolo et al., 1996) ; il est fort possible que cet effet stimulateur sur la libération d'Ach soit lié à une action indirecte, et notamment une désinhibition du système GABAergique expliquant ainsi la différence entre les résultats in vivo et in vitro.

Lors d'un traitement chronique (14 jours) par antidépresseur (i.e. citalopram ou tianeptine), aucune modification du relargage de l'Ach n'est observée au niveau des synaptosomes hippocampiques de rats. Cependant, l'effet inhibiteur du relargage d'acéthylcholine des agonistes des récepteurs 5-HT_{1B} (CGS 12066B) est diminué ; suggérant que l'hétérorécepteur 5-HT_{1B} situé sur les neurones cholinergiques est désensibilisé (Bolanos-Jimenez et al., 1994). Ces récepteurs sont également désensibilisés lors de l'exposition à un stress ; la diminution de relargage d'Ach consécutive à l'administration d'agonistes des récepteurs 5-HT_{1B} étant moins importante dans l'hippocampe et la substance noire d'animaux « stressés » (Bolanos-Jimenez et al., 1995).

Si les résultats obtenus in vitro sont très explicites et montrent bien le rôle inhibiteur du récepteur 5-HT_{1B} sur la libération d'Ach, il n'en est pas de même pour les résultats obtenus in vivo. En effet, la mise en place de mécanisme de compensation complique l'interprétation des résultats. Ainsi, dans l'hippocampe, l'augmentation de neurotransmission sérotoninergique induite par un traitement antidépresseur (type IRSS) permet une stimulation des récepteurs 5-HT_{1B} situés sur les neurones cholinergiques, diminuant ainsi le relargage d'Ach ; mais dans le même temps, l'augmentation de neurotransmission sérotoninergique s'accompagne d'une stimulation plus importante des récepteurs 5-HT_{2A} qui ont un rôle excitateur sur les interneurones contenant la substance P ; le relargage de substance P dans la fente synaptique entraîne une activation des récepteurs NK1 situés sur les neurones cholinergiques augmentant ainsi la neurotransmission cholinergique de façon indirecte (Feuerstein et al., 1996a). La variation de neurotransmission cholinergique résultante est donc soumise d'un coté à une composante inhibitrice directe liée à l'activité de la sérotonine sur le récepteur 5-HT_{1B}, et de l'autre à une composante excitatrice indirecte de la substance P sur les neurones cholinergiques.

1.4.3.2 Récepteur postsynaptique et glutamate

Comme cela a été largement démontré pour d'autres systèmes monoaminergiques, l'augmentation du relargage de sérotonine au niveau extracellulaire lors de l'administration locale ou systémique d'IRSS (citalopram) entraîne une diminution des concentrations extracellulaires de glutamate ($[Glu]_{EC}$) dans le cortex préfrontal de rats mesurée par microdialyse intracérébral in vivo chez le rat vigile prétraité par veratridine (utilisée pour augmenter la neurotransmission glutamatergique) (Golembiowska and Zylewska, 1999; Golembiowska and Dziubina, 2000) aussi bien après un traitement aigu que chronique. Afin d'établir quel pouvait être le récepteur sérotoninergique postsynaptique impliqué dans cet effet du citalopram, les mêmes auteurs ont réalisé des expériences complémentaires ; ils ont ainsi montré que l'augmentation de $[Glu]_{EC}$ dans le cortex préfrontal de rats mesurée par microdialyse intracérébral in vivo chez le rat vigile induite par la veratridine est inhibée lorsque la veratridine est coadministrée avec du CP93129 (agoniste des récepteurs 5-HT_{1B}); les effets du CP 93129 sont antagonisés par le SB 216641 (antagoniste des récepteurs 5-HT_{1B} qui ne possède pas d'effet propre sur la libération de glutamate) (Golembiowska and Dziubina, 2002). Ceci est en accord avec les travaux montrant une localisation des récepteurs 5-HT_{1B} sur les neurones glutamatergiques (Ma, 2001). Ainsi qu'avec les résultats d'électrophysiologie qui mettent en évidence que la neurotransmission glutamatergique dans le raphé et dans le locus coeruleus est inhibée par l'administration d'agoniste des récepteurs 5-HT_{1B} (Bobker and Williams, 1989; Li and Bayliss, 1998). Dans tous les cas, ces effets ne sont observables que sur des cellules dépolarisées et pas sur des cellules au repos. Les agonistes des récepteurs 5-HT_{1B} peuvent donc présenter un intérêt thérapeutique dans les pathologies associées à un hyperfonctionnement du système glutamatergique en régulant son fonctionnement.

1.4.3.3 Récepteur postsynaptique et GABA

Comme pour les autres systèmes monoaminergiques, le fait que le récepteur 5-HT_{1B} soit couplé négativement à une adénylate cyclase fait que sa stimulation aboutit à une inhibition des neurones sur lesquels il est localisé. Par conséquent, la stimulation de l'hétérorécepteur 5-HT_{1B} situé sur les terminaisons nerveuses des neurones GABAergiques aboutit à une diminution du relargage de GABA dans différentes aires cérébrales telles que le striatum et le noyau accumbens (Boschert et al., 1994). Certaines

études ont mis en évidence la présence de récepteurs 5-HT_{1B} sur des neurones GABAergiques au niveau de l'aire ventrale tegmentale (Bruinvels et al., 1993; Bruinvels et al., 1994) ; les expérimentations in vivo et in vitro réalisées ont permis de démontrer que la diminution de relargage du GABA dans ces aires cérébrales lors de l'administration de cocaïne est la conséquence de l'activation de l'hétérorécepteur 5-HT_{1B} (la cocaïne permettant d'accentuer le relargage de dopamine, de sérotonine et de noradrénaline) (Cameron and Williams, 1994; Harrison et al., 1999). Ceci est confirmé par de récentes expériences qui démontrent que l'administration locale de CP 93129 (agoniste des récepteurs 5-HT_{1B}) dans la VTA entraîne une diminution dose dépendante des concentrations extracellulaires de GABA (Yan et al., 2004). Etant donné que les neurones GABAergiques situés au niveau de la VTA sont des interneurones ayant un rôle inhibiteur sur les autres systèmes monoaminergiques, notamment dopaminergique, l'activation des hétérorécepteurs 5-HT_{1B} joue un rôle désinhibiteur sur la neurotransmission dopaminergique (Guan and McBride, 1989; Johnson et al., 1992; Klitenick et al., 1992; Cameron and Williams, 1994; Xi and Stein, 1998; Yan et al., 2004).

1.4.3.4 Récepteur postsynaptique et dopamine

L'apparition de techniques telles que l'électrophysiologie et la microdialyse a permis d'établir avec certitude que la sérotonine joue un rôle sur l'activité des neurones dopaminergiques. Les études in-vitro d'électrophysiologies ont ainsi montré une inhibition de l'activité électrique des neurones dopaminergiques de la VTA lors de l'administration locale de sérotonine (Pessia et al., 1994; Cameron et al., 1997), suggérant un rôle inhibiteur de la sérotonine sur l'activité neuronale dopaminergique. De même, des résultats obtenus lors d'autres travaux in-vitro ont suggéré une diminution de la libération de DA lors de la stimulation des récepteurs 5-HT_{1B} ; cette diminution a été mise en évidence au niveau des synaptosomes du striatum (Sarhan et al., 2000).

Les études in-vivo présentent des résultats opposés, ainsi la sérotonine libérée par les neurones de l'axe « raphé-substance noire » facilite le relargage de dopamine au niveau de l'axe nigrostriatal (Trent and Tepper, 1991), alors qu'une déplétion en sérotonine (induite par l'administration d'une dose unique de PCPA, 400 mg/kg i.p.) produit une diminution du nombre de neurones dopaminergiques actifs dans la VTA (Minabe et al., 1996). Cette diminution du nombre de neurones dopaminergiques actifs peut être liée à une diminution de la stimulation des hétérorécepteurs 5-HT_{1B} résultant de la diminution des concentrations extracellulaires de sérotonine à la suite du traitement par PCPA. Les

études de microdialyse sur animal vigile ont largement confirmé ces résultats en montrant une augmentation du relargage de dopamine dans le nucleus accumbens (Parsons and Justice, 1993), le striatum (Benloucif and Galloway, 1991; De Deurwaerdere et al., 1996; Iyer and Bradberry, 1996), et le cortex préfrontal (Iyer and Bradberry, 1996) lors de l'augmentation des concentrations extracellulaires de sérotonine (administration d'IRSS ou de sérotonine exogène).

La différence entre les résultats in-vivo et in-vitro s'explique par la mise en place de système de compensation, ainsi lors des études in-vitro, les résultats observés sont obtenus à la suite de la stimulation exclusive des récepteurs sérotoninergiques situés sur les neurones dopaminergiques, alors que les résultats obtenus in-vivo résultent de l'activité de la sérotonine sur l'ensemble des neurones présents.

Les études de microdialyse ont montré une augmentation de la libération de dopamine dans le noyau accumbens et la VTA après injection d'un agoniste sélectif des récepteurs 5-HT_{1B} (CP 93129) par voie intra accumbal et intra tegmental ; cette augmentation (dose dépendante) des concentrations extracellulaires de dopamine est associée à une diminution (également dose dépendante) des concentrations extracellulaires de GABA et est antagonisée par l'administration d'un antagoniste spécifique des récepteurs 5-HT_{1B}, montrant bien l'activité indirecte des récepteurs 5-HT_{1B} situés sur les neurones GABAergiques sur la libération de dopamine (Yan et al., 2004). Le WAY 100635 (antagoniste sélectif des récepteurs de type 5-HT_{1A}) n'exerce aucun effet sur l'augmentation des concentrations extracellulaires de dopamine induite par le CP 93129, alors que le cyanopindolol (antagoniste mixte des récepteurs 5-HT_{1A} et 5-HT_{1B}) et la tétrodotoxine antagonisent les effets du CP 93129 (Yan and Yan, 2001). Ces résultats montrent clairement que l'augmentation de la libération de dopamine au niveau du noyau accumbens consécutive à l'administration de CP 93129 est bien liée à l'activation des récepteurs 5-HT_{1B}. De plus, compte tenu des effets de la tétrodotoxine, il est fort possible que cet effet résulte de l'activation de l'hétérorécepteur GABAergique. De même, d'autres travaux (Hallbus et al., 1997; Boulenguez et al., 1998) ont également mis en évidence une augmentation du relargage de DA dans le noyau accumbens après injection de sérotonine ou d'agonistes des récepteurs 5-HT_{1B} (S-CM-GTNH2 par voie locale et RU 24 969 par voie sous cutanée) qui peut être antagonisée par la coadministration d'antagoniste spécifique de ce récepteur (GR 127935).

Cet effet facilitateur du relargage de dopamine par l'activation du récepteur 5-HT_{1B} a également été mis en évidence au niveau de différentes aires cérébrales telles que le cortex frontal de rat (Iyer and Bradberry, 1996). Dans cette étude, les auteurs démontrent

non seulement que les agonistes spécifiques des récepteurs 5-HT_{1B} (CP 93129 et CP 94253) permettent d'augmenter le relargage de dopamine, mais également que l'augmentation de dopamine induite par la perfusion de sérotonine est bloquée par l'administration d'un antagoniste spécifique du récepteur 5-HT_{1B} (GR 127935) alors que les antagonistes des récepteurs 5-HT_{2A} et 5-HT_3 sont dépourvus d'effets. L'administration locale de citalopram dans la substance noire chez le rat permet d'augmenter la $[5\text{-HT}]_{EC}$ de plus de 500%, cette augmentation s'accompagne d'une augmentation statistiquement significative de la $[DA]_{EC}$ de 45% (Thorre et al., 1998). Il semble fort probable que cette augmentation de la libération de DA soit liée à l'activation (indirecte) du récepteur 5-HT_{1B} par le citalopram ; en effet, bien que différents récepteurs sérotoninergiques soient présents dans la substance noire, il a été prouvé que la majorité d'entre eux sont du type 5-HT_{1B} (Hoyer et al., 1994). Dans cette même aire cérébrale, l'administration de CP 93129 (agoniste sélectif des récepteurs 5-HT_{1B}) induit une augmentation du relargage de dopamine de plus de 4500% ; tout en diminuant le relargage de sérotonine de 25% (Thorre et al., 1998).

Le rôle joué par les récepteurs 5-HT_{1B} situés sur les neurones GABAergiques dans la libération de dopamine a également été mis en évidence lorsqu'il a été démontré que la sérotonine (ou l'agoniste administré) active les hétérorécepteurs 5-HT_{1B} localisés sur les terminaisons axonales GABAergiques de la voie nigrostriée et que la stimulation de ces récepteurs aboutit à une diminution du relargage de GABA dans la substance noire (Stanford and Lacey, 1996) et cette inhibition du système GABAergique provoque une désinhibition du système dopaminergique, et donc une augmentation du relargage de dopamine.

De nombreuses études ont mis en évidence qu'il n'existait pas de différence au niveau des concentrations extracellulaires basales de dopamine et de 5-HT dans le **striatum** entre les animaux sauvages et des animaux mutants « knock-out » pour le gène codant pour le récepteur 5-HT_{1B}, suggérant ainsi que le récepteur 5-HT_{1B} striatal n'exerce aucun rôle sur le relargage de 5-HT et de DA dans les conditions basales (Shippenberg et al., 2000; Knobelman et al., 2001). Et ce malgré une altération de la neurotransmission dopaminergique (Ase et al., 2000). Toutefois, la technique du zéro net flux a permis de mettre en évidence un niveau basal de dopamine significativement plus élevé dans le **striatum** de souris mutantes privées du récepteur 5-HT_{1B} comparativement à des souris

sauvages (Shippenberg et al., 2000) ; suggérant ainsi une différence dans la régulation de la libération de dopamine entre les aires cérébrales.

Agoniste	Injection	Animal	[DA]	Structure	Auteur
5-HT	Locale	Rats	↗	Noyau accumbens	(Parsons and Justice, 1993)
	Locale	Cobayes	↗	Noyau accumbens	(Hallbus et al., 1997)
	Locale	Rats	↗	Striatum	(De Deurwaerdere et al., 1996)
	Locale	Rats	↗	Striatum	(Iyer and Bradberry, 1996)
	Locale	Rats	↗	Striatum	(Benloucif and Galloway, 1991)
	Locale	Rats	↗	Cortex frontal	(Iyer and Bradberry, 1996)
	Locale	Rat	↗	Substance noire	(Thorre et al., 1998)
CP 93129	Locale	Rats	↗	Noyau accumbens	(Yan and Yan, 2001)
	VTA	Rats	↗	Noyau accumbens	(Yan and Yan, 2001)
	VTA	Rats	↗	Noyau accumbens	(Yan et al., 2004)
	VTA	Rats	↗	VTA	(Yan et al., 2004)
	Locale	Rats	↗	Striatum	(Iyer and Bradberry, 1996)
	Locale	Rats	↗	Cortex frontal	(Iyer and Bradberry, 1996)
	Locale	Rat	↗	Substance noire	(Thorre et al., 1998)
RU 24969	Locale	Rats	↗	Striatum	(Benloucif et al., 1993)
	Locale	Rats	↗	Striatum	(Benloucif and Galloway, 1991)
S-CM-GTNH2	Subiculum dorsal	Rats	↗	Noyau accumbens	(Boulenguez et al., 1998)
TFMPP	Locale	Rats	↗	Striatum	(Benloucif and Galloway, 1991)
Sumatriptan	Locale	Cobayes	0	Noyau accumbens	(Hallbus et al., 1997)
IRSS					
Fenfluramine	Locale	Rats	↗	Striatum	(Benloucif and Galloway, 1991)
Fluoxétine					
Citalopram	Locale	Rat	↗	Substance noire	(Thorre et al., 1998)

Tableau 5 : Effets de différents agonistes des récepteurs 5-HT_{1B} sur les concentrations extracellulaires de dopamine mesurées dans différentes aires cérébrales par la technique de microdialyse intra-cérébrale in-vivo.

1.4.4 5-HT moduline

Une des particularités du récepteur 5-HT_{1B} est l'existence d'un modulateur allostérique spécifique de ce récepteur, la 5-HT moduline.

La 5-HT moduline est un tétrapeptide endogène (Leu-Ser-Ala-Leu) isolé et caractérisé par (Fillion and Fillion, 1981) qui interagit spécifiquement avec la liaison de 5-HT tritiée sur le récepteur 5-HT_{1B} et ce de façon non compétitive ; suggérant ainsi que son site de fixation sur le récepteur est différent de celui de la sérotonine (Massot et al., 1996; Rousselle et al., 1996). La 5-HT-moduline n'affecte pas la liaison de ligands spécifiques à d'autres récepteurs (sérotoninergiques, dopaminergiques, noradrénergiques, muscariniques, α et β adrénergiques, histaminergiques, récepteurs aux opiacés et aux benzodiazépines). L'étude autoradiographique de la distribution des sites de liaison de la 5-HT moduline tritiée a montré une répartition similaire à celle des récepteurs 5-HT_{1B} obtenu avec le [^{125}I] cyanopindolol confirmant le rôle de la 5-HT moduline dans l'activité des récepteurs 5-HT_{1B} (Cloez-Tayarani et al., 1997). La 5-HT moduline est ainsi fortement présente au niveau du cortex, du globus pallidus, de l'hippocampe, l'hypothalamus et la substance noire. Les études d'immunohistochimie ont montré que le striatum et le noyau caudé ne semblent quant à eux pas contenir de 5-HT moduline (Grimaldi et al., 1997). Ce neuropeptide est libéré à partir de synaptosomes de cortex de rat par un mécanisme Ca^{2+}-K^{+} dépendant. Les études pharmacologiques in-vitro ont mis en évidence la capacité de la 5-HT moduline à antagoniser les effets inhibiteurs des agonistes spécifiques des récepteurs 5-HT_{1B} sur la libération synaptosomale de [^{3}H]5-HT (Massot et al., 1996). Les résultats obtenus in-vitro ont été confirmés par les études in-vivo ; il a ainsi été démontré que:

L'injection intracérébroventriculaire de 5-HT moduline induit une action antagoniste sur les effets comportementaux d'un agoniste sélectif des récepteurs 5-HT_{1B} dans un test d'interaction sociale (Massot et al., 1996);

L'injection i.c.v. de 5-HT moduline provoque une désensibilisation des récepteurs 5-HT_{1B} présents dans la substance noire (Seguin et al., 1997);

La 5-HT moduline est libérée dans le milieu extracellulaire dès le début d'un stress aigu ; or il est clairement démontré qu'un stress aigu s'accompagne d'une désensibilisation des récepteurs 5-HT_{1B} ; cette désensibilisation pourrait être liée à l'activité de la 5-HT moduline. D'autant plus qu'une étude voltamétrique a mis en évidence l'augmentation de libération de sérotonine consécutive à un stress aigu d'immobilisation dans différentes aires cérébrales (cortex, hippocampe,…) (Clement et al., 1998) mais pas

dans le striatum qui ne contient pas de 5-HT moduline. Il est maintenant clairement établi que le relargage de 5-HT moduline consécutif à un exercice physique intensif entraîne une désensibilisation des récepteurs 5-HT_{1B} (Chennaoui et al., 2000)

1.4.5 Récepteur 5-HT_{1B} et activité de type antidépresseur

En se basant sur l'hypothèse monoaminergique de la dépression et en considérant le fait que les médicaments antidépresseurs sont efficaces en augmentant la neurotransmission sérotoninergique (Preskorn, 1994; Wong et al., 1995), les effets comportementaux des antidépresseurs devraient donc être potentialisés par les antagonistes des récepteurs 5-HT_{1B} puisque dans certaines études l'augmentation de $[5\text{-HT}]_{EC}$ obtenue lors de la coadministration d'antagonistes des récepteurs 5-HT_{1B} et d'antidépresseurs est le double de celle obtenue lors de l'administration de l'antidépresseur seul; alors que les agonistes de ces mêmes récepteurs devraient, théoriquement, limiter les effets de type antidépresseur puisque leur administration résulte en une diminution du relargage de la sérotonine dans le milieu extracellulaire.

De plus, il est très souvent rapporté dans la littérature qu'un traitement chronique par antidépresseur (i.e. antidépresseurs tricycliques ou SSRI) pendant deux semaines se traduit chez l'animal par une désensibilisation et une diminution (« down-regulation ») des autorécepteurs terminaux 5-HT_{1A} et 5-HT_{1B} et que ce délai nécessaire pour obtenir la désensibilisation des autorécepteurs correspond en moyenne au délai nécessaire pour obtenir les effets thérapeutiques des antidépresseurs (de Montigny and Blier, 1991; Hen, 1992; Pineyro and Blier, 1996; Davidson and Stamford, 1998; Sayer et al., 1999; Dremencov et al., 2000). Ainsi, il a été suggéré qu'il serait plus intéressant de coadministrer un antagoniste des récepteurs 5-HT_{1B} avec l'antidépresseur plutôt que d'attendre sa désensibilisation qui peut prendre deux à trois semaines ce qui permettrait d'obtenir un effet de type antidépresseur plus rapidement (Briley and Moret, 1993) et probablement plus important en augmentant la quantité de sérotonine extracellulaire disponible au niveau de la fente synaptique (Matzen et al., 2000).

De récentes études ont permis de mettre en évidence que l'administration d'antagonistes des récepteurs 5-HT_{1B} (GR 127935 ou NAS-181) potentialise la libération de sérotonine induite par l'administration aigue d'une dose d'IRSS (paroxétine, fluoxétine ou fluvoxamine) au niveau de certains tissus cérébraux (hippocampe et cortex) (Gobert et al., 1997; Malagie et al., 2001; De Groote et al., 2002b; Malagie et al., 2002; De Groote et al., 2003a) et que des résultats similaires sont obtenus lors de l'absence de récepteurs 5-

HT_{1B} chez des souris mutées génétiquement (Malagie et al., 2001; De Groote et al., 2002b; De Groote et al., 2002a; Malagie et al., 2002). Toutefois, bien que le blocage des récepteurs 5-HT_{1B} s'accompagne d'une augmentation des effets neurobiochimiques des IRSSs, cette augmentation n'est pas corrélée à une augmentation des effets de type antidépresseur du citalopram et de la fluoxétine (Tatarczynska et al., 2002; Tatarczynska et al., 2004a); au contraire, certaines études comportementales ont mis en évidence le fait que la coadministration d'un antagoniste sélectif des récepteurs 5-HT_{1B} et d'un IRSS, ou bien l'administration d'un IRSS seul chez des souris privées du gène codant pour le récepteur 5-HT_{1B}, se traduit par la disparition de l'activité antidépressive de cette molécule aussi bien dans le test de la nage forcée (Gardier et al., 2001; Tatarczynska et al., 2004a) que dans le test de suspension caudale (O'Neill et al., 1996).

Peu d'études reportent une potentialisation des effets de type antidépresseur de la fluoxétine dans le TST chez des souris ne possédant pas le récepteur 5-HT_{1B}; cet effet n'est observé que pour une dose de fluoxétine (2,5 mg/kg) sur les cinq doses testées (1,25 - 2,5 – 5 – 10 et 20 mg/kg). Pour les autres doses testées, l'effet de la fluoxétine seule est conservé (pas de potentialisation, ni d'antagonisme de l'effet de type antidépresseur). Dans cette même étude, la même dose de fluoxétine est potentialisée par la coadministration avec un antagoniste des récepteurs 5-HT_{1B} (GR 127935) alors que cet antagoniste est dépourvu d'effet sur les plus fortes doses d'IRSS (20 mg/kg) (Mayorga et al., 2001). Il est toutefois regrettable que les auteurs n'aient pas étudié les effets de la paroxétine, et ce d'autant plus que dans le même article la fluoxétine et la paroxétine ont été testées chez les animaux mutants privés du gène codant pour le récepteur 5-HT_{1A}. De même chez le rat une équipe polonaise a montré une potentialisation des effets comportementaux dans le FST de la paroxétine par des antagonistes des récepteurs 5-HT_{1B} (Tatarczynska et al., 2002), mais n'a pas retrouvé cette potentialisation pour la fluoxétine et le citalopram (Tatarczynska et al., 2002; Tatarczynska et al., 2004a). De plus, lors de l'étude réalisée en 2002, les auteurs trouvaient un effet propre de type antidépresseur du SB 216641 (Tatarczynska et al., 2002) chez le rat qu'ils n'ont pas retrouvé par la suite chez la souris (Tatarczynska et al., 2004b).

Par contre, les données portant sur les effets comportementaux consécutifs à la coadministration d'antagonistes des récepteurs 5-HT_{1B} et d'antidépresseurs appartenant à d'autres classes pharmacologiques telles que les tricycliques (imipramine), inhibiteurs de recapture de la noradrénaline (désipramine) et IMAO (moclobémide) sont beaucoup

moins hétérogènes que celles portant sur la coadministration IRSS-antagonistes 5-HT_{1B}. En effet, les effets de type antidépresseurs de ces molécules ne sont bloqués ni par l'utilisation d'antagonistes, ni chez les animaux mutants 5-HT_{1B}. Ainsi, l'administration de désipramine chez des souris dépourvues de récepteur 5-HT_{1B} ne permet pas de modifier de façon statistiquement significative le temps d'immobilité évalué dans le TST comparativement à des souris non mutées (Mayorga et al., 2001). Par contre, chez le rat, le GR 127935 et le SB 216641 permettent de potentialiser les effets de type antidépresseurs (observés dans le test de la nage forcée) de l'imipramine, la désipramine et le moclobémide (Tatarczynska et al., 2004a).
La différence au niveau des résultats observés entre l'imipramine et les IRSSs, qui théoriquement agissent d'une façon similaire (i.e. en augmentant la neurotransmission sérotoninergique), semble confirmer l'hypothèse selon laquelle certains antidépresseurs exercent directement une activité postsynaptique et pas seulement présynaptique (Chaput et al., 1991).

De plus, il est maintenant clairement établi que des agonistes des récepteurs 5-HT_{1B} (RU 24969, anpirtoline, CP94253), bien que diminuant la neurotransmission sérotoninergique, possèdent un effet de type antidépresseur dans différents tests comportementaux comme le FST chez la souris (Redrobe and Bourin, 1999; O'Neill and Conway, 2001; Tatarczynska et al., 2004b; Tatarczynska et al., 2005) ou bien peuvent être utilisés à doses subactives pour potentialiser les effets d'autres antidépresseurs dans ce même test (Redrobe et al., 1996; David et al., 2001). Alors que les antagonistes (GR 127935, isamoltane, SB 216641) semblent être dépourvus d'effet propre dans ce test de la nage forcée chez la souris (O'Neill and Conway, 2001; Tatarczynska et al., 2004b; Tatarczynska et al., 2005) et chez le rat (Tatarczynska et al., 2002; Tatarczynska et al., 2004a).

Il semble donc que l'activation des autorécepteurs 5-HT_{1B} limite les effets comportementaux des antidépresseurs, alors que l'activation des hétérorécepteurs induit ces effets.

1.5 Les modèles de dépression

La réalisation de tests comportementaux en psychopharmacologie permet lors de la phase d'essais préclinique de sélectionner les molécules présentant une potentielle activité thérapeutique. Ces tests peuvent également servir de moyen d'études pour mieux comprendre le mécanisme d'action des antidépresseurs.

La modélisation d'une pathologie à l'aide d'un modèle animal consiste à reproduire lors d'une expérimentation simple dont tous les paramètres sont maîtrisés un état beaucoup plus complexe qu'est la pathologie. Ainsi, un modèle animal idéal devrait non seulement reproduire tous les symptômes de la pathologie mais aussi être prédictif de l'activité antidépressive de nouveaux composés. Par conséquent, le développement d'un modèle animal nécessite nécessairement un compromis réductionniste. Cette « réduction » (modélisation) de la pathologie doit se faire en respectant trois grands critères de validité (Willner, 1984) :

- la validité créative, le comportement de l'animal induit par le modèle doit présenter des similarités avec la pathologie modélisée
- la validité théorique, le modèle animal doit permettre l'identification des mécanismes neurochimiques
- la validité prédictive, le modèle animal doit permettre d'identifier correctement les antidépresseurs des différentes classes pharmacologiques, sans faux-positifs, ni faux-négatifs.

A l'heure actuelle aucun test ne permet de reproduire chez l'animal tous les symptômes des épisodes dépressifs majeurs observés chez l'Homme.

La mise au point des tests a été réalisée de façon rétrospective (après avoir démontré que les médicaments antidépresseurs présentaient une activité dans les tests). Différents modèles ont ainsi été développés, et peuvent être classés en quatre catégories :

- **les modèles de comportements induits par des substances chimiques** ; le premier de ces modèles, fut l'antagonisme des effets de la réserpine. Ce modèle se base sur le fait que l'administration de réserpine permet d'induire un état dépressif chez l'Homme. L'administration de réserpine induit également chez l'animal une hypothermie, un ptosis et une diminution d'activité locomotrice ; les molécules administrées en dose unique et permettant la restauration de ces trois derniers paramètres seraient des antidépresseurs. Bien que présentant une bonne validité théorique (déplétion monoaminergique induite par la réserpine), ce test

présente une mauvaise validité prédictive (nombreux faux positifs) et n'est donc plus utilisé.

- **Modèle animal fondé sur un déficit neuronal** ; le seul modèle animal basé sur un déficit neuronal induit est la bulbectomie olfactive (BO) chez le Rat (Cairncross et al., 1977). Il repose sur l'hypothèse selon laquelle la dépression est un trouble biochimique développé par des individus prédisposés à un déficit neuronal. Les dommages cérébraux induits par l'ablation bilatérale des deux bulbes olfactifs, provoquent des changements comme l'irritabilité, l'hyperactivité et une élévation des concentrations de corticostéroïdes plasmatiques. L'altération du comportement de ces rats est corrigée après un traitement chronique par des antidépresseurs, mais pas par d'autres psychotropes. Ces rats présentent par ailleurs de nombreux dérèglements physiologiques, dont certains sont communément associés à la maladie dépressive, comme des altérations du comportement sexuel, des changements immunitaires et des perturbations de la fonction cardiaque (Kelly et al., 1997).

- **Modèles animaux fondés sur le stress** ; ces tests sont basés sur l'hypothèse que des conditions stressantes pourraient augmenter la vulnérabilité de l'Homme aux épisodes dépressifs et seraient donc un des facteurs étiologique de la dépression. Aussi, des modèles animaux basés sur l'hypothèse que la dépression est causée par un stress non contrôlé par l'animal, ont été proposés. Il s'agit du test de résignation acquise (Seligman and Beagley, 1975), du test de la nage forcée (FST) (Porsolt et al., 1977), du test de suspension caudale (TST) (Steru et al., 1987) et du stress modéré chronique (Willner et al., 1992).
- **Les Modèles animaux de prédisposition à la dépression** ; ces modèles ont été développés afin de reproduire chez l'animal des variations physiologiques observées lors d'un épisode dépressif (pour revue, voir Willner et Mitchell, 2002). Parmis ces modèles on trouve :

- des modèles génétiques (il s'agit du test de résignation acquise congénital, de souche sélectionnées pour l'hypersensibilité de leur système cholinergique),
- des modèles génomiques (souris dépourvues de transporteur de la sérotonine, de récepteurs au CRH ou aux tachykinines)
- des animaux pour lesquels le développement à été modifié (traitement néonatal par antidépresseur, soumission à un stress prénatal ou néonatal ainsi que des modèles de lésion)

2.0 MATERIELS ET METHODES

2.1 Animaux

Etude n°1 :

Choix de la souche de souris

De nombreuses études ont permis de mettre en évidence l'existence d'une réponse différente aux antidépresseurs dans les tests comportementaux en fonction de la souche de souris utilisée. Le choix du test et de la souche de souris à utiliser sont donc deux paramètres essentiels à prendre en compte lors de la réalisation d'études comportementales. Cette différence de **sensibilité** entre les animaux a fait l'objet de nombreuses expérimentations au sein du laboratoire. Afin d'être certain d'utiliser une souche de souris adaptée à nos études, nous avons réalisé une analyse rétrospective de l'ensemble des travaux réalisés dans notre laboratoire. Cette étude doit nous permettre de déterminer la souche répondant le mieux aux antidépresseurs ; c'est-à-dire qui, associé avec le test adéquat, présente une bonne prédictivité ainsi qu'une bonne validité créative.

Dans un second temps, nous avons voulu mettre à profit les différences de sensibilité aux antidépresseurs de certaines souches de souris (tels que l'absence de sensibilité des NMRI aux antidépresseurs tricycliques) afin de proposer une méthodologie fiable pour déterminer le mécanisme d'action d'un antidépresseur en se basant uniquement sur des tests comportementaux.

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Short communication

A proposal of decision tree to screen putative antidepressants using forced swim and tail suspension tests

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Abstract

Interstrain mice variability in response to antidepressant drugs has been reported in the most commonly utilized behavioural animal models of depression: the tail suspension test (TST) and the forced swimming test (FST). The behaviour of mice was examined in both tests for screening various antidepressants with different biochemical mechanism of action. Previous studies have revealed that drug sensitivity depends on the strain and test used. Swiss mice is the most sensitive strain to detect serotonin and/or noradrenaline antidepressants whereas C57BL/6J was the only strain sensitive to bupropion (dopaminergic agent) using the FST. In the TST, all antidepressants studied decreased the immobility time in Swiss and C57BL/6J strains.

Detection of an antidepressant-like activity could be performed using only one test (TST with Swiss mice or FST with Swiss and C57Bl/6 Rj mice), but both tests are necessary to conclude on the mechanism of action.

Keywords: Forced swimming test (FST); Tail suspension test (TST); Mouse; Antidepressants; Screening

Forced swimming test (FST) [21] and tail suspension test (TST) [28] are two of the most commonly animal models of depression used for antidepressant screening. In both tests, animals are placed in an inescapable situation and the antidepressant-like activity is expressed by the decrease of immobility duration. During the last decade, we have routinely used these models in our research laboratory not only to predict antidepressant-like activity of various compounds, but also to investigate their mechanism of action. Using various ligands and the FST, we demonstrated the important implication of serotonin 1A (5-HT_{1A}) and 1B (5-HT_{1B}) receptors in the mechanism of action of selective serotonin re-uptake inhibitors (SSRI) [23]. Recently, we published two studies establishing the impact of genetic factors in the efficiency of various antidepressants in both tests [11,24] as well as a review paper to clarify the use of the FST [19].

The aim of this paper is to summarise data obtained in previous studies, to propose a strategy that could be used for the development of new antidepressants via the determination of the potent antidepressant-like activity and investigation of the mechanism of action. Our objectives were to detect the antidepressant-like effect of each compound using low doses (better specificity of action), and secondly, to obtain the greater effect-size (response amplitude) for each type of antidepressant regarding their mechanism of action. This last point is crucial, as the greater the effect-size, the easier the possibility of antagonising the antidepressant effect and determining the implicated transporters or receptor subtype.

Four different naïve male strains of mice: Swiss, NMRI (outbred), C57Bl/6J and DBA/2 (inbred) (Janvier, France), weighing 20–24 g were employed. They were housed in

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normal conditions (18 per cage of 40 cm × 28 cm × 17 cm) on 12:12 light/dark cycle and had free access to food and water. The ambient temperature of the room was maintained at 21 ± 1 °C and the humidity was 50%. Each test was performed with a minimum of 10 animals.

In the FST mice are individually placed into glass cylinders (height 25 cm, diameter 10 cm), containing 10 cm of water maintained at 23–25 °C and left for 6 min. Immobility is measured during the last 4 min of the duration of the test (the animal is judged to be immobile when it floats in an upright position and makes only minimal movements to keep its head above water). [21].

The TST is a derivative of the FST and is based on the fact that a mouse suspended by the tail alternates periods of agitation and immobility similar (but not identical) to that observed in the FST [28]. Mice are suspended by the tail, using an adhesive scotch tape, to a hook connected to a strain gauge that picks up all movements of the mouse and transmits them to a central unit which calculates the total duration of immobility during a 6 min test. We used an automated version of the initial procedure [27].

A dose range (1–16 mg/kg) of imipramine (tricyclic antidepressant, TCA), desipramine (noradrenalin reuptake inhibitor, NRI), paroxetine, citalopram (SSRI) and bupropion (dopamine reuptake inhibitor, DRI) was tested in mice of each strain for motor activity in the locomotor's apparatus. Only the non-psychostimulant doses detected in this test for the same strain were subsequently tested in the TST and FST. Each drug was dissolved in distilled water and drugs were administered intraperitoneally 30 min prior to the test at a constant volume of 0.5 mL/20 g.

The antidepressants we used represent the main antidepressant classes proposed by Frazer [13] (Table 1).

These results indicate that only one test, the TST utilising Swiss mice, can consistently illustrate antidepressant-like activity. As the C57BL/6J mice attempted to redress their position (i.e. climbing up their tails previously reported) [17,24] it was difficult to conclude on their activity in this test. A similar problem to that obtained with the C57BL/6 Rj mice was observed for the DBA/2 strain in the TST [24].The use of Swiss mice is of greater interest due to the greater effect-size obtained (with the exception of citalopram: 68% versus 57%). In laboratories which do not use a TST apparatus, the antidepressant activity may be evaluated using FST in two strains of mice: Swiss and C57BL/6 Rj.

According to our research the majority of the drugs were efficient in the FST in Swiss mice. It was also largely demonstrated that various classes of ADs were efficient in the FST in this strain of mice such as SSRIs (citalopram: [3,6,9,11,16], fluoxetine: [5,9,25,29], fluvoxamine: [9,16,29], paroxetine: [3,6,9,11], sertraline: [9]); NRI (desipramine: [4,9]; oxaprotiline: [18]; viloxazine: [3,5,16]) and tricyclics ADs (amitriptyline: [3,29]; dothiepin: [7]; imipramine: [1,3–5,9,11,16,25]). To investigate the mechanisms of action of a new potent antidepressant drug, the FST is a more powerful tool in Swiss mice as the first step. A positive result indicates the potential AD properties of the compounds (TCAs, NRIs, SSRIs or serotonin and noradrenaline reuptake inhibitors, SNRIs) [23] in Swiss mice. Even if these results were obtained using Swiss mice from Janvier, similar results could be obtained using mice from an other breeder, i.e. CD1 from Charles River which is usually considered as being an equivalent of Swiss mice strain; various ADs showed an AD-like effect when tested in CD-1 mice: mianserin [8,12,21], desipramine [12,15], viloxazine [21], fluoxetine [32], paroxetine [10], sertraline [10], imipramine [2,8,20,21]. The major difference between CD1 and Swiss mice concerns the fact that DRI exerts an AD-like effect in the FST when tested in CD1 mice (bupropion: [32]; nomifensine: [8,12]) whereas they are devoid of effects when tested in Swiss mice. This last point seems to be crucial, but needs more investigation, because these studies do not include spontaneous locomotor evaluation; it is then highly presumable that the behavioural effect obtained should be linked to a psychostimulant activity of DRI.

In the case of a negative result, FST in C57Bl/6J mice must be performed, because dopaminergic antidepressants (e.g. bupropion) lack of effect in the first strategy purposed (FST with Swiss mice), but are efficient in the second test (decrease immobility time for more than 25% in C5BL/6 mice).

It appears that using only FST in two different strains of mice (Swiss and C57Bl/6) we can predict an antidepressant activity. If the FST demonstrates an antidepressant-like

Table 1
The values represent the maximal significant percentage of decrease of immobility time for each drug in each test for the optimal dose

	Imipramine		Desipramine		Paroxetine		Citalopram		Bupropion	
	FST	TST	FST	TST	FST	TST	FST	TST	FST	TST
Swiss	20% (16 mg/kg)	49% (16 mg/kg)	22% (16 mg/kg)	55% (16 mg/kg)	23% (16 mg/kg)	65% (16 mg/kg)	27% (8 mg/kg)	57% (16 mg/kg)	0	47% (16 mg/kg)
NMRI	0	66% (16 mg/kg)	0	0	16% (8 mg/kg)	56% (8 mg/kg)	0	47% (8 mg/kg)	0	0
C57Bl/6J	0	45% (16 mg/kg)	0	30% (16 mg/kg)	0	38% (2 mg/kg)	0	68% (16 mg/kg)	26% (4 mg/kg)	44% (8 mg/kg)
DBA/2	0	35% (16 mg/kg)	0	0	0	47% (2 mg/kg)	0	45% (1 mg/kg)	0	0

0 Indicate an absence of significant effect.

activity, other tests may be necessary to anticipate the impact of molecules on neurotransmitters.

The next test should be a TST in NMRI mice, as TCAs (e.g. imipramine), SSRIs (e.g. paroxetine and citalopram) and SNRIs (e.g. venlafaxine) were efficient in this test with an approximate time decrease of 50% (imipramine: 66%, paroxetine: 56%, citalopram: 47%). On the contrary, NRIs (e.g. desipramine) were devoid of effect [24]. If the test is negative, it suggests that the new drug is probably a NRI.

To distinguish between SSRIs and TCAs, we suggest the use of FST in Swiss mice, based on the fact that the effect could be antagonised, or potentate, using 5-HT_{1A} antagonist ((±)-pindolol) or agonist (8-OH-DPAT) or 5-HT1B antagonists (GR 127935):

- Prior administration of 8-OH-DPAT or GR 127935 potentate anti-immobility effects of TCAs (imipramine), but not those of SSRIs (fluoxetine, citalopram and fluvoxamine) [14,23,30]
- Pre-treatment with pindolol potentate the effect of SSRIs and was devoid of activity with imipramine [23].

To investigate the mechanism of action of a potential antidepressant drug, the use of both tests is required with only three strains of mice (Swiss, NMRI and C57Bl/6J). Some compounds with variable mechanisms of action (like TCAs and SSRIs), induce a similar response regardless of the test and the mouse strain used but with an effect size depending probably of the polymorphism in various genes such as those responsible for synthesis, metabolism and reuptake of 5-HT [26]. For these drugs, the mechanism of action may be investigated using additive compounds to potentate, or antagonise the response [22]. This methodology could be of interest when the binding and/or the mechanism of action is unclear but as well to choose the best strain and/or test, to confirm the previous results of biochemistry.

At the end a decision tree was established for screening purposes (Fig. 1).

Limitation of our proposal is linked with "seasonal" activity, but if the experiments following the decisional tree are performed into few days or weeks it is possible to escape this problem. In other hand, it was shown that the same strain of mice from different breeders may respond differently to antidepressants in the FST [20]. A gene interaction is possible and may account for some difference between laboratories [31]. For example, in our data set, animal of the same strain that received no treatments do not have the same immobility time (for CD-1 from 135 to 223 s of immobility time). The pharmacokinetics of antidepressants in between the different mice strains does not seem to induce a modification of the administration time (30 min) before the test (unpublished

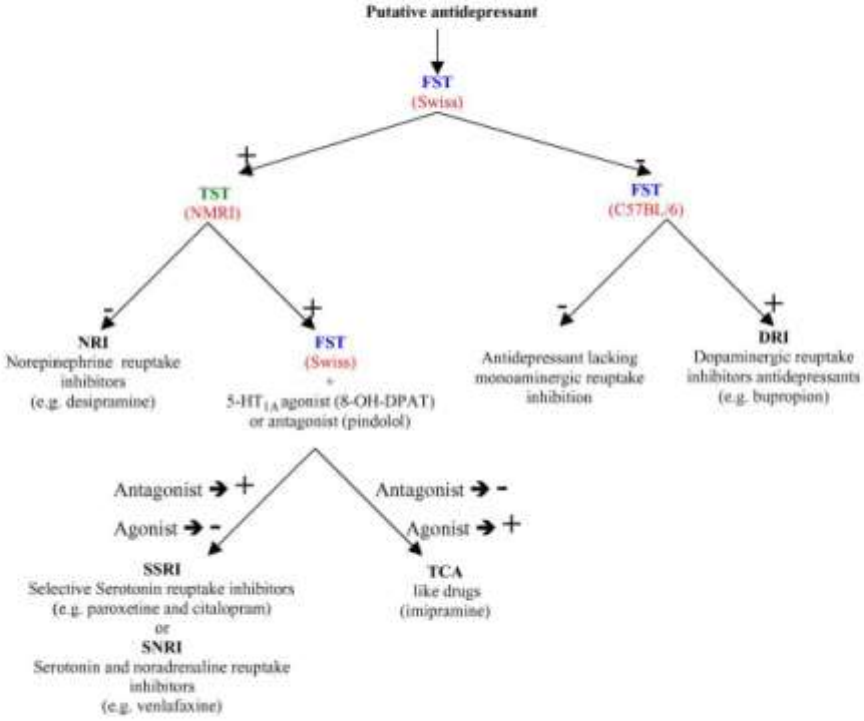

Fig. 1. A decision tree for screening putative antidepressants.

personal data). For all details on the parameters variations of the FST see our review [19]. Finally this decisional tree could be useful if biochemical studies do not provide any direct data on a specific neurotransmitter activity, the behavioural results will suggest an indirect action on a monoaminergic system.

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Résumé de l'étude n°1 :

Cette étude nous a permis de définir la souche de souris à utiliser pour les travaux expérimentaux : souris SWISS mâles (issues du centre d'élevage JANVIER, Le genest, France). Les animaux sont réceptionnés et hébergés dans l'animalerie du laboratoire pour une durée de 4 à 8 jours avant la réalisation des expérimentations ; ils sont placées par cage de 18 animaux (dimensions : 40 x 28 x 17 cm) et ont un accès libre à l'eau et à la nourriture. La température de l'animalerie est maintenue à 21±1°C et le cycle d'éclairage est un cycle standard (lumière entre 7h et 19h). Les expérimentations sont réalisées le matin entre 7h et 12h.
Les règles éthiques du Ministère Français de l'Agriculture concernant l'expérimentation animale ont été respectées tout au long de l'étude (décret n°87-848 du 19 octobre 1987).

A la suite de cette étude, nous avons également déterminé le test comportemental à utiliser pour la suite des travaux: le test de la nage forcée (FST) en se basant sur les deux principaux critères de choix d'un modèle animal de dépression : reproductibilité et robustesse. Nous aurions également pu utiliser le test de suspension caudale chez les souris Swiss, puisque les antidépresseurs sont également actifs dans ce test chez cette souche de souris. Toutefois, en se basant sur notre expérience nous avons remarqué que les résultats obtenus dans le FST sont plus reproductibles car moins soumis à des variations interindividuels. Par conséquent, le nombre d'animaux à utiliser pour chaque étude est restreint. (pour une revue sur le TST, voir Cryan et al., 2005).

2.2 Tests comportementaux

Test d'actimètrie (Boissier and Simon, 1965)

L'effet des différentes molécules utilisées sur l'activité locomotrice spontanée des animaux est déterminé à l'aide d'un actimètre (OSYS, Laval, France). L'appareil est constitué de 6 compartiments munis de cellules photoélectriques et contenant chacun une cage en plexiglas. Chaque compartiment est traversé par deux faisceaux. Les ruptures de ces faisceaux sont comptabilisées pour évaluer l'activité locomotrice horizontale de l'animal. Cette activité est enregistrée pendant une période de 10 min. Ce test est réalisé indépendamment du test de la nage forcée. Les résultats obtenus (nombre de coupure de faisceaux) permettent, en comparant l'activité locomotrice de chaque groupe de souris traitées avec un groupe contrôle, d'éliminer les doses psychostimulantes pouvant être à l'origine de « faux positifs ».

Figure 6 : Test d'actimètrie

Le test de la nage forcée chez la souris (Porsolt et al., 1977)

Etude n°2 :

Test originel ou test modifié ?

Le FST est un modèle largement utilisé pour détecter une activité de type antidépresseur tout d'abord parce qu'il présente une bonne prédictivité et une bonne validité créative, mais aussi puisqu'il est facile à mettre en place, peu coûteux et qu'il a été prouvé que ses résultats sont reproductibles.

Le FST chez la Souris apparaît comme un modèle animal de dépression adéquat pour définir une réponse antidépressive même si la symptomatologie induite n'est que difficilement comparable à la dépression chez l'Humain puisque les effets des molécules s'observent même lors d'une administration en aigu. Cependant ce modèle est sensible à toutes les classes d'antidépresseurs et induit peu de faux positifs lorsqu'il est couplé à un test d'activité locomotrice. Depuis quelques années, il a été démontré que le FST permet l'exploration des mécanismes d'action des molécules antidépressives à travers l'utilisation de coadministration d'antidépresseurs avec des ligands spécifiques tels que la buspirone (Redrobe and Bourin, 1998). Plusieurs auteurs ont cherché à modifier certains des paramètres du test décrit par Porsolt et al. afin d'optimiser les résultats obtenus. L'analyse bibliographique suivante a eu pour objectif de déterminer quel pouvaient être les modifications à apporter au test afin d'accroître sa sensibilité. Le test est réalisé chez la souris, car dans le FST chez la souris, il existe une très bonne corrélation entre l'activité de type antidépresseur prédite pour une molécule et son effet avéré en clinique puisque 94% des molécules, dont le test montre une baisse du temps d'immobilité, se révèlent être de futurs antidépresseurs alors que ce chiffre n'est que de 87% pour le FST chez le Rat (Bai et al., 2001).

Figure 7 : Test de Porsolt

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REVIEW

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Forced swimming test in mice: a review of antidepressant activity

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Abstract *Rationale:* Among all animal models, the forced swimming test (FST) remains one of the most used tools for screening antidepressants. *Objective:* This paper reviews some of the main aspects of the FST in mice. Most of the sensitivity and variability factors that were assessed on the FST are summarized. *Mechanisms:* We have summarized data found in the literature of antidepressant effects on the FST in mice. From this data set, we have extrapolated information on baseline levels of strain, and sensitivity against antidepressants. *Results:* We have shown that many parameters have to be considered in this test to gain good reliability. Moreover, there was a fundamental inter-strain difference of response in the FST. *Conclusions:* The FST is a good screening tool with good reliability and predictive validity. Strain is one of the most important parameters to consider. Swiss and NMRI mice can be used to discriminate the mechanisms of action of drugs. CD-1 seems to be the most useful strain for screening purposes, but this needs to be confirmed with some spontaneous locomotor activity studies.

Keywords Forced swimming test · Depression · Mouse · Antidepressants · Screening · Strain

Abbreviations

5-HT	Serotonin
8-OH-DPAT	8-Hydroxy-2-(di-*n*-propylamino)tetralin
Atypical	Antidepressants with an atypical activity
BDNF	Brain-derived neurotrophic factor
DRI	Dopamine re-uptake inhibitors
FST	Forced swimming test
MAO-I	Monoamine oxidase inhibitors
NA	Noradrenaline
NOS synthase	Nitric oxide synthase
NRI	Noradrenaline re-uptake inhibitors
SNRI	Serotonin and noradrenaline re-uptake inhibitors
SSRI	Selective serotonin re-uptake inhibitors
TCAs	Tricyclic agents
TST	Tail suspension test

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Introduction

Half a century ago, antidepressants were discovered by serendipity. In 1954, it was observed that some treatments for tuberculosis were exerting a beneficial effect in the sense of well-being (Selikoff and Robitzek 1952; Bloch et al. 1954). These results set iproniazid as the first antidepressant (Loomer et al. 1957) and the first member of the monoamine oxidase inhibitor (MAO-I) family (Zeller and Barsky 1952). At the same time, imipramine was found to be effective in treating depression (Kuhn 1957; Klerman and Cole 1965). At this point, a completely new approach was exposed: the monoamine theory of depression or biogenic amine hypothesis (Bunney and Davis 1965; Schildkraut 1965; Coppen 1967). The key role of monoamines is not discussed here but it does not fully describe the pathogenesis and aetiology of depression (Heninger et al. 1996; Hyman and Nestler 1996; Nestler 1998; Nestler et al. 2002).

In addition to clinical research, pre-clinical studies were necessary to test new drugs provided by pharmaceutical industry. Depression is defined clinically as a pathological complex of psychological, neuroendocrine and somatic symptoms that cannot be reproduced in animals and especially in mice. Only specific measurable behaviours (endophenotypes) can be assayed to be relevant in human depression (Holmes 2003a). During this 50-year period, numerous animal models of depression have been designed, tested and assessed (Willner 1984; Lucki

1997; Dalvi and Lucki 1999; Holmes 2003b; Cryan and Mombereau 2004). The reserpine effects reversal test, designed by Costa et al. (1960), was the first attempt to screen imipramine-like drugs and led to the isolation of desipramine and the demonstration of its antidepressant effect. To date, few models are commonly used for screening antidepressant effects or studying the mechanisms of action of these molecules. The aim of this paper is mainly to review the characteristics of one of these models: the forced swimming test (FST) in mice, and to discuss the main parameters that influence the sensitivity, specificity and reliability of this model.

Porsolt et al. (1977) described "a new behavioural method for inducing a depressed state in mice". The idea arose out of some learning experiments they were doing with rats in a water maze. Most rats were finding the exit within 10 min but they noticed that other rats ceased struggling altogether and remained floating passively (Porsolt et al. 1979). To describe this new behavioural model in mice, the following procedure was adopted "1 h after a single i.p. injection mice were dropped into the cylinder (height 25 cm, diameter 10 cm, 6 cm of water at 21–23°C) and left for 6 min. Because little immobility is observed during first 2 min, only that occurring during the last 4 min was counted. The duration of immobility occurring in each minute was scored. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only movements necessary to keep its head above water" (Porsolt et al. 1977). Male CD (Charles River) mice of 20–25 g, were housed ten to a cage with free access to food and water.

In the same paper, Porsolt tested a large range of antidepressants and showed a reduction of immobility of mice with all of them. The other usual clinical therapies were also effective (e.g. electroconvulsive shock or selective deprivation of REM sleep) (Porsolt et al. 1977, 1979).

The goal of the present paper is to summarize the advantages and drawbacks of the FST in mice, as well as the factors of variability of the test through an extensive review of the litterature.

FST validity, advantages and drawbacks

To evaluate the validity of an animal model, many criteria have to be explored. For example, we could consider reliability and different types of validity: predictive, face, construct, etiological, concurrent and discriminant. Undoubtedly, the more types of validity a model satisfies, the greater is its value, utility and relevance to the human condition. To establish the value of a model in basic neurobiological research, few of these parameters have to be satisfied (Geyer and Markou 2000). It was argued that there are only two criteria that a model must satisfy to establish its value in basic neurobiological research: reliability and predictive validity. Nevertheless, the process of construct validation is valuable in further development and refinement of the model; however, in practice it is difficult to determine this validity in animal models of depression (Geyer and Markou 2000; Willner and Mitchell 2002).

As semantic issues seem to exist between some authors (Geyer and Markou 2000; Willner and Mitchell 2002), it is fundamental to have a clear definition of used terms. For example, predictive validity and construct validity have not the same meaning for Willner and Mitchell than for Geyer and Markou. Predictive validity for Willner and Mitchell is assessed by whether a model correctly identifies antidepressant treatments without making errors of omission or commission, and whether potency in the model correlates with clinical potency. For Geyer and Markou, an animal model has predictive validity to the extent that it allows one to make predictions about phenomena based on the performance of the model. The narrow sense to refer to the ability of the model to identify drugs with potential therapeutic value in humans appears limited for Geyer and Markou. It does not include the identification of any variables that influence both the experimental preparation and the modelled phenomenon in similar ways. This wider definition includes much of what Willner and Mitchell would discuss under the rubric "construct validity". Bearing in mind this opposition, predictive value in our paper will accord to the definition of Willner and Mitchell.

Construct validity does not represent the same concept for these authors. Geyer and Markou defined it as the accuracy with which the test measures that which it is intended to measure. For Willner and Mitchell, it is a means of bringing the theoretical accounts of both the disorder itself and the disordered behaviour exhibited by the model into alignment. It includes neurobiological mechanisms, aetiology or psychosocial mechanisms. We will use this definition of construct validity that includes etiological validity previously evocated (Geyer and Markou 2000).

Reliability refers to the consistency and stability with which the variables of interest are observed, and is relevant to both independent and dependant variables (Geyer and Markou 2000).

Willner used a third parameter to describe some animal models of depression: face validity (Willner 1984).

Face validity for an animal model of depression, represents the analogy between the model and the disease (i.e. how well they apparently resemble the human depressive state). It refers to the phenomenological similarity between the behaviour exhibited by the animal model and the specific symptoms of the human condition. This criterion is often criticized because of its non-scientific aspect. It sums up specific patterns of depression and the model should not show features that are not seen clinically. Although it appears to be useful to validate models, such a criterion is actually not necessary (Geyer and Markou 2000). Because the pharmacotherapy of depression typically requires chronic drug treatment to obtain a full response, face validity (Willner 1984) takes account of the necessity, or not, to use chronic administration to have an antidepressant effect and the specificity

of observed features. Irrespective to how it responds to acute antidepressant treatment, to have face validity, an animal model if depression must respond to chronic treatment (Willner and Mitchell 2002).

These types of validity are discussed below for the FST. For other types of validity, the FST was estimated to have a lack of convergent validity and a possible etiological validity (Geyer and Markou 2000).

Reliability

The FST is currently a popular model, due to the low cost of the experiments and because it is arguably the most reliable model available (Holmes 2003b). Moreover, it has been reported to be reliable across laboratories (Borsini and Meli 1988).

Predictive validity

To evaluate predictive validity, correlating potencies between a model and the condition it models is possible (Willner 1984). In a comparative review of drug effects on immobility time in mice, Borsini and Meli adopted a limit of 20% reduction of immobility to consider an antidepressant effective on the test. They show that 94% of antidepressants decrease the immobility time in mice (Borsini and Meli 1988). In this study, they found that 83% of classes of drugs decrease immobility time in the mouse. This lack of specificity may be largely explained by methodological considerations. Some authors changed the scoring method, other authors recorded animal movements by using automated devices. Moreover, false positive effect of motor activity enhancing drugs would have been detected with an actimeter, where psychostimulant drugs could reduce immobility without antidepressant effect (Porsolt et al. 1978). Nevertheless, the FST is a suitable model to detect antidepressants due to the fact that it detects the majority of antidepressants and discriminates antidepressants from neuroleptics and anxiolytics (Borsini and Meli 1988).

Together, these data provide us with a broad spectrum of antidepressant effects with good reliability and some answers to the lack of specificity of the test, which has been discussed (Schechter and Chance 1979).

Face validity

A second characteristic of the FST is that acute drug treatments are effective in this model and do not correspond to the clinical time course of their action. One of the main arguments increasing the face validity is that chronic treatments reinforce the effects of antidepressants on immobility. It showed several differences between chronic and subchronic treatment of a SSRI, fluoxetine, in the FST with BALB/c mice weighting 25–35 g (Dulawa et al. 2004). Four days of treatment were ineffective, whereas 24 days were effective at 10 mg/kg per day and 18 mg/kg per day administered in drinking water. This delay of action provides further data to increase the face validity of FST in mice, even if their experimental paradigm was not the original method for the FST. (Dulawa et al. 2004). Moreover, we have to consider that some authors using standard methods showed that fluoxetine was effective acutely at 16 mg/kg in the FST with CD mice (Da-Rocha et al. 1997). This shows the preponderant place of methodological parameters in behavioural studies (e.g. strain, pretest session).

In addition, from an ethical point of view, this animal experiment requires only a single exposure to the stressful stimulus (Thierry et al. 1986). The used dose in the test as well as in other tests in mice are high doses compared to human but the pharmacokinetic parameters in mice are very different (i.e. half-life is about 1 h for mice compared to several hours in human). Face validity for the FST with mice is not strong; chronic administration remains to be fully studied in order to increase this face validity.

Construct validity

The construct validity of the FST is difficult to establish and questionable. Indeed, the onset of immobility observed during the test is hard to interpret. Porsolt et al. (1978) described this state as a behavioural despair "reflecting a state of lowered mood". He had also dissociated hypothermia induced by forced swimming from immobility occurring in these conditions and also from drug-induced hypothermia in rats (Porsolt et al. 1979). The anthroponorphic interpretation was assessed and replaced by other assumptions: a shift from active coping to passivity, a means to conserve energy (Arai et al. 2000; Holmes 2003b) or a psychological concept of "entrapment" described in clinical situations (Cryan and Mombereau 2004). This passive behaviour could also be considered as unwillingness to maintain effort in this inescapable situation. Immobility may be seen as an adaptative response to an inescapable situation. This strategy could be perceived as a successful coping rather than a failure of coping (West 1990).

Immobility observed in the swim test seems not to be related to behaviour in the tests used in anxiety models (elevated-plus maze, hole-board test, locomotor activity) (Hilakivi et al. 1989). Even if the onset of immobility remains hard to interpret, the aetiological part of construct validity could be highly relevant. The stress leading to the behavioural despair may be involved in the aetiology of some types of depression in humans (Geyer and Markou 2000). Nevertheless, the FST has a very little construct validity due to this acute and non-ecologically relevant stressor that produces this behaviour (Willner and Mitchell 2002).

To summarize, FST has strong predictive validity, good reliability, some face validity and poor construct validity. In a review of the causes of immobility in the FST, West (1990) concluded that FST "no longer appears to be a

valid model of depression. Nonetheless the forced swim test is still likely to be useful in understanding antidepressants treatments". This point of view should be moderated by a consideration on "what is a valid model of depression?"

When pre-clinical tests were created to study the depressive state, the first role for models of depression was to predict antidepressant potency. Moreover, the validity of these tests was largely based on an empirical observation, namely that the two major groups of antidepressants, MAO-I and tricyclic drugs (TCAs), are active (Bourin 1990).

The FST, as described by Porsolt et al. (1977), has been designed to be "a primary screening test for antidepressants". For this purpose, FST is a good model for screening antidepressants, maybe the best one. FST shows a strong sensitivity to monoamine alterations, but it should not be forgotten that other antidepressants treatments, such electroconvulsive shock, are efficient (Porsolt et al. 1977). To summarize these ideas, we can consider that "The FST models a very specific cluster of stress-induced behaviours that have no direct, empirical relation to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations" (Holmes 2003b). Additional possibilities for the FST should be considered on a more neuropharmacological point of view. This test also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses (Porsolt 2000; Lucki et al. 2001; Nestler et al. 2002).

Moreover, new approaches of research for antidepressant treatments continue to use the FST as a preliminary test. For example, some authors work on neurotrophic factor that could potentially be used in the treatment of depression. They used the FST and showed that brain-derived neurotrophic factor (BDNF) infusion in the ventral tegmenta area resulted in 57% shorter latency to immobility relative to control animals, in the FST in rats (Eisch et al. 2003). This use of the FST had already been described previously with a 70% decrease in the immobility time compared to vehicle-infused controls after BDNF infusion (Siuciak et al. 1997). Other ways of investigation for depression use the FST as model of depression. Acute antidepressant treatment attenuates swim stress-induced corticosterone release in the rat (Baez and Volosin 1994). NK2-receptor antagonists, K^+ channel openers and K^+ channel blockers were considered for their antidepressant-like properties in the forced swim test (Guo et al. 1996; Redrobe et al. 1996; Slattery et al. 2004). Nitric oxide synthase (NOS) or neurosteroids have been tested in the FST with mice to look for an antidepressant-like effect (Harkin et al. 1999; Khisti et al. 2000). Many studies keep using the FST, not only for screening for antidepressant effects, but for a more neuropsychological purpose. This utilization of the FST differ from the monoaminergic purpose it is often used. Nevertheless, this model of depression is not only linked to monoamine. The uncontrollable stress involved during the test may implicate many mechanisms of reaction that could be considered as possible investigation ways. The fact that electroconvulsive seizures are effective in the test argues for its ability to pick up broader mechanisms of action (Nestler et al. 2002). The relevance of using the FST for this new ways of research needs clinical correlations to validate also the FST for this utilization. The development of clinically effective antidepressant

Table 1 Summary of some modifications tested on the FST in mice

Factor	Sensitivity	Variability	Reference
Acute vs chronic administration		X	Dulawa et al. (2004)
Age of mice		X	Bourin et al. (1998a); David et al. (2001a)
Automated device/water waves	X		Browne (1979); Denenberg et al. (1990)
Circadian rhythm		X	Dubocovich et al. (1990)
Cylinder diameter	X		Sunal et al. (1994)
Depth of water	X		Aley and Kulkarni (1989)
Environment of the laboratory		X	Crabbe et al. (1999)
Food restriction		X	Cabib et al. (2000); Cabib et al. (2002)
Gender		X	Alonso et al. (1991); David et al. (2001b); Voikar et al. (2001)
Housing of animals		X	Karolewicz and Paul (2001)
Isolation of animals			Hilakivi et al. (1989); Yates et al. (1991)
Interval of observation	X		Sunal et al. (1994); Lucki (1997)
Observer		X	
Revised scoring	X		Lucki (1997)
Scoring on categorized behavior			Schramm et al. (2001)
Side preference in rotation		X	Krahe et al. (2002)
Strains		X	Lucki et al. (2001); Voikar et al. (2001); Bai et al. (2001); David et al. (2003)
Test / retest		X	Alcaro et al. (2002)
Time between treatment and FST	X		
Water temperature	X		Arai et al. (2000); Tathavull et al. (2003)
Wheel water tank	X		Nomura et al. (1982)

drugs with novel mechanisms should give answers to this question.

Another point is the utilization of the FST for genetically modified animals that is applicable to study mechanisms of action of antidepressant on the test. For example, the decrease of immobility observed after paroxetine administration in wild-type mice is absent in 5-HT1B knockout in the test (Gardier et al. 2001). Other data with knock-out mice can be useful to determine the role of NA or 5-HT in the test; for example with mice lacking serotonin transporter (Holmes et al. 2002) or dopamine-beta-hydroxylase deficient mice (Cryan et al. 2001). This new employment of the test permits to better know the mechanisms of action of drugs on the FST involving or not the monoamine, i.e. for new possible therapies for example, BDNF+/– mice (MacQueen et al. 2001) or inducible BNDF knock-out (Monteggia et al. 2004).

Modifications of the FST

There have been many modifications of the FST but improvements of the test are often poorly validated (Bourin et al. 2001). Many parameters have been assessed in order to increase the sensitivity, specificity and reliability of detection of antidepressant activity. The following list describes some of these modifications of FST (Table 1). The two columns of Table 1 separate each modification between variability and sensitivity. A "variability factor" is assessed to check what parameter could increase or decrease reliability of the test between different laboratories.

Sensitivity factors

Automated device/water waves Different procedures have been elaborated to automate the FST. Video-tracking, computer analysis or wave analysis were used to score the immobility of rodents. From the data set, one can extract full or partial turns, clockwise or counter-clockwise rotations, total activity, and speed of swimming clockwise and counter-clockwise (Denenberg et al. 1990). Another author used an apparatus consisting of a transparent plastic cylinder (10×20 cm) containing 7 cm of water (23°C). Movement by the animal created a waveform in the water, resulting in a converted digital signal (Browne 1979). The ease of use of these systems appears not to counterbalance the cost of the equipment. Few studies use an automated video-tracking device, and mainly as a confirmation tool (Eisch et al. 2003). Nevertheless, some automated devices employed in FST studies were reported to be reliable for antidepressant screening (Yoshikawa et al. 2002).

Cylinder diameter To test this parameter, mice were forced to swim for 15 min in tanks of 10 (the original diameter of the Porsolt's forced swimming chamber), 20, 30, and 50 cm diameter in 20 cm deep water. Modifications of this parameter provide a way to distinguish the antidepressant drugs from caffeine, anticholinergics, and antihistaminics, which gave a false positive response in 10 cm diameter cylinders. The selective effect of antidepressants, namely, the rotatory locomotor activity during swimming can also be studied (Sunal et al. 1994). In our laboratory, we use a cylinder with the closest available diameter to the original test's diameter, associated with a check of variation of locomotor activity that can discriminate false-positive effects (Porsolt et al. 1978).

Depth of water This parameter had to be considered as mice should not sense a limit under the level of water. Their tails should not touch the bottom of the cylinder or the behaviour of the mice would be altered. Increased depth of water decreases the time spent immobile. No paper clearly described this process in mice; this parameter was shown to alter the behaviour of the rat (Borsini and Meli 1988; Detke and Lucki 1996). The original description of the FST by Porsolt et al. (1977) explains that 6 cm of water is sufficient. But mice can sense the bottom of the cylinder with this level of water. In our laboratory, the water level is at least 10 cm. Some modifications of Porsolt's paradigm have often been used; one of the most quoted is the method of Aley and Kulkarni (1989). They measure immobility in a glass jar (21×12 cm) containing 12 cm of water maintained at 22±1°C, during a 6-min period. It is important to consider that the only main modification of the original test is the increased depth of water. This procedure is consistent with the one we use and should be considered as the actual standard method.

Interval of observation/scoring Porsolt's paradigm has been modified by some researchers in order to increase the sensitivity or the specificity of the FST. Some authors have created a totally new analysis procedure for scoring immobility. The observation interval can be separated into 5-s parts in which the main behaviour is scored (Lucki 1997). Analysis of the behaviour of the mice can be totally different with categorization of a specific behaviour (Schramm et al. 2001). Some authors made a series of observations at 30 s. intervals and the mouse was rated as immobile (score 0) or not (score 1) for each observation period (Borsini and Meli 1988).

Time between treatment and FST This factor is not often considered but may explain some of the differences between FST results. Two possibilities seem to be available: acute injection 1 h before the FST as described by Porsolt et al. (1977) or acute injection then the FST when the maximal effect is intended. This requires a time-course study of the drug effect.

Water temperature The influence of water temperature on immobility time of the mice was studied. An effect of water temperature was revealed; a higher temperature (35°C) resulted in shorter immobility time after 10 min of forced swimming (Arai et al. 2000). Other data suggest

that immobility, which develops rapidly during forced swimming in cold water, may result from dramatic inhibition of neural functions because of severe brain hypothermia (Taltavull et al. 2003). Currently, most studies use warmer water between 23°C and 28°C. In our laboratory, we choose a temperature between 23°C and 25°C.

Wheel water tank Some authors have tried to measure immobility time in another way. A wheel was immersed in the water tank. Mice placed on this apparatus keep turning the wheel vigorously; when they abandon their attempts to escape from the water, the wheel stops turning. The number of rotations of the water wheel is counted. All antidepressants tested increased the number of rotations as tranquillizers, anticholinergics and antihistaminics were not effective. It was suggested that this water wheel test was more appropriate as screening test for antidepressants than Porsolt's test with regard to both objectivity and specificity (Nomura et al. 1982).

Variability factors

Acute versus chronic administration The effectiveness of acute treatment is a particularity of the FST. Useful for a screening test, it appears to decrease the face validity of this model, as the clinical time course requires chronic administration to be active. Experiments were made to find out the effects of chronic administration on the FST. Subchronic or acute effects were increased by chronic administration (Dulawa et al. 2004).

Age of the mice This parameter should be considered in parallel with weight. Our team has already shown a strong difference between younger and older mice groups. Sensitivity to some antidepressants is profoundly altered. Tricyclic, noradrenaline reuptake inhibitors (NRI) and serotonin reuptake inhibitors were more active in 4-week-old mice than 40-week-old Swiss mice (Bourin et al. 1998; David et al. 2001a). In our laboratory experiments, we choose mice weighting 20–25 g.

Circadian rhythm An effect of circadian rhythm was shown in response to antidepressants in the FST. FST was carried with three strains of mice: C3H, C57BL/6J and ND/4. Immobility time was scored at noon (1200–1400 hours) and midnight (0000–0200 hours). For C3H/Hen mice, duration of immobility was greater at midnight (Dubocovich et al. 1990). Another study did not show any difference between the FST made at noon (1100–1200 hours), early dark (2000–2100 hours) and at midnight (0100–0200 hours) for BALB/c and C57BL/6J mice (Raghavendra et al. 2000). Genetics studies on the Clock gene, implicated in circadian rhythm, revealed an effect of this parameter on immobility time (Easton et al. 2003). Studies in our laboratory are only made between 0800 and 1200 hours to avoid any risk of behavioural modification throughout the experiments.

Environment of the laboratory Interactions with laboratory environment have been studied in several strains of mice on few behavioural tests (open field, elevated plus maze, water maze, alcohol preference) (Crabbe et al. 1999). Despite standardization, there were systematic differences in behaviour across three different laboratories. In our opinion, FST is less sensitive to variation of laboratory environment (noise, air temperature, light, atmosphere pressure).

Food restriction Food restriction can strongly modify behavioural responses, as shown with amphetamine or the FST. The authors used FST for two sessions with two groups of DBA/2 mice. One group was isolated and food restricted, the other group was isolated but had free access to food. Immobility time was significantly decreased in the food-restricted group compared to the other group (Cabib et al. 2000; 2002).

Gender Differences of sensitivity between male and female mice were revealed by some studies depending on the strain used. David et al. (2001b) described a different sensitivity to antidepressants in the FST related to gender. Imipramine and paroxetine were active on CD1 male and female but at different doses. Another study showed a difference between male and female mice but only in some strains; FVB females, for example, had a shorter floating time than males (Voikar et al. 2001). Sexual differences have also been described in another study of immobility, which was higher in males than in females (Alonso et al. 1991).

Housing of animals/isolation of animals All studies have shown that housing was a critical parameter. In the above mentioned study of Cabib (see food restriction section), a group of DBA/2 mice was isolated for 13 days and compared with group-housed mice in the FST. They showed a significant increase of the immobility time in the isolated group (Cabib et al. 2002). Yates et al. (1991) linked this difference with the age of the mice. After having isolated mice for 24 h prior to a 15-min FST, they showed an increase in immobility time in 17- to 21-day-old Swiss Webster mice but not in 26- to 30-day-old mice. In another study, the immobility time in the FST was shortened in NIH Swiss mice isolated for 2 or 5 days, suggesting an improved ability to cope with stressful situations (Hilakivi et al. 1989; Yates et al. 1991). Isolation seems to have strain-dependent effects on the FST, but none of these studies had the same isolation time. If isolated for a longer period (8 weeks), mice displayed lower levels of immobility time when exposed to this test (Karolewicz and Paul 2001). Nevertheless, isolation, e.g. for surgery, had to be specified in methods of a paper, as it may modify dramatically immobility time of the FST.

Observer The most important source of variability (and the best way to consider in order to increase the sensitivity of the FST), with identical environmental parameters, is the observation. Like all behavioural studies, the observer is

the main actor of the test and reproducibility between laboratories is a matter that affects all these tests. The scoring of the immobility time should be strongly considered and assessed by all teams. The mouse is judged to be immobile when it makes only movements necessary to keep its head above water. It can move in the cylinder but without struggling movements. The analysis of active behaviours in the FST has strengthened the possibility of replicating the experiments.

Side preference in rotation A study was made on side rotational preference of mice during the FST. Krahe et al. (2002) concluded that side preferences of spontaneous rotational behaviour may account for inter-individual differences.

Strains Strain is one of the most important parameters to deal with (Lucki et al. 2001). Important differences exist between strains in both immobility observed and effects of imipramine (Porsolt et al. 1978). Genetic background could modify response by providing an inappropriate baseline level of behaviour (Holmes 2003a). There is a maximal tenfold difference in baseline immobility scores in control animals between strains and baseline level does not correlate with antidepressant sensitivity (Lucki et al. 2001). Several gender dissociations suggest the strain and task specificity (Voikar et al. 2001). Intra-strain and inter-strain comparisons indicate that the biological substrates mediating performance in the FST and the tail suspension test (TST) are not identical. For example, in NIH-Swiss mice, a 7-fold difference in baseline immobility was observed between the FST and TST. By contrast, the baseline immobility in C57BL/6 mice was similar in both procedures (Bai et al. 2001). There is a continuum of variation in basal responses from almost no time spent immobile by DBA/2J mice to more than 210 s of immobility in a 360-s test session with Balb/cJ mice (O'Neil and Moore 2003). In one of our studies, we have shown that drug sensitivity is genotype dependent. FST results have shown that Swiss mice were the most sensitive strain to detect serotonin (5-HT) and/or noradrenaline (NA) treatment. The use of DBA/2 inbred mice may be limited, as an absence of antidepressant-like response was observed in the FST (David et al. 2003). Control mice from the same breeders with comparable housing conditions should have the same immobility time in all laboratories. However, a gene-environment interaction is possible and may account for some difference between laboratories (Wahlsten et al. 2003). For example, in our data set, animals of the same strain that received no treatments do not have the same immobility time (for CD-1 from 135 s to 223 s of immobility time).

Test/retest This method is used normally for rats. In a first session, the animal is able to discover the test, rat usually explore the water surface and dive. In a second session were they will be scored, rats are familiarized to the test and do not try to dive. Mice do not have this behaviour and this explain the easy use of mice that do not need a second session. This second session has been assessed for the construct validity of the FST. Memory process was involved to explain immobility of the rat. The absence of second session with mice removes this problem and simplifies the test. In their experiments, Alcaro et al. (2002) evaluated behavioural responses to FST in naive animals and in animals pre-exposed to the FST 14 days before the test session. They showed a major effect of the pre-session FST in mice on immobility time with a dramatic increase after pre-exposure. For Andreatini and Bacellar (2000), "this test showed a very low intra-class correlation coefficient in the test-retest design, which suggests a poor reliability of these measures". These results suggest that the behavioural parameters of the behavioural despair are not stable. Therefore, they are possibly more related to state than trait characteristics, this test is not appropriate to evaluate trait characteristics which are supposed to be stable over time without treatment. Some authors use the test/retest paradigm to avoid variations and to maintain consistency in the immobility time between different groups (Hirani et al. 2002).

Discussion

Many antidepressants have been tested with the FST on mice. Some results available for all classes of antidepressants with different strains of mice are reviewed here.

In the literature, the lowest control immobility time was obtained with FVB/NJ (13 s) mice and highest with ddY (220 s).

Table 2 summarises the results for three inbred strains and four outbred strains that are compared over their results in the FST. A more detailed version of this table, with more antidepressants and strains, is available as Electronic Supplementary Material (ESM).

Inbred strains have been found very defective in the FST with antidepressants. Only one type of antidepressant (DRI), bupropion, was significantly effective in the FST with C57BL/6Rj. For C57BL6j and DBA/2, no positive result coupled with a locomotor test was found in papers we analysed. Outbred strains of mice are more responsive to antidepressants in the FST than inbred strains. These four outbred strains may be used for at least three classes of treatments. The most frequently used strains, CD1, NMRI and Swiss, have positive results with most of the antidepressants in the FST. HaM/ICR seems to be very responsive to drugs in the FST but it is a rare strain. Only one paper was found to use this strain on the FST (De Graaf et al. 1985). There are many differences between strains; DBA/2, for example, does not have an appropriate response to the FST. This strain should not be used for behavioural studies with the FST. CD-1 is not useful to discriminate different mechanisms of action in the test. It could be used as a screening model but, to recommend this strain, we need to know if Dopamine Reuptake Inhibitors (DRI, e.g. bupropion), NRI and MAO-I are effective in the

Table 2 Antidepressant effects on the FST with different strains of mice (taken from the literature)

	Inbred			Outbred			
	C57 BL/6Rj	C57 BL6J	DBA/2	CD-1	HaM/ICR	NMRI/EC	Swiss/Janvier
Atypical				*	+	*	*
DRI	*		–	+	+	–	–
NRI	–	+	–	+	+	–	*
SNRI							*
SSRI	–		–	*	+	*	*
MAO-I				+			–
TCAs	–		–	*	+	*	*

*, treatment is effective and locomotor activity was tested without significant variation
+, treatment is effective but locomotor activity was not tested
–, treatment has no potency and does not increase locomotor activity
Different categories of drugs are listed in the first column. For example, DRI includes bupropion, nomifensine or amineptine. Atypical antidepressants include mianserin, iprindole and others. A positive result, represented by a star, signifies that at least one study showed a significant effect of one drug of the considered category. For a more detailed table, Electronic Supplementary Material is available with all drugs and effects reported in different studies

FST with CD-1 without increasing spontaneous locomotor activity of the animals.

Even with a very precise binding of antidepressants, it is often difficult to understand the mechanisms of action of antidepressants. Some of our previous works showed that dopaminergic activity compounds are not easy to be active on the FST. On the other hand, the binding or the drug activity at the synaptic level is only an indirect understanding of the activity of drug in a whole animal. It was showed in our lab (David et al. 2003) as well as in Lucki's laboratory that depending on the strain, the effect size is quite different (from 0% effect for desipramine in C3H/HeJ to almost 60% of decrease of immobility time with BALB/cJ) (Lucki et al. 2001).

FST was designed by Porsolt as a primary screening test for antidepressants. It is still one of the best models for this procedure. This is a low-cost, fast and reliable model to test potential antidepressant treatments with a strong predictive validity. However, the low face and construct validities should not forbid the use of this model for neurophysiological studies. It has a great sensitivity with all the antidepressant classes and all the mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully. Studying the method of action of an antidepressant is different from studying aetiology and how to cure depression.

For this reason, some authors decided to abandon the term "model" of depression. They prefer the word "test", which corresponds to an examination of a critically key aspect of either the response to stress or to antidepressant drug action. It could help to reconsider their true role in the process of discovery of novel antidepressants (O'Neil and Moore 2003).

We totally agree that the FST is "a very specific cluster of stress-induced behaviours that have no direct, empirical relation to depression symptoms in humans" (Holmes 2003b). Care must be taken on the strain used for the test and all the experimental parameters involved. For a screening test, CD-1 can be a good strain to use to find out if a treatment has an antidepressant-like activity.

Despite their intrinsic limitations, the full potential of animal models of depression has not yet been realized and they represent an under-explored opportunity for drug development. Such opportunities arise from the molecular dissection of the biological features of the models (Wong and Licinio 2004).

Uncited references

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Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
Atypical	Cor 32-24			110	NS	8	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
Atypical	iprindole	207	94	45	0,01	40	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
Atypical	iprindole	207	81	39	0,01	80	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
Atypical	iprindole			<50	0,05	50	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
Atypical	iprindole			103	NS	64	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
Atypical	iprindole	156	156	100	NS		?	M	20-32	Swiss/Janvier	(Malinge et al. 1988)
Atypical	medifoxamine			44	0,05	128	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
Atypical	mianserin	190	106	56	0,01	20	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
Atypical	mianserin	210	185	88	0,05	20	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
Atypical	mianserin	190	89	47	0,01	40	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
Atypical	mianserin			44	0,05	10	NO	M	20-30	CD-1/Charles River	(Luttinger et al. 1985)
Atypical	mianserin			51	0,05	32	NO	M	25-30	CD-1/Charles River	(Browne 1979)
Atypical	mianserin			19	0,01	56	NO	M	25-30	CD-1/Charles River	(Browne 1979)
Atypical	mianserin			<50	0,05	5	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
Atypical	mianserin	180	124	69	0,01	8	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
Atypical	mianserin	194	112	58	0,01	20	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
Atypical	mianserin	142	97	68	0,001	15	YES(<)	M	18-33	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
Atypical	mianserin	148	96	65	0,05	32	YES(**)	M	20-24	Swiss/Janvier	(Bourin et al. 1996)
Atypical	mianserin	148	96	65	0,05	32	?	M	20-27	Swiss/Janvier	(Malinge et al. 1988)
Atypical	mianserin			0	0,05	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
Atypical	minaprine	180	123	68	0,01	5	YES	F	18-23	CD-1/Charles River	(Biziere et al. 1982)
Atypical	trazodone					1->30	NO	M	20-30	CD-1/Charles River	(Luttinger et al. 1985)
Atypical	trazodone			50	0,05	13	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
Atypical	trazodone			101	NS	16	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
DRI	amineptine			?	0,05	3	YES(**)	F	18-23	CD-1/Charles River	(Biziere et al. 1985)
DRI	bupropion			74	0,05	4	YES	M	20-24	C57 BL/6Rj	(David et al. 2003)
DRI	bupropion	200	20	10	0,01	30	NO		20-26	CD-1/Charles River	(Zocchi et al. 2003)
DRI	bupropion			100		1-8	YES	M	20-24	DBA/2	(David et al. 2003)
DRI	bupropion			50	0,05	10	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
DRI	bupropion			100		1-16	YES	M	20-24	NMRI	(David et al. 2003)
DRI	bupropion			100		1-16	YES	M	20-24	Swiss/Janvier	(David et al. 2003)
DRI	bupropion	230	179	78	0,01	16	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)
DRI	bupropion	200	169	85	0,05	16	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
DRI	bupropion	215	50	23	0,01	32	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
DRI	bupropion	230	86	37	0,01	32	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)

Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
DRI	bupropion	200	88	44	0,01	32	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
DRI	bupropion	200	93	47	0,01	32	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
DRI	nomifensine	210	70	33	0,05	10	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
DRI	nomifensine			7	0,01	10	NO	M	25-30	CD-1/Charles River	(Browne 1979)
DRI	nomifensine			50	0,05	10	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
NRI	(+)oxaprotiline	155,2	111	72	0,01	5	YES	M	20-25	Swiss	(Mogilnicka et al. 1987)
NRI	desimipramine	204	66	32	0,01	15	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
NRI	desimipramine	204	62	30	0,01	30	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
NRI	desipramine	85	30	35	0,01	20	NO	M		129/SvemJ(Jackson)	(Lucki et al. 2001)
NRI	desipramine	90	30	33	0,01	20	NO	M		A/J(Jackson)	(Lucki et al. 2001)
NRI	desipramine	102	30	29	0,01	20	NO	M		BALC/cJ(Jackson)	(Lucki et al. 2001)
NRI	desipramine	72	65	90	NS	20	NO	M		C3H/HeJ Swiss Webster(Jackson)	(Lucki et al. 2001)
NRI	desipramine			100		1-16	YES	M	20-24	C57 BL/6Rj	(David et al. 2003)
NRI	desipramine	143	90	63	0,05	10	NO	M		C57BL/6J(Jackson)	(Lucki et al. 2001)
NRI	desipramine	143	110	77	0,05	20	NO	M		C57BL/6J(Jackson)	(Lucki et al. 2001)
NRI	desipramine	210	165	79	0,05	30	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
NRI	desipramine	118	65	55	0,05	20	NO	M		CD-1/Charles River	(Lucki et al. 2001)
NRI	desipramine	88	60	68	NS	20	NO	M		CF-1/Charles River	(Lucki et al. 2001)
NRI	desipramine			100		1-16	YES	M	20-24	DBA/2	(David et al. 2003)
NRI	desipramine	100	55	55	0,01	20	NO	M		DBA/2J(Jackson)	(Lucki et al. 2001)
NRI	desipramine	13					NO	M		FVB/NJ(Jackson)	(Lucki et al. 2001)
NRI	desipramine			50	0,05	25	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
NRI	desipramine	52	20	38	0,05	20	NO	M		NIH-Swiss Harlan	(Lucki et al. 2001)
NRI	desipramine			100		1-16	YES	M	20-24	NMRI	(David et al. 2003)
NRI	desipramine			78		16	YES	M	20-24	Swiss/Janvier	(David et al. 2003)
NRI	desipramine	221	181	82	0,05	4	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)
NRI	desipramine	219	193	88	0,05	4	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
NRI	desipramine	218	86	39	0,01	16	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
NRI	desipramine	200	81	41	0,01	16	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
NRI	desipramine	219,3	152,5	70	0,01	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
NRI	desipramine	81	55	68	NS	20	NO	M		Swiss-Webster/ Charles River	(Lucki et al. 2001)
NRI	viloxazine	176	72	41	0,05	30	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
NRI	viloxazine			50	0,05	45	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
NRI	viloxazine	174	64	37	0,01	16	YES	M	20-24	Swiss/Janvier	(Bourin et al. 1996)
NRI	viloxazine	174	64	37	0,01	16	?	M	20-28	Swiss/Janvier	(Malinge et al. 1988)
NRI	viloxazine			37	0,01	16	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)

Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
NSRI	midalcipram			?	0,05	10	NO	M	20-26	Swiss	(Stenger et al. 1987)
NSRI	midalcipram			71	0,05	20	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
NSRI	midalcipram	158	109	69	0,05	30	YES	M	25-30	Swiss	(Rogoz et al. 1999)
NSRI	venlafaxine	240	186	78	0,01	8	YES	M	20-24	Swiss/Janvier	(Redrobe et al. 1998)
SSRI	citalopram			100		1-16	YES	M	20-24	C57 BL/6Rj	(David et al. 2003)
SSRI	citalopram	162	141	87	NS	4	NO	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	citalopram			100		1	YES	M	20-24	DBA/2	(David et al. 2003)
SSRI	citalopram			100		1-8	YES	M	20-24	NMRI	(David et al. 2003)
SSRI	citalopram			73	0,05	8	YES	M	20-24	Swiss/Janvier	(David et al. 2003)
SSRI	citalopram	179	136	76	NS	4	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
SSRI	citalopram	211	58	27	0,05	16	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
SSRI	citalopram	170	57	34	0,001	16	?	M	20-29	Swiss/Janvier	(Malinge et al. 1988)
SSRI	citalopram	215	113	53	0,01	16	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
SSRI	citalopram	226	155	69	0,01	32	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)
SSRI	citalopram			34	0,01	16	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
SSRI	citalopram	231,1	178	77	0,01	16	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
SSRI	clovoxamine			21	0,01	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
SSRI	fluoxetine	120	70	58	0,05	5	NO	M	20-30	Laka / Panjab University	(Anjaneyulu et al. 2003)
SSRI	fluoxetine	204	172	84	NS	8	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	186	174	94	NS	8	NO	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	194	190	98	NS	8	YES(**)	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	194	172	89	NS	16	YES(**)	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	198	72	36	0,01	32	YES(**)	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	189	90	48	0,01	32	YES(**)	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluoxetine	170	110	65	0,01	30	NO		20-26	CD-1/Charles River	(Zocchi et al. 2003)
SSRI	fluoxetine			<50	0,05	20	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
SSRI	fluoxetine	260,4	140	54	0,01	32	NO	2	30 à 40	Swiss	Rosa AO et al., 2002
SSRI	fluoxetine	139	99	71	0,001	40	YES(<)	M	18-34	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
SSRI	fluoxetine	232	150	65	0,01	16	YES	M	20-24	Swiss/Janvier	(Bourin et al. 1998)
SSRI	fluoxetine	231,7	187,8	81	0,01	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
SSRI	fluvoxamine	171	138	81	NS	4	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluvoxamine	177	169	95	NS	4	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluvoxamine	177	140	79	NS	16	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	fluvoxamine			<50	0,05	20	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
SSRI	fluvoxamine	129	93	72	0,001	20	YES(<)	M	18-35	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
SSRI	fluvoxamine	181	79	44	0,01	32	?	M	20-31	Swiss/Janvier	(Malinge et al. 1988)

Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
SSRI	fluvoxamine			44	0,01	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
SSRI	fluvoxamine	235,2	199,9	85	0,01	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
SSRI	indalpine	170	100	59	0,01	16	NO	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	indalpine	207	93	45	0,01	32	?	M	20-30	Swiss/Janvier	(Malinge et al. 1988)
SSRI	indalpine			44	0,01	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
SSRI	paroxetine			100		1-2	YES	M	20-24	C57 BL/6Rj	(David et al. 2003)
SSRI	paroxetine	170	146	86	NS	1	NO	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	paroxetine	164	77	47	0,01	8	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	paroxetine	168	111	66	0,05	8	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	paroxetine			100		1-2	YES	M	20-24	DBA/2	(David et al. 2003)
SSRI	paroxetine			84	0,05	8	YES	M	20-24	NMRI	(David et al. 2003)
SSRI	paroxetine			77	0,05	16	YES	M	20-24	Swiss/Janvier	(David et al. 2003)
SSRI	paroxetine	179	116	65	NS	2	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
SSRI	paroxetine	224	100	45	0,01	8	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)
SSRI	paroxetine	215	110	51	0,01	8	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
SSRI	paroxetine	222	131	59	0,01	8	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
SSRI	paroxetine			71	0,05	16	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
SSRI	paroxetine	224,7	162,8	72	0,01	16	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
SSRI	sertraline	185	49	26	0,01	8	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	sertraline	158	57	36	0,01	8	YES	M	22-25	CD-1/Charles River	(Da-Rocha et al. 1997)
SSRI	sertraline	231,7	170,6	74	0,01	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
MAO-I	clorgyline	135	65	48	0,05	60	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	clorgyline	210	120	57	0,05	60	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
MAO-I	Lilly 51641	223	70	31	0,01	60	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	moclobémide	modif	modif	50	0,01	10	NO	M	20-25	Balb/ca / SPF	(Miura et al. 1996)
MAO-I	moclobémide	230	223	97	NS	8	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
MAO-I	nialamide	192	138	72	0,05	150	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	nialamide	178	178	0	NS		?	M	20-33	Swiss/Janvier	(Malinge et al. 1988)
MAO-I	pargyline	210	72	34	0,05	300	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
MAO-I	pargyline	172	61	35	0,01	300	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	T-794			60	0,05	30	NO	M	5 week	ddY/SLC	(Kato et al. 1998)
MAO-I	toloxatone	202	100	50	0,01	200	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	toloxatone	202	29	14	0,01	400	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
MAO-I	tranylcypromine	193	58	30	0,01	20	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	amitriptyline	166	60	36	0,01	15	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	amitriptyline	166	45	27	0,01	30	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	amitriptyline			25	0,01	32	NO	M	25-30	CD-1/Charles River	(Browne 1979)

Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
TCA	amitriptyline	129	90	70	0,02	10	YES(<)	M	18-31	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
TCA	amitriptyline	179	136	76	0,05	4	?	M	20-25	Swiss/Janvier	(Malinge et al. 1988)
TCA	amitriptyline			1	0,05	4	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
TCA	amitryptiline			50	0,05	4	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	amoxapine			?	0,05	6	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	chlorimipramine	175	84	48	0,01	20	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	chlorimipramine			43	0,01	32	NO	M	25-30	CD-1/Charles River	(Browne 1979)
TCA	ciclazindol			50	0,05	12	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	clomipramine	180	115	64	0,05	20	NO	M	35-45	CD-1/Charles River	(Eschalier et al. 1983)
TCA	clomipramine	215	153	71	0,05	20	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1982)
TCA	clomipramine	210	180	86	0,05	30	NO	M	30-35	CD-1/Charles River	(Devoize et al. 1984)
TCA	clomipramine			50	0,05	20	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	dosulepine	227	72	32	0,01	32	YES	M	20-25	Swiss/Janvier	Bourin et al., 1995 "Profil psychopharmacologique de la Dosulepine"
TCA	dothiepin	227	72	32	0,01	32	YES	M	20-24	Swiss/Janvier	(Bourin et al. 1996)
TCA	doxepine			44	0,05	10	NO	M	25-30	CD-1/Charles River	(Browne 1979)
TCA	doxepine			<50	0,05	10	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	doxepine	182	146	80	NS	2	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
TCA	doxepine	182	90,3	50	0,05	10	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
TCA	imipramine HCl	120	70	58	0,05	15	NO	M	20-30	Laka / Panjab University	(Anjaneyulu et al. 2003)
TCA	imipramine HCl	149	97	65	0,05	30	NO	F	20-25	BABL/cki/Kirschbaum Memorial	(Schechter and Chance 1979)
TCA	imipramine HCl			100		1-16	YES	M	20-24	C57 BL/6Rj	(David et al. 2003)
TCA	imipramine HCl	152,7	84	55	0,05	5	N	M	25	C57 Harlan S-D	(Bai et al. 2001)
TCA	imipramine HCl	189,2	39,5	21	0,01	30	NO	M		CD-1/Charles River	(Porsolt et al. 1978)
TCA	imipramine HCl	189	40	21	0,01	30	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	imipramine HCl	180	80	44	0,01	1	YES	F	18-23	CD-1/Charles River	(Bizière et al. 1982)
TCA	imipramine HCl			39	0,01	10	NO	M	25-30	CD-1/Charles River	(Browne 1979)
TCA	imipramine HCl			100		1-16	YES	M	20-24	DBA/2	(David et al. 2003)
TCA	imipramine HCl			74	0,05	10	NO	M	5 week	ddY/SLC	(Kato et al. 1998)
TCA	imipramine HCl			50	0,05	3	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985).
TCA	imipramine HCl	137,9	25	18	0,05	45	NO	M	25	NIH-Swiss Harlan	(Bai et al. 2001)
TCA	imipramine HCl			100		1-16	YES	M	20-24	NMRI	(David et al. 2003)

Class	AD	control (sec)	treated (sec)	% control	P <	dose (mg/kg)	Actimetry	Gender	Weight (g)	Strain	Ref
TCA	imipramine HCl	182	151	83	NS	5	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
TCA	imipramine HCl	192	100	52	0,01	30	YES	F	24-30	NMRI / Evic-Ceba	(Scotto di Tella and Mercier 1981)
TCA	imipramine HCl	89,1	42,5	48	NS	30	NO	M		NMRI/Evic Ceba	(Porsolt et al. 1978)
TCA	imipramine HCl	94,6	62,8	66	NS	30	NO	M		OF-1/Iffa Credo	(Porsolt et al. 1978)
TCA	imipramine HCl	260,4	164,7	63	0,01	15	NO	2	30 à 40	Swiss	Rosa AO et al., 2002
TCA	imipramine HCl	138	91	66	0,001	20	YES(<)	M	18-30	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
TCA	imipramine HCl			80	0,01	16	YES	M	20-24	Swiss/Janvier	(David et al. 2003)
TCA	imipramine HCl	200	64	32	0,01	16	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
TCA	imipramine HCl	215	106	49	0,01	16	NO	M	35-45	Swiss/Janvier	(Bourin et al. 1998)
TCA	imipramine HCl	224	48	21	0,01	32	NO	M	18-22	Swiss/Janvier	(Bourin et al. 1998)
TCA	imipramine HCl	139	35	25	0,01	32	?	M	20-24	Swiss/Janvier	(Malinge et al. 1988)
TCA	imipramine HCl	218	117	54	0,05	32	NO	M	41-51	Swiss/Janvier	(Bourin et al. 1998)
TCA	imipramine HCl	224	147	66	0,01	32	YES(*)	M	20-24	Swiss/Janvier	(Bourin et al. 1996)
TCA	imipramine HCl			25	0,01	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
TCA	imipramine HCl	230,8	131,7	57	0,01	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
TCA	maprotiline	139	109	78	0,01	10	YES(<)	M	18-32	Swiss	(Szymczyk and Zebrowska-Lupina 2000)
TCA	maprotiline	150	54	36	0,01	16	?	M	20-26	Swiss/Janvier	(Malinge et al. 1988)
TCA	maprotiline	150	53	35	0,01	32	YES(**)	M	20-24	Swiss/Janvier	(Bourin et al. 1996)
TCA	maprotiline			26	0,01	32	NO	M	22-26	Swiss/Janvier	(Bourin et al. 1991)
TCA	maprotiline	226,1	195	86	0,05	32	YES	M	20-24	Swiss/Janvier	(Clenet et al. 2001)
TCA	nortriptyline	197	130	66	0,05	15	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	nortriptyline	197	13	7	0,01	30	NO	M	20-25	CD-1/Charles River	(Porsolt et al. 1977)
TCA	nortriptyline			50	0,05	5	NO	M		HaM/ICR (Broekman inst.)	(De Graaf et al. 1985)
TCA	pizotifen			33	0,05	3	NO	M	20-30	CD-1/Charles River	(Luttinger et al. 1985)

Class	AD	129/SvemJ	A/J	BALB/cki	Balb/ca	BALC/cJ	C3H/HeN	C3H/HeJ	C57BL/6Rj	C57H	C57BL/6J	CD-1	CF-1	DBA/2	DBA/2J	ddY/SLC	FVB/NJ	HaM/ICR	Laka / Panjab University	NIH-S-H	NMRI/EC	OF-1/Iffa	Swiss	Swiss-Web/CR
Atypical	Cor 32-24																						-	
	iprindole											+						+					-	
	medifoxamine																						+	
	mianserin											+						+			*		*	
	minaprine											▫												
	trazodone											-						+					-	
DRI	amineptine											-												
	bupropion								*			+		-				+			-		+	
	nomifensine											+						+						
NRI	(+)oxaprotiline																						▫	
	desimipramine											+												
	desipramine	+	+			+	+	-	-		+	+	-	-	+		-	+		+	-		▫	-
	viloxazine											+						+					▫	
SNRI	midalcipram																						+	
	venlafaxine																						▫	
SSRI	citalopram								-			-		-							-		▫	
	clovoxamine																						+	
	fluoxetine											▫						+	+				▫	
	fluvoxamine											-						+					▫	
	indalpine											+											+	
	paroxetine								-			▫		-							*		▫	
	sertraline											▫											▫	
MAO-I	T-794															+								
	tranylcypromine											+												
	clorgyline											+												
	Lilly 51641											+												
	moclobémide				+																		-	
	nialamide											+											-	
	toloxatone											+												
	pargyline											+												
TCA	amitriptyline											+						+					*	
	amoxapine																	+						
	chlorimipramine											+												
	ciclazindol																	+						
	clomipramine											+						+						
	dosulepine																						▫	
	dothiepin																						▫	
	doxepine											+						+			▫			
	imipramine HCl			+					-	+		▫		-		+		+	+	+	▫	-	▫	
	maprotiline																						▫	
	nortriptyline											+						+						
	pizotifen											+												

Résumé de l'étude n°2 :

Ce travail a permis d'isoler différents paramètres (traitement aigue ou chronique, age des souris, restriction alimentaire, température et hauteur d'eau, …) pouvant être modifiés pour augmenter l'amplitude de la réponse observée à la suite de l'administration d'antidépresseurs. Cependant, certains points doivent attirer notre attention :

- Les modifications de paramètre n'ont été évalué que sur un nombre restreint d'antidépresseurs et ne permettent donc pas de conclure à la **validité** du modèle en fonction de la classe d'antidépresseur testé.
- En général la modification d'un paramètre n'est évaluée que dans un laboratoire, ce qui pose le problème de la **reproductibilité** interlaboratoire.
- Seul des produits possédant une activité de type antidépresseur ayant été testé ; on ne peut donc exclure une augmentation du nombre de résultats « **faux positifs** » lors de la réalisation de ces tests modifiés.

Par conséquent, la procédure choisie pour le test est celle décrite par Porsolt et al. ; aucune modification n'y est apportée dans nos études. Le test consiste à placer individuellement des souris dans des bocaux de verre (hauteur = 25 cm, diamètre = 10 cm) contenant 10 cm d'eau maintenue entre 23°C et 25°C et à les y laisser pendant six minutes. Le test en lui-même dure 6 minutes. Après une phase d'activité vigoureuse de deux minutes (temps d'adaptation), l'animal contrôle cesse de nager et se fige adoptant un comportement de désespoir. On considère que la souris est immobile lorsqu'elle flotte en position horizontale et ne réalise que des mouvements de faible amplitude, suffisant à maintenir sa tête hors de l'eau. L'administration en aiguë d'antidépresseurs 30 min avant le test, diminue la durée du temps d'immobilité, qui est comptabilisée pendant les quatre dernières minutes du test.

2.3 Produits administrés

Lors des tests comportementaux, les administrations systémiques de produit se font par voie intra-péritonéale (i.p.) sous un volume de 25mL/kg. Les prétraitements sont administrés 45 min avant le début du test et le traitement 30 minutes avant le test. Tous les produits utilisés dans ces études sont solubles dans l'eau distillée.

2.3.1 Antidépresseurs

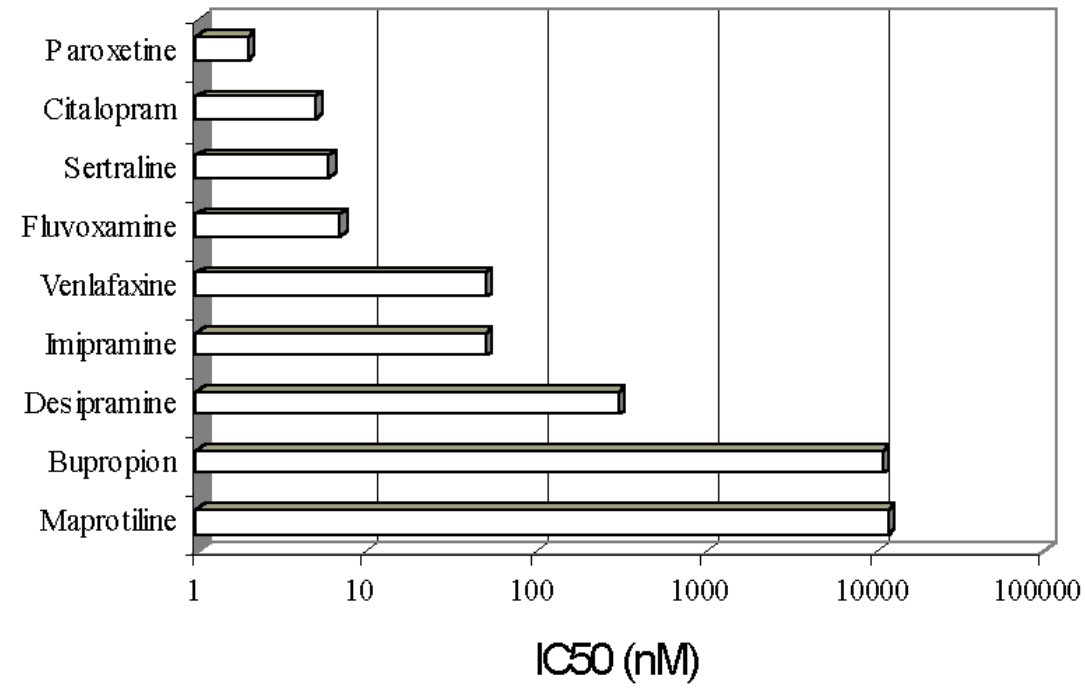
Les antidépresseurs utilisés appartiennent à différentes classes en fonction de leur mécanisme d'action :

Les inhibiteurs de la recapture sélectifs de la sérotonine (IRSSs) : paroxétine, HCl (GSK, France) ; citalopram, HBr (Lundbeck, Danemark).
Les antidépresseurs tricycliques : Imipramine HCl (RBI, USA).
Les inhibiteurs de la recapture noradrénaline : désipramine HCl (Sigma, France).

L'affinité de chacun de ces produits pour les transporteurs à la noradrénaline et à la sérotonine est représentée sur la figure 8.

2.3.2 Ligands des récepteurs 5-HT_{1B}

Agoniste : Anpirtoline HCl (Tocris, France). L'anpirtoline possède une affinité plus forte pour les récepteurs 5-HT_{1B} que pour les récepteurs 5-HT_{1A} et 5-HT_2 (Ki respectifs de 28 nM, 151 nM et 1,48 µM ; Metzenauer et al., 1992)
Antagoniste : GR127935 (*N*-[4-methoxy-3-(4,methyl-1-piperazinyl)phenyl]-2-methyl-4′-(methyl-1,2,4-oxadiozol-3-yl)-[1,1-biphenyl]-4-carboxamide) (GSK, France).



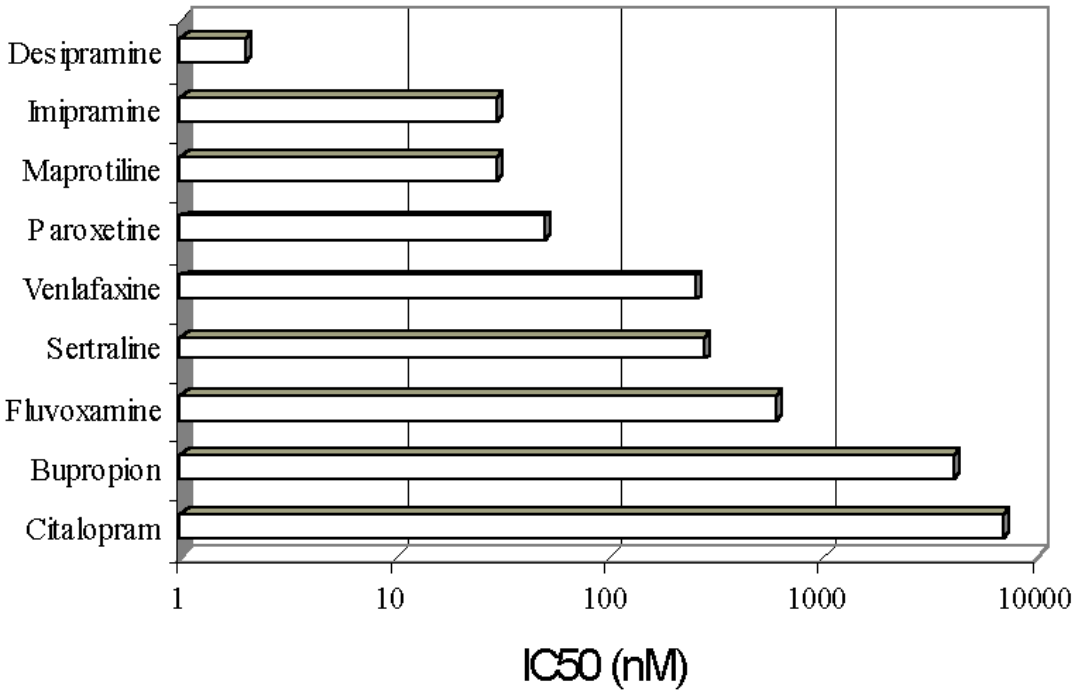


Figure 8a : Puissance d'inhibition (IC50) de différents antidépresseurs pour les transporteurs à la sérotonine et à la noradrénaline.

D'après Frazer et al., 2001

Table III. Biogenic Amine Reuptake Inhibitory Potencies *in Vitro* of Antidepressants and Their Metabolites Expressed as IC_{50} or K_i* Values, and *in Vivo* 5-HT and NA Reuptake Inhibition Measured as Potentiation of 5-HTP-Induced 5-HT Syndrome and Reversal of Tetrabenazine-Induced Ptosis in Mice, Respectively (Method Described by Hyttel *et al.*, 1992).

Compound	Major metabolite	Reuptake inhibition *in vivo*, IC_{50} or K_i* (n*M*)			Reuptake inhibition *in vivo*, ED_{50} (μmol/kg, s.c.)	
		5-HT	NA	DA	5-HT	NA
Amitriptyline		39[a]	24[a]	5,300[a]	17[a]	20[a]
	Nortriptyline	570[a]	3.4[a]	3,500[a]	89[a]	2.9[a]
Dothiepin		78*[b]	70*[b]	5,185*[b]		65[b]
	Northiaden	192*[b]	25*[b]	2,539*[b]		9.8[b]
	Dothiepin sulfoxide	5,402*[b]	4,912*[b]	77,387*[b]		
	Northiaden sulfoxide	534*[b]	1,948*[b]	58,727*[b]		
Lofepramine		880[a]	2.7[a]	3,300[a]	88[a]	1.9[a]
	Desipramine	200[a]	0.83[a]	9,100[a]	>130[a]	1.6[a]
Fluoxetine		6.8[a]	370[a]	5,000[a]	88[a]	>29[a]
	Norfluoxetine	3.8[a]	580[a]	4,300[a]		
Paroxetine		0.29[a]	81[a]	5,100[a]	0.63[a]	>54[a]
Sertraline		0.19[a]	160[a]	48[a]	5.4[a]	>54[a]
	Demethylsertraline	450[a]	4,600[a]	3,800[a]		
Fluvoxamine		3.8[a]	620[a]	42,000[a]	18[a]	>92[a]
Citalopram		1.8[a]	6,100[a]	40,000[a]	1.8[a]	>99[a]
	Demethylcitalopram	14[a]	740[a]	28,000[a]	170[a]	>120[a]
Bupropion		19,000	1,400	570	130	>140
	Hydroxybupropion	105,000[a]	7,000[a]	23,000[a]		
	Threohydrobupropion	67,000[a]	16,000[a]	47,000[a]		
Venlafaxine		210[a]	640[a]	2,800	13	29
	O-Demethylvenlafaxine	180[a]	1,160[a]	13,400[a]		
Nefazodone		68[d]	110[d]	470[d]	96	37[f]
	α-Hydroxynefazodone	165[d]	376[d]			39[f]

[a] Hyttel (1994).
[b] Heal *et al.* (1992).
[c] Muth *et al.* (1986, 1991).
[d] Eison *et al.* (1990).
[e] Hyttel *et al.* (1992).
[f] Reversal of reserpine-induced ptosis in mice, p.o. administration (Taylor *et al.*, 1986).
[g] Ascher *et al.* (1995).
[h] mg/kg, p.o.

Figure 8b : Puissance d'inhibition (IC50) de différents antidépresseurs pour les transporteurs à la sérotonine, à la noradrénaline et à la dopamine.

D'après Sanchez et Hyttel, 1997.

2.3.3 Substances entraînant une déplétion en monoamine

Pour chacun des produits utilisés pour induire des déplétions, ou lésions, monoaminergiques chez l'animal, des gammes de doses ont été réalisées au préalable. Les deux principaux critères utilisés pour définir la dose de produit à utiliser sont le niveau de déplétion, et la sélectivité d'action de la neurotoxine à la dose considérée. La 5,7-DHT (neurotoxine spécifique du système sérotoninergique) et la 6-OHDA (neurotoxine spécifique du système dopaminergique) sont injectées par voie intracérébroventriculaire, la procédure d'administration est détaillée dans la partie chirurgie stéréotaxique. Le DSP-4 (neurotoxine spécifique du système noradrénergique) et le p-CPA (inhibiteur de la synthèse de sérotonine) sont administrés par voie i.p.

5,7-Dihydroxytryptamine

La 5,7-DHT (Tocris, France) est une neurotoxine qui, du fait de son analogie de structure avec la 5-HT, possède une forte affinité pour les sites de recapture des monoamines (principalement ceux des neurones sérotoninergiques). Sa toxicité repose sur sa capacité à s'oxyder en composés dicétoniques sous l'action de monoamines oxydases. Ces composés dicétoniques conduisent à l'hypoxie intraneuronale et à la formation de radicaux libres.

6-Hydroxydopamine

La 6-OHDA (Sigma, France) est un analogue hydroxylé de la dopamine qui va induire une destruction des neurones dopaminergiques, par une production importante de radicaux libres : d'une part, le peroxyde d'hydrogène, producteur d'ions hydroxyles, serait produit par la désamination de la 6-OHDA par la monoamine oxydase, d'autre part de fortes quantités de radicaux hydroxyles seraient formées par l'auto oxydation de la 6-OHDA. De plus la 6-OHDA inhibe l'activité du complexe I de la chaîne respiratoire mitochondriale et induit une chute du potentiel membranaire mitochondriale. La 6-OHDA entre dans les cellules dopaminergiques probablement du fait de son analogie structurale avec la dopamine.

DSP-4

Le DSP-4 ([N-(2-chloroéthyl)-N-éthyl-2-bromobenzylamine HCl] ; Sigma, France) va former de l'aziridium, un cation qui va endommager un élément de la membrane des

neurones noradrénergiques issus du locus coeruleus. La lésion du système noradrénergique est réalisée par l'administration d'une dose unique de DSP-4 (50 mg/kg) par voie intra-péritonéale (i.p.) 168 heures avant le test. Après le traitement les animaux sont replacés par cage de 18 animaux dans l'animalerie.

p-CPA

Le *p*-CPA ([para-chlorophénylalanine] ; Sigma, France) exerce son activité en inhibant une enzyme intervenant dans la synthèse de la sérotonine, la tryptophane hydroxylase. La déplétion en sérotonine est obtenue par l'administration répétée de *p*-CPA 72, 48 et 24 heures avant le test (300 mg/kg.) L'action de ce produit est réversible dans le temps, d'où le terme de déplétion et non de lésion. Le produit est utilisé sous forme de sel (méthyl-esther) soluble dans l'eau distillée.

2.4 Chirurgie Stéréotaxique

Les souris sont anesthésiées avec de l'hydrate de chloral (400 mg/kg ; i.p.) puis placées sur un appareil de stéréotaxie, maintenues à l'aide de barres d'oreilles et d'un étau buccal fixant la mâchoire supérieure. L'appareil stéréotaxique est équipé d'un système de 3 axes avec vis micrométriques permettant le déplacement dans les 3 axes (antéro-postérieur : AP, latéral : L et ventral : V) du bras portant les canules d'injection (ou le nanoinjecteur). La peau du crâne est incisée en suivant un axe antéropostérieur ; l'application d'eau oxygénée (10 volumes) sur l'os crânien permet alors la mise en évidence des lignes de suture. Le Bregma (point d'intersection entre ces lignes) sert de point de référence pour l'implantation des canules. Les coordonnées des structures sont déterminées à partir de l'atlas stéréotaxiques (Swiss : Franklin et Paxinos 1997 ; 129/Sv : *Comparative Cytoarchitectonic atlas of the C57BL/6 and 129/Sv Mouse Brains,* Elsevier Science). L'os du crâne est ensuite percé en deux points (droite et gauche) pour permettre l'implantation des guides canules (injection locale d'anpirtoline) ou la descente de la seringue Hamilton (lésion des systèmes sérotoninergiques et dopaminergiques).
Les coordonnées stéréotaxiques utilisées sont :
-pour l'hippocampe : AP : - 2,5 mm, L : ± 1 mm, V : ± 1,5 mm.
-pour le cortex frontal : AP : + 1 mm, L : ±2 mm, V : -1,7 mm.
-pour le Caudate Putamen : AP : 0 mm, L : ± 2 mm, V : - 3 mm.
-pour la Substance Noire : AP : -3,3 mm, L : ± 1,5 mm, V : – 4,5 mm.
-pour les ventricules (souris Swiss) : AP :- 0,6 mm ; L : ± 1,2 mm, V : – 2,2 mm.
-pour les ventricules (souris 129/Sv) : AP :- 0,7 mm ; L : ± 1 mm, V : – 3,5 mm.

2.4.1 Injection i.c.v. de neurotoxine

2.4.1.1 Injection de 5,7-DHT

La lésion du système sérotoninergique est réalisée à l'aide d'une neurotoxine sélective, la 5,7-DHT. La 5,7-DHT est injectée après dissolution dans un mélange eau ultra pure/acide ascorbique (0,1%) (afin d'éviter son auto oxydation). 1,5 µL de solution de 5,7-DHT (soit 11,4 µg de 5,7-DHT pure) sont injectés par voie i.c.v. dans les ventricules droits et gauches à l'aide d'un nanoperfuseur motorisé KDS 310 (Phymep, Paris, France).

Afin d'augmenter la sélectivité d'action de la neurotoxine, l'animal est préalablement traité avec de la désipramine et de la nomifensine:

La désipramine a pour but d'empêcher l'entrée de la neurotoxine dans les neurones noradrénergiques et donc d'éviter la déplétion en NA en bloquant son transporteur. La désipramine (20 mg/kg) est dissoute dans de l'eau distillée, puis est administrée par voie intra-péritonéale (i.p., 25 ml/kg) 30 minutes avant l'injection de 5,7-DHT.

La nomifensine a pour but d'empêcher l'entrée de la neurotoxine dans les neurones dopaminergiques et donc d'éviter la déplétion des concentrations cérébrales de DA en bloquant son transporteur. La nomifensine (15 mg/kg) est dissoute dans de l'eau distillée, puis est administrée par voie i.p. (25 ml/kg) 30 minutes avant l'injection de 5,7-DHT.

Le protocole que nous avons suivi est sensiblement le même que celui utilisé par Harrison et al. (1997).

L'animal est anesthésié 5 minutes après l'administration i.p. de désipramine et de nomifensine. Il est ensuite placé sur un appareil stéréotaxique afin de réaliser une injection intracérébroventriculaire (i.c.v.) bilatérale précise de la neurotoxine. L'aiguille du nanoinjecteur est descendue aux coordonnées souhaitées (la descente et la remontée de l'aiguille se font lentement, 1mm/min, afin d'éviter de léser le tissu cérébral). L'injection de la 5,7-DHT est alors effectuée à un débit de 0,2 μL/min pendant 7,5 minutes, puis le perfuseur est laissé en place pendant 2 minutes après l'arrêt de l'injection afin d'éviter le reflux de la neurotoxine.

Après injection, les animaux sont placés en cage individuelle pendant 14 jours afin de laisser la toxine exercer ses effets.

Au 14ème jour, les souris sont soit sacrifiées (détermination de la dose de toxine active par dosage des taux résiduels de neurotransmetteurs et de leurs principaux métabolites sur les homogénats de parties de cerveaux de souris ou par autoradiographie), soit utilisées dans un test comportemental, le FST.

2.4.1.2 Injection de 6-OHDA

Le protocole suivi est similaire à celui de la 5,7-DHT. La 6-OHDA (6-hydroxydopamine) est également dissoute dans de l'acide ascorbique (0,1%), puis administrée par voie i.c.v. bilatéral (30µg) grâce au nanoinjecteur. 30 minutes avant l'injection, les systèmes noradrénergiques et sérotoninergiques sont protégés par l'administration de désipramine (25 mg/kg) et de paroxétine (16 mg/kg).

2.4.2 Injection locale d'anpirtoline

L'injection est réalisée chez des animaux vigiles libres de tout mouvement.

Système d'injection

Le système d'injection est constitué de deux parties :

- un guide canule (tube en acier inoxydable de 0,60 mm de diamètre externe et de 0,35 mm de diamètre interne, de 7 à 11 mm de long selon la structure cérébrale visée) (UNIMED, Suisse) implanté directement dans les tissus cérébraux.
- une canule d'injection constituée d'un tube en acier inoxydable (0,30 mm de diamètre externe et 0,15 mm de diamètre interne, 18 à 25 mm de long) coulissant parfaitement dans le guide canule. La longueur de la canule est ajustée, à l'aide d'un guide canule, de façon à ce qu'elle ne dépasse pas du guide canule lorsqu'elle y est introduite.

Chirurgie stéréotaxique

Le guide canule est fixé avec un ciment dentaire verre ionomère GC Fuji IX (Henri Shein, France) qui est appliqué sur la boite crânienne de l'animal. Puis un fil d'acier est inséré dans le guide canule afin d'éviter son obturation. L'animal est ensuite placé dans une cage individuelle pendant 3 jours pour la récupération puis replacé par groupe de 6 animaux. Le passage dans le test comportemental se fait 7 jours après l'opération.

Préparation de la solution

L'anpirtoline est dissoute dans une solution de liquide cérébro-spinal artificiel (aCSF) et la concentration de la solution est adaptée en fonction du volume injecté. Les souris contrôles reçoivent une injection locale d'aCSF.

Procédure d'injection

La canule d'injection est reliée à une seringue Hamilton de 2 µl par un cathéter. La seringue et le cathéter sont remplis soit d'aCSF pour les souris contrôles ou d'anpirtoline dissout dans l'aCSF pour les souris traitées. Avant l'injection le fil d'acier est retiré du guide canule et remplacé par la canule. Le volume injecté est de 0,4 µl par coté (d'où un volume par souris de 0,8 µl) avec une vitesse d'injection de 0,2 µl/min. Les souris sont soumises au test comportemental immédiatement après la fin de l'injection.

2.5 Vérification des déplétions monoaminergiques

2.5.1 Prélèvement des structures

Les souris sont euthanasiées par dislocation cervicale. Le cerveau est prélevé et placé sur une plaque réfrigérante (Leica EG 1130, Nussloch, Allemagne) dont la température est réglée à -7°C (Figure 8). Quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex) sont isolées selon la technique d'Iversen et Glowinski (1966) développée pour le rat et que nous avons adapté à la souris. Le cervelet est retiré puis le cerveau est posé sur la face dorsale afin de prélever l'hypothalamus. Ensuite les deux hémisphères sont séparés pour pouvoir prélever l'hippocampe de chaque hémisphère. Le corps calleux est ôté pour pouvoir prélever le striatum et le tissu restant correspond au cortex (Figure 9).

Figure 9 : Plaque réfrigérante

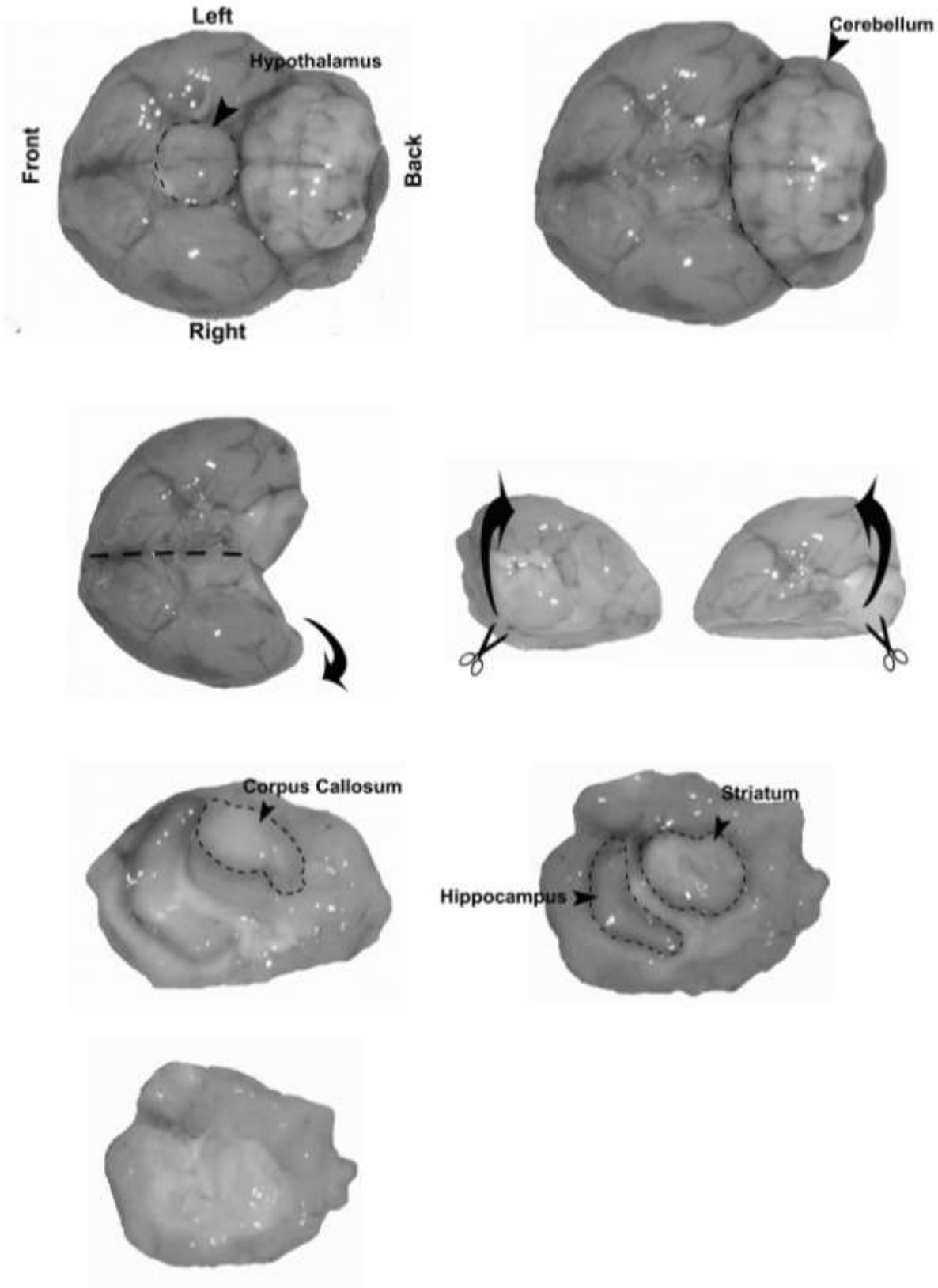


Figure 10 : Prélèvements des tissus cérébraux
D'après (Chenu et al., 2006)

2.5.2 Mesure des concentrations tissulaires de monoamines

Etude n°3 :

Mise au point de la technique de dosage des monoamines tissulaires

Chaque structure est placée dans un tube de polypropylène d'une capacité de 1,5 ml préalablement pesé. 600µl, pour les tubes contenant l'hypothalamus et l'hippocampe, et 1200µl, pour les tubes contenant le striatum et le cortex, d'une solution acide contenant 8,8 mg d'acide ascorbique, 122mg d'EDTA dans 1litre d'acide perchlorique 0,1 M sont ajoutés dans chaque tube. Les tissus sont ensuite disloqués par ultrasons (Branson Sonifer) puis centrifugés à 12000g pendant 10min à une température de + 4°C (Hareus Biofuge). Le surnageant est enfin prélevé est stocké à – 80°C avant l'analyse HLPC (chromatographie en phase liquide à haute performance).

L'intégralité de la technique de dosage a fait l'objet d'une publication dans un article de méthodologie. Cet article reprend également les gammes de dose de deux des produits utilisés pour induire des déplétions monoaminergiques, le DSP-4 et le p-CPA.

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Specificity and efficacy of noradrenaline, serotonin depletion in discrete brain areas of Swiss mice by neurotoxins

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Abstract

The aim of this work is to define neurotoxins doses to have efficient and specific depletion of noradrenaline (NA), serotonin (5-HT) neurotransmission in cortex, striatum, hippocampus and hypothalamus of Swiss mice after intraperitoneal administration of, respectively, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) and *para*-chlorophenylalanine methyl ester hydrochloride (PCPA). The neurotransmitters concentrations were determined by high performance liquid chromatography with amperometric detection. The minimal single dose necessary to produce a highly significant decrease of NA levels ($p < 0.01$ in comparison with control group) in hypothalamus (−44%), hippocampus (−91%), striatum (−40%) and cortex (−68%) was 50 mg/kg but DA and 5-HT levels were modified, respectively, in hypothalamus and striatum. Three doses of PCPA 300 mg/kg over 3 consecutive days involve a profound depletion of 5-HT transmission in all discrete brain areas but NA and DA levels were also significantly reduced. In conclusion, DSP-4 has a different efficacy in discrete brain areas with a noradrenergic specificity which is not absolute, PCPA has a similar efficacy in all brain areas but is unspecific of 5-HT transmission.

Keywords: Noradrenaline; Serotonin; Swiss mice; Depletion

1. Introduction

The depletion of noradrenaline (NA) or serotonin (5-HT) neurotransmission in brain by neurotoxins are methods commonly used in particular to investigate models of neuropsychiatric diseases in rodents. Thus, Srinivasan and Schmidt (2004) showed the importance of noradrenergic pathophysiology in Parkinson's disease thanks to a Parkinson's animal model with additional noradrenergic lesions. Recently, Slattery et al. (2005) demonstrated that the antidepressant-like properties of GABA (B) receptor antagonists in the forced swin test was abolished by 5-HT depletion. In our laboratory, depletion of NA and 5-HT systems by neurotoxins is used to investigate the mechanism of action of drug in animal models of anxiety, such as the four-plate test (Bourin et al., 2005). However, the specificity of depletion for a monoamine system is frequently not absolute and the degree of depletion is variable between discrete brain areas, strains and species of rodents which affects the interpretation of results obtained with neurotransmitters depleted animals (Fornai et al., 1996). Moreover, most of these studies which investigated the specificity and efficacy of depletion by neurotoxins were conducted in rats. Swiss mice are a commonly used strain of mice and detailed studies describing the efficacy and specificity of neurotoxins in discrete brain areas in this strain of mice are lacking. The aim of this work is to define optimal doses of neurotoxins to obtain the more efficient and specific depletion of NA or 5-HT system by neurotoxins in Swiss mice. The neurotoxins which were investigated are *para*-chlorophenylalanine methyl ester hydrochloride (PCPA) which crosses blood brain barrier better than *para*-chlorophenylalanine, a serotoninergic neurotoxin (Fratta et al., 1973) and *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4) (Hallman and

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Jonsson, 1984) which is usually presented as a selective noradrenergic neurotoxin. Efficacy and specifity of depletion in hippocampus, hypothalamus, striatum and cortex were evaluated by a biochemical approach after intraperitoneal (i.p.) administration of PCPA and DSP-4.

2. Materials and methods

2.1. Chemicals

Ascorbic acid, citric acid monohydrate and sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). DA, 5-HT, NA, octanesulfonic acid sodium salt, PCPA and DSP-4 were from Sigma (Saint Louis, USA), ethylendiaminetetracetic acid tetrasodium salt (EDTA) and dibutylamine were from Fluka Chemie (Buchs, Germany) and methyl alcohol for analysis from Carlo Erba (Val de Reuil, France).

2.2. Animals

Experiments were carried out on male Swiss mice (Centre d'élevage Janvier, Le Genest, France) 4 weeks old and weighing 18–20 g. Mice were housed in groups of 18 per cage (40 cm × 28 cm × 17 cm), in the standard conditions of the animal room (20 ± 1 °C, standard light/dark cycle light on at 7 a.m., off at 7 p.m.) with free access to food and water for a period of 1 week before use. Each experimental group consisted of naive randomly grouped mice of the same weight, which were used only once.

2.3. NA and 5-HT depletions

PCPA and DSP-4 were dissolved extemporaneously in distilled water. PCPA (100, 300, 500, 700 and 900 mg/kg) and DSP-4 (10, 30, 50, 70 and 90 mg/kg) were administered i.p., respectively, 72, 48 and 24 h for PCPA and 168 h for DSP-4 before brain dissection under a volume of 25 ml/kg. According to neurotoxins solubility in water, ranges of PCPA and DSP-4 doses were selected to try to reach, respectively, a total depletion of 5-HT and NA systems in brain sections. The control group received only distilled water (three injections for PCPA control group and one injection for DSP-4 control group). For control groups and for each dose of PCPA or DSP-4, the group was composed of 10 animals.

2.4. Preparation of samples

Mice were killed by cervical dislocation without anaesthesia. The brain was rapidly removed from the cranium and dissected on a cooled aluminium apparatus. The brain sections (cortex, striatum, hippocampus and hypothalamus) were weighed into a 1.5 ml polypropylene tube. Six hundred microlitres (for tubes containing hippocampus or hypothalamus) of an acid solution (8.8 mg of ascorbic acid and 122 mg of EDTA in 1000 ml of perchloric acid 0.1 M) and 1200 μl (for tubes containing cortex and striatum) were added to each tube. Tissue was then disrupted by sonication. After sonication, the solution was centrifuged at 12,000 × *g* for 10 min at +4 °C. The supernatant was stored at −80 °C before use.

2.5. HPLC analysis

The HPLC system was composed of an isocratic Varian Prostar model 210 pump (Sunnyvale, USA), a cooled Waters Wisp model 717 autosample injector (Milford, USA), a Decade amperometric detector (Leiden, The Netherlands) with an electrochemical Antec Leyden model VT-03 Flow cell (Zolterwoude, The Netherlands) and a C18 column (Nucleosil, 5 μm particule size, 15 cm, Colochrom, Gagny, France). The chromatographic conditions were (i) a mobile phase composed of 4.2 g/l of citric acid monohydrate, 6.8 g/l of sodium acetate trihydrate, 0.8 g/l of octanesulphonic acid sodium salt, 0.05 g/l of EDTA, 0.02% (v/v) dibutyl amine, 7% (v/v) methyl alcohol (ii) a rate flow of 1.6 ml/min (iii) a total runtime of 25 min, (iv) 20 μl of the supernatant (half diluted in the acid solution for striatum and cortex to avoid the saturation of the detector by DA) were injected into the HPLC system and (v) the potential of detection was 0.48 V. For each analysis, a set of standards containing various concentrations of each compound (NA, DA and 5-HT) was prepared in the acid solution. The calibration curves were calculated by a linear regression. The concentrations of compounds in the supernatant were determined from the peak area of each compound and compared with the standard curve.

2.6. Statistical analysis

Statistical comparisons of the average monoamines levels were performed initially via an one-way analysis of variance (ANOVA) for independent groups, after verifying the normality of distribution by a Kolmogorof–Smirov non-parametric test. If any statistical change was observed, data was further analysed using post hoc comparisons, with a Fisher test, to detect eventual differences between control and depleted groups.

3. Results

The results of NA, DA and 5-HT depletion by DSP-4 are present in Fig. 1 and Table 1. The minimal dose necessary to produce a highly significant decrease ($p < 0.01$) of NA levels in all discrete brain areas is 50 mg/kg. NA levels decrease in comparison with controls of 44% in hypothalamus, 91% in hippocampus, 40% in striatum and 68% in cortex after administration of DSP-4 50 mg/kg. With this dose, a significant decrease of DA level (−38%) was observed in

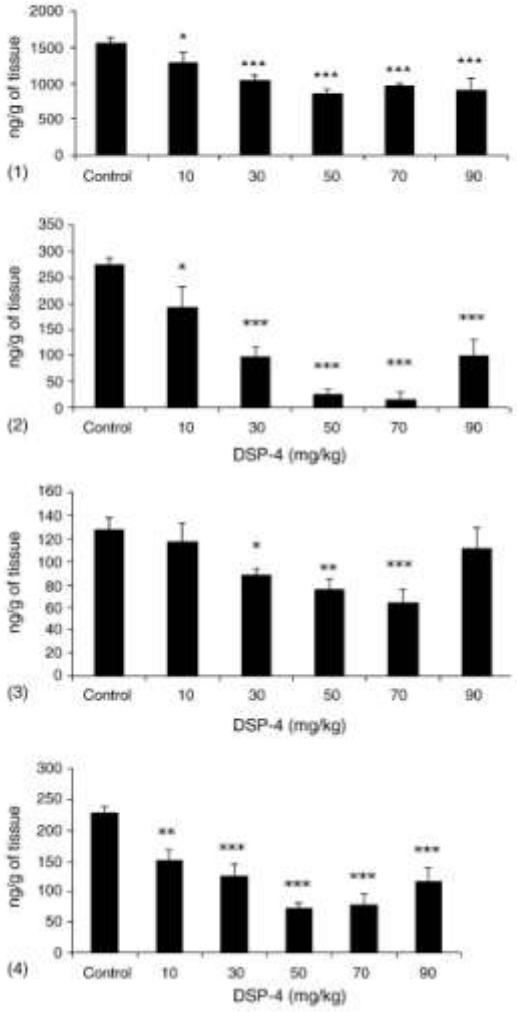

Fig. 1. Effect of DSP-4 (10, 30, 50, 70 and 90 mg/kg, i.p.) on NA levels in hypothalamus (1), hippocampus (2), striatum (3) and cortex (4) of Swiss mice: bar and error bar correspond with mean and S.E. mean. Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ in comparison with control.

hypothalamus and an increase of 5-HT level was observed in striatum (+81%). The effect of PCPA on NA, DA and 5-HT depletions are present in Fig. 2 and Table 2. With PCPA 300 mg/kg, a total 5-HT depletion was obtained in hypothalamus, hippocampus and an almost total depletion in striatum (−95%) and in cortex (−83%), moreover, significant decreases of NA levels (−30% in hypothalamus) and DA levels (−62% in hypothalamus and −17% in striatum) were also observed.

Table 1
Effect of DSP-4 (10, 30, 50, 70 and 90 mg/kg, i.p.) on DA and 5-HT levels in hypothalamus, hippocampus, striatum and cortex of Swiss mice

Tissue	Dopamine (ng/g of tissue)						Serotonin (ng/g of tissue)					
	Control	DSP-4 (mg/kg, i.p.)					Control	DSP-4 (mg/kg, i.p.)				
		10	30	50	70	90		10	30	50	70	90
Hypothalamus	363.39 ± 22.47	283.53 ± 43.76	288.79 ± 37.22	224.81 ± 17.50**	228.64 ± 39.56**	271.24 ± 27.42	600.00 ± 69.58	578.54 ± 82.48	439.90 ± 34.69	717.71 ± 57.69	726.67 ± 30.49	803.12 ± 58.87*
Hippocampus	/	/	/	/	/	/	454.30 ± 20.63	459.69 ± 46.03	420.24 ± 22.59	441.68 ± 29.10	372.41 ± 34.86	326.79 ± 31.21
Striatum	5614.34 ± 237.32	5803.04 ± 481.32	4816.08 ± 382.73	5254.53 ± 586.37	4674.18 ± 340.86	5220.62 ± 593.03	299.58 ± 31.89	312.59 ± 32.90	270.95 ± 28.05	543.78 ± 43.67***	456.83 ± 49.46**	588.87 ± 53.64***
Cortex	542.68 ± 44.27	570.27 ± 46.04	551.98 ± 51.86	597.66 ± 49.64	589.03 ± 57.73	563.65 ± 81.27	571.39 ± 19.69	560.65 ± 20.54	590.04 ± 52.84	450.85 ± 31.05	520.28 ± 26.33	572.01 ± 30.34

Values are expressed as the mean ± S.E.M. of 10 animals per group. Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant. Dopamine levels are under the limit of detection in hippocampus.

* Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p<0.05$ in comparison with control.

** Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p<0.01$ in comparison with control.

*** Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p<0.001$ in comparison with control.

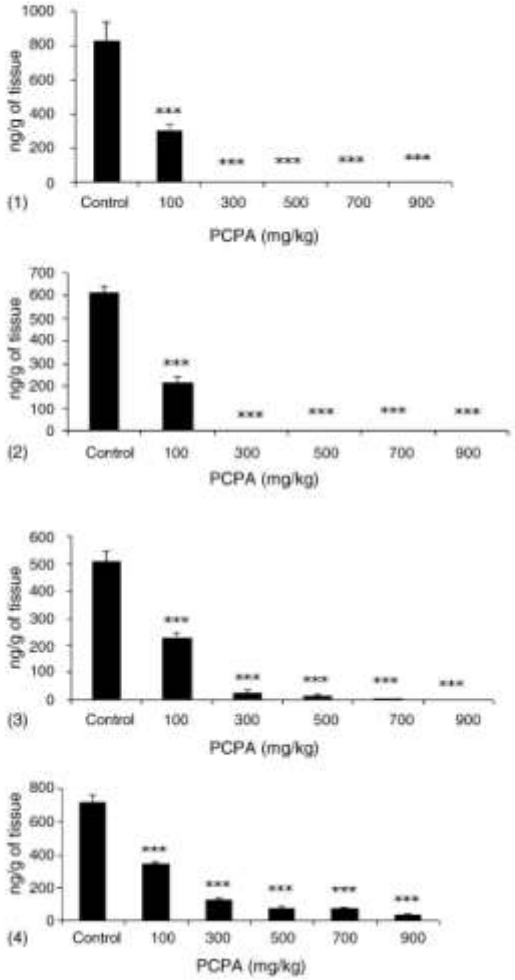

Fig. 2. Effect of PCPA (100, 300, 500, 700 and 900 mg/kg, i.p.) on 5-HT levels in hypothalamus (1), hippocampus (2), striatum (3) and cortex (4) of Swiss mice. bar and error bar correspond with mean and S.E. mean. Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $^{***}p < 0.001$ in comparison with control.

4. Discussion

According to previous studies in mice and rats (Fornai et al., 1996), the minimal doses of DSP-4 necessary to have a highly significant depletion of NA is a single i.p. injection of 50 mg/kg in Swiss mice. However, the selectivity of DSP-4 on noradrenergic neurons is not absolute since a significant increase of 5-HT was observed in striatum and a significant decrease of DA level was observed in hypothalamus. According to Fornai et al. (1996), a variable extent of NA depletion was observed in discrete brain

Table 2
Effect of PCPA (100, 300, 500, 700 and 900 mg/kg, i.p.) on NA and DA levels in hypothalamus, hippocampus, striatum and cortex of Swiss mice

Tissues	Noradrenaline (ng/g of tissue)						Dopamine (ng/g of tissue)					
	Control	PCPA (mg/kg, i.p.)					Control	PCPA (mg/kg, i.p.)				
		100	300	500	700	900		100	300	500	700	900
Hypothalamus	1170.19 ± 85.52	111.291 ± 73.69	827.31 ± 69.85***	523.16 ± 82.55***	373.06 ± 43.90***	272.69 ± 17.32***	356.14 ± 33.50	211.25 ± 21.64	96.81 ± 8.69***	34.25 ± 5.87***	/	/
Hippocampus	209.93 ± 19.89	223.15 ± 22.99	220.38 ± 11.02	172.97 ± 8.55	160.42 ± 15.32*	177.09 ± 16.72	/	/	/	/	/	/
Striatum	96.12 ± 10.27	88.59 ± 6.50	103.95 ± 9.60	86.88 ± 7.24	76.87 ± 4.68	80.57 ± 7.32	4588.36 ± 372.84	4685.84 ± 444.58	5230.10 ± 581.59*	4722.92 ± 532.91**	4504.94 ± 353.81***	3915.98 ± 317.25***
Cortex	240.64 ± 16.10	211.78 ± 10.85	216.61 ± 16.77	197.29 ± 14.21*	160.53 ± 6.79***	147.05 ± 9.85***	665.73 ± 48.06	669.30 ± 28.80	657.98 ± 46.77	631.30 ± 73.79	459.88 ± 35.93*	472.30 ± 47.87*

Values are expressed as the mean ± S.E.M. of 10 animals per group. Dopamine levels are under the limit of detection in hypothalamus (PCPA 700 and 900 mg/kg, i.p.) and hippocampus.
* Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p < 0.05$ in comparison with control.
** Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p < 0.01$ in comparison with control.
*** Differences between groups were analysed via a one-way ANOVA followed by a Fisher test if ANOVA was significant: $p < 0.001$ in comparison with control.

areas. A dramatic (over −90%) NA depletion occurred in hippocampus, a significant depletion in cortex and a limited depletion in striatum and hypothalamus. The variation of NA decrease between brain areas could be the consequence of a higher affinity for DSP-4 of noradrenergic axons arising from locus coeruleus compared with extra-coeruleus noradrenergic terminals and a higher relative amount of coeruleus versus extra-coeruleus noradrenergic axons in the hippocampus arising from locus coeruleus compared with extra-coeruleus noradreneric terminals (Zaczek et al., 1990). The dramatic decrease in the hippocampus is consistent with previous results obtained with C57 Black mice (over −90%). However, this depletion is higher than depletion obtained in Swiss–Webster mice (−71%) (Fornai et al., 1996). Similarly, these differences between strains could be explained by a relative higher amount of coeruleus versus extra-coeruleus noradrenergic axons in the hippocampus of C57 Back and Swiss mice in comparison with Swiss–Webster mice.

PCPA, which is usually presented as a 5-HT synthesis inhibitor induced a decrease of 5-HT level in discrete brain areas. This result is consistent with the decrease of 5-HT (−71.7%) observed by Dursun and Handley (1993) in the whole brain of Aston Bred male MFI mice treated with three doses of PCPA (300 mg/kg, i.p.) 24, 48 and 72 before killing mice. However, no detailed study describing the effects of PCPA on 5-HT, NA and DA in different brain areas of mice was available. Our study showed that the specificity of this depletion was not absolute in Swiss mice. A significant decrease of NA in hypothalamus, DA in hypothalamus and striatum was found with three doses of PCPA 300 mg/kg. This result in Swiss mice is consistent with a previous study in Long-Evans rats: Dringenberg et al. (1995) showed that PCPA could induce a profound depletion (over −90%) of 5-HT level and a significant decrease of NA and DA levels in the whole brain of rats treated with 500 mg/kg PCPA 2 consecutive days.

In conclusion, our study (i) showed that DSP-4 (a single 50 mg/kg administration i.p.) had a different efficacy in discrete brain areas with a noradrenergic specificity which was not absolute and (ii) showed that PCPA (300 mg/kg, i.p., 3 consecutive days) had a similar efficacy in all brain areas and was an unspecific neurotoxin of 5-HT system in Swiss mice.

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Analyse statistique

Les analyses statistiques ont été réalisées à l'aide du logiciel SIGMASTAT. Les résultats sont exprimés sous forme de moyenne des valeurs mesurées ± écart standard à la moyenne (S.E.M.). Les paramètres suivis sont :

- le nombre de coupures de faisceau dans le test d'actimètrie,
- le temps d'immobilité en seconde dans le FST
- les concentrations (en ng/g de tissus) monoaminergiques lors des dosages tissulaires.

Les résultats des tests comportementaux ont été analysés à l'aide d'une analyse de variance (ANOVA) à deux facteurs (prétraitement ou souche x traitement) ; alors que les études de neurochimie ont été analysées par une ANOVA à 1 facteur (déplétion).
Si il y a lieu, l'ANOVA est suivi d'un test a posteriori de Student Newman Keuls.
Le seuil de significativité est fixé à $p<0.05$.

3.0 RESULTATS

Etude n°4 :

Evaluation des effets de type antidépresseur consécutifs à l'activation des hétérorécepteurs 5-HT_{1B}

5-HT_{1B} heteroreceptor activation induces an antidepressant-like effect in 5,7-DHT-treated mice

Franck Chenu, Denis J.P. David, Cédric Przybylski, Isabelle Leroux-Nicollet, Erwan Le Maître, René Hen, Alain M. Gardier, and Michel Bourin

Soumis dans Psychopharmacology

Objectif de l'étude n°4 :

Différents travaux ont mis en évidence le fait que les agonistes des récepteurs 5-HT_{1B} permettent d'induire chez l'animal un effet de type antidépresseur. Cependant, il est maintenant clairement établi que l'activation de ces récepteurs s'accompagne d'une diminution de la libération de sérotonine, d'une diminution de l'activité électrique des neurones sérotoninergiques, ainsi que d'une diminution de la synthèse de sérotonine. Ces modifications de paramètres biochimiques et électrophysiologiques n'étant pas compatibles avec les effets comportementaux observés, nous avons émis l'hypothèse que l'effet de type antidépresseur pourrait être lié à l'activation des hétérorécepteurs 5-HT_{1B}.

Toutefois, aucune étude ne permettait de confirmer cette hypothèse. Les récepteurs 5-HT_{1B} pré et postsynaptiques ayant la même structure, il n'existe donc pas de ligands sélectifs de l'un, ou l'autre de ces deux sous-types de récepteurs.

Nous avons évalué les effets de l'anpirtoline chez des animaux mutants ne présentant pas de récepteurs 5-HT_{1B} (souris « knockout », KO 5-HT_{1B}) afin de démontrer que les effets observés sont bien imputables au récepteur sérotoninergique de type 1B. Afin d'évaluer les effets comportementaux relatifs à l'activation des seuls hétérorécepteurs 5-HT_{1B}, nous avons réalisé une destruction des afférences sérotoninergiques (et donc des autorécepteurs 5-HT_{1B} situés sur ces neurones) à l'aide d'une neurotoxine spécifique, la 5,7-DHT. Le niveau de déplétion en sérotonine à été évalué à l'aide de deux techniques différentes, le dosage tissulaire et l'autoradiographie du transporteur à la sérotonine. Une fois la déplétion réalisée, nous avons administré un agoniste de ces récepteurs (l'anpirtoline) par voie i.p..

5-HT_{1B} heteroreceptor activation induces an antidepressant-like effect in 5,7-DHT-treated mice

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Abstract

Rationale: It was demonstrated that absence, or blockade, of 5-HT_{1B} receptors, potentiates the increase in extracellular serotonin (5-HT) levels in the hippocampus induced by a single i.p. administration of Selective Serotonin Reuptake Inhibitors (SSRI) in mice. However, this increase has not been correlated with antidepressant-like (AD-like) activity in the mice forced swim test.

Objectives: We postulated that activation of postsynaptic 5-HT_{1B} heteroreceptors could mediate an AD-like effect.

Methods: This hypothesis was explored by inducing a selective destruction of presynaptic 5-HT neurons (and 5-HT_{1B} autoreceptors) with a selective neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) administered by intracerebroventricular route in 129/Sv mice. Depletion level and selectivity of action of 5,7-DHT were estimated by measurements of neurotransmitters and metabolite tissue levels in discrete brain regions.

Results: An intracerebroventricular administration of 5,7-DHT led to 5-HT and 5-HIAA depletions of 76.5% and 75.4% in the hippocampus and 42.2% and 45.3% in the cortex, respectively. The serotonergic lesions by 5,7-DHT was confirmed by the decreased binding of [^{3}H]-Citalopram to serotonin transporter in both areas (-78% and -45% in the hippocampus and cortex, respectively). A single i.p. administration of active doses of anpirtoline (5-HT_{1B} receptor agonist) in sham-operated and in 5,7-DHT-treated-mice induced a dose-dependent AD-like activity with a greater effect in 5,7-DHT-treated-mice (-19% and -26%) than in sham-operated-mice (-5% and -10%) at 4 and 8 mg/kg, respectively. As expected, anpirtoline was devoid of effects in 5-HT_{1B} -/- mice.

Conclusion: These data suggest that the AD-like activity of anpirtoline measured in 5,7-DHT-lesioned-mice depends on 5-HT_{1B} heteroreceptors stimulation (autoreceptors being destroyed by the neurotoxin).

Keywords: 5-HT_{1B} receptors, 5,7-DHT, FST, monoamines, depletion, mice.

Introduction

By using intracerebral *in vivo* microdialysis, our team and others demonstrated that the absence of 5-HT_{1B} receptors in knockout mice, or its blockade by a selective 5-HT_{1B} receptor antagonist (GR127935) potentiates the increase in the extracellular serotonin levels ($[5\text{-HT}]_{EC}$) induced by a single intra-peritoneal (i.p.) administration of Selective Serotonin Reuptake Inhibitors (SSRIs: citalopram, fluoxetine and paroxetine) (De Groote et al. 2002a; b; Gobert et al. 1997; Malagie et al. 2002; Malagie et al. 2001). These data suggest that activation of 5-HT_{1B} autoreceptors limit the effects of SSRIs on dialysate 5-HT levels at serotonergic nerve terminals in both hippocampus and cortex areas.

Surprisingly, this increase in $[5\text{-HT}]_{EC}$ was not correlated with an increase of the antidepressant-like (AD-like) activity evaluated by using the forced swimming test (FST) in mice (Gardier et al. 2001). At the opposite, we found that SSRI-induced decrease in immobility time in the FST is absent in 5-HT_{1B} knockout mice and blocked by GR127935 in wild-type suggesting therefore that activation of postsynaptic 5-HT_{1B} heteroreceptors mediate the AD-like effects of SSRIs. We postulated that the AD-like effect of 5-HT_{1B} receptor agonists, and probably those of SSRIs, might be mediated by activation of 5-HT_{1B} heteroreceptors.

The aim of the present study was to demonstrate that activation of these heteroreceptors by a direct 5-HT_{1B} receptor agonist, anpirtoline, can mediate AD-like effects in mice, similarly to the results obtained with SSRIs (O'Neill and Conway 2001; Redrobe and Bourin 1999). This was explored by performing several experiments in mice lesionned with a neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), thus lacking presynaptic 5-HT_{1B} autoreceptors, but still having postsynaptic 5-HT_{1B} heteroreceptors (Maroteaux et al. 1992). Postsynaptic 5-HT_{1B} receptors are located on non serotonergic neurons such as dopaminergic (Galloway et al. 1993; Sarhan et al. 1999; Sarhan et al. 2000), glutamatergic (Bobker and Williams 1989; Boeijinga and Boddeke 1996), acetylcholinergic (Cassel et al. 1995; Maura et al. 1989; Maura and Raiteri 1986), and GABAergic neurons

(Feuerstein et al. 1996), and are not affected by the neurotoxin: in 1997, Harrison et al. induced a depletion of 5-HT brain levels using 5,7-DHT and demonstrated that other monoamine levels were not significantly affected by the lesion. We thus induced a selective destruction of presynaptic 5-HT axons and cell bodies with 5,7-DHT administered by an intracerebroventricular (i.c.v.) route to 129/Sv mice.

Similarly to D'Souza et al. (2002), two sets of experiments were performed on the brain to test for the selectivity of the neurotoxin 5,7-DHT to destroy 5-HT-containing nerve terminals, 14 days after its i.c.v. injection. First, some mice were killed by cervical dislocation to determine the depletion of brain tissue levels of serotonin (Harrison et al. 1997; Nelson et al. 1978). Second, a [^{3}H]-citalopram binding study was performed to analyze, in several brain areas, the degree of occupancy of the serotonin transporter by a SSRI and the extent of impairment of the high affinity serotonin uptake site in these mice (Montanez et al. 2003; Romero et al. 1998). These two experiments studied the degree of serotonergic neurotransmission impairment following neurotoxin injection to mice.

Behavioural effects obtained after injection of anpirtoline (chosen because of its ability to cross the *blood-brain-barrier*; Schlicker et al. 1992; Swedberg et al. 1992) on lesionned mice should be considered as resulting from the activation of 5-HT$_{1B}$ heteroreceptors only (some mice have been then sacrified and monoamine levels have been evaluated to confirm the depletion level on tested mice).

Materiels and Methods

Animals

The founders of the wild-type and mutant colonies used in the present study were the product of heterozygous matings made at the animal facility of Columbia University. These founders were shipped to France and their offspring were bred and reared in

independent colonies as already described (Malagie et al. 2001). Wild-type and KO 5-HT_{1B} mice were obtained from a pure 129/Sv genetic background.

They were housed in groups of 20 per cage (40 cm x 28 cm x 17 cm) on a 12:12h light: dark cycle (lights on 07.00 hours) and had free access to food and water. The ambient temperature of the room was maintained at 21 ± 1°C and the humidity was 50%. All experiments were performed within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law 87 848).

Surgical procedures

All mice were pretreated 30 minutes prior to i.c.v. administration of 5,7-DHT (or vehicle) with i.p. injections of 20 mg/kg desipramine HCl (Ciba Geigy) and 25 mg/kg of nomifensine maleate (Ciba Geigy) to protect norepinephrine (NE) and dopamine (DA)-containing neurons (Bjorklund et al. 1975). They were then anaesthetised with chloral hydrate (400 mg/kg, i.p.) and were placed in a ASI stereotaxic instrument fitted with atraumatic earbars. 5,7-Dihydroxytryptamine creatinin sulphate (5,7-DHT) dissolved in 0.1% ascorbic acid was injected using an infusion pump (KDScientific) at a flow rate of 0.2 µL/min for 7.5 min, and the needle was left in place for a further 2 min before removal to prevent efflux of the injected solution. Treated mice received a bilateral intracerebroventricular (i.c.v.) injection of 11.4 µg of 5,7-DHT (free base); coordinates from Bregma (mm): anterior = -0.7, lateral = ± 1, ventral = -3.5. After surgery, a post operative period of 14 days was necessary to allow the degeneration of 5-HT containing neurons (delay of action of neurotoxin; Bjorklund et al. 1975).

After these 14 days, two groups of treated mice were killed by cervical dislocation to determine the serotonin depletion levels and the selectivity of action of the neurotoxin (HPLC brain tissue dosage and [^{3}H]-citalopram binding). Two other groups were used for behavioural studies (locomotor activity and forced swimming test; the depletion level was

confirmed on these mice by HPLC brain tissue dosage the day after the test). Anpirtoline, a 5-HT_{1B} receptor agonist, was dissolved in water and administered i.p. 30 minutes before both behavioural tests.

Determination of depletion level and selectivity of action of 5,7-DHT

Measurements of neurotransmitters and metabolite concentrations in discrete brain region:

Preparation of samples and HPLC analysis

Mice were killed by cervical dislocation without anaesthesia. The brain was rapidly removed from the cranium and dissected on a cooled aluminium apparatus as we previously described (Chenu et al. 2006). The brain sections (cortex and hippocampus) were weighed into a 1.5 ml polypropylene tube. 600 µL (for tubes containing hippocampus) and 1200 µL (for tubes containing cortex) of an acid solution (8.8 mg of ascorbic acid and 122 mg of EDTA in 1000 ml of perchloric acid 0.1 M) were added to each tube. Tissue was then disrupted by sonication and, the solution was centrifuged at 12000 g for 10 min at + 4°C. The supernatant was stored at –80°C before use.

The preparation of samples and the HPLC analysis were fully described in a recent methodological article (Dailly et al. 2006).

Autoradiographic studies

After sacrifice, the brains were quickly removed, frozen in isopentane at -30°C and stored at -80°C until used. Coronal 20µm tissue sections were cut in a cryostat at -20°C (Microm HM 560) throughout the hindbrain and thaw-mounted onto chrome-alum 5%-gelatine 0.5%-coated slides. Slides were dried and stored dessicated at -80°C until used (Naudon et al. 2001). Then, we performed binding of [^{3}H]-citalopram on the serotonin neuronal transporter. Before autoradiographic experiments, brain sections were thawed under vacuum for 20 min, then stored at room temperature until incubation.

Autoradiographic study with ligand was performed on adjacent slices from the same animals.

[^{3}H]-citalopram binding (D'Amato et al. 1987)

Slides were preincubated for 15 min at room temperature in Tris buffer (Tris HCl 50 mM, NaCl 120 mM, KCl 5 mM; pH 7.4). To assess total binding, incubation was performed at room temperature for 1h in coplin-jars containing 30 ml for 10 slides of the same buffer with 1 nM [^{3}H]-citalopram (Spec. Act. 84.2 Ci/mmol, NEN-Perkin Elmer). Non specific binding was evaluated on adjacent sections incubated in the same conditions plus 10^{-5} M fluoxetine (Lilly). Then, the slides were dipped into buffer, rinsed twice in the same buffer at 4°C during 10 min, and dipped in distilled water. Slides were dried under a cold air stream for 30 min and allowed to dry overnight. The sections were then apposed on Biomax MR films (Kodak) for 4 weeks, with tritiated standard strips (Amersham). Quantification of autoradiograms was performed using a computerized image analysis system (Samba Technologies, Meylan, France).

Since 5-HT_{1B} autoreceptors are located on presynaptic 5-HT nerve terminals, the decrease in [^{3}H]-citalopram binding sites following 5,7-DHT injection should be therefore linked to a decrease in the number of 5-HT neurons and to a decrease in the density of 5-HT_{1B} autoreceptors (Compan et al. 1998).

Behavioural tests

Measurement of locomotor activity in mice (Boissier and Simon 1965)

Animals were kept in the darkened test-room at least 1 hour before the test for habituation. After injection (vehicle or treatment), mice were replaced in their holding cages for the required injection-test interval, and then individually transfer to the actimeter for the 10 min test. These animals were different from those used in the forced swimming

test. The spontaneous activity of naive animals was recorded using a photoelectric actimeter (OSYS, Laval France). This actimeter consists of a stainless steel apparatus containing transparent cages in which the animals' horizontal activity is measured by light beams connected to a photo-electric cell. The activity is recorded during a 10 minutes period. The actimeter test is performed independently of the mouse FST to examine the effect of the drugs on the spontaneous locomotor activity of mice.

Measurement of immobility time in the forced swimming test

The forced swimming test employed was essentially similar to that described elsewhere (Porsolt et al. 1977). Mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm water, maintained at 23-25°C, and remained there for 6 min. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Six mice were tested simultaneously, and the time of immobility was recorded during the last 4-min of the 6-min testing period, thus after 2 min of habituation. The test was performed by the same well trained experimenters, blind to the treatment administered. Anpirtoline (4 and 8 mg/kg) was injected i.p. 30 minutes before the test. Results are expressed as the immobility time during the 240-sec test period (mean ± S.E.M.).

Data analysis and statistics

Statistical analyses were performed by the use of the computer software Sigmastat. In behavioural tests, a two-way ANOVA on either the immobility time (FST) or the number of beams broken (locomotor activity) was performed with the 5-HT depletion (lesionned or sham) and the drug treatment as main factors followed by a *post-hoc* Newman-Keuls test. The depletion in monoamine level and [^{3}H]-citalopram binding were analyzed by a one-way ANOVA followed by a Newman-Keuls test. Statistical significance was set at $p<0.05$.

Results:

1) Effects of anpirtoline treatment on 5-HT$_{1B}$ receptor knockout and wild-type non-operated mice in behavioural studies:

The two-way ANOVA performed (mice genotype x treatment) on the immobility time of mice (FST) revealed statistically significant main effects of genotype (Wild-Type or 5-HT$_{1B}$ Knockout; $F_{1.36} = 29.29$; $p<0.001$), treatment (saline or anpirtoline; $F_{1.36} = 28.67$; $p<0.001$) and a significant interaction between these two factors ($F_{1.36} = 25.11$; $p<0.001$). Anpirtoline injected i.p. in WT mice induced a significant decrease in the immobility time in the FST (by 15%: 236.7 $\pm$ 1.8 versus 200.5 $\pm$ 6.4 sec; $p<0.001$). Whereas in 5-HT$_{1B}$ KO mice, a 4 mg/kg dose of anpirtoline did not alter the immobility time in the FST (238.1 $\pm$ 1.1 versus 236.9 $\pm$ 1.6 sec; $p=0.81$). Moreover, there was a statistically significant difference between strains of mice further to administration of anpirtoline ($p<0.001$), whereas no changes were obtained following vehicle administration ($p=0.78$) (Figure 1).

The two-way ANOVA performed (mice genotype x treatment) on the locomotor activity of mice did not reveal statistically significant effects of genotype (Wild-Type or 5-HT$_{1B}$ Knockout; $F_{1.36} = 0.37$; $p=0.545$), treatment (saline or anpirtoline; $F_{1.36} = 0.217$; $p=0.644$) factors, nor a significant interaction between these two factors ($F_{1.36} = 1.141$; $p=0.293$) (Data not show).

These results suggest that the decrease in immobility time in the FST obtained in WT mice was not linked to a psychostimulant effect of anpirtoline and that the absence of effects in KO mice was not linked to a sedative effect of this drug. Antidepressant-like effect of anpirtoline appears to be mediated by activation of 5-HT$_{1B}$ receptors.

The 4 and 8 mg/kg doses were then chosen for the studies in sham-operated and neurotoxin-treated mice.

2) Effects of 5,7-DHT treatment in mice:

The i.c.v. perfusion of 22.8 µg of 5,7-DHT (freebase) per mice induced a significant decrease in 5-HT and of 5-HIAA levels in both cortex (Figure 2a) and hippocampus (Figure 2b). In the cortex, the decrease in 5-HT levels (one-way ANOVA: lesion, $F_{1.16}$=33.6; p<0.001) was of 42.2 % (non lesionned = 797.8 ng/g vs lesionned = 461.15 ng/g of wet weight of tissue), and the decrease in 5-HIAA levels (one-way ANOVA: lesion, $F_{1.16}$=33.58; p<0.001) was of 45.3 % (non lesionned = 549.66 ng/g vs lesionned = 300.5 ng/g of wet weight of tissue). In the hippocampus, the decrease in 5-HT levels (one-way ANOVA: lesion, $F_{1.17}$=52.51; p<0.001) was of 76.5 % (non lesionned = 354.16 ng/g vs lesionned = 83.14 ng/g of wet weight of tissue), and the decrease in 5-HIAA levels (one-way ANOVA: lesion, $F_{1.18}$=19.96; p<0.001) was of 75.4 % (non lesionned = 460.4 ng/g vs lesionned = 113.1 ng/g of wet weight of tissue).

Levels of other neurotransmitters (noradrenaline, dopamine) were not affected by the 5,7-DHT lesion, neither in the hippocampus nor in the cortex (dopamine levels were non detectable in the hippocampus similarly to that we previously obtained). These results indicate that the lesion was selective and was limited to the brain serotonergic systems. The brain turnover of serotonin (5-HIAA/5-HT ratio, mean ± SEM; Figure 2c) was not significantly different between sham-operated and 5,7-DHT lesionned mice (Cortex: sham = 0.72 ± 0.05 and lesionned = 0.74 ± 0.08, p=0.77; Hippocampus: sham = 1.43 ± 0.16 and lesionned = 1.46 ± 0.19, p=0.92).

Figure 2A 2B & 2C

We also used an autoradiographic method with [^{3}H]-citalopram to check whether monoamines depletion was linked to nerve terminal destruction. The autoradiography showed a decrease in the number of ligand binding sites by -45% in the cortex ($F_{1.14}$=22.53; p<0.001) (non lesionned = 49.25 fmol/mg vs lesionned = 27.20 fmol/mg) and by -78% in the hippocampus ($F_{1.14}$=210.19; p<0.001) (non lesionned = 58.73 fmol/mg vs

lesionned = 12.95 fmol/mg) (Figure 3). These results indicate that the destruction of serotonergic nerve terminals paralleled the decreases in indolamines levels in these two different brain regions.

Figure 3

3) Effects of anpirtoline on the FST in 5,7-DHT treated mice:

Anpirtoline (4 and 8 mg/kg) was injected i.p. in sham-operated and lesionned mice. The two-way ANOVA on the immobility time show a significant effect of lesion (sham or 5,7-DHT; $F_{1.45}$=19.64; p<0.001), treatment (saline or anpirtoline; $F_{2.45}$=20.81; p<0.001) factors and a significant interaction between these two factors ($F_{2.45}$=4.27; p<0.05). In sham-operated mice, anpirtoline was devoid of antidepressant-like activity at 4 mg/kg (p=0.161), but induced an AD-like effect at 8 mg/kg (p<0.05). In 5,7-DHT-treated mice, anpirtoline induced a significant decrease in the immobility time at both doses (p<0.001 at 4 and 8 mg/kg). For each doses of anpirtoline, the decrease in the immobility time obtained is significantly greater in 5,7-DHT-treated mice than in sham-operated mice (p<0.01 and p<0.001 at 4 and 8 mg/kg respectively).

The two-way ANOVA performed on the locomotor activity showed a significant effect of lesion (sham or 5,7-DHT; $F_{1.53}$=7.99; p<0.01), treatment (saline or anpirtoline; $F_{2.53}$=5.72; p<0.01), but no significant interaction between these two factors ($F_{2.45}$=0.52; p=0.60). At 4 mg/kg, anpirtoline induced a significant increase in locomotor activity of 5,7-DHT treated mice (p<0.05). The increase in the number of light beams broken was significantly greater in 5,7-DHT-treated mice than in sham operated mice receiving 4 mg/kg of anpirtoline (p<0.05).

Taken together, these results suggest that the increase in the swimming attempt of 5,7-DHT-treated mice receiving 4 mg/kg (in comparison to sham-operated mice) obtained

in the FST could be linked to a psychostimulant effect of this drug; however, the absence of psychostimulant effect of the 8 mg/kg dose suggest an enhancement of the antidepressant-like effect of anpirtoline in serotonin depleted mice.

After these tests the monoamine levels were evaluated in both sham operated and 5,7-DHT treated mice. The depletion levels were similar to that we previously obtained (data not shown).

Figure 4 & 5

Discussion:

The first step of this study, i.e. the destruction of the brain serotonergic neurons was performed to evaluate behavioural consequences of 5-HT_{1B} heteroreceptors activation only (5-HT_{1B} autoreceptors being destroyed by the lesion of serotonin-containing nerve terminals). Evaluation of monoamines levels showed that the lesion was selective since noradrenaline and dopamine tissue levels in the cortex and hippocampus were not statistically affected by 5,7-DHT lesion. 5,7-DHT lesion also induced a partial serotonin depletion (42% and 76% in the cortex and hippocampus, respectively), which paralleled the partial impairment of the high affinity serotonin uptake site in several brain areas as measured by autoradiography with [^{3}H]-citalopram in mice (in the cortex -42% vs -45% and hippocampus: -76% vs -78%); moreover 5-HT turnover (5-HIAA/5-HT ratio) was not significantly different between sham and lesionned mice in the cortex (sham: 0.72$\pm$0.05; Lesionned: 0.74$\pm$0.08) and in the hippocampus (sham: 1.43$\pm$0.16; Lesionned: 1.44$\pm$0.13). Our results obtained in mice are different from those described by Stachowiak et al. (1986), in which the 5-HIAA/5-HT ratio in the hippocampus significantly increased following i.c.v. injection of 5,7-DHT in rats. The reasons for these discrepancies are currently unclear. However, two points must be discussed:

(i)-it is possible that a compensatory increase in the synthesis and release of 5-HT from nerve terminals occurred in rats, but not in mice. Moreover, some lesion studies performed with 5,7-DHT in rats revealed no differences in basal levels of extracellular 5-HT evaluated by microdialysis (Romero et al. 1998), while others revealed significant differences (Daszuta et al. 1989). It is highly presumable that these different parameters (brain tissue depletion, extracellular monoamines levels) are linked to compensatory mechanisms such as upregulation of receptors and/or variation of its intrinsic activity.

(ii)-the limited loss of 5-HT nerve terminals in 5,7-DHT-treated mice in some brain areas (e.g. here in the cortex) raises the possibility that some serotonergic axons were spared by the neurotoxin. Consequently, the presence of nerve terminal 5-HT_{1B} autoreceptors cannot be excluded: it would imply that their activation by anpirtoline could participate to its AD-like effects in neutoxin-treated mice. To test for this hypothesis, we should have increased the icv dose of 5,7-DHT in mice in order to induce a more profound damage of 5-HT axons and confirm our conclusions regarding the preferential postsynaptic 5-HT_{1B} receptor effects of anpirtoline: however, decreasing the remaining 5-HT innervation by increasing the 5,7-DHT dose, we would have lost the serotonergic selectivity of this neurotoxin.

The second part of this study analyzed the potent AD-like effect of anpirtoline evaluated in the FST (Porsolt et al. 1977). Similarly to what we found in the present study, it had been previously demonstrated by Chia et al. (1996 and 1999) that treatment with the neurotoxin 5,7-DHT did not increase the basal locomotor activity of animals. In our hand, these neurotoxin-treated mice were also able to swim as previously described by Matsuda et al. (1995) and Luscombe et al. (1993). A systemic administration of anpirtoline (4 mg/kg) induced a statistically significant decrease in the immobility time in the FST evaluated in non-operated mice without affecting their locomotor activity. This decrease should also be considered as an AD-like effect of anpirtoline. This result agrees with other

studies demonstrating that anpirtoline induces an AD-like effect in a Swiss strain of mice (O'Neill and Conway 2001; Redrobe and Bourin 1999), or can be used at subactive doses to potentiate subactive doses of various antidepressant drugs (David et al. 2001; Redrobe et al. 1996). Since anpirtoline has a higher affinity for 5-HT_{1B} receptors than for 5-HT_{1A} and 5-HT_2 receptor subtypes (Ki= 28 nM, 151 nM and 1.48 µM, respectively) (Metzenauer et al. 1992) and had no significant effects in 5-HT_{1B} KO mice (KO for pre- and postsynaptic receptors) (Figure 1), the AD-like effect measured in WT mice is likely linked to the 5-HT_{1B} receptor activation. At the same dose, anpirtoline was devoid of activity in sham-operated mice (but is still active at 8 mg/kg), thus suggesting that the absence of anpirtoline's effect might be explained by the isolation of mice for two weeks after 5,7-DHT injection: indeed, it has been previously demonstrated that isolation decreases the AD-like effect of an acute dose of either a SSRI or anpirtoline (Rilke et al. 2001). At the opposite, the antidepressant-like effect of anpirtoline at 4 mg/kg persisted in 5,7-DHT treated mice. Moreover, at both doses, anpirtoline was more effective in 5,7-DHT treated mice than in sham-operated mice. The 5,7-DHT lesion seems then to potentiate anpirtoline's effect (by 15% and 18% at 4 and 8 mg/kg, respectively). This increase in the AD-like effect of anpirtoline could be explained by compensatory mechanisms enabling surviving 5-HT nerve terminals to maintain basal neurotransmitter activity in 5,7-DHT treated mice. Accordingly, either an upregulation of 5-HT_{1B} heteroreceptors (Manrique et al. 1993), or an increase in their intrinsic activity were described in 5,7-DHT lesionned rats (Eide and Hole 1988; Nelson et al. 1978). This latter explanation is the more likely to occur because an upregulation of 5-HT_{1B} receptors appears only in rats having 95% of brain 5-HT depletion (Compan et al. 1998), while here we observed only ≈70% of brain 5-HT depletion in mice. A previous study (Gardier et al. 2001) demonstrated that 5-HT_{1B} receptor antagonist can be used to block SSRIs effects in a behavioural test, the FST. The present study demonstrates that the postsynaptic serotonin receptor responsible for the AD-like effect of SSRIs could be, at least in part, the 5-HT_{1B} heteroreceptors. These

results are in line with a recent study showing that administration of a 5-HT_{1B} receptor antagonist (GR 127935) potentiates the behavioural effect of subactive doses of tricyclic antidepressant (imipramine), NE reuptake inhibitor (desipramine) and monoamine oxidase A inhibitor (moclobemide), but is devoid of activity with a subactive dose of a SSRI (citalopram) (Tatarczynska et al. 2004).

All these results taken together indicate that 5-HT_{1B} heteroreceptor activation induces an AD-like effect in mice, while its genetic inactivation inhibits the AD-like effect of SSRIs (Gardier et al. 2001). Therefore, 5-HT_{1B} heteroreceptors could be considered as a promising target for antidepressant drugs (as already described by Moret and Briley 2000 and Chenu et al. 2005). Numerous studies demonstrated a decrease in the number of high affinity serotonin uptake sites in depressed patients following chronic SSRIs treatment (for review see Arango et al. (2003). However, no variation in the density of 5-HT_{1B} receptors was observed in these patients (Turecki et al. 2003). Drugs selectively acting on these receptors can be an alternative treatment to SSRIs in these patients.

Despite causing severe 5-HT depletions of brain 5-HT content, pre-treatment with 5,7-DHT potentiated anpirtoline-induced decrease in the immobility time in the FST, which should be associated with a decrease in basal extracellular levels of 5-HT in several mice brain areas. This latter effect should be carried out by remaining *presynaptic* nerve terminals originating in raphe nuclei, and spared by the neurotoxin. Recent *in vivo* microdialysis studies showed that, two weeks after i.c.v. or intra-raphé injection of 5,7-DHT, basal extracellular levels of 5-HT in rats were either unchanged (in the dorsal raphe nucleus and ventral hippocampus: Romero et al. 1998) or reduced (in the hippocampus: Daszuta et al. 1989). These results must be interpreted in line with the fact that lesionned serotonin-containing nerve terminals are known to activate compensatory mechanisms such as increase in 5-HT synthesis, decrease in 5-HT release and reuptake (Stachowiak et al. 1986) and decrease in the density of [^{3}H]-citalopram or [^{3}H]-paroxetine binding sites in various brain areas (D'Souza et al. 2002; Romero et al. 1998). In the present study, we

found decreases in the density of presynaptic [^{3}H]-citalopram binding sites in various mice brain areas following 5,7-DHT icv injection.

Following 5,7-DHT intra-raphé injections in rats, a severe 5-HT depletion in brain tissue (over 95%) was associated with a large increase in 5-HT_{1B} (hetero ?) receptor binding in the substantia nigra (Compan et al. 1998). It is thus possible that a reduced number of presynaptic terminal 5-HT_{1B} autoreceptors (as well as somatodendritic 5-HT_{1A} autoreceptors) which restrain 5-HT release and an increase sensitivity of postsynaptic 5-HT_{1B} heteroreceptors may influence the potentiation of the AD-like effects of anpirtoline following 5,7-DHT infusion. Based on the location of 5-HT_{1B} heteroreceptors (i.e. on dopaminergic, GABAergic, glutamatergic and cholinergic neurons), further studies are necessary to conclude on the nature of the main monoaminergic system involved in the mediation of antidepressant-like effect of 5-HT_{1B} receptor agonist.

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Figure 1:

Effects of anpirtoline on immobility time in the FST in Wild-Type (■) and 5-HT_{1B} Knockout (□) mice.

Asterisks indicate significant difference of treated group from the respective control group. Data were analysed by a two way ANOVA followed by a Newman-Keuls test: ***p< 0.001. $ indicate a significant effect of genotype between groups receiving the same treatments; $$$p<0.001

Figure 2:

Effects of 5,7-DHT treatment on monoamine levels in the cortex (2A) and hippocampus (2B) of 129/Sv mice and in the serotonin turnover (2C) in these two brain areas. $ indicate statistically significant differences between sham-operated (■) and lesionned (□) mice. Data were analyzed by a one-way ANOVA followed by a Newman-Keuls test: $$$p< 0.001.

Figure 3:

Effects of 5,7-DHT treatment on [^{3}H]-citalopram binding sites in the cortex, and hippocampus of 129/Sv mice. $ indicate statistically significant differences between sham-operated (■) and lesionned (□) mice. Data were analyzed by a one-way ANOVA followed by a Newman-Keuls test: $$$p< 0.001.

Figure 4:

Effects of anpirtoline on the immobility time in the FST in sham-operated and 5,7-DHT lesionned 129/Sv mice. Data are expressed as mean ± S.E.M (in seconds), and were analyzed by a two-way ANOVA (lesion x treatment) followed by a Newman-Keuls test. Asterisks indicate statistically significant differences between anpirtoline-treated mice and

its respective control group (saline); ***p<0.001. $ indicates statistically significant difference between sham-operated and lesionned mice for the same treatment dose ($$p<0.01; $$$p<0.001).

Figure 5:

Effects of anpirtoline on locomotor activity in sham operated (■) and 5,7-DHT treated-mice (□). Data are expressed as mean of locomotor activity (±SEM) and were analyzed by a two-way ANOVA (lesion x treatment) followed by a Newman-Keuls test. Asterisks indicate statistically significant differences between anpirtoline-treated mice and its respective control group (saline); *p<0.05. $ indicates statistically significant difference between sham-operated and lesionned mice for the same treatment dose ($p<0.05).

Figure 1

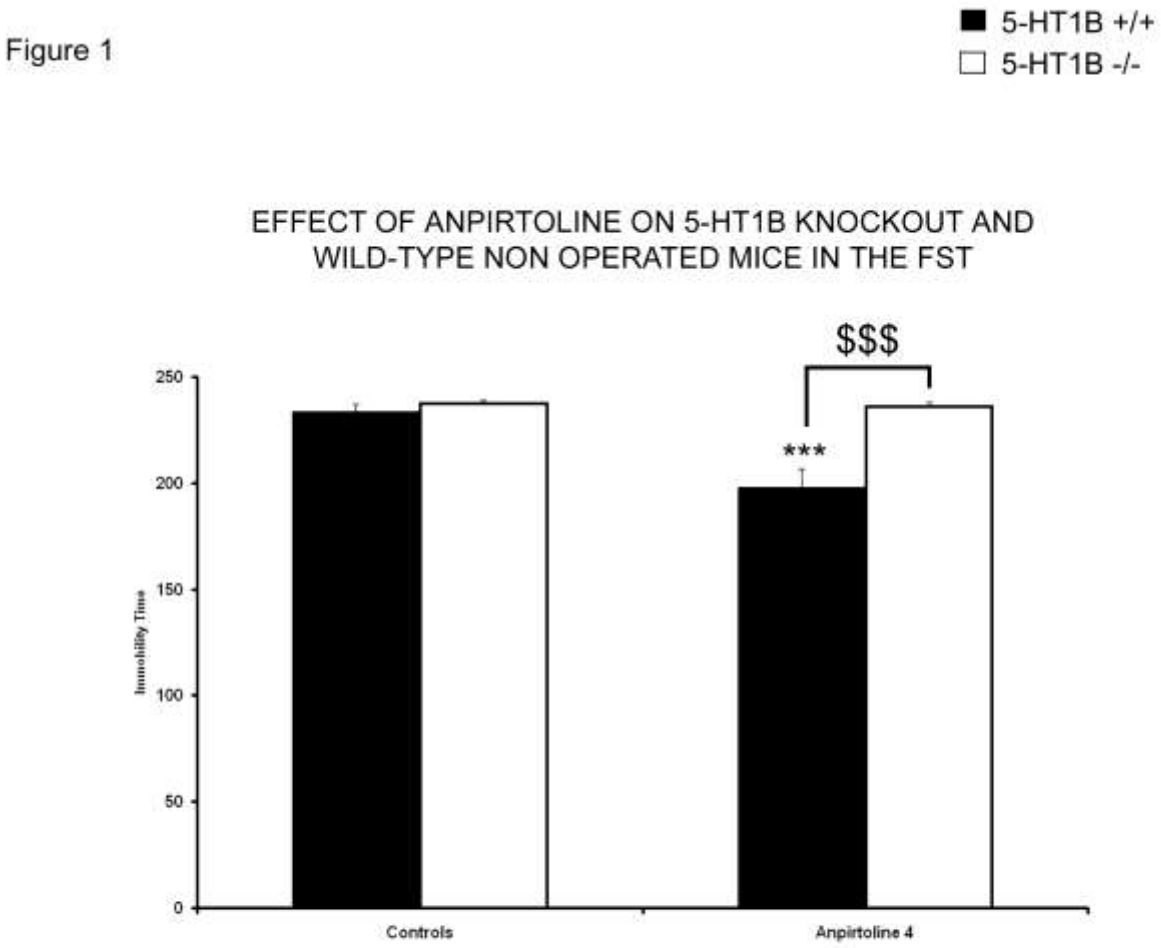

5-HT1B +/+
5-HT1B -/-
EFFECT OF ANPIRTOLINE ON 5-HT1B KNOCKOUT AND WILD-TYPE NON OPERATED MICE IN THE FST
$$$
***
Immobility Time
250
200
150
100
50
0
Controls
Anpirtoline 4

FIGURE 2a EFFECT OF 5,7-DHT TREATMENT ON MONOAMINE LEVELS IN THE CORTEX

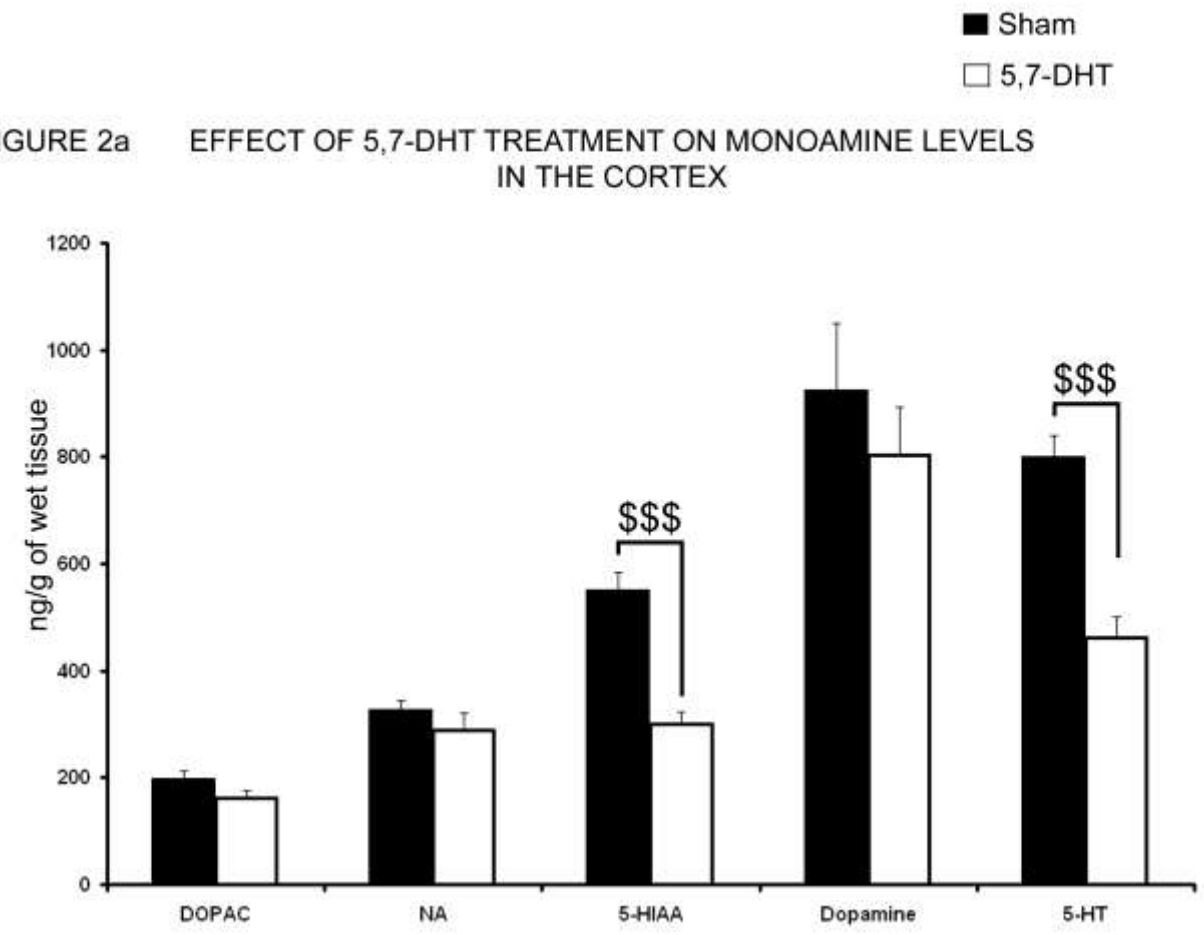


FIGURE 2b EFFECT OF 5,7-DHT TREATMENT ON MONOAMINE LEVELS IN THE HIPPOCAMPUS

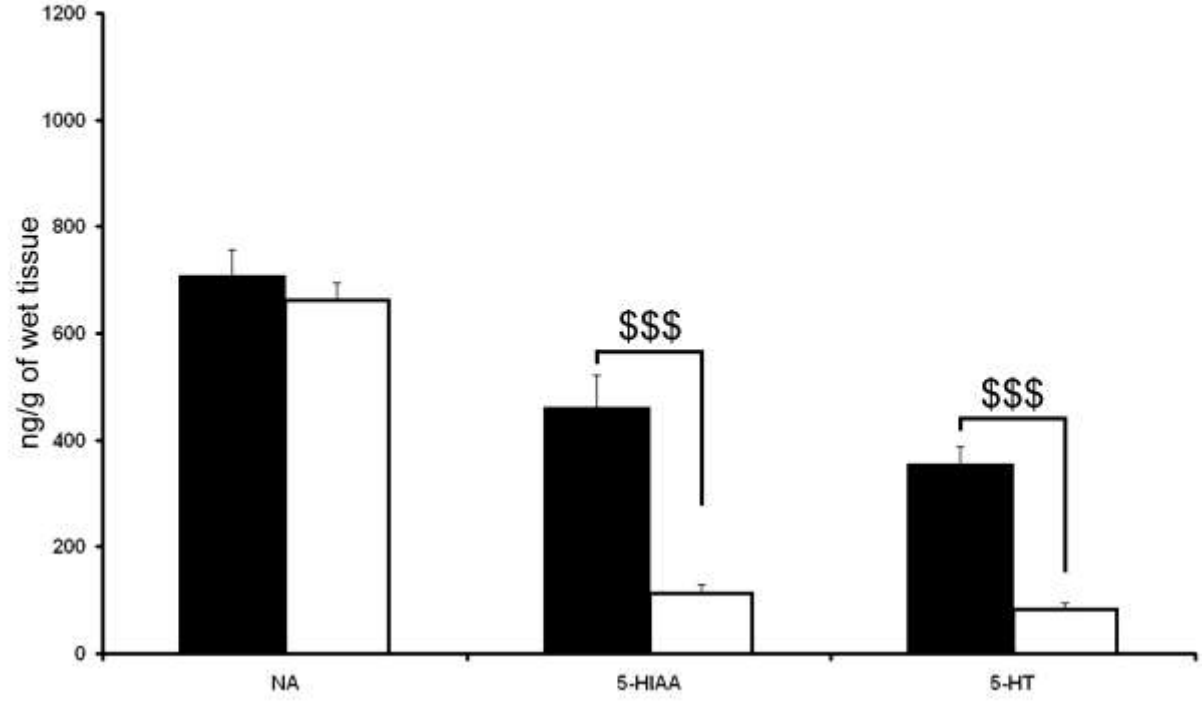


FIGURE 2c EFFECT OF 5,7-DHT TREATMENT ON SEROTONIN TURNOVER IN MICE CORTEX AND HIPPOCAMPUS

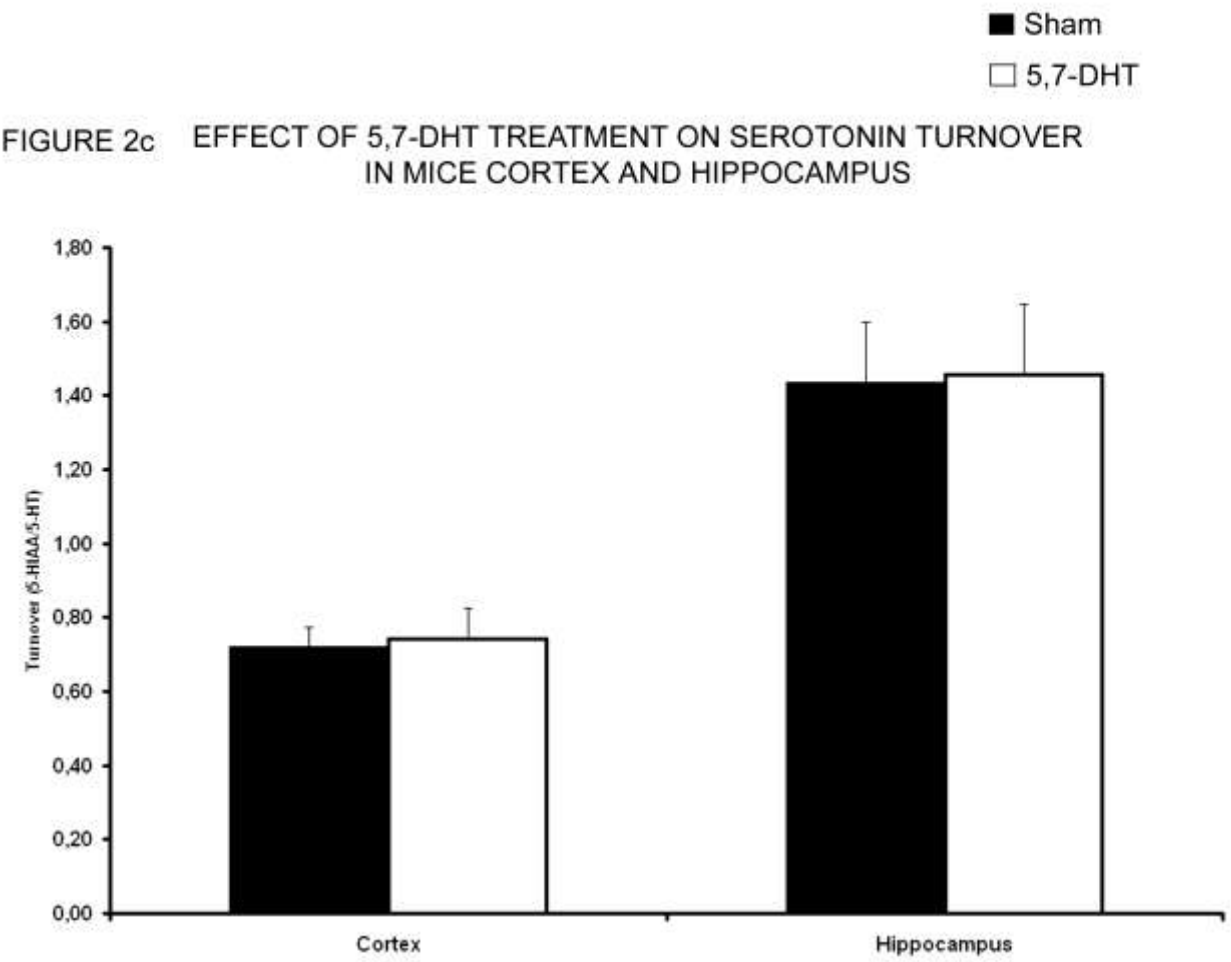
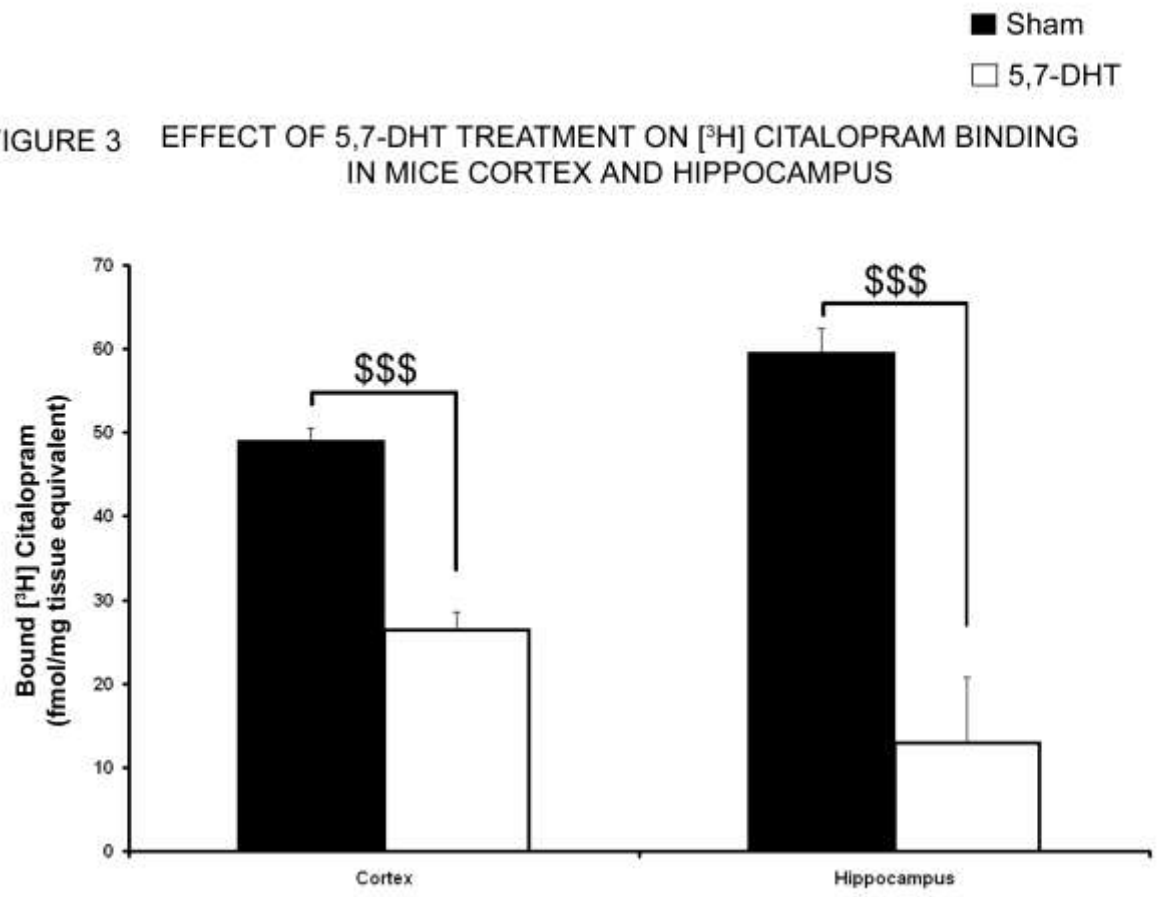


FIGURE 3 EFFECT OF 5,7-DHT TREATMENT ON [^{3}H] CITALOPRAM BINDING IN MICE CORTEX AND HIPPOCAMPUS



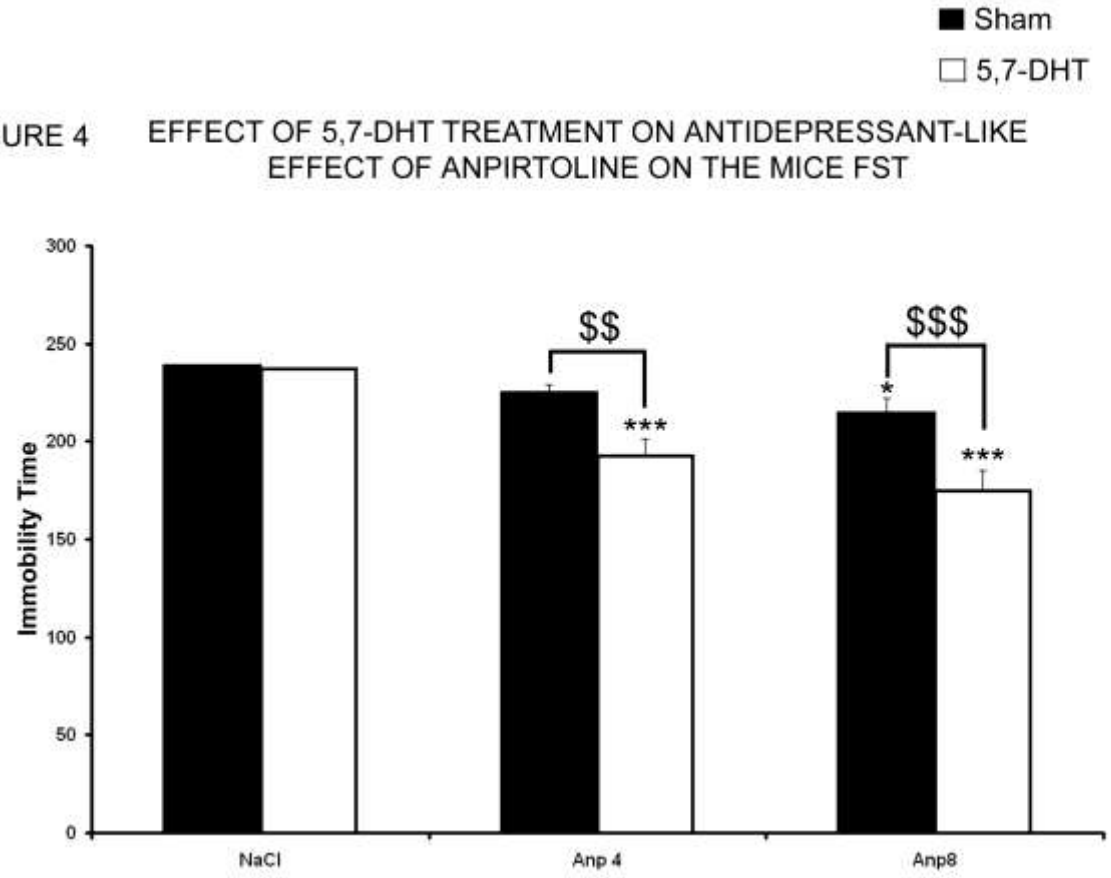


FIGURE 4 EFFECT OF 5,7-DHT TREATMENT ON ANTIDEPRESSANT-LIKE EFFECT OF ANPIRTOLINE ON THE MICE FST

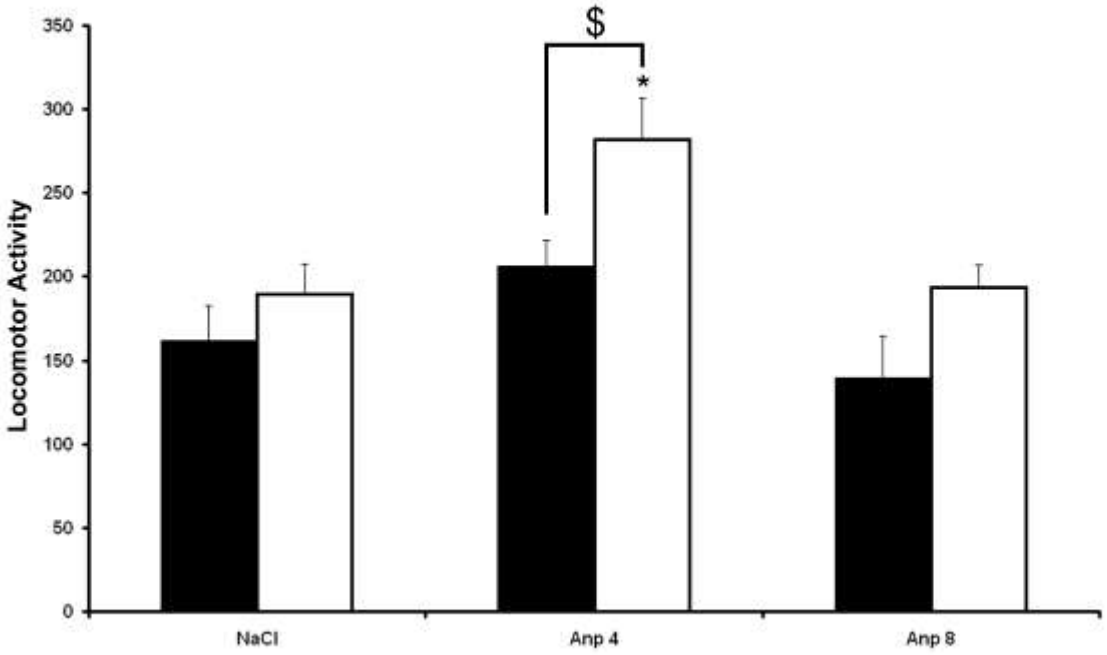


FIGURE 5 EFFECT OF ANPIRTOLINE ON THE LOCOMOTOR ACTIVITY OF SHAM OPERATED AND 5,7-DHT TREATED MICE

Résumé de l'étude n°4 :

Dans cette étude l'effet de type antidépresseur de l'anpirtoline a été étudié dans le FST chez des souris 129/Sv -contrôles,
-lésées par la 5,7-DHT
-mutées privées du récepteur 5-HT_{1B}

Nos résultats montrent un effet de type antidépresseur chez les souris présentant un récepteur 5-HT_{1B} intact (5-HT_{1B} +/+) qui n'est pas retrouvé chez les animaux mutés génétiquement (5-HT_{1B} -/-). Ceci suggère que les effets comportementaux observés dans le FST suite à l'administration d'anpirtoline sont liés à l'activation des récepteurs 5-HT_{1B} et pas à la fixation du produit sur d'autres types de récepteurs.
Dans la seconde partie de l'étude, nous avons réalisé une lésion du système sérotoninergique à l'aide d'une neurotoxine la 5,7-DHT. Le niveau de déplétion a été évalué à l'aide de deux méthodes : le dosage tissulaire et l'autoradiographie. En effet, le dosage tissulaire nous permet de vérifier la déplétion en monoamines (et donc la sélectivité d'action de la neurotoxine), alors que l'autoradiographie à l'aide de citalopram tritié nous permet d'évaluer la destruction des neurones sérotoninergiques.
Les résultats de déplétion obtenus par la méthode de dosage tissulaire (concentration tissulaire de 5-HT : hippocampe : -77 % ; cortex : -42 %) et par l'autoradiographie (fixation de citalopram tritié : hippocampe : -78 % ; cortex : -45 %) étant similaires, pour la suite de nos études la seule technique utilisée sera le dosage tissulaire afin de pouvoir également vérifier la sélectivité d'action de la neurotoxine.
Dans cette étude, nous avons également mis en évidence le fait que les effets comportementaux de l'anpirtoline persistent chez les animaux lésés, suggérant ainsi que les effets de type antidépresseurs des agonistes des récepteurs 5-HT_{1B} dans le FST sont liés à l'activation des hétérorécepteurs.
Compte tenu de la localisation de ces hétérorécepteurs, les effets de l'anpirtoline peuvent donc être lié à une activité de ce ligand sur les systèmes GABAergiques, Glutamatergiques, Dopaminergiques ou Cholinergiques.

Etude n°5 :

Mise en évidence du rôle du récepteur 5-HT_{1B} dans le FST chez la souris par l'administration locale d'un agoniste de ce récepteur (l'anpirtoline) et par la coadministration systémique d'un antagoniste de ce récepteur avec des antidépresseurs.

Evidence for a role of 5-HT_{1B} receptor in the mice FST through local perfusion of the 5-HT_{1B} receptor agonist anpirtoline and the systemic coadministration of 5-HT_{1B} receptor antagonist with antidepressants

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Soumis dans International Journal of Neuropsychopharmacology

Objectif de l'étude n°5 :

L'étude précédente nous ayant permis de montrer que l'effet antidépresseur de l'anpirtoline obtenu dans le FST est lié à l'activation des récepteurs 5-HT_{1B} ; dans cette étude nous avons dans un premier temps cherché à identifier les aires cérébrales impliquées dans l'apparition des effets comportementaux de l'anpirtoline. Nous avons donc réalisé une injection locale d'anpirtoline chez des animaux vigiles dans des tissus cérébraux contenant une forte densité de récepteur 5-HT_{1B}. Compte tenu du fait que parmi tous les neurotransmetteurs dont la libération est modifiée par les ligands des récepteurs 5-HT_{1B} la dopamine et la sérotonine sont fortement impliquées dans les troubles de l'humeur, les aires cérébrales choisies sont celles contenant une forte densité de ces monoamines (caudate putamen et substance noire pour la dopamine puis hippocampe et cortex pour la sérotonine).

Dans la seconde partie de l'étude, nous avons voulu évaluer les effets d'un antagoniste des récepteurs 5-HT_{1B} (GR127935) sur l'effet de type antidépresseur des IRSSs dans le FST. En effet, lors de précédentes études, nous avions émis l'hypothèse que l'activation des récepteurs 5-HT_{1B} permet l'apparition des effets de type antidépresseur des IRSSs. Par conséquent, le blocage de ce récepteur devrait antagoniser leurs effets. Des antidépresseurs appartenant à différentes classes thérapeutiques ont été testés afin de voir s'il existe des profils d'actions différents entre ces substances.

Evidence for a role of 5-HT_{1B} receptor in the mice FST through local perfusion of the 5-HT_{1B} receptor agonist anpirtoline and the systemic coadministration of 5-HT_{1B} receptor antagonist with antidepressants

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ABSTRACT

In the present study, we evaluated the antidepressant-like activity of anpirtoline (5-HT_{1B} receptors agonist) when locally perfused in brain areas containing 5-HT_{1B} receptors. We also examined effects of coadministration of the selective serotonin (5-HT) reuptake inhibitors (SSRIs) paroxetine and citalopram, noradrenalin reuptake inhibitor (NRI) desipramine and tricyclic antidepressant (TCA) imipramine in combination with a 5-HT_{1B} receptors antagonist (GR127935) in the mice forced swimming test (FST). When infuse in Caudate Putamen (CPu) and Substantia Nigra (SN), anpirtoline induce an antidepressant-like effect whereas it was devoid of effect in both prefrontal cortex and ventral hippocampus. When given alone (systemic infusion) all antidepressant decrease the immobility time in a dose dependant manner, whereas the 5-HT_{1B} antagonist, GR127935, was devoid of effect. On the other hand, administration of GR127935 in combination with both SSRIs antagonized the anti-immobility effect of drugs given alone but did not modify the duration of immobility time obtained with desipramine and imipramine. Taken together, these results suggest that activation of 5-HT_{1B} receptors located in CPu and SN could be involved in the AD-like effects of SSRIs but not those of NRIs and TCAs in the mice FST.

Key words: 5-HT_{1B} receptor, antidepressant, anpirtoline, GR 127935, mice FST

I- INTRODUCTION

We recently demonstrated that dopamine (DA) depletion is associated with a loss of antidepressant-like activity of Selective Serotonin (5-HT) Reuptake Inhibitors (SSRIs, citalopram and paroxetine) in the mice FST. By contrast, behavioral activity of both Noradrenalin Reuptake Inhibitor (NRI, desipramine) and tricyclic antidepressant (TCA, imipramine) was not altered by the loss of DA neurons (Chenu et al. 2006). It is thus of interest to assess whether antidepressant-like effect of SSRIs in the mice FST requires an activation of DA pathways to occur. Numerous microdialysis studies have demonstrated an increase in DA release following systemic or local administration of SSRIs in various brain areas such as substantia nigra (SN) (Thorre et al. 1998) and striatum (Benloucif and Galloway 1991). So far, although the control of DA system activity by 5-HT has been proposed from various studies in different brain areas (Parsons and Justice 1993, De Deurwaerdere et al. 1996, Hallbus et al. 1997), the 5-HT receptors types involved are not clearly identified. Among all 5-HT receptors indirectly activated by SSRIs (further to increase in $[5\text{-HT}]_{EC}$), 5-HT_{1A} and 5-HT_{1B} subtypes are strongly involved in DA release process. Indeed, as serotonin infusion, the local infusion of 5-HT_{1B} receptor agonist also induces an increase in dopamine release in rat nucleus accumbens (Yan and Yan 2001), VTA (Yan et al. 2004), SN (Thorre et al. 1998) striatum and frontal cortex (Iyer and Bradberry 1996). Moreover, autoradiographic and *in-situ* hybridization studies have largely demonstrated a high rate of 5-HT_{1B} receptors in brain areas of interest for DA neurotransmission such as substantia nigra, nucleus accumbens and caudate putamen (Bruinvels et al. 1993, Boschert et al. 1994, Bruinvels et al. 1994, Bonaventure et al. 1998). Many studies have shown that systemic administration of 5-HT_{1B} receptor agonists such as anpirtoline and RU 24969 exert an AD-like effect in mice FST (Redrobe and Bourin 1999, O'Neill and Conway 2001, Tatarczynska et al. 2005) or can be used to

potentiate the effect of antidepressants in this test (Redrobe et al. 1996, David et al. 2001) even if these pharmacological treatments induce a decrease in $[5\text{-HT}]_{EC}$ outflow in various brain area (Roberts et al. 2000, De Groote et al. 2003) and also a decrease in 5-HT synthesis when acutely administered (Watanabe et al. 2006). Taken together these results suggest that an AD-like effect should be obtained even if $[5\text{-HT}]_{EC}$ levels were decreased whereas usually antidepressant drugs are effective by increasing serotonergic neurotransmission (Preskorn 1994, Wong et al. 1995).

On the other hand, it is now well established that the lack, or blockade, of 5-HT_{1A} and/or 5-HT_{1B} receptors potentiates the increase in 5-HT extracellular level (evaluated by microdialysis intracerebral in-vivo) induced by a single i.p. administration of selective serotonin reuptake inhibitors (SSRI), whereas 5-HT_{1A} receptors antagonist are devoid of effects when administered alone. Therefore it suggests that $5\text{-HT}_{1A/B}$ receptors antagonists are devoid of neurochemical effect on basal conditions in dorsal raphe nucleus (Adell et al. 2001), hippocampus (Gardier et al. 2001) and cortex (Adell et al. 2001, Gardier et al. 2001). It was then postulated that 5-HT_{1B} receptors antagonist effects only appears when 5-HT neurotransmission is enhanced (Hjorth 1993).

Thus, the present study was first designed to investigate the antidepressant-like effect resulting from local infusion of 5-HT_{1B} receptor agonist, anpirtoline, in brain areas containing 5-HT_{1B} receptors (hippocampus, median prefrontal cortex, substantia nigra and caudate putamen) using the mice forced swimming test (Porsolt et al. 1977). In the second part of the study we tested for the impact of pharmacological blockade of 5-HT_{1B} receptors (using a 5-HT_{1B} receptor antagonist: GR127935) in the antidepressant-like effect of various antidepressant drugs evaluated in the mice FST. Antidepressant chosen included the selective serotonin reuptake inhibitors (SSRIs) paroxetine and citalopram; the noradrenalin reuptake inhibitor, desipramine and the tricyclic antidepressant, imipramine.

II- MATERIELS AND METHODS

Animals

Male Swiss mice (Centre d'élevage Janvier, Le Genest, France) 4 weeks old and weighing 18–20 g at the treatment day were housed in groups of 18 per cage (40 cm × 28 cm × 17 cm), in the standard conditions of the animal room (20 ± 1 °C, standard light/dark cycle light on at 7:00 h, off at 19:00 h) with free access to food and water for a period of 1 week before use. Each experimental group consisted of naïve randomly grouped mice of the same weight, which were used only once. All experiments were performed between 7h00 and 12h00 within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law 87 848)

Drugs

Range doses (4-16 mg/kg) of paroxetine, (GSK, France), citalopram (4-16 mg/kg) (Lundbeck laboratory, Copenhagen, Denmark), desipramine (8-32 mg/kg) (Sigma, France) and imipramine (8-32 mg/kg) (RBI, USA) were dissolved in distilled water and administered intraperitoneally (i.p.) (25 mL/kg). GR 127935 (or vehicle) (4 mg/kg) was injected s.c. (25 mL/kg) 45 minutes before the test. Range doses of antidepressant were administered 15 minutes after GR 127935 (30 minutes before FST). Anpirtoline was dissolved in aCSF and administered by local infusion at a flow rate of 0.2µL/min.

Behavioural tests

Measurement of locomotor activity in mice (Boissier and Simon 1965)

The spontaneous locomotor activity was performed independently of the mouse FST to determine whether or not the change in the immobility time in the FST could be linked or not to a change in locomotor activity. Animals were kept in the test-room at least 1 hour before the test for habituation. After injection (vehicle or treatment), mice were replaced in their holding cages. The spontaneous activity of naive animals was recorded

using a photoelectric actimeter (OSYS, Laval France). This actimeter consists of a stainless steel apparatus containing transparent cages in which the animals' horizontal activity is measured by light beams connected to a photo-electric cell. The activity is recorded during a 10 minutes period.

1.1.1.1 Measurement of immobility time in the forced swimming test

The forced swimming test employed was similar to that described elsewhere (Porsolt et al. 1977). Mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm water, maintained at 23-25°C, and remained there for 6 min. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Six mice were tested simultaneously, and the time of immobility was recorded during the last 4-min of the 6-min testing period, thus after 2 min of habituation. The test was performed by the same well trained experimenters, blind to the treatment administered. Results are expressed as the immobility time during the 240-sec test period (mean ± S.E.M.). Only doses of antidepressant that do not significantly increase locomotor activity were used for this test.

Local Infusion of anpirtoline / Surgery

Mice were anaesthetized with chloral hydrate (400 mg/kg; i.p.) prior to surgery and placed in an ASI stereotaxic instrument (Bioseb, France) fitted with atraumatic earbars. A guide-cannulae (diameter: internal = 0.35 mm, external = 0.60 mm) (Unimed, Switzerland) was stereotaxically implanted in the brain area studied. Coordinates from bregam (in mm) were: AP: +1.0, L: ±2.0, V: -1.7 for the prefrontal cortex (PFC); AP: -1.7, L: ±1.0, V: -1.5 for the ventral hippocampus ; AP: +0.0, L: ±2.0, V: -3.0 for caudate putamen (CPu) and AP: -3.3, L: ±1.5, V: -4.5 for substantia nigra (SN). Guide-cannulae were secured with dental cement (GC Fuji Europe, Belgium). After 7 days of recovery, the injection cannulae

(diameter: internal = 0.15 mm, external = 0.30 mm) (Unimed, Switzerland) was lowered into the guide-cannulae in freely moving mice. Anpirtoline was locally perfused at a flow rate of 0.2µL/min during 2 minutes using a PHD 2000 infuse pump (Harvard Apparatus, France) and then the cannulae was removed and the animal was directly tested in the mice FST.

At the end of the experiment, mice were killed and the brain was quickly removed, frozen in isopentane at -30°C, stored at -80°C and the exact guide-cannulae location in brain regions was determined by using a digital photomicrograph of coronal sections of frozen brain (Bert et al. 2004). Data were omitted if guide-cannulae was not in the targeted brain site.

Data analysis and statistics

Statistical analyses were performed by the use of the computer software Sigmastat.

For combination studies, a two-way ANOVA (pre-treatment x treatment) was performed on either the immobility time (FST) or the number of beams broken (locomotor activity), followed by a Newman-Keuls test when appropriate.

For local infusion study, a one-way ANOVA (effect of Anpirtoline) was performed on the immobility time and followed by a Newman-Keuls test when appropriate.

Statistical significance was set at $p<0.05$.

III- RESULTS

III-1 Local perfusion of anpirtoline

Caudate Putamen (Figure 1a):

The one-way ANOVA performed revealed a significant effect of anpirtoline ($F_{2,27}$=10.35; p<0.001). The immobility time of mice was significantly reduce further to the infusion of the higher dose of the 5-HT_{1B} receptor agonist (20µg, p<0.001). Moreover, the antidepressant-like effect was significantly different between the two doses of anpirtoline perfused (p<0.01).

Substantia Nigra (Figure 1b):

The one-way ANOVA performed revealed a significant effect of anpirtoline ($F_{2,27}$=4.59; p<0.05). The immobility time of mice was significantly reduce further to the infusion of both doses of the 5-HT_{1B} receptor agonist (p<0.05). No difference was obtained between the two treated groups.

Frontal Cortex (Figure 1c):

The one-way ANOVA performed does not revealed any significant effect of anpirtoline ($F_{2,27}$=0.72; p=0.50), suggesting that infusion of anpirtoline in the prefrontal cortex does not change immobility time of mice in the FST

Hippocampus (Figure 1d):

The one-way ANOVA performed does not revealed a significant effect of anpirtoline ($F_{2,27}$=0.65; p=0.53). The perfusion of anpirtoline in the hippocampus does not induce AD-like effect in the mice FST.

III-2 Effect of GR 127935

When given alone, GR127935 was devoid of effect on the immobility time of mice.

Effect of combined administration of GR127935 and paroxetine in the mouse FST (Figure 2)

A range dose of paroxetine (4, 8 and 16 mg/kg) co-administered with GR 127935 (or vehicle) was tested in the forced swimming test. The two-way ANOVA analysis on the immobility time revealed a significant effect of pre-treatment (GR127935 or NaCl: $F_{1,72}$=5.21; p<0.05), treatment (Paroxetine or NaCl: $F_{3,72}$=8.84; p<0.001) and a significant interaction between these two factors ($F_{3,72}$=4.19; p<0.01). Paroxetine alone significantly reduced the immobility time for the two higher doses tested (p<0.05 and p<0.001 for 8 and 16 mg/kg, respectively), indicating an antidepressant-like effect. By contrast, the combination of GR127935 and paroxetine was devoid of antidepressant-like effect, but moreover GR 127935 significantly antagonized the behavioural effect of 16 mg/kg of paroxetine as compared to the corresponding group of mice receiving paroxetine alone 16 mg/kg (p<0.001).

Effect of combined administration of GR127935 and citalopram in the mouse FST (Figure 3)

A range dose of citalopram (4, 8 and 16 mg/kg) co-administered with GR 127935 (or vehicle) was tested in the forced swimming test. The two-way ANOVA analysis on the immobility time revealed a significant effect of pre-treatment (GR127935 or NaCl: $F_{1,72}$=9.56; p<0.01), treatment (citalopram or NaCl: $F_{3,72}$=3.91; p<0.05) and interaction ($F_{3,72}$=4.68; p<0.05). All doses of citalopram given alone significantly reduced the immobility time (p<0.05, p<0.01 and p<0.001 at 4, 8 and 16 mg/kg respectively). GR alone

or co-administered with citalopram was devoid of antidepressant-like effect. At 8 and 16 mg/kg of citalopram, GR 127935 significantly antagonize the AD-like effects of this drugs ($p<0.05$ and $p<0.001$ respectively).

Effect of combined administration of GR127935 and desipramine in the mouse FST (Figure 4)

A range dose of desipramine (8, 16 and 32 mg/kg) combined with GR 127935 (or vehicle) was tested in the forced swimming test. The two-way ANOVA analysis on the immobility time revealed a significant effect of treatment ($F_{3,72}=15.58$; $p<0.001$), but neither effect of pre-treatment ($F_{1,72}=0.86$; $p=0.36$) nor interaction ($F_{3,72}=0.39$; $p=0.76$). All doses of desipramine significantly reduced the immobility time ($p<0.05$, $p<0.01$ and $p<0.001$ at 8, 16 and 32 mg/kg respectively). No differences were obtained between groups that received desipramine alone or in combination with GR.

Effect of combined administration of GR127935 and imipramine in the mouse FST (Figure 5)

A range dose of imipramine (8, 16 and 32 mg/kg) co-administered with GR 127935 (or vehicle) was tested in the forced swimming test. The two-way ANOVA analysis on the immobility time revealed a significant effect of treatment ($F_{3,72}=23.878$; $p<0.001$) but neither effect of pre-treatment ($F_{1,72}=0.09$; $p=0.76$) nor interaction between the two factors ($F_{3,72}=0.60$; $p=0.62$). At 8, 16 and 32 mg/kg imipramine significantly reduced the immobility time ($p<0.01$ at 8 mg/kg and $p<0.001$ at higher doses). No differences were obtained between groups that received imipramine alone or in combination with GR.

Effect of coadministration of antidepressant and GR 127935 on spontaneous locomotor activity (Table 1)

Prior administration of GR 127935 (4 mg/kg) with citalopram, desipramine and imipramine did not modify statistically the locomotor activity of mice compared to antidepressant

alone. A decrease in the locomotor activity was obtained when GR 127935 was coadministered with 16 mg/kg of paroxetine ($p<0.05$). Antidepressants were used at doses that were devoid of psychostimulant activity.

IV- Discussion

The present work was carried out to evaluate the antidepressant-like activity of local infusion of 5-HT_{1B} receptor agonist anpirtoline in various brain areas containing a high rate of 5-HT_{1B} receptors. The second part of the study was carried out to evaluate the behavioural effects of conventional antidepressant drugs, SSRIs, NRIs and TCAs, when combined with the 5-HT_{1B} receptor antagonist GR127935. Our results provide evidence that activation of 5-HT_{1B} receptors located in both SN and CPu induce an antidepressant-like effect in the mice FST and that the association of GR127935 with both SSRIs (paroxetine and citalopram) significantly antagonized the antidepressant-like effect of those drugs in the mice FST, whereas no change were obtained when GR127935 was associated with imipramine or desipramine.

Previous studies have suggested that the antidepressant-like effect of selective serotonin reuptake inhibitors could be mediated through 5-HT_{1B} receptor activation (Redrobe et al. 1996), as specific activation of this receptor type by a selective agonist such as RU 24969, CP 94253 or anpirtoline (Redrobe et al. 1996, Redrobe and Bourin 1999, O'Neill and Conway 2001, Tatarczynska et al. 2005) led to an antidepressant-like effect in the mice FST after systemic infusion. Also the constitutive lack (Gardier et al. 2001) of this receptor antagonized the antidepressant-like effect of SSRIs. These results are surprising since microdialysis studies have indicated that the inactivation of 5-HT_{1B} autoreceptors strengthen the neurochemical activity of SSRIs (Cremers et al. 2000, Hervas et al. 2000, Gardier et al. 2001, Malagie et al. 2001, Gardier et al. 2003, Stenfors et al. 2004). Discrepencies between neurochemical and behavioural studies may account from the fact that 5-HT release and antidepressant-like activity would not be mediated by the same 5-HT_{1B} receptors. (autoreceptors vs heteroreceptors). This appears to be confirmed by the local infusion of anpirtoline; indeed, many studies have demonstrated that combined

administration of [SSRI+5-HT_{1B} receptors antagonist] induce an elevation of 5-HT release in both cortex and hippocampus (Gobert et al. 1997, Cremers et al. 2000, Hervas et al. 2000, Malagie et al. 2001, Stenfors et al. 2004) suggesting that 5-HT_{1B} receptors located in these two brain areas are of the autoreceptor subtype. This is consistent with our finding that demonstrates an absence of antidepressant-like effect when anpirtoline was injected in these brain areas. On the other hand, it was demonstrated that local infusion of 5-HT_{1B} agonist on both SN and striatum induce an increase in DA release (Benloucif and Galloway 1991, Thorre et al. 1998, Yan and Yan 2001) suggesting therefore that 5-HT_{1B} receptors in these brain areas are some heteroreceptors. Since we shown that local infusion of anpirtoline in CPu and SN induce an AD-like effect, it could then be confirmed that activation of 5-HT_{1B} heteroreceptors induce an antidepressant-like effect in the mice FST.

Since it was demonstrated that GR127935 is a selective 5-HT_{1B} receptor antagonist (De Vries et al. 1997) our results provide evidences that the anti-mobility effect of GR127935, when co-administrated with SSRIs, is only linked to its activity on 5-HT_{1B} receptor and could not be associated to an activity on 5-HT_{1A} receptors. Indeed, although 5-HT_{1A} receptors agonists (flesinoxan, 8-OHDPAT) inhibit the firing rate of DRN 5-HT neurons, they also produce a dose dependent increase in the firing rate of dopaminergic neurons in the both ventral tegmental area (VTA) and/or the frontal cortex (Arborelius et al. 1993b, Arborelius et al. 1993a, Lejeune and Millan 1998). However, in previous studies it was described that pharmacological (WAY 100635 and NAN 190), or constitutive, inactivation of 5-HT_{1A} receptor does not antagonize the antidepressant-like effect of SSRIs (paroxetine, citalopram and fluvoxamine) in the mice FST (Redrobe et al. 1996, Guilloux et al. 2006). Interestingly, it the study performed by Guilloux et al., the swimming duration was increased suggesting that the increase in the firing of dopamine neurons following 5-HT_{1A} receptors activation is not necessary for the mediation of antidepressant-like effect

of SSRIs in the mice FST. On the other hand, in this study, the mix 5-$HT_{1A/1B}$ receptors antagonist pindolol antagonized the antidepressant-like effect of paroxetine in 5-HT_{1A} receptor knockout mice thus underlying the putative impact of 5-HT_{1B} receptor activation in the mice FST. Considering that 5-HT_{1B} autoreceptor blockade would enhance the neurobiochemical effects of antidepressant (Gobert et al. 1997, Cremers et al. 2000, Malagie et al. 2001, Stenfors et al. 2004), we suggested that the opposite result obtained in our behavioural study could be linked to the activity of GR127935 on heteroreceptors.

Taken together our results suggest that antidepressant-like effect of SSRIs in the mice FST appears further to activation of 5-HT_{1B} receptors (and most likely 5-HT_{1B} heteroreceptors) which are, at least, located in both CPu and SN. Since we have demonstrated that both dopamine depletion (Chenu et al. 2006) and 5-HT_{1B} receptor blockade inhibit the antidepressant-like effect of SSRIs, it could be suggested that the activation of 5-HT_{1B} heteroreceptors by SSRIs induce an increase in dopaminergic neurotransmission. This increase in dopaminergic neurotransmission is probably responsible of the antidepressant-like effect of SSRIs in the mice FST.

It is also of interest to notice that both dopamine depletion and 5-HT_{1B} receptor blockade are devoid of effect on imipramine and desipramine. It suggests that those drugs do not involve the same final monoaminergic pathways for their antidepressant-like effect to occur.

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Statement of Interest: None

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Figure 1:

Effects of local perfusion of anpirtoline on the immobility time in the mice FST. Data were analysed by a one way ANOVA followed by a Student Newman Keuls test when necessary. Asterisks indicate significant difference between NaCl and anpirtoline treated group. *p<0.05; ***p< 0.001. § indicate a significant difference between groups treated with anpirtoline. §§p<0.01.

Figure 2:

Effects of paroxetine on immobility time in saline (■) and GR 127935 (□) pretreated mice in the FST. Data were analysed by a two way ANOVA followed by a Student Newman Keuls test when necessary. Asterisks indicate significant difference between NaCl x paroxetine treated group and control. § indicate a significant difference between saline and GR 127935 pretreated group. *p<0.05; ***p< 0.001, §§§p<0.001.

Figure 2:

Effects of citalopram on immobility time in saline (■) and GR 127935 (□) pretreated mice in the FST. Data were analysed by a two way ANOVA followed by a Student Newman Keuls test when necessary. Asterisks indicate significant difference between NaCl x citalopram treated group and control. § indicate a significant difference between saline and GR 127935 pretreated group. *p<0.05; **p<0.01, ***p< 0.001, §p<0.05 §§§p<0.001.

Figure 3:

Effects of desipramine on immobility time in saline (■) and GR 127935 (□) pretreated mice in the FST. Data were analysed by a two way ANOVA followed by a Student Newman Keuls test when necessary. Asterisks indicate significant difference between NaCl x desipramine and control and GR 127935 x paroxetine treated group and control. § indicate a significant difference between saline and GR 127935 pretreated group. *p<0.05; **p<0.01, ***p< 0.001, §p<0.05 §§§p<0.001.

Figure 4:

Effects of imipramine on immobility time in saline (■) and GR 127935 (□) pretreated mice in the FST. Data were analysed by a two way ANOVA followed by a Student Newman Keuls test when necessary. Asterisks indicate significant difference between NaCl x imipramine and control and GR 127935 x paroxetine treated group and control *$p<0.05$; **$p<0.01$, ***$p< 0.001$. No significant differences were obtained between the NaCl and GR 127935 pretreated groups.

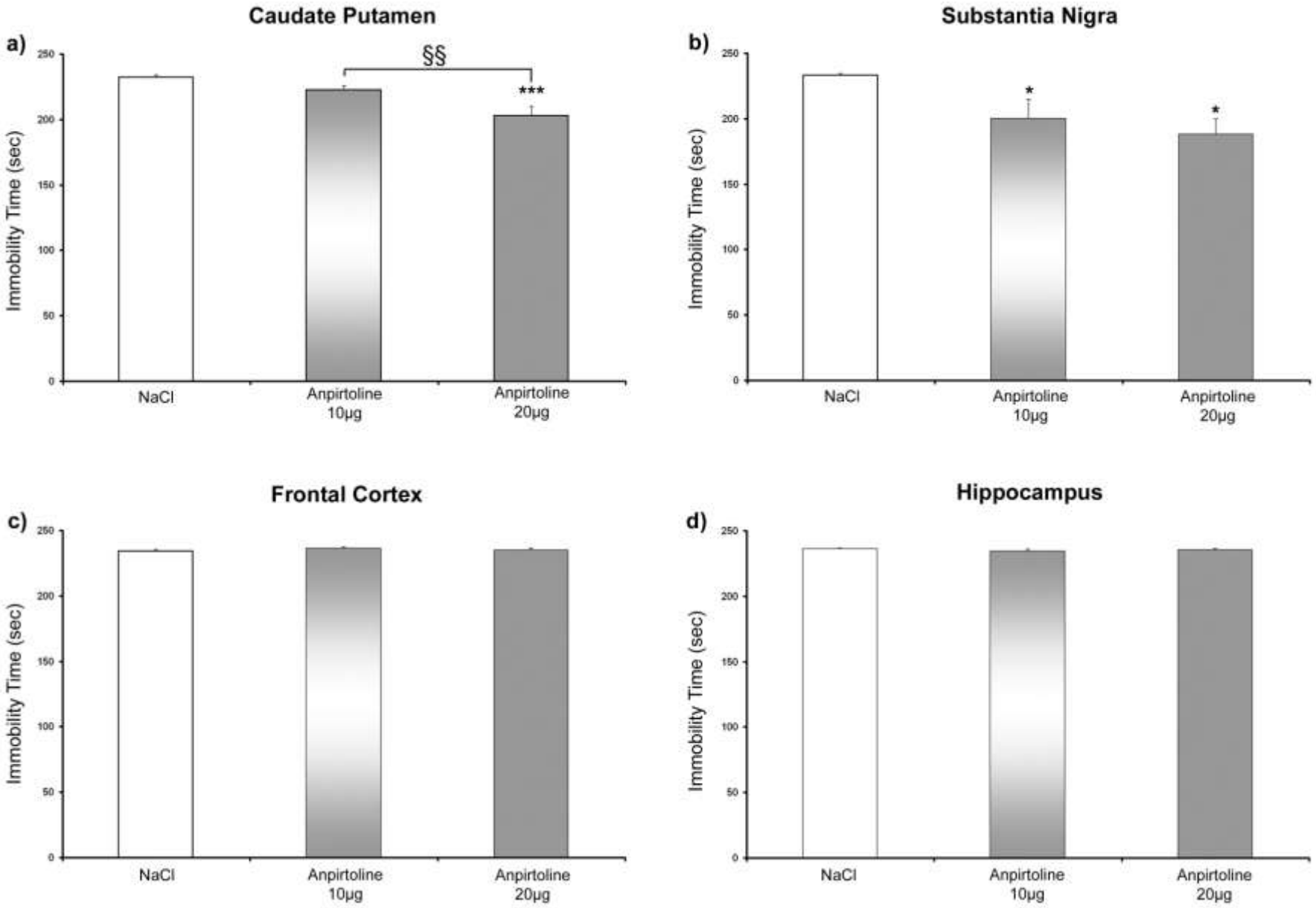
Table 1

Effect of antidepressants +/- GR127935 on the spontaneous locomotor activity of mice. Data are expressed as mean of locomotor activity (±SEM). All the statistical analyses were calculated by *Fisher t-test* following significant one-way ANOVA (pre-treatment). *Asterisks* show significant difference between GR and NaCl pre-treated animals ($p<0.05$).

Table 1:

	Dose	AD alone	AD + GR127935
Citalopram	4 mg/kg	223.3 +/- 12.3	225.1 +/- 13.5
	8 mg/kg	252.9 +/- 14.9	237.9 +/- 12.7
	16 mg/kg	309.8 +/- 28.2	254.7 +/- 18.7
Paroxetine	4 mg/kg	225.6 +/- 18.5	230.4 +/- 9.5
	8 mg/kg	262.3 +/- 20.2	242.2 +/- 19.0
	16 mg/kg	294.0 +/- 15.3	237.9 +/- 15.6 *
Desipramine	8 mg/kg	115.7 +/- 12.5	107.0 +/- 10.2
	16 mg/kg	98.7 +/- 10.9	107.3 +/- 10.8
	32 mg/kg	81.4 +/- 12.3	96.4 +/- 8.7
Imipramine	8 mg/kg	143.1 +/- 14.9	143.7 +/- 11.3
	16 mg/kg	129.2 +/- 15.6	124.8 +/- 8.9
	32 mg/kg	136.8 +/- 9.0	128.6 +/- 12.8

Figure 1


Caudate Putamen
a)
§§
***
Immobility Time (sec)
0
50
100
150
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250
NaCl
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Anpirtoline 20µg
Substantia Nigra
b)
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NaCl
Anpirtoline 10µg
Anpirtoline 20µg
Frontal Cortex
c)
Immobility Time (sec)
NaCl
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Hippocampus
d)
Immobility Time (sec)
NaCl
Anpirtoline 10µg
Anpirtoline 20µg

Figure 2

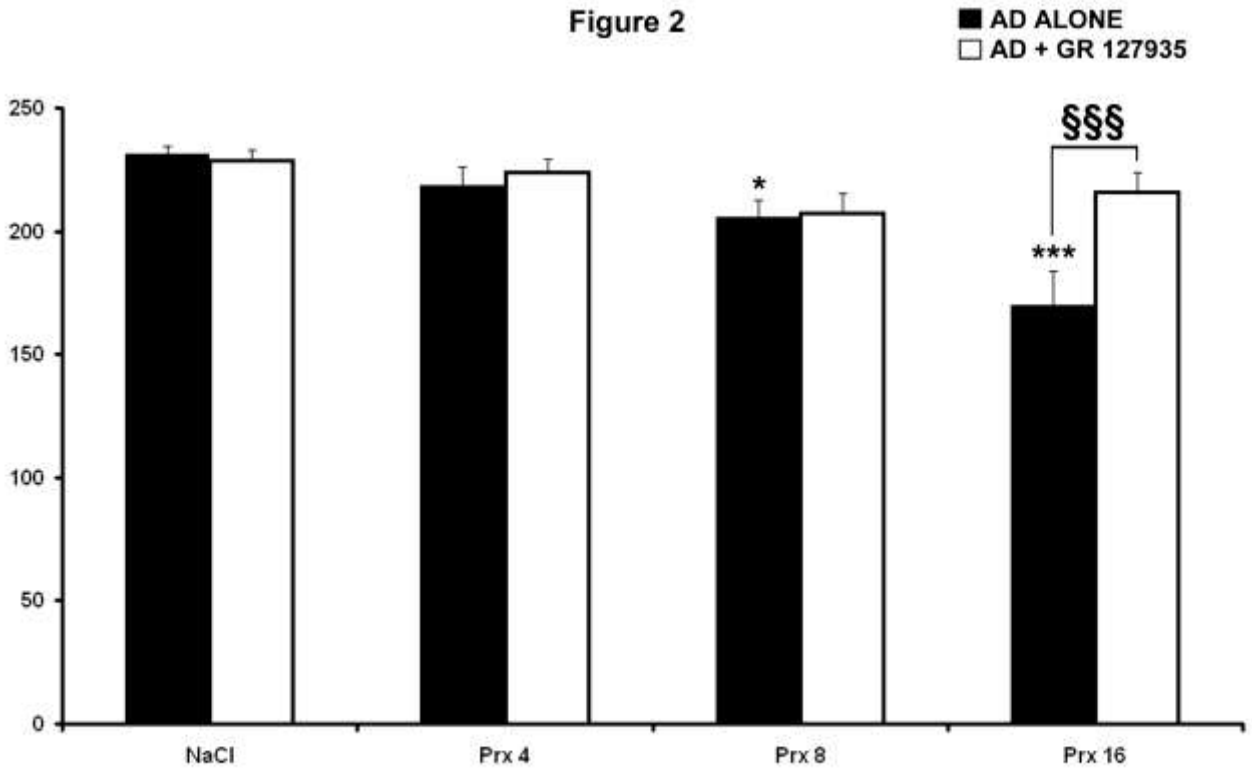


Figure 3

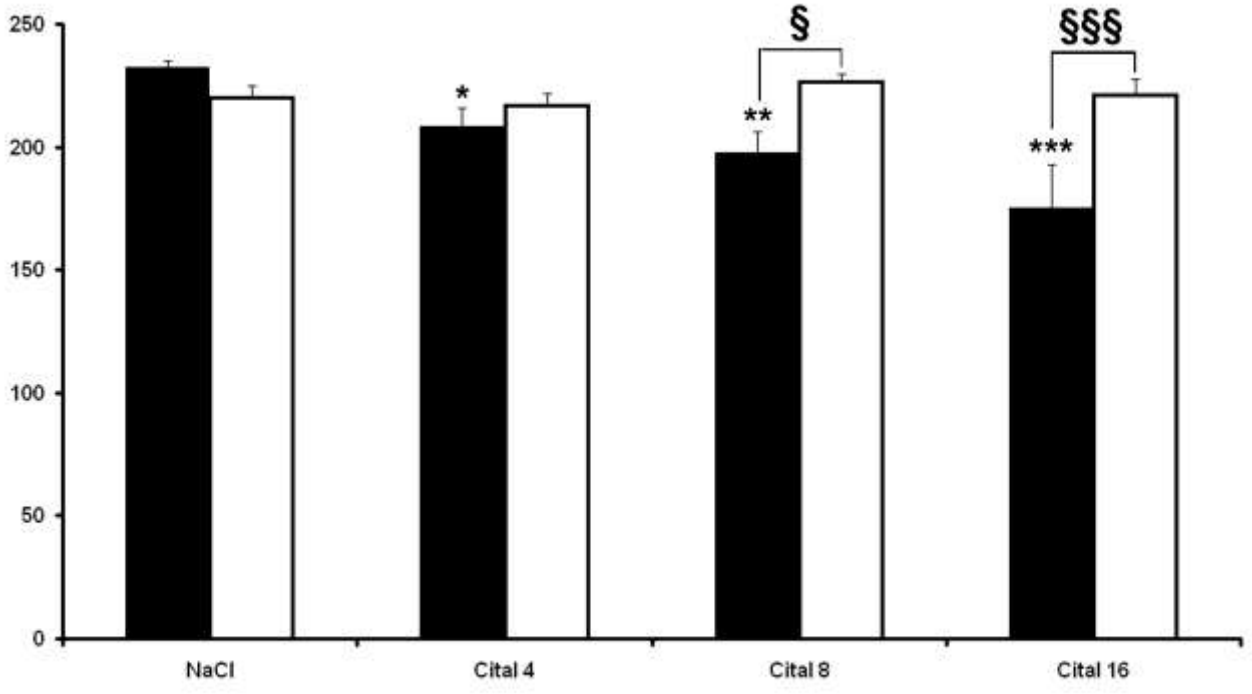


Figure 4

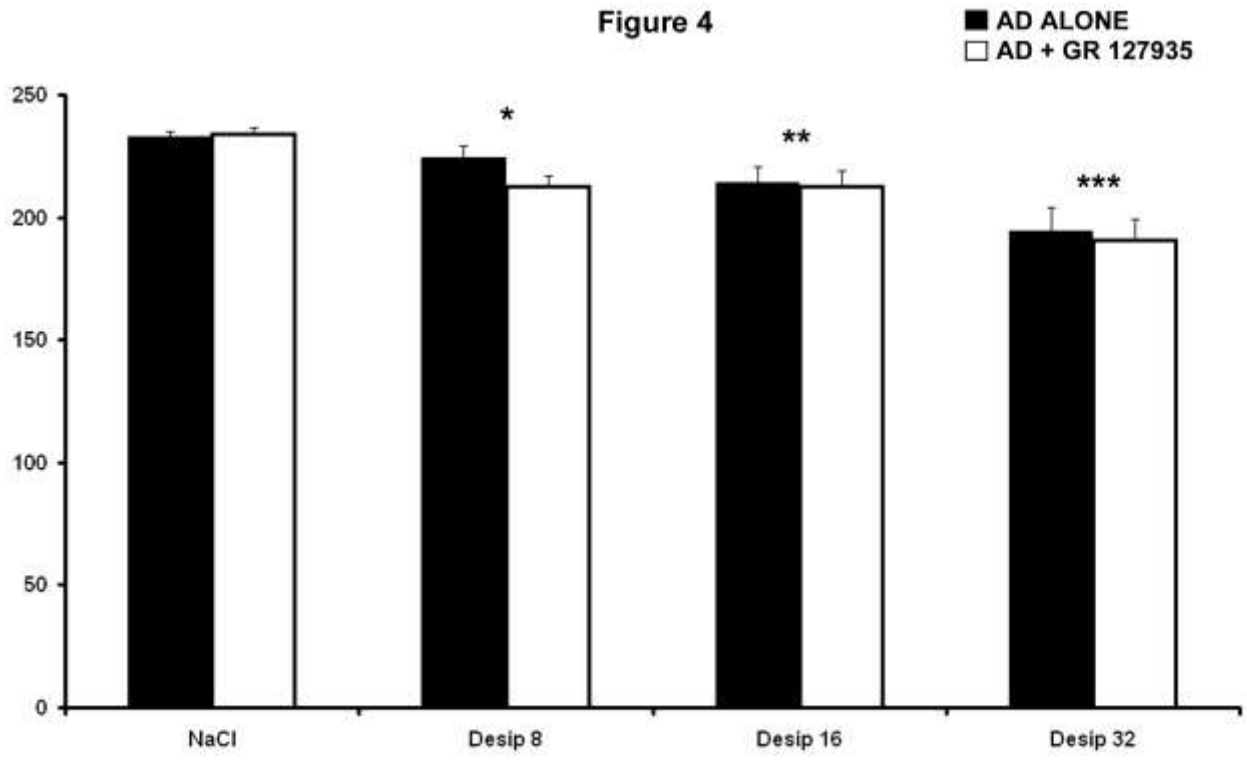
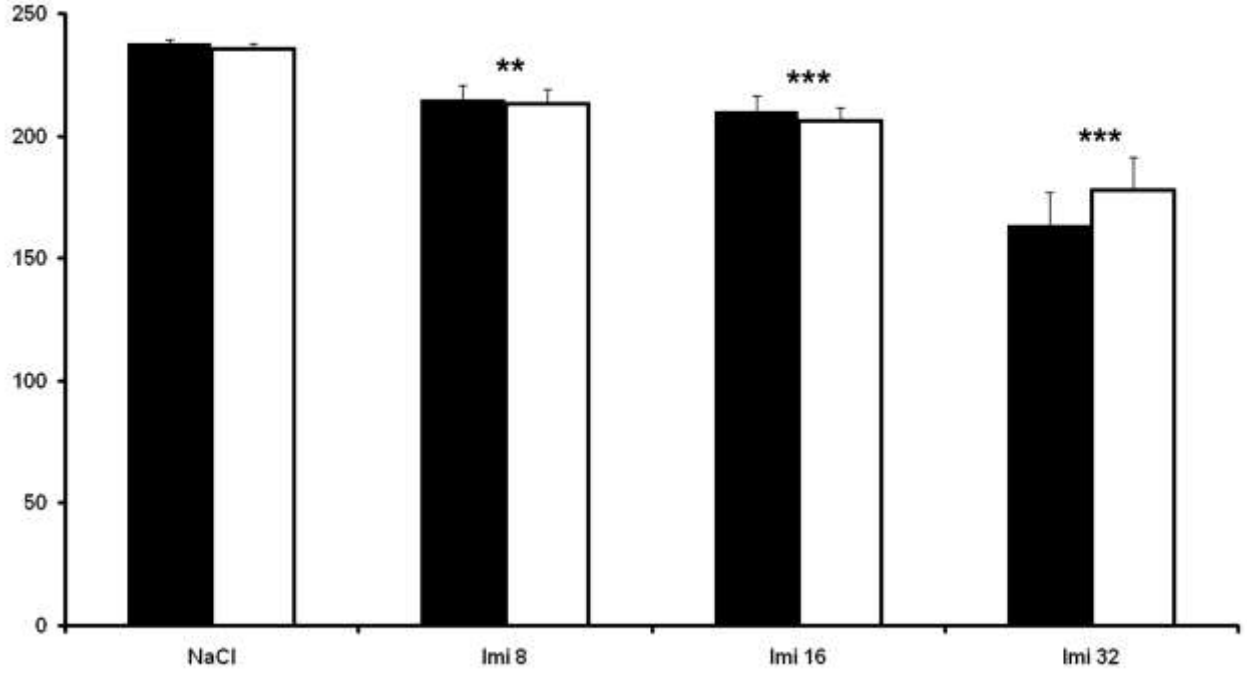


Figure 5



Résumé de l'étude n°5 :

La première partie de cette étude nous a permis de confirmer les résultats préalablement obtenus ; en effet, l'administration d'anpirtoline dans le cortex et l'hippocampe ne permet pas d'obtenir d'effet de type antidépresseur chez la souris. Les récepteurs situés dans ces deux structures étant principalement des autorécepteurs, ces résultats sont compatibles avec ceux de l'étude n°4. A l'inverse, l'administration d'anpirtoline dans le caudate putamen et la substance noire entraîne chez l'animal l'apparition d'un effet de type antidépresseur ; confirmant notre hypothèse selon laquelle l'activation des hétérorécepteurs est responsable de l'apparition des effets comportementaux de l'anpirtoline dans le FST chez la souris.

La seconde partie de l'étude a mis en évidence le fait que les effets comportementaux des IRSSs (paroxétine et citalopram) dans le FST mais pas ceux de l'imipramine ni de la désipramine sont transmis par l'activation des récepteurs 5-HT_{1B}. Compte tenu du profil pharmacologique de ces différents produits, il semble probable que les effets des IRSSs passent par l'activation indirecte des hétérorécepteurs 5-HT_{1B} (consécutivement à l'augmentation des concentrations de sérotonine extracellulaire), alors que les effets de l'imipramine et de la désipramine sont probablement plus liés à un effet noradrénergique.

Etude n°6 :

Effet de la lésion du système dopaminergique sur l'activité de différents antidépresseurs dans le test de la nage forcée chez la souris.

Effect of antidepressant drugs on 6-OHDA treated mice in the FST

Franck CHENU, Eric DAILLY, Michel BOURIN

Publiés dans European Neuropsychopharmacology

Objectif de l'étude n°6

Les résultats de l'étude n°5, ont mis en évidence que le blocage des récepteurs 5-HT_{1B} par un antagoniste spécifique, le GR127935, entraîne la disparition de l'effet antidépresseur des IRSSs dans le test de la nage forcée chez la souris. A l'inverse, dans le même test, l'activation des hétérorécepteurs 5-HT_{1B} par l'anpirtoline, permet l'apparition d'un effet de type antidépresseur (étude n°4). De plus, nous avons également montré que l'injection locale d'anpirtoline dans des aires cérébrales riches en dopamine (substance noire et caudate putamen) permet d'entraîner un effet de type antidépresseur chez l'animal. Il semble donc fortement probable que lors de l'administration d'IRSSs, l'élévation de la concentration extracellulaire de sérotonine permette l'activation indirecte des récepteurs 5-HT_{1B} situés sur les neurones GABAergiques dans le caudate putamen et la substance noire, entrainant ainsi une diminution de la libération de GABA et une désinhibition du relargage de dopamine (voir introduction) qui puisse être responsable de l'apparition des effets comportementaux des IRSSs dans le FST.

Le but de l'étude n°6 est d'évaluer les effets résultants d'une administration systémique d'antidépresseurs chez des animaux dont le système dopaminergique a été préalablement lésé à l'aide d'une neurotoxine sélective, la 6-hydroxydopamine (6-OHDA).

L'étude n°5 ayant permis de mettre en évidence des profils d'action différents entre les différents antidépresseurs testés (IRSS, tricyclique et IRN), nous avons donc décidé d'utiliser les mêmes antidépresseurs lors de l'étude n°6.

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Effect of antidepressant drugs on 6-OHDA-treated mice in the FST

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6-OHDA;
FST;
Depletion

Abstract There is growing evidence suggesting that dopamine could be indirectly involved in the appearance of behavioural effects of antidepressants. In this study, we induced a partial (over 70%) and non-reversible depletion of dopamine-containing neurons in mice by i.c.v. infusion of 6-OHDA. Then, we compared the antidepressant-like effect of drugs (citalopram, paroxetine, desipramine and imipramine) with or without dopamine depletion in the mice forced swimming test. Our results clearly show that lesion with 6-OHDA does not modify the response of mice to desipramine and imipramine, whereas dopamine depletion abolished the antidepressant-like effect of citalopram and paroxetine. It could then be suggested that antidepressant-like effect of selective serotonin reuptake inhibitors (paroxetine and citalopram) in the mice FST requires the activation of dopaminergic pathways to occur.

1. Introduction

It is now well established that a local infusion of a selective serotonin reuptake inhibitors (SSRI) such as citalopram or fluoxetine induces an increase in dopamine release in the rat striatum (Benloucif and Galloway, 1991) and the rat substantia nigra (Thorre et al., 1998). This increase in dopamine release can also been obtained further to local 5-HT perfusion in rat striatum (De Deurwaerdere et al., 1996), nucleus accumbens (Hallbus et al., 1997), frontal cortex (Iyer and Bradberry, 1996) and substantia nigra (Thorre et al., 1998). However, to the best of our knowledge it is not clearly demonstrated whether this increase in dopamine levels could mediate the antidepressant effect of drugs. However, it has been shown that dopaminergic ligands (agonist or antagonist) could modify the antidepressant-like effect of SSRIs evaluated in the mice forced swimming test (Renard et al., 2001). On the other hand it has also been largely demonstrated that 5-HT_{1B} receptor agonists as well as decreasing 5-HT extracellular levels in mice hippocampus (De Groote et al., 2002), mice striatum (De Groote et al., 2003b) and rat cortex (De Groote et al., 2003a) induce an increase in DA release in rat nucleus accumbens (Yan and Yan, 2001), ventral tegmental area (Yan et al., 2004), substantia nigra (Thorre et al., 1998) striatum and frontal cortex (Iyer and Bradberry, 1996). This activity of 5-HT_{1B} receptors agonist on monoamine brain levels is associated with an antidepressant-like effect in the mice (O'Neill and Conway, 2001; Redrobe and Bourin, 1999; Tatarczynska et al., 2005) and the rat FST (Tatarczynska et al., 2004) or is able to potent such effect in mice (David et al., 2001;

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Redrobe et al., 1996). Moreover, authors suggest that antidepressant-like effects of SSRI (paroxetine) in the mice forced swimming test require the activation of 5-HT_{1B} heteroreceptors in order to occur (Gardier et al., 2001). Interestingly these results suggest that indirect dopamine neurotransmission activation (consecutively to the increase in serotonin release) could be necessary to obtain the behavioural effects of SSRIs.

The present study was designed to investigate the impact of dopaminergic depletion, further to infusion of 6-OHDA, on the antidepressant-like effects of various drugs evaluated in the mice forced swimming test. Antidepressant chosen included the selective serotonin reuptake inhibitors paroxetine and citalopram; the noradrenaline reuptake inhibitor, desipramine and the tricyclic antidepressant, imipramine.

2. Experimental procedures

2.1. Animals

Male Swiss mice (Centre d'élevage Janvier, Le Genest, France) 4 weeks old and weighing 18–20 g at the surgery day were housed in groups of 18 per cage (40 cm × 28 cm × 17 cm), in the standard conditions of the animal room (20 ± 1 °C, standard light/dark cycle light on at 7:00 h, off at 19:00 h) with free access to food and water for a period of 1 week before use. Each experimental group consisted of naive randomly grouped mice of the same weight, which were used only once. All experiments were performed within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law 87 848).

2.2. Drugs

Range doses (4–32 mg/kg) of paroxetine (GSK, France), citalopram (Lundbeck, Denmark), desipramine (Sigma, France) and imipramine (RBI, USA) were dissolved in distilled water and administered intraperitoneally (i.p.) (25 ml/kg). Chloral hydrate (400 mg/kg) was dissolved in distilled water and injected i.p. (5 ml/kg). 6-Hydroxydopamine (Sigma, France) was dissolved in ascorbic acid 0.1% and administered by i.c.v. route.

2.3. Monoamines depletions

2.3.1. Surgical procedures

All mice were pre-treated 30 min prior to i.c.v. administration of 6-OHDA (or vehicle) with i.p. injections of 20 mg/kg desipramine HCl and 16 mg/kg of paroxetine HCl to help to protect noradrenaline (NA) and serotonin (5-HT)-containing neurons respectively. They were then anaesthetised with chloral hydrate (400 mg/kg, i.p.) and placed in an ASI stereotaxic instrument (Bioseb, France) fitted with atraumatic earbars. 6-OHDA dissolved in 0.1% ascorbic acid was injected using an infusion pump (KDS Scientific) at a flow rate of 0.2 μl/min for 2.5 min, and the needle was left in place for a further 2 min before removal to prevent efflux of the injected solution. Treated mice received a bilateral intracerebroventricular (i.c.v.) injection of 10 to 30 μg of 6-OHDA; coordinates from Bregma (mm): anterior = −0.6, lateral = ± 1.2, ventral = −2.2. After surgery, a post-operative period of 14 days was necessary to allow the degeneration of DA-containing neurons (corresponding to the delay of action of 5,7-DHT, another neurotoxin acting on 5-HT system with similar mechanism of action; Björklund et al., 1975). Control groups (sham) received i.c.v. perfusion of vehicle. Every animals were housed individually during 48 h post-surgery; then they were housed by groups of 10 animals (each group = 1 dose of 6-OHDA).

After these 14 days, 4 groups of 10 treated mice (receiving 0, 10, 20 or 30 μg of 6-OHDA respectively) were killed by cervical dislocation to determine the dopamine depletion levels and the selectivity of action of the neurotoxin (HPLC brain tissue dosage). The brain sections were removed on a cold apparatus (Leica) in the following order (mean weight ± S.E.M. in mg): hypothalamus (8.8 ± 0.3), hippocampus (22.9 ± 0.7), striatum (44.6 ± 0.9) and cortex (177.2 ± 2.2) (see Picture 1). Other groups were used for behavioural studies. Antidepressants were dissolved in distilled water and administered i.p. 30 min before the forced swimming test. The 30 μg dose of 6-OHDA was chosen for the behavioural test because of the large decrease in dopamine it induces.

2.3.2. Determination of depletion level and selectivity of action of 6-OHDA

The preparation of samples and the HPLC analysis were fully described in a recent methodological article (Dailly et al., 2006).

2.4. Behavioural tests

2.4.1. Measurement of immobility time in the forced swimming test

The forced swimming test employed was essentially similar to that described elsewhere (Porsolt et al., 1977). Mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 12 cm water, maintained at 25 ± 1 °C, and remained there for 6 min. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Six mice were tested simultaneously, and the time of immobility was recorded during the last 4 min of the 6-min testing period, thus after 2 min of habituation. The test was performed by the same well trained experimenters, blind to the treatment administered. Antidepressants were injected i.p. 30 min before the test. Results are expressed as the immobility time during the 240-s test period (mean ± S.E.M.).

2.5. Data analysis and statistics

Statistical analyses were performed by the use of the computer software SIGMASTAT. In behavioural tests, a two-way ANOVA on the immobility time (FST) was performed with the drug treatment and the monoamine depletion (lesioned or sham) as main factors followed by a Newman–Keuls test. The depletion in monoamine level was analyzed by a one-way ANOVA followed by a Newman–Keuls test. Statistical significance was set at $p < \pm 0.05$.

3. Results

3.1. Monoamines depletions

Only 3 doses were used for this treatment, higher doses induced a high mortality (over 50%).

3.1.1. Effect of 6-OHDA treatment on monoamines brain levels in mice

In the *hypothalamus* all monoamines were decreased further to the 6-OHDA treatment; however only DA ($F_{3,29}$=15.6; $p<0.001$) and 5-HIAA ($F_{3,29}$=4.57; $p<0.01$) levels were significantly decreased. The DA level decrease was dose dependent (not significant at 10 μg) and was maximum at the higher dose tested (30 μg: −77%; $p<0.001$) compared to controls. The one-way ANOVA performed on NA

Table 1a Basal value (ng/g of wet tissue) of neurotransmitters in sham operated mice

	NA	DA	5-HIAA	5-HT
Hypothalamus	3485 (±107)	554 (±63)	662 (±29)	1633 (±140)
Hippocampus	583 (±45)	ND	433 (±40)	585 (±30)
Striatum	340 (±24)	7385 (±426)	149 (±13)	813 (±33)
Cortex	508 (±23)	582 (±49)	159 (±6)	703 (±34)

Values are expressed as the mean ± S.E.M. (ng/g of wet tissue) of each monoamine in each brain area (n = 12 animals per group). ND = not detectable.

levels was significant ($F_{3,29}$=3.79; p<0;05), but the Newman–Keuls Pairwise Comparison performed failed to demonstrate significant difference between groups (Tables 1a and b).

In the *hippocampus* DA levels were undetectable, but the 6-OHDA perfusion induced a significant ($F_{3,29}$=19.4; p<0.001) dose dependent decrease in NA (from 28% at 10 μg, p<0.05 to 79% at 30 μg, p<0.001). 5-HT and 5-HIAA levels were not significantly affected by monoamine depletion.

In the *striatum*, as in the hypothalamus, DA levels were dose dependently (except 10 μg) and significantly ($F_{3,29}$=15.83; p<0.001) decreased (p<0.001) such as NA ($F_{3,29}$=10.3; p<0.001). 5-HIAA levels were increased ($F_{3,29}$=4.95; p<0.01) further to infusion of 20 and 30 μg of 6-OHDA (p<0.05) whereas 5-HT levels were not modified ($F_{3,29}$=1.08; p=0.37).

In the *cortex*, 6-OHDA did not decrease 5-HT and 5-HIAA levels; at higher doses (20 and 30 μg) both DA ($F_{3,29}$=10.99; p<0.001) and NA ($F_{3,29}$=6.92; p<0.01) levels were decreased (maximum 77 and 38% decrease, respectively).

Table 1b % of basal value of each monoamine in various brain areas further to treatment with 6-OHDA

	NA	DA	5-HIAA	5-HT
Hypothalamus				
10 μg	99	77	76*	79
20 μg	83	71*	91	84
30 μg	83	23***	72*	82
Hippocampus				
10 μg	72	ND	101	102
20 μg	40***	ND	119	96
30 μg	21***	ND	90	91
Striatum				
10 μg	96	81	109	92
20 μg	73*	56***	168*	106
30 μg	54***	29***	151*	97
Cortex				
10 μg	94	74	100	96
20 μg	72*	56*	107	90
30 μg	62**	23***	108	100

Values are expressed as percentage of basal value. Differences between groups were analyzed by a one-way ANOVA followed by a Newman–Keuls test if ANOVA was significant. *p<0.05, **p<0.01 and ***p<0.001 in comparison with sham operated group. ND = not detectable.

3.2. Behavioural test

3.2.1. Effect of paroxetine on 6-OHDA-treated (30 μg) and sham operated mice in the FST

The two-way ANOVA performed revealed a significant effect of treatment ($F_{3,72}$=11.0; p<0.001) and interaction ($F_{3,72}$=3.72; p<0.05) but not of lesion ($F_{1,72}$=0.001; p=0.97). Paroxetine dose dependently decreased the immobility time in the FST in sham operated mice, with a statistically significant decrease at 16 mg/kg (p<0.01) indicating an AD-like effect. At the same dose paroxetine did not reduce immobility time in 6-OHDA-treated mice. The antidepressant-like effect of 16 mg/kg of paroxetine was significantly reduced by 6-OHDA lesion (p<0.05) (Fig. 1).

3.2.2. Effect of citalopram on 6-OHDA-treated (30 μg) and sham operated mice in the FST

The two-way ANOVA revealed significant effect of both lesion ($F_{1,72}$=22.6; p<0.001), treatment ($F_{3,72}$=8.05; p<0.001) and interaction ($F_{3,72}$=3.48; p<0.05). Citalopram reduced immobility time in sham operated mice in a dose dependent manner (p<0.01 at 8 and 16 mg/kg) but was devoid of effect on dopamine depleted mice. More-

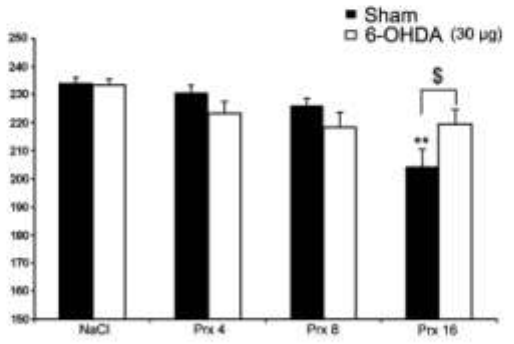


Figure 1 Antidepressant-like effect of paroxetine on the FST in sham operated (■) and 6-OHDA (□) treated mice (n=10 per group). Data are expressed as mean of immobility time (in seconds) (±S.E.M.). Asterisks show a significant difference compared to control group with same pre-treatment (vehicle or 6-OHDA). **p<0.01. $ indicate a significant difference between Sham and 6-OHDA lesioned mice. $^{\$}p$<0.05.

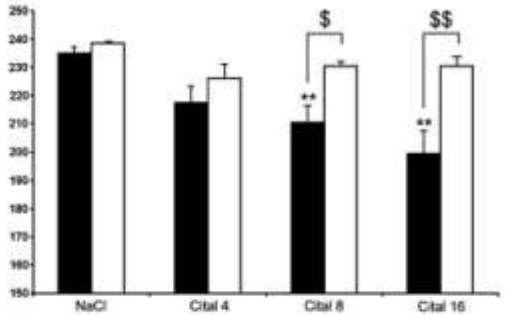


Figure 2 Antidepressant-like effect of citalopram on the FST in sham operated (■) and 6-OHDA (□) treated mice ($n=10$ per group). Data are expressed as mean of immobility time (in seconds) (±S.E.M.). Asterisks show a significant difference compared to control group with same pre-treatment (vehicle or 6-OHDA). $^{**}p<0.01$. $ indicate a significant difference between Sham and 6-OHDA lesioned mice. $^{\$}p<0.05$; $^{\$\$}p<0.01$.

over, the 6-OHDA lesion significantly antagonized the behavioural effect consequently to the administration of 8 and 16 mg/kg of citalopram ($p<0.05$ and $p<0.01$, respectively) (Fig. 2).

3.2.3. Effect of desipramine on 6-OHDA-treated (30 μg) and sham operated mice in the FST

The two-way ANOVA revealed significant effect of both lesion ($F_{1,72}=5.77$; $p<0.05$) and treatment ($F_{3,72}=40.2$; $p<0.001$) but not of interaction suggesting therefore that antidepressant-like effect of the drug is not significantly different in the vehicle and 6-OHDA-treated mice. Desipramine reduced immobility time in sham operated mice and 6-OHDA-treated animals only at the higher dose ($p<0.01$ in both groups at 32 mg/kg). At lower doses desipramine was devoid of effect on both groups (Fig. 3).

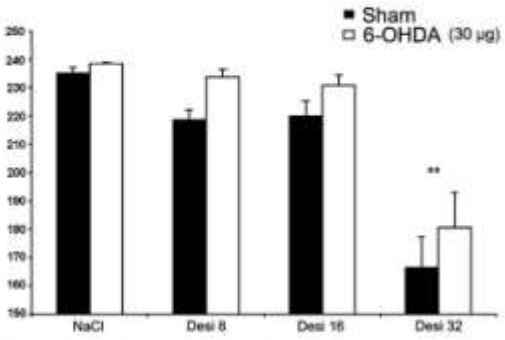


Figure 3 Antidepressant-like effect of desipramine on the FST in sham operated (■) and 6-OHDA (□) treated mice ($n=10$ per group). Data are expressed as mean of immobility time (in seconds) (±S.E.M.). Asterisks show a significant difference compared to control group with same pre-treatment. $^{**}p<0.01$.

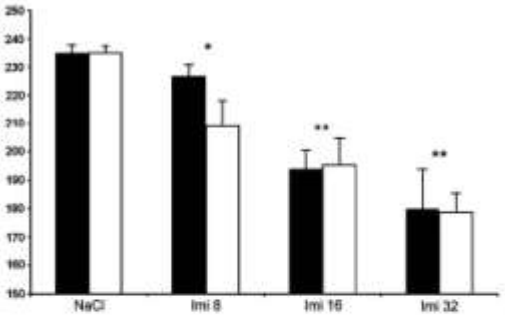


Figure 4 Antidepressant-like effect of imipramine on the FST in sham operated (■) and 6-OHDA (□) treated mice ($n=10$ per group). Data are expressed as mean of immobility time (in seconds) (±S.E.M.). Asterisks show a significant difference compared to control group with same pre-treatment. $^{*}p<0.05$; $^{**}p<0.01$.

3.2.4. Effect of imipramine on 6-OHDA-treated (30 μg) and sham operated mice in the FST

The statistical analysis only revealed a significant effect of treatment ($F_{3,72}=20.7$; $p<0.001$) suggesting therefore that dopaminergic system depletion did not change the behavioural response of mice in the FST further to the administration of imipramine. In both groups imipramine induced an antidepressant-like effect at 8 ($p<0.05$), 16 ($p<0.01$) and 32 ($p<0.01$) mg/kg (Fig. 4).

4. Discussion

The behavioural results obtained in the present study indicate that SSRIs (paroxetine and citalopram), but not desipramine and imipramine, lose their antidepressant-like effect in 6-OHDA-treated mice.

The 6-OHDA treatment induces a non-reversible depletion of both dopamine (71% to 77%) and noradrenaline (17% to 79%). Dopaminergic and noradrenergic-containing neurons being destroyed by the neurotoxin treatment, the loss of efficiency of SSRIs could then be associated to the decrease in dopamine levels as well as to the decrease in noradrenaline levels. However, numerous studies demonstrate that specific depletion of noradrenergic system (using DSP-4) does not modify the antidepressant-like effect of inositol (Einat et al., 2001), venlafaxine (Redrobe et al., 1998) and fluoxetine (Gavioli et al., 2004) in the mice FST. In these studies, DSP-4 was used at the dose of 50 mg/kg and induced a higher depletion in noradrenaline levels than the depletion obtained further to the administration of 6-OHDA (Dailly et al., 2006). Moreover, in the present study we also demonstrate that the noradrenaline reuptake inhibitor desipramine is still efficient by producing antidepressant-like effect in 6-OHDA-lesioned mice (Fig. 3), suggesting therefore that noradrenaline reuptake blockade can still mediate an antidepressant-like effect (even if noradrenergic system is partially depleted by neurotoxin treatment). Taken together, these results seem to indicate that the loss of efficiency of SSRIs obtained in the mice forced swimming

test after infusion of 6-OHDA is only linked to dopaminergic neurons destruction.

There is substantial evidence that SSRIs increase dopamine extracellular levels in rat substantia nigra (Thorre et al., 1998), and prefrontal cortex (Pozzi et al., 1999; Tanda et al., 1994). This effect of SSRIs probably appears further to an indirect activation of postsynaptic receptors ensuing from facilitated serotonergic neurotransmission in the brain (Goodwin, 1996; Stanford, 1996) since such effect can be mimicked by the local infusion of 5-HT in frontal cortex (Iyer and Bradberry, 1996) and substantia nigra (Thorre et al., 1998). However the augmentation effect of SSRIs on dopamine levels in the prefrontal cortex of rats can also be obtained in PCPA pre-treated animals suggesting therefore that SSRIs (fluoxetine and citalopram) could exert a direct effect on dopamine release (Pozzi et al., 1999). Nonetheless it is also presumable that dopamine release in the prefrontal cortex comes from noradrenaline-containing neurons because Devoto's team have largely demonstrated that a co-release of dopamine and noradrenaline from noradrenergic neurons occurs in the cerebral cortex (Devoto et al., 2001, 2003, 2005). Then the effect of SSRIs on dopamine levels in the cerebral cortex could be linked to an inhibitory effect on noradrenaline reuptake transporters. But, even if the increase in dopamine release appears to be fundamental for mediation of antidepressant-like effect of SSRIs (paroxetine and citalopram being devoid of effect on dopamine depleted mice, Figs. 1 and 2), it appears that activation of dopamine release must be indirect because SSRIs are devoid of antidepressant-like effect on PCPA (tryptophan hydroxylase inhibitor) treated rats (Page et al., 1999) and mice (Redrobe et al., 2005). Behavioural effects of selective serotonin reuptake inhibitors in the mice FST could then be mediated by indirect activation of 5-HT heteroreceptors which would induce an elevation of dopamine release. Among all 5-HT receptors subtypes indirectly activated by SSRIs, 5-HT_{1A} and 5-HT_{1B} receptor subtypes are strongly involved in mediation of dopamine release. It has then been demonstrated that 5-HT_{1A} receptors agonist (flesinoxan) even if inhibiting the firing rate of 5-HT neurons in the DRN (which is in line with data showing a tonic activation of 5-HT_{1A} autoreceptors in the rat DRN; Haddjeri et al., 2004), it also increases dose dependently the firing rate of dopaminergic neurons in the VTA (Lejeune and Millan, 1998). It has also been demonstrated that another 5-HT_{1A} receptors agonist, 8-OH-DPAT, is able to increase firing rate of dopaminergic neurons in both VTA and frontal cortex (Arborelius et al., 1993a,b). However, in a recent study we have found that pharmacological, or constitutive, blockade of 5-HT_{1A} receptor does not antagonize the antidepressant-like effect of SSRIs (paroxetine); on the opposite, the swimming duration is increased (Guilloux et al., in press). Suggesting therefore that activation of 5-HT_{1A} receptors, even if increasing dopaminergic neurons firing, is not necessary for the mediation of antidepressant-like effect of SSRIs in the mice FST. On the other hand, the mix 5-$HT_{1A/1B}$ receptors antagonist pindolol antagonizes the antidepressant-like effect of paroxetine in 5-HT_{1A} receptor knockout mice thus suggesting the impact of 5-HT_{1B} receptor activation in the mice FST. Such as serotonin infusion, the local infusion of 5-HT_{1B} receptor agonist also induces an increase in dopamine release in rat nucleus accumbens (Yan and Yan, 2001), ventral tegmental area (Yan et al., 2004), substantia nigra (Thorre et al., 1998) striatum and frontal cortex (Iyer and Bradberry, 1996). This leads us to hypothesize that activation of this serotonin receptor subtype could mediate the antidepressant-like effects of SSRIs in the mice forced swimming test. Moreover, it has been found that 5-HT_{1B} receptor agonists (such as anpirtoline, RU 24969 and CP 94253) exert an antidepressant-like effect in the mice FST (O'Neill and Conway, 2001; Redrobe and Bourin, 1999; Tatarczynska et al., 2005) or can be used to potent the effect of antidepressants in this test (David et al., 2001; Redrobe et al., 1996). It has also been demonstrated that the pharmacological blockade, or the constitutive lack, of this receptors is associated with the loss of efficiency of SSRIs in the same test (Bourin et al., 1998; Gardier et al., 2001). On the other hand authors have recently found that in the mice tail suspension test, SSRIs are efficient in 5-HT_{1B} receptors knockout mice (low doses of fluoxetine are potentiated compared to control animals) (Mayorga et al., 2001). This difference seems to indicate that these two tests do not involve the same postsynaptic receptors activation.

In conclusion, our results suggest that the antidepressant-like effect of SSRIs in the mice FST might be due to indirect excitatory effect of drugs on dopaminergic neurons and are probably mediated by the activation of 5-HT_{1B} postsynaptics receptors (heteroreceptors); whereas different monoaminergic systems are implicated in the mediation of behavioural effects of imipramine and desipramine. Taken together, these results would indicate that in the mice FST, but not in the TST, antidepressant-like effect of SSRIs could be mediated by the activation of 5-HT_{1B} receptors, and therefore by an increase in dopamine levels in the mice forced swimming test, whereas imipramine and desipramine effects could be mediated by others receptors.

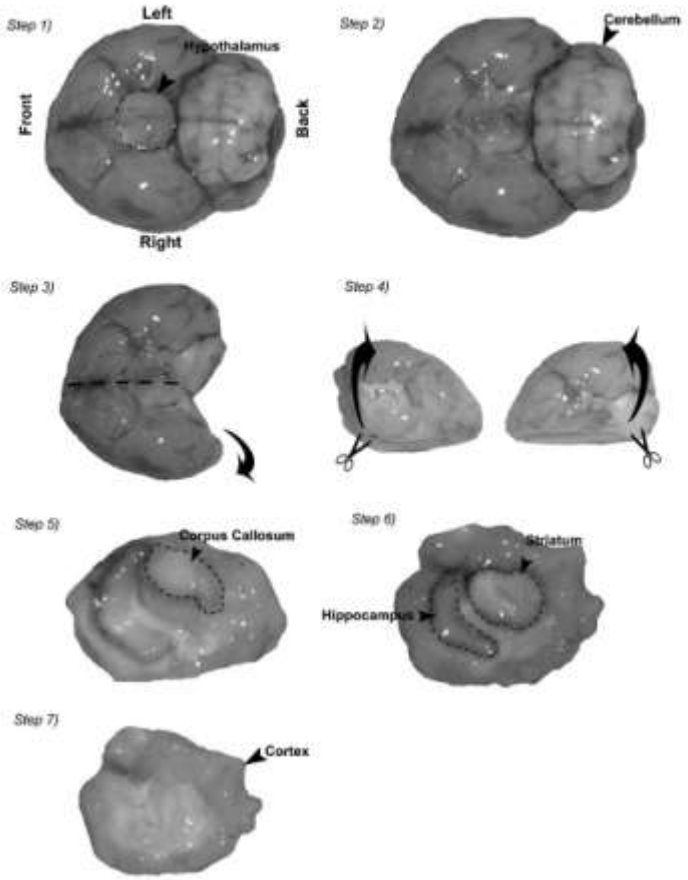
Appendix A. Brain dissection

Step 1 The brain is removed from the cranium and rapidly placed on a cool apparatus (Leica) maintained at −8 °C; ventral section is placed upside down on the top in order to detect easily the hypothalamus area which is then removed using a micro dissecting tweezer. The hypothalamus is circled by a dotted line on picture 1.

Step 2 Cerebellum is removed with a scalpel blade (picture 2).

Step 3 The brain is cut following the anteroposterior axis. Each half brain is then placed as shown on picture 4.

Step 4 A small incision is performed on the basis of corpus callosum (between corpus callosum and cortex). The half brain is then laid on the cold plate (lateral cortex facing the plate) following the black arrow on picture 4. Pictures 5, 6 and 7 represent the right half brain (the part which is on the left on picture 4).The other half brain is symmetric to the right one (hippocampus would



Picture 1 Brain Dissection.

then be on the right, striatum and corpus callosum on the left).

Step 5 Removal of corpus callosum. The corpus callosum (slightly white) is just localized on the top of the striatum (gray with some striae) and must be removed carefully (picture 5).

Step 6 The hippocampus and striatum are then removed using a micro dissecting tweezer. The hippocampus looks like a "crescent", whereas the striatum is like a "bowl".

Step 7 What is left then on the cold plate is the cortex.

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Résumé de l'étude n°6

Le prétraitement des souris par 6-OHDA a permis d'obtenir une déplétion partielle en dopamine dans l'hypothalamus (-77%; $p<0.001$), le striatum (-71%; $p<0.001$) et le cortex (-77%; $p<0.001$). Dans l'hippocampe les concentrations de dopamine ne sont pas détectables. Cette déplétion en dopamine s'accompagne de la disparition de l'activité antidépressive des IRSSs (citalopram et paroxétine), suggérant ainsi qu'une activation des systèmes de neurotransmission dopaminergique à la suite de l'administration d'IRSSs est nécessaire pour obtenir les effets de ces molécules dans le FST.

A l'inverse, l'activité de l'imipramine et de la désipramine n'est pas modifiée par une déplétion en dopamine, suggérant donc que les voies de neurotransmission impliquées dans l'apparition des effets thérapeutiques de ces différentes molécules ne sont pas les mêmes Ces résultats sont donc en accord avec ceux de l'étude précédente montrant que les récepteurs 5-HT_{1B} ne sont impliqués que dans les effets des IRSSs.

Etude n°7 :

Effet d’une déplétion en sérotonine sur les effets dans le FST du citalopram et de la paroxétine

Objectif de l'étude n°7:

Dans l'étude n°6, nous avons démontré qu'une lésion du système dopaminergique s'accompagne d'une disparition de l'effet antidépresseur du citalopram et de la paroxétine dans le FST chez la souris ; suggérant ainsi que l'administration d'IRSSs induit une activation des systèmes de neurotransmission dopaminergiques nécessaires à l'apparition de leurs effets.

Toutefois, bien que cette étude montre le rôle prépondérant de la dopamine dans l'activité des IRSSs, elle ne permet pas de déterminer le type d'activation nécessaire. Deux mécanismes d'actions principaux peuvent être proposés : une activation indirecte de la voie dopaminergique (via une augmentation de la libération de sérotonine qui entraîne une activation des récepteurs sérotoninergiques situés sur les neurones dopaminergiques) ou bien une activation directe (fixation des antidépresseurs sur les transporteurs dopaminergiques). L'objectif de cette étude est de définir le type d'activation nécessaire.

Nous avons réalisé une déplétion du système sérotoninergique avec du *p*-CPA. L'inhibition de la tryptophane hydroxylase permet de bloquer la synthèse de la sérotonine et donc d'entraîner une déplétion en monoamine tout en conservant l'intégrité des neurones (et donc des transporteurs de la sérotonine). Le p-CPA a été administré pendant 3 jours consécutifs (72, 48 et 24H avant le test) à la dose de 300 mg/kg.

Chez des animaux ainsi prétraités l'administration d'IRSS ne permet pas d'augmenter les concentrations extracellulaires de sérotonine.

La dose de *p*-CPA à utiliser a été définie lors de la réalisation de l'étude n°3 pour laquelle nous avons établit une relation effet-dose pour le *p*-CPA et le DSP-4.

Résultats de l'étude 7 :

Effet de l'administration de p-CPA sur l'activité du citalopram dans le FST chez la souris

L'ANOVA à deux facteurs met en évidence l'existence d'un facteur prétraitement ($F_{1,72}$=37,60 ; p<0.001), d'un facteur traitement ($F_{3,72}$=4,56 ; p<0.01) et une intéraction significative entre ces deux facteurs ($F_{3,72}$=6,29 ; p<0,001). Ces résultats montrent que le prétraitement par le p-CPA empêche l'apparition de l'effet de type antidépresseur du citalopram dans le FST (p<0.001 à 4 et 8 mg/kg ; p<0.01 à 16 mg/kg), alors que cet effet est présent dans les groupes prétraités avec le véhicule (p<0.001 à 4 et 8 mg/kg ; p<0.01 à 16 mg/kg).

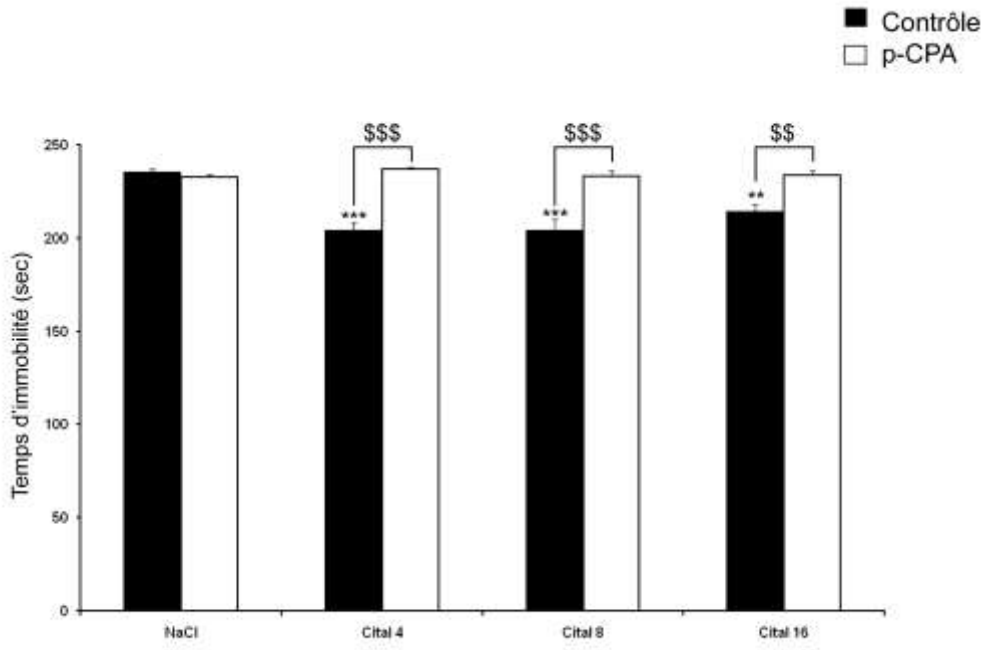

Figure 11 : Effet de l'administration de *p*-CPA (i.p.72, 48, 24h avant le test) et de citalopram (i.p. 30min avant le test) sur le temps d'immobilité chez les souris dans le FST.

Les résultats sont exprimés en moyenne (± ESM) du temps d'immobilité (en secondes) des souris (n=10). L'analyse statistique est réalisée par une ANOVA à deux facteurs suivie d'un test de Student Newman Keuls (*p<0.05, **p<0.01, ****p*<0,001 versus groupe contrôle et $p<0.05, $$p<0.01, $$$*p*<0,001 animaux déplétés en sérotonine versus animaux non déplétés).

Effet de l'administration de p-CPA sur l'activité de la paroxétine dans le FST chez la souris

L'ANOVA à deux facteurs met en évidence l'existence d'un facteur prétraitement ($F_{1,72}$=20,26 ; p<0.001), d'un facteur traitement ($F_{3,72}$=5,10 ; p<0.01) et une interaction statistiquement significative entre ces deux facteurs ($F_{3,72}$=6,64 ; p<0,001). Quand administrée seule, la paroxétine induit un effet de type antidépresseur à 8 et 16 mg/kg (p<0.001 et p<0.01 respectivement). A l'inverse, la paroxétine est dépourvue d'effet chez les souris prétraitées par p-CPA, quelque soit la dose d'antidépresseur considéré. Aux deux doses actives, le prétraitement par p-CPA empêche l'apparition des effets de la paroxétine.(p<0.001 à 8 mg/kg et p<0.05 à 16 mg/kg).

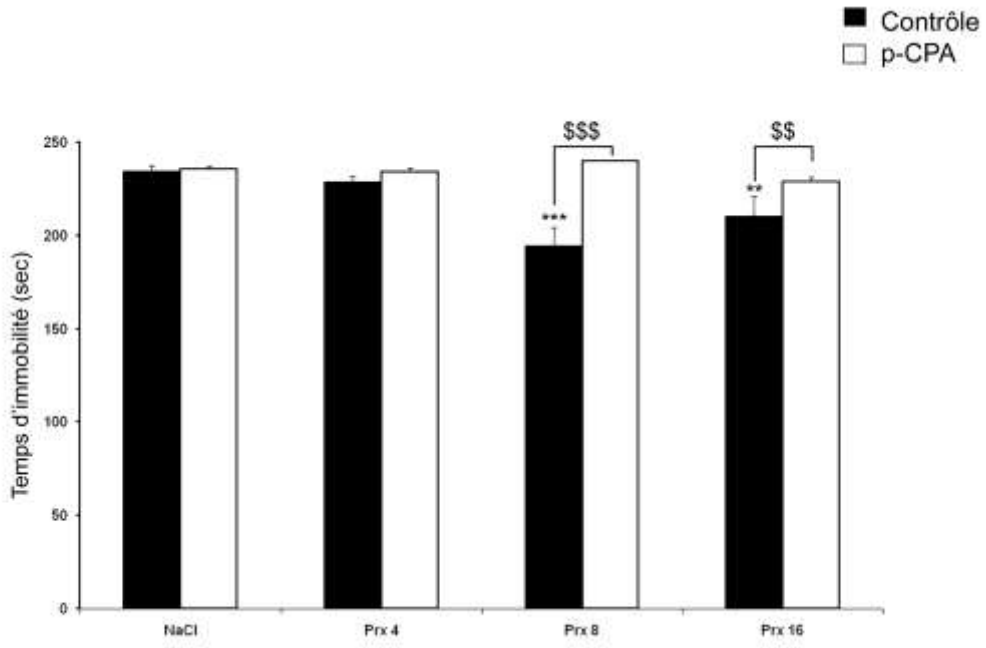

Figure 12 : Effet de l'administration de *p*-CPA (i.p. 72, 48, 24h avant le test) et de paroxétine (i.p. 30min avant le test) sur le temps d'immobilité chez les souris dans le FST.

Les résultats sont exprimés en moyenne (± ESM) du temps d'immobilité (en secondes) des souris (n=10). L'analyse statistique est réalisée par une ANOVA à deux facteurs suivie d'un test de Student Newman Keuls (*p<0.05, **p<0.01, ****p*<0,001 versus groupe contrôle et $p<0.05, $$p<0.01, $$$*p*<0,001 animaux déplétés en sérotonine versus animaux non déplétés).

Résumé de l'étude n°7 :

Dans cette étude nous avons montré que les IRSSs sont dépourvus d'effet dans le FST chez des souris préalablement traitées par du *p*-CPA ; démontrant ainsi que l'activation du système dopaminergique nécessaire pour obtenir l'apparition de l'effet de type antidépresseur de ces substances (voir étude n°6) passe par une élévation des concentrations extracellulaires de sérotonine. Le niveau de déplétion en sérotonine a été vérifié à la suite du test pour confirmer l'activité du *p*-CPA (résultats non montrés). Nos résultats sont en accord avec ceux montrant une disparition des effets de la fluoxétine dans le FST chez la Souris (Redrobe et al., 2005) et le Rat prétraités par du *p*-CPA (Page et al., 1999).

Parmis les 14 sous-types de récepteurs sérotoninergiques indirectement activés par l'administration systémique d'IRSSs, deux sont particulièrement impliqués dans la modulation du relargage de la dopamine, les récepteurs 5-HT_{1A} et 5-HT_{1B}. Lors d'une étude réalisée récemment en collaboration avec le laboratoire du Pr Alain Gardier (Guilloux et al., 2006), il a été démontré que les effets des IRSSs (paroxétine) sont toujours présents chez des souris knockout pour le gène codant pour le récepteur 5-HT_{1A} et que l'absence de ce récepteur permet une potentialisation des effets de la paroxétine. Suggérant ainsi que l'activation du récepteur 5-HT_{1A} n'est pas nécessaire à l'apparition des effets des IRSSs dans le FST, contrairement à l'activation du récepteur 5-HT_{1B} qui est primordiale.

En 2001 une équipe américaine a démontré que l'absence de récepteur 5-HT_{1A} empêchait l'apparition des effets de la fluoxétine dans le TST, alors que l'absence de récepteur 5-HT_{1B} potentialisait les effets de la paroxétine et de la fluoxétine (Mayorga et al., 2001). Ces résultats sont très intéressants, car ils sous-entendent que, selon le test auxquels sont soumis les animaux, l'apparition de l'effet antidépresseur ne nécessite pas l'activation de la même voie monoaminergique.

Etude n°8 :

Effet de deux tests comportementaux (FST et TST) sur les concentrations tissulaires monoaminergiques (5-HT, 5-HIAA, NA et DA) dans différentes structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Objectif de l'étude 8 :

Le test comportemental utilisé pour la réalisation de cette thèse (le FST), permet de mettre en évidence le profil antidépresseur de certaines molécules. Bien que ce test soit largement utilisé lors des études précliniques, il n'existe aucune étude permettant d'expliquer pourquoi les antidépresseurs, à l'inverse des autres substances, modifient le comportement de l'animal. En effet, bien que les systèmes monoaminergiques (5-HT, NA et DA) aient largement été étudiés dans la neurobiologie de la dépression, les changements neurochimiques qui ont lieu lors d'une exposition de l'animal à un test comportemental (FST et TST) n'ont pas encore été étudiés de façon précise (les seules études disponibles portent sur le cerveau entier Renard et al., 2003). De plus, comme nous l'avons évoqué précédemment, il semble que les deux tests les plus utilisés (FST et TST) ne mettent pas en jeu les mêmes mécanismes neurochimiques. Afin d'essayer de mieux comprendre ces tests, nous avons mesuré les variations en monoamines dans quatre régions cérébrales (hypothalamus, hippocampe, striatum et cortex) avant et après une exposition aux tests. Ces quatre structures sont largement reconnues comme étant impliquées dans les troubles de l'humeur et dans la réponse aux antidépresseurs. Le métabolite de la 5-HT, le 5-HIAA a permis après dosage de mesurer l'activité monoaminergique dans ces quatre structures. Le rapport 5-HIAA/5-HT, « turnover » ou taux de renouvellement de la 5-HT a été calculés afin de servir d'index de l'activité de la 5-HT après exposition aux tests.

Résultats de l'étude 8 :

Concentrations de 5-HT dans les quatre structures étudiées

Les deux tests comportementaux induisent une diminution des concentrations tissulaires de sérotonine dans l'hypothalamus ($p<0,05$ et $p<0,01$ dans le FST et le TST respectivement) et une augmentation dans le cortex ($p<0,05$) en comparaison avec des souris naïves non testées. La concentration de sérotonine est augmentée (non significativement) dans l'hippocampe et le striatum chez des souris testées dans le FST. A l'inverse, les concentrations de sérotonine dans ces deux aires cérébrales ont tendance à diminuer chez les souris testées dans le TST (non significatif). La concentration de sérotonine tissulaire dans l'hippocampe et le striatum est statistiquement plus élevée chez les souris testées dans le FST que chez les souris testées dans le TST.

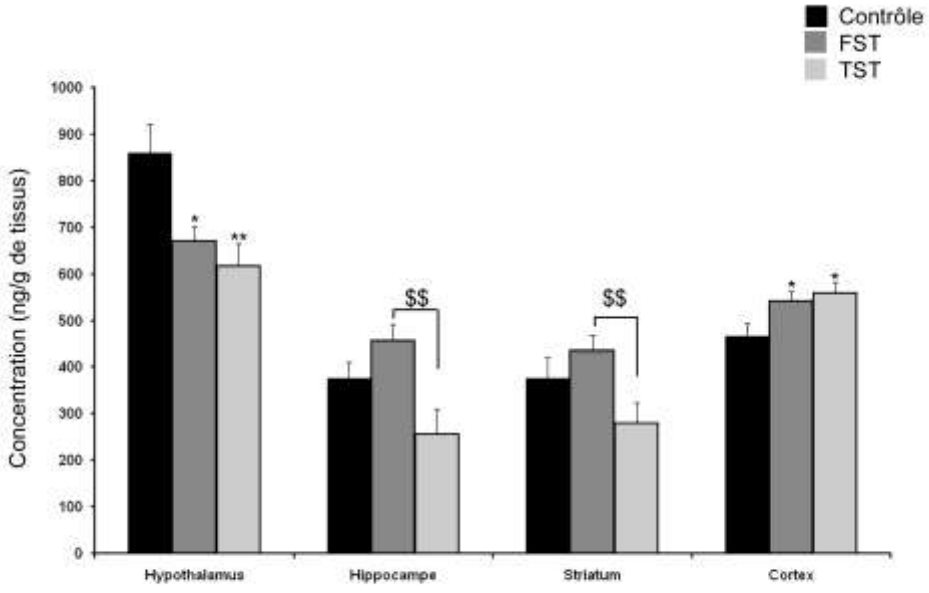

Figure 13 : Effet d'une exposition des animaux au FST et au TST sur la concentration de 5-HT dans quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Les résultats sont exprimés sous forme de moyenne ± ESM (ng/g) avec n=10. L'analyse statistique est réalisée grâce à un test de Student Newman Keuls. * indique une différence significative animaux testés versus animaux non testés, $ indique une différence significative entre les groupes testés (FST versus TST) (* $p<0,05$; **p<0,01 ; ***p<0,001 ;$ $p<0,05$; $$p<0,01 ; $$$p<0,001).

Concentrations de 5-HIAA dans les quatre structures étudiées

Le FST entraîne une diminution de la concentration hypothalamique de 5-HIAA, et une élévation de la concentration de 5-HIAA dans le cortex en comparaison avec des souris naïves non testées (p<0.05). La concentration de 5-HIAA dans le striatum et le cortex est augmentée chez les souris ayant été testées dans le TST (p<0,001 et p<0.05). A l'inverse, la concentration de 5-HIAA dans l'hippocampe diminue significativement chez des souris testées dans le TST en comparaison avec des souris naïves non testées (p<0,05). Les concentrations de 5-HIAA sont significativement différentes entre les animaux testés dans le FST et ceux testés dans le TST dans l'hippocampe, l'hypothalamus et le striatum (p<0,05 ; p<0,01 et p<0,001 respectivement).

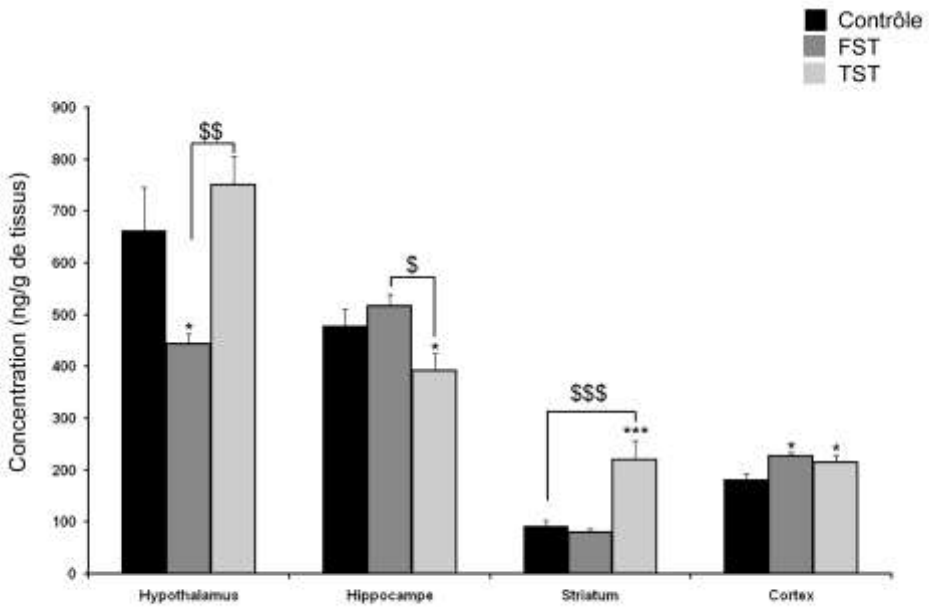

Figure 14 : Effet d'une exposition des animaux au FST et au TST sur la concentration de 5-HIAA dans quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Les résultats sont exprimés sous forme de moyenne ± ESM (ng/g) avec n=10. L'analyse statistique est réalisée grâce à un test de Student Newman Keuls. * indique une différence significative animaux testés versus animaux non testés, $ indique une différence significative entre les groupes testés (FST versus TST) (* p<0,05 ; **p<0,01 ; ***p<0,001 ;$ p<0,05 ; $$p<0,01 ; $$$p<0,001).

Mesure du taux de renouvellement de la 5-HT dans les quatre structures étudiées

Le rapport 5-HIAA/5-HT ou taux de renouvellement sérotoninergique n'est modifié dans aucune structure après le FST. Ce rapport augmente significativement dans le striatum et l'hypothalamus après un TST.

Taux de renouvellement 5-HT	Hypothalamus		Hippocampe		Striatum		Cortex	
Avant test	0,84 ± 0,13		1,41 ± 0,19		0,25 ± 0,02		0,40 ± 0,03	
Après FST	0,67 ± 0,03	P<0,01	1,18 ± 0,09	NS	0,18 ± 0,01	P<0,001	0,42 ± 0,02	NS
Après TST	**1,26 ± 0,09****		2,34 ± 0,55		**0,78 ± 0,04*****		0,39 ± 0,02	

Tableau 6 : Taux de renouvellement de la 5-HT avant et après une exposition des animaux au FST et au TST dans quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Les résultats sont exprimés sous forme de moyenne ± ESM avec n=10. L'analyse statistique est réalisée grâce à un test de Student Newman Keuls pour comparer les groupes deux à deux (NS: non significatif).

Concentrations de NA dans les quatre structures étudiées

La concentration de NA dans l'hypothalamus, l'hippocampe, le striatum et le cortex reste inchangée chez des souris qui ont été testées dans le FST en comparaison avec des souris naïves non testées. En revanche, la concentration de NA dans l'hippocampe et le cortex est significativement modifiée chez des souris testées dans le TST en comparaison avec des souris naïves non testées ; on observe une diminution de la concentration de NA dans l'hippocampe ($p<0,05$) et une augmentation dans le cortex ($p<0,01$).

Concentration de NA (ng/g)	Hypothalamus		Hippocampe		Striatum		Cortex	
Avant test	3148 ± 142		465 ± 30		195 ± 25		324 ± 20	
Après FST	3068 ± 122	NS	454 ± 27	P<0,05	165 ± 13	NS	354 ± 15	P<0,01
Après TST	2717 ± 248		**347 ± 29***		242 ± 24		**449 ± 32****	

Tableau 7 : Effet d'une exposition des animaux au FST et au TST sur la concentration de NA dans quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Les résultats sont exprimés sous forme de moyenne ± ESM (ng/g) avec n=10. L'analyse statistique est réalisée grâce à un test de Student Newman Keuls. * indique une différence significative entre les animaux testés et les animaux non testés (* *p*<0,05 ; **p<0,01 ; ***p<0,001 ; NS : Non Significatif)

Concentrations de DA dans les quatre structures étudiées

La concentration de DA dans l'hippocampe est trop faible pour être détectée par notre méthode de dosage. Quelque soit l'aire cérébrale considérée, aucun de ces tests n'entraîne de variation de la concentration tissulaire de dopamine.

Concentration de DA (ng/g)	Hypothalamus		Hippocampe		Striatum		Cortex	
Avant test	380 ± 20		ND		6392 ± 488		526 ± 31	
Après FST	346 ± 42	NS	ND		6279 ± 404	NS	550 ± 20	NS
Après TST	312 ± 22		ND		5597 ± 479		625 ± 54	

Tableau 8 : Effet d'une exposition des animaux au FST et au TST sur la concentration de DA dans quatre structures cérébrales (hypothalamus, hippocampe, striatum et cortex).

Les résultats sont exprimés sous forme de moyenne ± ESM (ng/g) avec n=10. L'analyse statistique est réalisée grâce à un test de Student Newman Keuls. (NS : Non Significatif)

Résumé de l'étude 8 :

L'exposition des souris à un FST entraîne des changements dans les concentrations tissulaires monoaminergiques. La concentration de 5-HT est significativement diminuée dans l'hypothalamus et augmentée dans le cortex. Dans ces même structures, le métabolite de la 5-HT, le 5-HIAA, est également diminué significativement. La diminution simultanée de la concentration de 5-HT et de son métabolite font qu'il n'y a aucune modification du taux de renouvellement de la 5-HT dans ces aires cérébrales en comparaison à des souris non testées ; suggérant ainsi que le FST n'entraîne pas de modification du métabolisme de la sérotonine. Ces travaux n'ont pas mis en évidence de modification des concentrations tissulaires de noradrénaline et de dopamine après un FST.

A l'inverse, l'exposition des animaux au TST entraîne une augmentation du taux de renouvellement de la sérotonine suggérant une modification du métabolisme sérotoninergique. Cette modification s'accompagne de changements dans les concentrations extracellulaires de noradrénaline dans l'hippocampe et le cortex.

Les modifications physiologiques induites par les tests comportementaux permettent certainement d'expliquer les différences observées sur le plan comportemental.

Etude n°9 :

Evaluation de l'effet antidépresseur résultant de la coadministration d'un IRSS avec un antagoniste des récepteurs NK1 dans le FST chez la souris

Antidepressant-like activity of Selective Serotonin Reuptake Inhibitors combined with a NK1 receptor antagonist in the mouse Forced Swimming Test

Chenu F[1*], Guiard BP[2*], Bourin M[1], Gardier AM[2].

Publié dans Behavioural Brain Research

Objectif de l'étude n°9 :

Cette étude a été réalisée en collaboration avec le Laboratoire du Pr Alain Gardier. Le but de cette étude était de confirmer des résultats de microdialyse qui montraient que l'administration combinée d'un antidépresseur (IRSS) et d'un antagoniste des récepteurs NK1 (GR205171) potentialise le relargage de sérotonine dans le cortex frontal de souris (Guiard et al., 2004; Guiard et al., 2006).

Toutefois, cette étude présente également un intérêt dans la détermination des voies monoaminergiques impliquées dans l'apparition de l'effet antidépresseur des IRSSs dans le FST.

En effet, des études réalisées *in-vitro* sur des coupes d'hippocampe de rats ont mis en évidence que l'administration de sérotonine permet de diminuer le relargage d'acétylcholine, et que cet effet de la sérotonine dépend de l'activation du récepteur 5-HT_{1B} (Maura and Raiteri, 1986; Maura et al., 1989). A l'inverse, les études *in-vivo* de microdialyse ont mis en évidence que l'administration d'IRSS se traduit par une augmentation du relargage d'acétylcholine dans le cortex frontal de rat et que cet effet peut être antagonisé par l'administration d'un antagoniste des récepteurs 5-HT_{1B} (Consolo et al., 1996). Une explication possible pour justifier la différence entre les études in-vitro et in-vivo est l'activation des récepteurs 5-HT_{1B} situés sur les neurones GABAergiques qui peut entraîner une désinhibition du système cholinergique. Par conséquent, il semble possible que le blocage de l'effet antidépresseur de la paroxétine et du citalopram lors de la coadministration avec un antagoniste des récepteurs 5-HT_{1B} (le GR127935, voir étude n°5) ne soit pas seulement lié à une activité sur la dopamine, mais soit également lié à une diminution du relargage d'acétylcholine.

Or en 1996, il a été démontré que l'administration d'IRSS permet l'activation des récepteurs 5-HT_{2A} situés sur les neurones contenant la substance P ; cette activation entraîne une augmentation du relargage de substance P ; ce qui permet une activation des récepteurs NK1 responsables d'une augmentation de la neurotransmission cholinergique (Feuerstein et al., 1996a).

Par conséquent, les coadministrations [IRSS + antagoniste des récepteurs NK1] et [IRSS + antagoniste des récepteurs 5-HT_{1B}] permettent de produire les mêmes effets sur la libération d'acétylcholine (blocage de l'augmentation de la libération d'acéthylcholine) et de sérotonine (potentialisation de l'augmentation de 5-HT extracellulaire).

Dans cette étude, nous avons donc réalisé des coadministrations [antidépresseur + antagoniste des récepteurs NK1 (GR205171)] nous permettant, même si ce n'était pas

l’objectif initial de cette étude, d’évaluer les effets comportementaux résultant de l’absence d’augmentation de relargage d’acétylcholine lors d’un traitement par antidépresseur dans le FST chez la souris.

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Research report

Antidepressant-like activity of selective serotonin reuptake inhibitors combined with a NK1 receptor antagonist in the mouse forced swimming test

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Abstract

Substance P antagonists of the neurokinin-1 receptor type (NK1) have growing interest as new antidepressant therapies. It has been postulated that these drugs exert this putative therapeutic effect without direct interactions with serotonin (5-HT) neurons. In line with this assumption, previous intracerebral *in vivo* microdialysis experiments provided evidence that the NK1 receptor antagonists did not change basal cortical 5-HT levels. However, we found that increases in cortical 5-HT overflow caused by systemic injection of the selective serotonin reuptake inhibitor (SSRI), paroxetine was higher in freely moving (C57BL/6x129sv) NK1−/− mutants than in wild-type NK1+/+ mice [17]. More recently, a pharmacological study has led to a similar conclusion since GR205171, a NK1 receptor antagonist, potentiated paroxetine-induced increases in cortical 5-HT dialysate following its acute systemic or intra-raphe administration to wild-type mice [25]. In the present study, we tested whether an acute combination of SSRI and NK1 receptor antagonist could display antidepressant-like activity using the forced swimming test in Swiss mice. We found that a single systemic dose of GR205171 (10 and 30 mg/kg, i.p.) had no effect by itself. However, it selectively potentiated the antidepressant-like activity of subactive doses of two serotonergic antidepressant drugs, citalopram and paroxetine (without psychomotor stimulant activity), but not that of noradrenaline reuptake inhibitor, desipramine. In agreement with neurochemical data, the present study confirms that co-administration of a NK1 receptor antagonist with an antidepressant drug such as a SSRI may have a therapeutic potential to improve the treatment of major depressive episodes in human compared to SSRI alone.

Keywords: Forced swimming test; Mouse; NK1 receptor antagonist; Selective serotonin reuptake inhibitor; Substance P

1. Introduction

To the present knowledge, antidepressant drugs used in the treatment of major depressive disorders are believed to act on the central monoaminergic systems mainly serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (NA) synaptic neurotransmissions. Selective serotonin reuptake inhibitors (SSRIs: paroxetine, fluoxetine, citalopram, escitalopram, fluvoxamine, sertraline) and noradrenaline reuptake inhibitors (NRIs: reboxetine, desipramine) are the most common prescribed antidepressant drugs [37]. They exert their therapeutic effects by increasing availability of 5HT and NA neurotransmitters in the synapses of different limbic areas including the frontal cortex. Although SSRIs and NRIs are effective in treating most depressive episodes, a significant proportion of depressed patients do not display signs of mood improvement until 2–3 weeks after the start of the treatment [3]. Furthermore, about one third of these patients show only partial or no response to the treatment [56]. In addition, some side effects are reported during the chronic treatment such as gain weight and sexual dysfunction [16,53]. Thus, multiple strategies are in progress to improve the activity of these conventional antidepressant drugs (for reviews, see [2,52]).

Abbreviations: CNS, central nervous system; DRN, dorsal raphe nucleus; [5-HT]ext, extracellular levels of 5-HT; FST, forced swimming test; KO, knock-out; LC, locus coeruleus; NK1, neurokinin 1 receptor; NA, noradrenaline; SSRIs, selective serotonin reuptake inhibitors; NRI, noradrenalin reuptake inhibitors; SP, substance P; 5-HT, serotonin; TST, tail suspension test

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Substance P (SP), a member of tachykinin family, is widely distributed within the brain and exerts its biological effects mainly through the activation of the G protein-coupled neurokinin 1 (NK1) receptor [51]. During the past 10 years, clinical trials carried out in depressed patients revealed a putative new class of antidepressant drugs known as the NK1 receptor antagonists. Initial phase II studies of the NK1 receptor antagonists MK689, L759274 and CP122721 have reported a higher rate of symptoms remission than that of paroxetine, fluoxetine or placebo [10,31,32]. However, these compounds exhibit the same delay of therapeutic effects as SSRIs (for review, see [36]). A recent clinical study has compared the effects of a NK1 receptor antagonist alone and citalopram on anxiety symptoms in social phobia [18] and demonstrated that short-term administration of either GR205171 or citalopram similarly alleviated social anxiety. Despite these encouraging results, a progressive decreased interest for NK1 receptor antagonism in the treatment of major depression is observed. This lack of interest is most likely due to the fact that approximately half of the antidepressant clinical trials performed with NK1 receptor antagonists failed to differentiate these new drugs from placebo or comparators [29,39,42].

Although the antidepressant properties of NK1 receptor antagonists were first attributed to a new mechanism, without interfering with central monoamine systems [31], various preclinical approaches have revealed that NK1 receptor antagonists can modulate both 5-HT and NA neurotransmission in the brain (for reviews, see [1,4,19,24]). For example, it was reported that the genetic inactivation of NK1 receptors causes a functional desensitisation of 5-HT1A autoreceptors and alpha-2 adrenoceptors [17,28,50] as observed with SSRIs and NRIs [33,45]. As a direct consequence, microdialysis experiments have demonstrated that the increase in cortical extracellular 5-HT and NA levels induced by paroxetine and desipramine respectively, were higher in NK1−/− KO mice than in their wild-type littermates [17,28]. Likewise, paroxetine-induced increase in cortical [5-HT]ext was potentiated in wild-type mice receiving a single or repeated administration (for 3 weeks) of the NK1 receptor antagonist, GR205171 [22,25] suggesting that NK1 receptor antagonism may strengthen the antidepressant-like activity of SSRIs. This, hypothesis postulates that a high levels of [5-HT]ext at serotonergic nerve terminals, would predict a high degree of antidepressant effect by stimulating post-synaptic 5-HT receptor sub-types in brain regions involved in mood disorders. Interestingly, many studies demonstrated the antidepressant-like effect of various NK1 receptor antagonists given alone in both the forced swimming test [13,57] and the tail suspension test [55]. However, to our knowledge, no combination studies have been performed using co-administration of NK1 receptor antagonist and antidepressant drugs.

Thus, the present study was aimed to evaluate the antidepressant-like effects of paroxetine, citalopram and the NRI desipramine in association with a non peptidic, selective and brain penetrant NK1 receptor antagonist GR205171 [46], using the mouse forced swimming test (FST). This behavioural test is one of the most widely used preclinical paradigms for predicting antidepressant-like activity of drugs after their acute administration [41] alone or in combination.

2. Material and methods

2.1. Animals

Male Swiss mice (Centre d'élevage Janvier, Le Genest, France) 4 weeks old and weighing 20 ± 2 g at the treatment day were housed in groups of 18 per cage (40 cm × 28 cm × 17 cm) in the standard conditions of the animal room (21 ± 1 °C, standard light/dark cycle light on at 7:00 h, off at 19:00 h) with free access to food and water for a period of 1 week before use. Each experimental group consisted of naïve randomly grouped mice of the same weight, which were used only once (evaluation of locomotor activity or forced swimming test). All experiments were performed within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law 87 848).

2.2. Drugs and treatment

GR205171 and paroxetine hydrochloride were generous gifts from GlaxoSmithKline laboratory (Harlow, UK), and citalopram hydrobromide from Lundbeck (Copenhagen, Denmark). Range doses of SSRIs (4–16 mg/kg) and that of desipramine HCl (2–8 mg/kg) (Sigma, France) were dissolved in NaCl 0.9% and administered intraperitoneally (i.p.) (25 ml/kg) 30 min before testing. GR205171 (10 and 30 mg/kg) was dissolved in NaCl 0.9% and injected i.p. (25 ml/kg) 15 min before behavioural test.

Only subactive doses of antidepressant drugs (citalopram, desipramine and paroxetine) were co-administered with GR205171; these doses were defined the day of test. Doses of GR205171 were chosen according to our previous results: we have already demonstrated that this compound administered by an intraperitoneal route at the dose of 30 mg/kg in mice, was able to modify the central 5-HT neurotransmission [25], and reversed the effects of centrally administered substance P [23].

2.3. Behavioural tests

2.3.1. Measurement of locomotor activity in mice [5]

Animals (nine mice per group) were kept in the darkened test-room at least 1 h before the test for habituation. After injection (pretreatment and/or treatment), mice were replaced in their holding cages for the required injection-test interval, and then individually transferred to the actimeter for the 10 min test. These animals were different from those used in the forced swimming test. The spontaneous activity of naive animals was recorded using a photoelectric actimeter (OSYS, Laval France). This actimeter consists of a stainless steel apparatus containing transparent cages in which the animals horizontal activity is measured by light beams connected to a photo-electric cell. Results are expressed as the number of light beams broken during the 10 min test period (mean ± S.E.M.) for each group.

2.3.2. Measurement of immobility time in the forced swimming test [41]

The forced swimming test employed was essentially similar to that described elsewhere [40]. Briefly, mice were dropped individually into glass cylinders (height: 25 cm; diameter: 10 cm) containing 10 cm water, maintained at 25 ± 1 °C, and remained there for 6 min. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Six mice were tested simultaneously, and the time of immobility was recorded during the last 4 min of the 6-min testing period, thus after 2 min of habituation. The test was performed by the same well-trained experimenters, blind to the treatment administered. Results are expressed as the immobility time during the 240-s test period (mean ± S.E.M.) for the 10 mice tested in each group.

2.4. Statistical analyses

Statistical analyses were performed using the computer software Sigmastat (Systat software, Erkrath, Germany). A two-way ANOVA on the immobility time (FST) or the locomotor activity was performed with pre-treatment and treatment as main factors. In case of significant interaction factor (pre-treatment × treatment), ANOVA was followed by a Newman–Keuls test (mul-

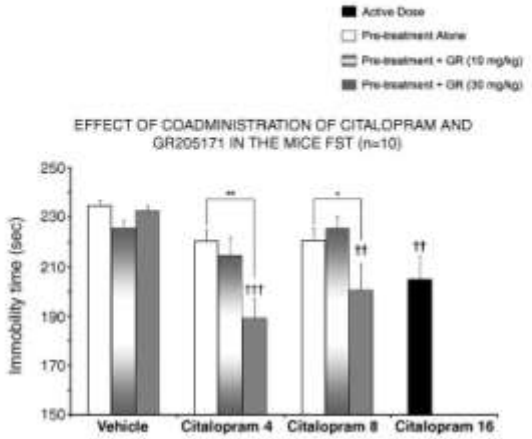

Fig. 1. Interaction of subactive doses of citalopram and GR205171 (10 and 30 mg/kg) in mice FST. All groups consisted of 10 mice. Results are expressed as mean immobility time ± S.E.M. † indicate a significant difference with control group receiving the same treatment (NaCl or GR205171). Asterisks show a significant difference compared to group receiving same dose of antidepressant. ††$p<0.01$; †††$p<0.001$; *$p<0.05$; **$p<0.01$.

tiple pairwise comparison). In case of significant effect of pre-treatment and/or treatment factors (without significant effect of interaction), means were compared to their own control by a Newman–Keuls post hoc test. Statistical significance was set at $p<0.05$.

3. Results

3.1. The forced swimming test

In all groups, GR205171 was found to be devoid of antidepressant-like activity by itself in Swiss mice FST since it did not significantly modified the immobility time as compared to the NaCl-treated control group (Figs. 1–3: $p>0.4$ in each group).

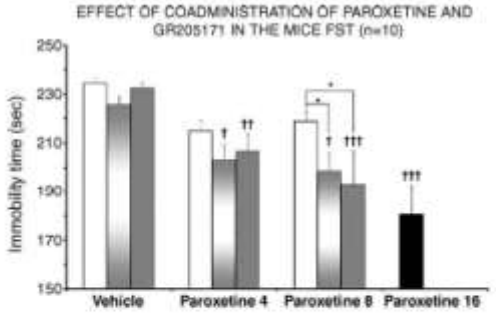

Fig. 2. Interaction of subactive doses of paroxetine and GR205171 (10 and 30 mg/kg) in mice FST. All groups consisted of 10 mice. Results are expressed as mean immobility time ± S.E.M. † indicate a significant difference with control group receiving the same treatment (NaCl or GR205171). Asterisks show a significant difference compared to group receiving same dose of antidepressant. †$p<0.05$; ††$p<0.01$; †††$p<0.001$; *$p<0.05$.

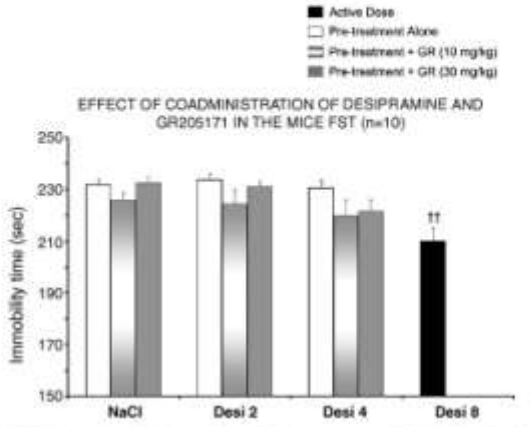

Fig. 3. Interaction of subactive doses of desipramine and GR205171 (10 and 30 mg/kg) in mice FST. All groups consisted of 10 mice. Results are expressed as mean immobility time ± S.E.M. † indicate a significant difference with control group receiving the same treatment (NaCl or GR205171). ††$p<0.01$.

3.1.1. Effect of combined administration of GR205171 and sub-active doses of citalopram in the mouse FST

The two-way ANOVA (pre-treatment × treatment) performed revealed significant main effects of pre-treatment (saline or citalopram: $F_{2,81}=11.56$; $p<0.001$), treatment (saline or GR205171: $F_{2,81}=7.75$; $p<0.001$) and an interaction between the two factors ($F_{4,81}=2.92$; $p<0.05$). Citalopram given alone induced a significant antidepressant-like effect at 16 mg/kg ($p<0.01$), but not at lower doses (4 and 8 mg/kg) as compared to the vehicle-treated control group (Fig. 1).

The lowest dose of GR205171 (10 mg/kg) did not modify the immobility time in mice when combined with inactive doses of the SSRI.

However, the highest dose of GR205171 (30 mg/kg) co-administered with inactive doses of citalopram induced an antidepressant-like effect (in comparison with the corresponding dose of GR205171 given alone ($p<0.001$). The NK1 receptor antagonist also significantly enhanced the antidepressant-like effect of citalopram alone 4 and 8 mg/kg ($p<0.01$ and $p<0.05$, respectively).

3.1.2. Effect of combined administration of GR205171 and subactive doses of paroxetine in the mouse FST

The two-way ANOVA performed revealed significant main effects of pre-treatment (saline or paroxetine: $F_{2,81}=15.18$; $p<0.001$) and treatment (saline or GR205171: $F_{2,81}=3.88$; $p<0.05$), but no interaction between the two factors ($F_{4,81}=0.891$; $p=0.47$). Paroxetine given alone induced a significant antidepressant-like effect at 16 mg/kg ($p<0.001$), but not at lower doses (4 and 8 mg/kg) as compared to the vehicle-treated control group (Fig. 2). The co-administration of GR205171 (at both doses) and paroxetine (4 and 8 mg/kg) induced an antidepressant-like effect when compared to the corresponding doses of GR205171 alone. Interestingly, the NK1 receptor antagonist (10 and 30 mg/kg) significantly enhanced

the antidepressant-like effect of paroxetine only when the SSRI was given at the dose of 8 mg/kg ($p < 0.05$ in comparison to the corresponding doses of paroxetine alone).

3.1.3. Effect of combined administration of GR205171 and subactive doses of desipramine in the mouse FST

The two-way ANOVA performed did not reveal significant effects of pretreatment (saline or desipramine; $F_{2,81} = 2.157$; $p = 0.12$), treatment (saline or GR205171; $F_{2,81} = 1.77$; $p = 0.18$) nor interaction between these two factors ($F_{4,81} = 0.86$; $p = 0.49$) suggesting that GR205171 did not potentiate the antidepressant-like effect of subactive doses of desipramine (2 and 4 mg/kg) in the mouse FST. Desipramine given alone induced a significant antidepressant-like effect at 8 mg/kg only ($p < 0.001$) (Fig. 3).

3.2. Measurement of locomotor activity

3.2.1. Effect of GR205171 on spontaneous locomotor activity of antidepressant drugs

GR205171 (10 and 30 mg/kg) was tested alone or in combination with various antidepressant drugs in the locomotor activity apparatus in order to test whether or not the variation in the immobility time evaluated in the mouse FST could be linked to a modification of the antidepressant-like effect of drugs rather than a modification of the spontaneous locomotor activity.

3.2.2. GR205171 given alone

The results showed that GR205171 did not induce any psychostimulant effects at the doses employed (Table 1). At the opposite, when given alone, GR205171 (10 mg/kg) significantly decreased the locomotor activity in mice ($F_{2,24} = 2.73$; $p < 0.05$).

3.2.3. Citalopram

The two-way ANOVA revealed significant effects of both pre-treatment ($F_{2,72} = 8.59$; $p < 0.001$) and treatment ($F_{2,72} = 7.02$; $p < 0.01$) factors, but no interaction between the two factors ($F_{4,72} = 1.44$; $p = 0.23$). Thus [Cital 8 + NaCl], [Cital 4 + GR10] and [Cital 8 + GR10] induced a psychostimulant effect in comparison with their own control ([NaCl + NaCl] and [NaCl + GR10], respectively). However, although these data would represent a confounding factor in the interpretation of our results, we have reported in the present study that the abovementioned combinations did not produce antidepressant-like effects. Conversely, at the highest dose tested, GR205171 decreased the locomotor activity when co-administered with citalopram 4 mg/kg ($p < 0.05$) suggesting therefore that the enhancement of the mobility time of mice obtained in the FST, following combined administration, was only linked to an enhancement of antidepressant-like activity of the SSRI citalopram.

3.2.4. Paroxetine

The two-way ANOVA performed revealed significant effects of both pre-treatment ($F_{2,72} = 3.57$; $p < 0.05$) and treatment ($F_{2,72} = 5.64$; $p < 0.01$) factors, but no interaction between the two factors ($F_{4,72} = 1.56$; $p = 0.19$). When combined with paroxetine 4 mg/kg, GR205171 (10 mg/kg) increased the locomotor activity ($p < 0.05$), and the effect of treatment revealed a significant sedative effect of [Prx 4 + GR30], when compared to [Prx 4 + NaCl] ($p < 0.05$). The enhancement of the antidepressant-like activity of paroxetine 8 mg/kg by GR205171 was not associated with changes in the spontaneous locomotor activity.

3.2.5. Desipramine

The two-way ANOVA performed revealed significant effects of both pre-treatment ($F_{2,72} = 10.33$; $p < 0.001$) and treatment ($F_{2,72} = 5.13$; $p < 0.01$) factors, but no interaction between the two factors ($F_{4,72} = 1.56$; $p = 0.50$). Thus, desipramine 4 mg/kg induced a sedative effect when given alone ($p < 0.01$). The locomotor activity of mice was not decreased further to administration of GR205171 (in combination with desipramine). The absence of enhancement of antidepressant-like activity of desipramine could then not be linked to a sedative effect of the NK1 receptor antagonist.

Table 1
Effect of two doses of GR205171 on the spontaneous locomotor activity of antidepressants

Pre-treatment	Doses (mg/kg)	Pre-treatment alone	Pre-treatment + GR205171 (10)	Pre-treatment + GR205171 (30)
Vehicle (NaCl)		185.8 ± 13.8	139.8 ± 12.8*	153.2 ± 9.7
SSRI				
Citalopram				
	4	197.9 ± 21.2	**203.7 ± 11.6**	151.2 ± 11.3*
	8	**251.9 ± 12.8**	**209.7 ± 24.4**	182.1 ± 21.4
Paroxetine				
	4	202.3 ± 18.4	**208.0 ± 25.7**	133.0 ± 15.2*
	8	225.0 ± 16.1	194.3 ± 20.6	177.9 ± 24.3
NRI				
Desipramine				
	2	159.4 ± 13.1	134.3 ± 12.1	119.7 ± 10.9
	4	**121.6 ± 9.9**	102.1 ± 9.7	116.1 ± 18.0

Effect of co-administration of antidepressants and GR205171 on spontaneous locomotor activity. All groups consist of nine mice. Data are expressed as numbers of light beams broken during the 10-min testing period. All the statistical analyses were calculated by Newman–Keuls test following significant ANOVA. Asterisks indicate difference between animals receiving (pre-treatment + GR205171) and animals receiving (pre-treatment alone) (*$p < 0.05$). Data in bold indicate difference between animals receiving (Antidepressant ± GR205171) and animals receiving (NaCl ± GR205171) ($p < 0.05$).

4. Discussion

The present study was carried out to evaluate the antidepressant-like activity of the conventional antidepressant drugs, SSRIs and NRIs, when combined with the NK1 receptor antagonist GR205171. Our results provide evidence that the association of both paroxetine and citalopram, but not desipramine, with GR205171 produced a significant antidepressant-like effect in the mouse FST, whereas, each pharmacological agent given separately had no activity. The potentiated efficacy of this combination suggests that the blockade of two distinct targets, the 5-HT transporter and the NK1 receptor, may improve the treatment of depressive episodes in humans.

4.1. NK1 receptor antagonists alone

A dysregulation of the SP neurotransmission in limbic structures has been proposed to play a role in stress-related paradigms and disorders. Accordingly, SP and its preferred NK1 receptors have been identified within the regions of CNS that are traditionally associated with stress (DRN, LC, frontal cortex, hippocampus, amygdala) (for review, see [1]). Acute or chronic stressors are also known to increase SP content in these brain regions [15,31,54]. Moreover, in guinea-pigs, central infusion of SP agonists causes long lasting audible stress vocalizations which can be abolished by pretreatment with NK1 receptor antagonists or antidepressant drugs such as imipramine or fluoxetine [31]. Together, these preclinical data raise interesting questions regarding the putative role of SP and related-NK1 receptors in the pathophysiology of depression and its interaction with central monoamenergic systems. Here it was shown that GR205171 (10 and 30 mg/kg, i.p.) per se, did not produce antidepressant-like activity in the mouse FST (Figs. 1–3). Even if GR205171 alone tended to decrease the spontaneous locomotor activity (Table 1), the lack of antidepressant-like effect could probably not be related to a sedative activity since it was demonstrated that many antidepressant drugs with sedative effects (such as imipramine, desipramine and clonidine) exhibit an antidepressant-like activity in the mice FST [14,27]. Contrasting with the present findings, it was previously shown that a single administration of various NK1 receptor antagonists was reported to reduce the time of immobility in mice ([57]: GR205171 30 mg/kg, i.p.), rats ([13]: CP96345 5 mg/kg, i.p.) and gerbils ([55]: MK869, L742694 and L733060 10 mg/kg, p.o., CP99994 and CP122721 30 mg/kg, p.o.) submitted to FST. Species differences need to be considered, in the pharmacology of the antagonists, given that many of the compounds have reduced affinity for the rodent NK1 receptor, and thus often require high doses to observe an effect [46,47]. Discrepancies may also be attributed to methodological considerations such as distinct time interval between drug administration and the test itself (30 and 15 min), the level of stress between species or the use of different strains of mice since genotype is an important factor determining sensitivity to antidepressant drugs in behavioural tests [7,14,44]. For example, Swiss mice are the most sensitive strain to detect antidepressant-like activity of SSRI or SNRI in the FST. Finally, it can be assumed that in our experimental conditions, a single administration of GR205171 is not sufficient to induce an antidepressant-like effect. Nevertheless, the lack of antidepressant-like activity of GR205171 reported here is consistent with *in vivo* and *in vitro* data showing that an acute administration of NK1 receptor antagonist did not modify the spontaneous firing of DRN 5-HT neurons and the basal extracellular concentration of 5-HT ([5-HT]ext) in the frontal cortex of mice [22,25,57] or rats [12,26,34,38]. Thus, although the behavioural effects of NK1 receptor antagonists remain somewhat equivocal, there is now a large body of evidence suggesting that acute NK1 receptor antagonism does not modify 5-HT neurotransmission within brain regions involved in mood disorders.

4.2. NK1 receptor antagonist in combination

4.2.1. With selective serotonin reuptake inhibitor

It was shown here that a single intraperitoneal administration of the SSRIs citalopram and paroxetine (16 mg/kg), decreased the time of immobility in the mouse FST as compared to the control group receiving vehicle alone (Figs. 1 and 2). Surprisingly, lower doses have failed to produce antidepressant-like effects and these results diverge from previous dose-response studies performed in the mouse FST [14]. Such differences likely result from different factors (housing conditions, testing procedures) known to alter the sensitivity to SSRIs [6,40]. Nevertheless, the main finding of the present study is the significant reduction of time of immobility when subactive doses of citalpopram (4 and 8 mg/kg) and GR205171 (30 mg/kg) were combined as compared to the corresponding groups of mice treated either with citalopram alone ($p < 0.01$ and $p < 0.05$, respectively) or GR205171 (30 mg/kg) alone ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 1). Similarly, a significant reduction of the time of immobility was obtained from the association of the subactive dose of paroxetine (8 mg/kg) with GR205171 (10 and 30 mg/kg) as compared to the corresponding groups of mice treated either with paroxetine alone ($p < 0.05$ for each dose of GR2051171) or GR205171 alone ($p < 0.05$ and $p < 0.001$, respectively) (Fig. 2). It is noteworthy that the combination of SSRIs and GR205171 did not produce psychomotor stimulant effects as compared to SSRI alone (Table 1), suggesting that the effects observed in the mouse FST are specifically related to antidepressant-like activity. The neurochemical changes that could underlie these behavioural effects remain unclear and likely complex. Nevertheless, since an increase in 5-HT availability at nerve terminals is essential for antidepressant-like efficacy, we could infer that the combination of SSRIs and NK1 receptor antagonist have produced an enhancement of 5-HT neurotransmission in the brain regions involved in mood disorders. In line with this assumption, promising results were initially described by using a microdialysis approach in freely moving mice [25].

An important question raised by these microdialysis and behavioural results concerns the mechanism of action of NK1 receptor antagonists and whether these pharmacological agents act via a common molecular target similar to SSRIs? We previously demonstrated that NK1 receptor antagonists act by stimulating the release of 5-HT at nerve terminals rather than

inhibiting its reuptake [25]. In agreement, recent *in vitro* observations using human neocortical synaptosomes indicated that the selective blockade of NK1 receptors did not affect basal 5-HT uptake or the inhibition of 5-HT uptake induced by the SSRI fluvoxamine [35] sugesting that NK1 receptor antagonists do not interfere with the 5-HT transporter protein. Reverse microdialysis experiments with local intra-raphe injection of NK1 receptor antagonist suggested that the main interaction site between Substance P-ergic and serotonergic systems is located in the dorsal raphe nucleus (DRN) indicating that serotonin pathways are playing a critical role in the potentiation of the antidepressant-like activity. These results strongly suggest that co-administration of SSRI and a NK1 receptor antagonist can enhance 5-HT neurotransmission presumably through a lower inhibitory feedback control of the serotonergic neurons by 5-HT1A autoreceptors [24,25].

So far, two mechanisms can be proposed: NK1 receptor antagonists may reduce SSRI-induced activation of the negative feedback on 5-HT system. Otherwise, an effective clearance of 5-HT from the synapse may mask the increase in 5-HT release induced by NK1 receptor antagonists alone. In these conditions, SSRIs might unveil the activity of NK1 receptor antagonists by preventing the 5-HT reuptake process.

4.2.2. *With selective noradrenalin reuptake inhibitor*

In agreement with our previous dose-response study performed in Swiss mice [14], it was shown here that a single administration of the SNRI, desipramine at the dose of 8 mg/kg, i.p., decreased the time of immobility in the mouse FST as compared to the control group receiving vehicle alone (Fig. 3). In addition, GR205171 (10 and 30 mg/kg, i.p.) failed to potentiate the antidepressant-like activity of subactive doses (2 and 4 mg/kg, i.p.) of desipramine (Fig. 3). Although this effect could be related to a sedative activity of GR205171, we have shown that addition of the NK1 receptor antagonist did not modify desipramine-induced decrease in spontaneous locomotor activity (Table 1). It would be a relevant interest to determine whether or not a pharmacological inactivation of NK1 receptors could enhance the antidepressant-like activity of an active dose of desipramine. To our knowledge, no experiments have examined the neurochemical and behavioural changes produced by the association of SNRIs and NK1 receptor antagonists. Therefore, although previous observations have reported that the basal cortical efflux of noradrenaline increased two to four-fold in NK1−/− mice compared to NK1+/+ mice, the net increase in noradrenaline efflux in the cerebral cortex following injection of desipramine did not differ in NK1−/− and NK1+/+ genotype mice [28].

Taken together, our results interestingly suggest that a change in basal 5-HT neurotransmission is probably necessary to obtain an augmentation effect of NK1 receptor antagonist on activity of antidepressant drugs.

5. Conclusion

In conclusion, the present behavioural data in response to the co-administration of SSRIs with a NK1 receptor antagonist paralleled changes measured in cortical [5-HT]ext [24]. It is interesting to note that small molecules with dual NK1 antagonism and serotonin reuptake inhibition properties (NK1/SSRI) exhibit a robust neurochemical and antidepressant-like activities in animal models [48,49]. This new potentiating antidepressant strategy should now arouse psychiatrists towards clinical trials to determine the extend to which this chronic combination would display advantages over existing therapies with conventional antidepressant drugs, particularly to reduce the long delay of action and resistant rate of depressed patients to the SSRIs. It is also interesting to note that many augmentation strategies have been proposed to enhance the effects of currently prescribed antidepressant drugs. The most commonly used augmenting agents (lithium and buspirone; for review, see [30]) have also been demonstrating as being augmenting agents in the mouse FST [9,43] and in intracerebral microdialysis studies in rodents [8,11,20,21]. Consequently, these findings suggest that correlation between microdialysis and FST data had a predictive value on clinical results.

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Résumé de l'étude n°9

Dans cette étude, nous avons mis en évidence une potentialisation des effets de type antidépresseur de la paroxétine et du citalopram lors de la coadministration avec un antagoniste des récepteurs NK1, le GR 205171. Les résultats obtenus dans cette étude sont conformes à ceux obtenus lors des travaux de microdialyse : la potentialisation des effets neurochimiques des IRSSs est associée à une potentialisation de leurs effets comportementaux. Le développement des antagonistes des récepteurs NK1 en tant qu'antidépresseur ayant été abandonné pour cause de manque d'efficacité (en monothérapie), ces résultats peuvent relancer l'intérêt du développement de ces produits sous forme de coadministration.

La potentialisation de l'activité antidépressive des IRSSs par le GR205171 montre que l'augmentation de la concentration extracellulaire d'acéthylcholine induite par l'administration d'IRSSs n'est pas nécessaire à l'apparition de leur effet antidépresseur. En effet, cette augmentation du relargage d'acétylcholine n'est pas présente lorsque les IRSSs sont coadministrés avec un antagoniste des récepteurs NK1. De ce fait, l'absence d'effet de la paroxétine et du citalopram lors de la coadministration avec le GR127935, ne peut être liée à un effet de l'antagoniste des récepteurs 5-HT_{1B} sur la neurotransmission cholinergique.

4.0 DISCUSSION GENERALE

L'objectif de ce travail était d'étudier l'implication du récepteur 5-HT_{1B} dans l'apparition de l'effet antidépresseur des IRSSs dans le FST en démontrant le rôle joué par l'activation des hétérorécepteurs 5-HT_{1B}. Dans un second temps, nous avons cherché à localiser au niveau cérébral les récepteurs 5-HT_{1B} dont l'activation par un agoniste sélectif, l'anpirtoline, permet d'obtenir un effet anti-immobilité dans le FST ; puis nous avons déterminé les voies monoaminergiques dont l'activation est nécessaire pour obtenir les effets thérapeutiques des IRSSs.

4.1 Récepteur 5-HT_{1B} et effet antidépresseur

Plusieurs études ont mis en évidence que l'anpirtoline administrée par voie systémique permet d'induire un effet de type antidépresseur chez des souris Swiss dans le FST (Redrobe and Bourin, 1999; O'Neill and Conway, 2001). Utilisée à dose subactive les agonistes des récepteurs 5-HT_{1B} (anpirtoline ou RU24969) permettent de potentialiser les effets comportementaux de différents antidépresseurs appartenant à différentes classes (imipramine, maprotiline, venlafaxine, fluvoxamine et sertraline) dans le FST chez la Souris (Redrobe et al., 1996; David et al., 2001). De façon surprenante, ces résultats sont obtenus alors que dans le même temps les agonistes des récepteurs 5-HT_{1B} induisent des effets neurochimiques opposés à ceux des antidépresseurs : diminution du relargage, de la synthèse et de l'activité électrique des neurones sérotoninergiques. Par conséquent si sur le plan comportemental les agonistes des récepteurs 5-HT_{1B} permettent d'induire un effet anti-immobilité comparable à celui des antidépresseurs, ils s'opposent à leurs effets physiologiques. Nous avons donc émis l'hypothèse que l'activité de l'anpirtoline est liée à l'activation des hétérorécepteurs 5-HT_{1B}. L'absence de ligands spécifique pour les hétérorécepteurs nous a amené à développer une technique nous permettant de n'activer que les récepteurs 5-HT_{1B} postsynaptiques (hétérorécepteurs). En effet, l'administration de 5,7-DHT (22,8µg) entraîne une destruction des neurones sérotoninergiques ; d'où également une destruction des autorécepteurs 5-HT_{1B}. L'administration d'un agoniste sélectif, l'anpirtoline, permet alors de n'activer que les hétérorécepteurs 5-HT_{1B} chez des souris préalablement prétraitées par cette neurotoxine. Bien que l'anpirtoline possède une affinité pour les récepteurs 5-HT_{1B}, 5-HT_{1A} et 5-HT_2 (Ki respectifs de 28 nM, 151 nM et 1,48 µM ; Metzenauer et al., 1992), l'effet antidépresseur observé dans le FST ne peut être lié qu'à l'activation du récepteur 1B, puisque cet effet antidépresseur induit par l'administration d'une dose active d'anpirtoline est absent chez les souris mutantes

privées du récepteur 5-HT_{1B} alors qu'il est présent chez leurs congénères non mutées (étude n°4). La conservation de cet effet chez les animaux dont le système sérotoninergique a été préalablement lésé (prétraitement par 5,7-DHT) démontre avec certitude l'implication des récepteurs 5-HT_{1B} situés sur des neurones non sérotoninergiques (hétérorécepteurs). Il est intéressant de noter également que l'hypofonctionnement du système sérotoninergique provoqué par la 5,7-DHT entraîne une potentialisation des effets de l'anpirtoline (diminution de plus forte amplitude du temps d'immobilité de 15% par rapport aux animaux non lésés) ainsi qu'une diminution de la dose minimale active dans le test (4 mg/kg au lieu de 8 mg/kg).

La première hypothèse permettant d'expliquer cette potentialisation des effets de l'anpirtoline serait que la destruction des terminaisons neuronales sérotoninergiques présynaptiques par la 5,7-DHT s'accompagne d'une augmentation du nombre d'hétérorécepteurs 5-HT_{1B}, signifiant une « upregulation » (préalablement décrite par Manrique et al., 1993). La seconde hypothèse possible, serait une hypersensibilité des récepteurs consécutive à l'hypofonctionnement du système sérotoninergique, correspondant à une augmentation de leur activité intrinsèque (décrite chez les rats lésés avec de la 5,7-DHT par Nelson et al., 1978; Eide et al., 1988). L'hypothèse la plus probable permettant d'expliquer la potentialisation de l'effet antidépresseur est la variation d'activité intrinsèque. En effet, la lésion maximale observée dans nos études est d'environ 75% et une étude a montré que l' « upregulation » du récepteur 5-HT_{1B} n'apparait qu'à partir de 95% de déplétion (Compan et al., 1998).

Dans une seconde série d'expérimentation, nous avons réalisé une administration locale d'anpirtoline (10 et 20 µg par souris) chez des animaux vigiles que nous avons ensuite directement soumis à un test comportemental (FST). Lorsqu'un agoniste des récepteurs 5-HT_{1B} (l'anpirtoline) est injecté dans les aires cérébrales riches en sérotonine (hippocampe et cortex) et donc en autorécepteurs, aucune diminution du temps d'immobilité n'est observée. Bien qu'aucune « cartographie » ne soit disponible pour différencier la localisation des autorécepteurs et des hétérorécepteurs, les études de microdialyse montrent que l'élévation de 5-HT extracellulaire induite par les IRSSs (citalopram, fluoxétine, fluvoxamine ou paroxétine) dans l'hippocampe et le cortex est potentialisée par des antagonistes des récepteurs 5-HT_{1B} (GR127935, SB224289 ou AR-A000002), démontrant ainsi qu'il s'agit d'autorécepteurs (Gobert et al., 1997; Cremers et al., 2000a; Hervas et al., 2000; Malagie et al., 2001; Stenfors et al., 2004). A l'inverse, lorsque l'anpirtoline est administrée dans des aires cérébrales riches en neurones dopaminergiques et en interneurones GABAergiques (substance noire et caudate

putamen), l'activation des récepteurs 5-HT_{1B} (hétérorécepteurs) permet une augmentation du temps de nage ; confirmant ainsi les résultats obtenus après l'injection de la 5,7-DHT. Une des limites de cette étude est l'absence de détermination de la zone de diffusion de l'anpirtoline. En effet, il aurait été intéressant d'injecter de l'anpirtoline radiomarquée puis de réaliser des coupes cérébrales afin de vérifier que les effets comportementaux sont bien associés à une localisation de l'anpirtoline dans l'aire cérébrale étudiée. Toutefois, compte tenu du faible volume injecté (0,4 µL par coté) et de l'absence de temps de latence entre la fin de l'injection et le début du test, nous pouvons supposer que la diffusion est négligeable ; ce qui est confirmée par l'absence d'effet de l'anpirtoline après injection corticale et hippocampique (le produit ne diffuse pas dans les zones cérébrales permettant d'obtenir un effet).

Les résultats montrant un effet de l'anpirtoline chez les souris prétraitées par la neurotoxine sont d'autant plus intéressants que l'utilisation de 5,7-DHT chez l'animal permet de se rapprocher des modifications physiologiques générées par la dépression (i.e. diminution des concentrations cérébrales de sérotonine). Or nous avons montré qu'une diminution des concentrations tissulaires de sérotonine s'accompagne d'une disparition des effets des IRSSs (paroxétine et citalopram) dans le FST après administration aiguë (voir étude n°8).

Il semble donc possible que l'utilisation d'agonistes des récepteurs 5-HT_{1B} permette une réponse plus rapide des patients dans le traitement de la dépression en comparaison aux traitements par IRSSs. L'hypofonctionnement du système sérotoninergique chez les personnes souffrant de dépression pouvant être associé à une augmentation d'activité intrinsèque des récepteurs postsynaptiques.

Cette étude nous a également permis de constater un changement de la dose minimale active d'anpirtoline dans le FST entre les animaux non opérés (4 mg/kg) et les animaux opérés-non-lésés (8 mg/kg). L'analyse de la bibliographie nous a permis de constater que l'isolement des souris (i.e. pendant 15 jours dans le cas de la 5,7-DHT) diminue les effets d'une dose aiguë d'anpirtoline et d'IRSSs (Rilke et al., 2001). Nous avons donc décidé pour la suite des expérimentations de placer les souris en cage individuelle pendant une durée de 6 jours après la chirurgie puis de les replacer par cage de 6 animaux jusqu'au jour du test.

4.2 Implication du récepteur 5-HT_{1B} dans l'apparition de l'effet de type antidépresseur des IRSSs

Lors de l'administration d'antidépresseurs, les effets de ces médicaments sur l'activité pré-synaptique peuvent être déterminés à l'aide de la technique de microdialyse intra-cérébrale. A l'inverse, les tests comportementaux sont le reflet de l'activation des récepteurs postsynaptiques. D'où l'intérêt de ces tests dans la détermination du mécanisme d'action des antidépresseurs. Toutefois, lors de l'administration d'IRSSs, la réponse observée est la résultante de l'activation de plusieurs systèmes car l'augmentation de sérotonine entraîne l'activation indirecte des 14 sous types de récepteurs sérotoninergiques (Barnes and Sharp, 1999) et donc l'activation des différents systèmes monoaminergiques. Parmis ces 14 sous types de récepteurs, le récepteur 5-HT_{1B} pourrait jouer un rôle primordial dans l'apparition de l'effet de type antidépresseur des IRSSs dans le FST. En effet, deux études réalisées précédemment nous avaient permis d'émettre l'hypothèse selon laquelle les effets des IRSSs dans le FST seraient liés à l'activation des hétérorécepteurs 5-HT_{1B} (Trillat et al., 1998; Gardier et al., 2001). Cependant, dans une étude publiée en 2001, les auteurs montrent que le blocage du récepteur 5-HT_{1B} (blocage pharmacologique et constitutif) permet une potentialisation des effets de la fluoxétine (à dose subactive) dans le test de suspension caudale (Mayorga et al., 2001). Bien que n'excluant pas que la différence observée puisse être liée au test choisi, les auteurs avaient suggérés que l'absence d'effet des IRSSs dans le FST que nous avions obtenu provenait de la souche de souris utilisée (129/Sv). En effet, dans l'étude n°1, nous avons démontré que le choix de la souche de souris à utiliser est primordial puisque des résultats obtenus avec une souche donnée ne sont pas forcément reproductibles avec une autre souche de souris. Par conséquent, l'ensemble des travaux présentés ici a donc été effectué chez des souris Swiss, que nous avons démontré être la souche la plus adaptée pour la mise en évidence d'une activité antidépressive dans le FST. Seule l'étude n°4 n'a pas été réalisée chez ces animaux. En effet, dans cette étude une partie des expérimentations ayant été réalisée chez des animaux mutants n'exprimant pas le gène codant pour le récepteur 5-HT_{1B}, il était donc nécessaire d'utiliser comme contrôle des souris ayant le même fond génétique (129/Sv).

Les études d'interactions entre le GR 127935 (antagoniste sélectif des récepteurs 5-HT_{1B}, De Vries et al., 1997) et les antidépresseurs (imipramine, désipramine, paroxétine et citalopram) chez des souris Swiss ont confirmé nos résultats préliminaires obtenus chez des souris 129/Sv nous permettant ainsi de démontrer avec certitude que le blocage

pharmacologique du récepteur 5-HT_{1B} entraîne la disparition des propriétés antidépressives des inhibiteurs de recapture sélectifs de la sérotonine dans le test de la nage forcée chez la souris ; suggérant ainsi que la différence entre nos données et celles de Mayorga et al. vient du test choisi. Il est également intéressant de noter que dans cette étude, Mayorga et al. ont démontré que le blocage pharmacologique du récepteur 5-HT_{1A} (à l'aide d'un antagoniste spécifique, le WAY 100635) antagonise l'effet anti-immobilité de la fluoxétine dans le TST, alors que dans le FST l'absence de récepteurs 5-HT_{1A} (souris mutées génétiquement) induit une potentialisation de l'effet antidépresseur de la paroxétine utilisée à dose active (Guilloux et al., 2006).

Cette différence entre le FST et le TST est également retrouvée au niveau neurochimique, puisque le TST entraîne une modification du taux de renouvellement de la sérotonine dans l'hypothalamus et le striatum ainsi qu'une modification des concentrations tissulaires de noradrénaline ; alors que ces changements ne sont pas retrouvés après un FST ; suggérant que les deux tests ne mettent pas en jeu les mêmes mécanismes physiologiques. Cette différence dans les modifications neurochimiques est en accord avec le fait que les modèles animaux de dépression ne permettent pas seulement de mettre en évidence l'activité antidépressive de nouvelles molécules, mais peuvent également être utilisés pour déterminer les mécanismes d'action de ces substances (voir notre étude n°1 : Bourin et al., 2005) en faisant varier le test, les souches de souris et en coadministrant des ligands spécifiques.

Il est intéressant de noter que cette différence entre les deux tests peut avoir un impact sur la capacité des molécules à montrer un effet de type antidépresseur. Ainsi, lors de la réalisation d'un test de la nage forcée, les concentrations basales de sérotonine sont augmentées dans le cortex ($p<0.05$), l'hippocampe et le striatum (NS). Cette élévation des concentrations tissulaire de 5-HT pourrait donc masquer les effets de faible doses d'IRSSs dans ce test ; les fortes doses restant pour leur part active. A l'inverse, les concentrations de 5-HT sont diminués dans l'hippocampe et le striatum de souris après exposition à un test de suspension caudale ; l'administration d'une faible dose d'IRSS s'oppose donc à l'effet du test sur les concentrations tissulaires de sérotonine. Ceci est en accord avec les résultats montrant que l'administration d'une dose unique de citalopram (l'IRSS le plus sélectif pour le transporteur à la sérotonine ; cf Figure 8) induit un effet de type antidépresseur dans le FST chez les souris Swiss à partir de 8 mg/kg (voir le tableau récapitulatif de l'étude n°2) alors que ce produit est actif dans le TST à partir de 2 mg/kg chez la même souche de souris (Ripoll et al., 2003).

Des travaux réalisés chez le rat ont quant à eux mis en évidence que la coadministration d'imipramine, de désipramine ou de moclobémide avec un antagoniste des récepteurs 5-HT_{1B} (GR127935 ou SB216641) permet la potentialisation des effets de doses subactives de ces antidépresseurs (Tatarczynska et al., 2004a) mais ne permet pas de potentialiser les effets d'une dose subactive de citalopram. La différence entre nos résultats (absence de potentialisation des effets de l'imipramine et de la désipramine) et ceux obtenus par Tatarczynska et al. peut s'expliquer par l'espèce animale utilisée (Souris versus Rat) ou bien également par la dose de GR127935 administrée (4 mg/kg versus 10 et 20 mg/kg).

4.3 Voies monoaminergiques impliquées dans le mécanisme d'action des IRSSs

Nous avons démontré que l'activation indirecte des hétérorécepteurs 5-HT_{1B} par les IRSSs permet l'apparition de l'activité antidépressive de ces molécules. Ces récepteurs jouant un rôle sur l'activité des neurones GABAergiques, glutamatergiques, cholinergiques et dopaminergiques, nous avons voulu déterminer le(s)quel(s) de ces systèmes étai(en)t impliqué(s) dans l'effet anti-immobilité des antidépresseurs dans le FST.

Système dopaminergique :

De plus en plus d'études ont mis en évidence l'implication du système dopaminergique aussi bien dans la dépression que dans son traitement (Dailly et al., 2004) ; il a ainsi été suggéré que l'altération du système dopaminergique pourrait jouer un rôle important dans la dépression. Lors d'études précédentes réalisées au sein du laboratoire, il a été démontré que l'utilisation de ligands dopaminergiques permet de moduler la réponse au traitement par antidépresseurs dans le FST et dans le TST (Renard et al., 2001). De même, une augmentation du relargage de la dopamine dans le cortex préfrontal (Tanda et al., 1994; Pozzi et al., 1999) et la substance noire (Thorre et al., 1998) est observée chez le Rat après administration d'IRSSs. Afin de déterminer quel pouvait être le rôle précis de la dopamine dans l'apparition des effets comportementaux des antidépresseurs, nous avons étudié les effets de la paroxétine, du citalopram, de la désipramine et de l'imipramine chez des animaux déplétés en dopamine (prétraitement par de la 6-OHDA). Nous avons ainsi pu mettre en évidence que la lésion du système dopaminergique entraîne la disparition des propriétés anti-immobilité des IRSSs dans le test de la nage

forcée. L'augmentation de la neurotransmission dopaminergique observée suite à l'administration de ces antidépresseurs est donc nécessaire à l'apparition de leur activité antidépressive. La lésion par la 6-OHDA entraîne également une lésion du système noradrénergique qui pourrait expliquer la perte d'activité des IRSSs dans le FST. Toutefois, cette explication semble peu probable du fait de la faible lésion du système noradrénergique et de la conservation de l'activité antidépressive de la désipramine qui agit en inhibant le recaptage de cette monoamine. De plus, il a été montré que la lésion du système noradrénergique à l'aide de DSP-4 ne modifie pas l'activité d'un autre antidépresseur, la fluoxétine (Gavioli et al., 2004). Par conséquent, la perte d'efficacité des IRSSs dans le FST chez des animaux prétraités par la 6-OHDA est uniquement liée à la destruction des neurones dopaminergiques.

La similitude des résultats obtenus après administration soit de GR127935 soit de la 6-OHDA (i.e. conservation des effets de l'imipramine et de la désipramine mais pas des IRSSs) confirme le lien existant entre les récepteurs 5-HT_{1B} et la dopamine (voir tableau 5). Nous pouvons donc conclure que l'administration d'IRSS entraîne une augmentation de la libération de sérotonine qui entraîne une activation des récepteurs 5-HT_{1B} permettant ainsi une diminution du relargage de GABA et une activation de la neurotransmission dopaminergique (désinhibition) et donc l'apparition des effets des IRSSs. Ces résultats sont en accord avec l'apparition d'un effet antidépresseur suite à l'injection locale d'anpirtoline dans la substance noire et le caudate putamen ; ces aires cérébrales étant riches en dopamine et en GABA.

Système cholinergique :

Lors d'un traitement par un IRSS, l'augmentation de la neurotransmission sérotoninergique s'accompagne d'une augmentation de la libération d'acétylcholine (Maura and Raiteri, 1986; Cassel et al., 1995; Feuerstein et al., 1996a). Cet effet des IRSSs sur la neurotransmission cholinergique est dépendant des récepteurs 5-HT_{1B} (désinhibition cholinergique suite à la diminution du relargage de GABA) et des récepteurs NK1 (augmentation du relargage de substance P suite à l'activation des récepteurs 5-HT2) (Feuerstein et al., 1996a). Par conséquent, la coadministration de GR127935 (ou de GR205171) avec un IRSS limite l'augmentation de la neurotransmission cholinergique qui serait observée si l'IRSS avait été administré seul. Contrairement à ce qui est observé lors de l'administration de l'antagoniste des récepteurs 5-HT_{1B}, le GR205171 permet de

potentialiser les effets de la paroxétine et du citalopram dans le FST. Les résultats obtenus dans l'étude n°9 démontrent ainsi que l'augmentation de la neurotransmission cholinergique observée suite à l'administration systémique d'IRSSs n'est pas nécessaire à l'apparition de l'effet anti-immobilité dans le test de la nage forcée chez la souris.

4.4 Différence de profil entre les antidépresseurs

De façon surprenante, dans ces expérimentations la désipramine et l'imipramine présentent des profils d'actions distincts de ceux des IRSSs ; suggérant ainsi l'implication de voies monoaminergiques postsynaptiques différentes. En effet, la déplétion en dopamine et l'utilisation d'antagonistes des récepteurs 5-HT_{1B} permettent d'antagoniser les effets des IRSSs mais pas de la désipramine ni de l'imipramine. De même, le GR205171 (antagoniste des récepteurs NK1) permet la potentialisation des effets de la paroxétine et du citalopram, mais pas de l'imipramine. Bien que ces produits présentent tous une activité dans le FST chez la souris (pour revue voir (Petit-Demouliere et al., 2005), les voies monoaminergiques impliquées semblent différentes. Compte tenu de la forte affinité de ces produits pour les transporteurs de la noradrénaline, il est possible que ces produits exercent leurs effets dans le FST par une augmentation de la neurotransmission noradrénergique (pour les affinités respectives de ces produits pour les transporteurs à la sérotonine et à la noradrénaline voir Frazer, 2001). Ceci serait en accord avec des résultats préliminaires obtenus au sein du laboratoire qui montrent que le prétraitement par *p*-CPA ne modifie pas de façon significative l'effet anti-immobilité de l'imipramine et de la désipramine. La composante noradrénergique étant plus importante que la composante sérotoninergique lors de l'utilisation de ces produits, leurs effets ne peuvent donc pas être antagonisés par le GR127935.

Ces travaux posent une question quant à l'implication du système noradrénergique dans le mécanisme d'action des IRSSs. En effet, comme nous l'avons montré dans la partie analyse bibliographique, l'administration d'agonistes des récepteurs 5-HT_{1B}, de sérotonine ou d'IRSSs, s'accompagne d'une augmentation du relargage de la dopamine dans différentes aires cérébrales ; or il a été démontré que l'augmentation de neurotransmission dopaminergique dans le noyau accumbens entraîne une augmentation du relargage de noradrénaline (Vanderschuren et al., 1999). De récents travaux ont également mis en évidence que les effets du CP94253 (agoniste des récepteurs 5-HT_{1B}) peuvent être antagonisés par l'idazoxan, un antagoniste des récepteurs α_2

noradrénergiques (Tatarczynska et al., 2005). Ceci suggère, même si aucune donnée n'a encore été publiée, que l'administration d'un antagoniste des récepteurs α_2 noradrénergiques pourrait permettre d'antagoniser les effets des IRSSs, alors qu'il a été montré que les agonistes des récepteurs α_2 noradrénergiques (i.e. la clonidine) exercent une activité de type antidépresseur, ou peuvent être utilisés pour potentialiser les effets comportementaux d'autres antidépresseurs (Malinge et al., 1988; Bourin et al., 1991; Bourin et al., 1996). Ceci est en accord avec une étude récente qui met en évidence une disparition des effets des IRSSs (fluoxétine, sertraline et paroxétine) dans le TST chez des souris mutées ne pouvant plus synthétiser de noradrénaline (mutation du gène codant pour la dopamine ß-hydroxylase).

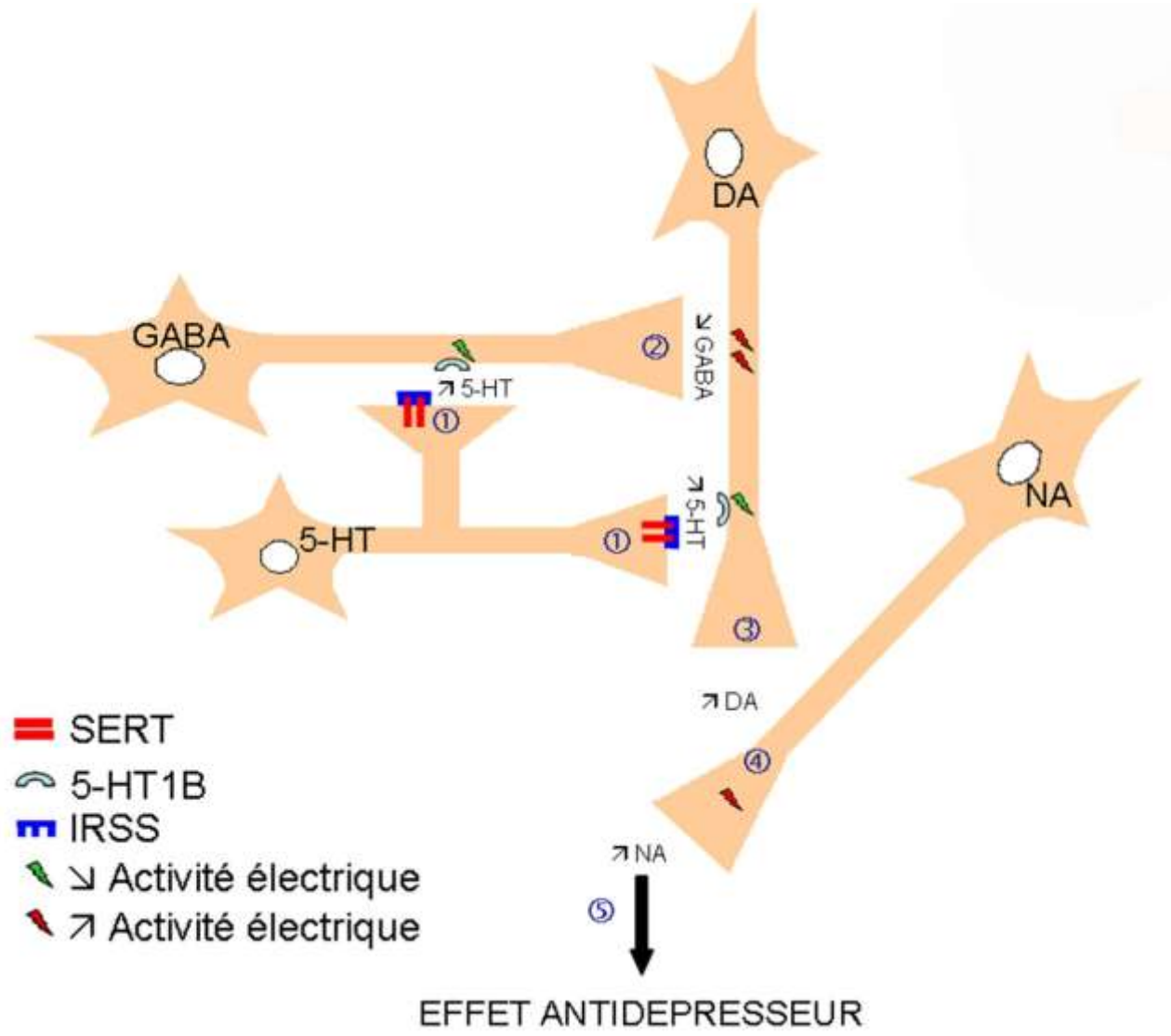

Figure 15 : Représentation schématique des voies monoaminergiques postsynaptiques impliquées dans le mécanisme d'action des IRSSs.

① L'administration d'un IRSS induit une augmentation du relargage de 5-HT et l'activation des récepteurs 5-HT_{1B} situés sur les neurones GABAergiques et dopaminergiques

② L'activation du récepteur 5-HT_{1B} GABAergique entraîne une diminution du relargage de GABA responsable d'une désinhibition de la neurotransmission DAergique

③ La neurotransmission dopaminergique est donc soumise à 2 composantes :
- excitatrice via le GABA
- inhibitrice via le récepteur 5-HT_{1B} situé sur les neurones dopaminergiques

La résultante est une augmentation du relargage de DA

④ L'augmentation de la libération de DA entraîne une augmentation de la libération de NA

⑤ L'augmentation de la neurotransmission noradrénergique induit un effet antidépresseur

5.0 CONCLUSION

Grâce à ce travail, nous avons tenté de comprendre les mécanismes neurobiologiques qui sont mis en œuvre dans le FST après une administration d'IRSS pour induire un effet de type antidépresseur.

A la fin de ce travail, nous pouvons conclure que l'effet de type antidépresseur des IRSSs dans le FST, après administration par voie intra-péritonéale, est induit par l'activation de récepteurs 5-HT_{1B} présents au niveau du caudate putamen et de la substance noire sur des neurones GABAergiques. L'activation de ces récepteurs permet une diminution du relargage de GABA et une désinhibition des voies dopaminergiques. La dopamine est donc un facteur limitant dans l'activité des IRSSs dans le FST.

Les antidépresseurs permettant d'inhiber la recapture de la noradrénaline (imipramine et désipramine) conservant une activité chez les animaux déplétés en dopamine, il apparaît que si les voies noradrénergiques sont impliquées dans l'apparition de l'effet antidépresseur des IRSSs dans le FST, cette implication est consécutive à l'augmentation de la neurotransmission dopaminergique.

Afin de confirmer cet effet des IRSSs sur les neurones noradrénergiques, il serait intéressant de mettre en place des études électrophysiologiques et/ou de microdialyse pour observer l'effet local d'agonistes des récepteurs 5-HT_{1B} sur l'activité des neurones noradrénergiques chez des animaux contrôles et déplétés en dopamine. De même, il serait également intéressant d'évaluer l'effet antidépresseur dans le FST des IRSSs et de l'anpirtoline chez des animaux déplétés en noradrénaline. Ceci nécessite la mise en place d'une méthode de déplétion avec le 6-OHDA en protégeant le système dopaminergique, le DSP-4 (neurotoxine spécifique du système noradrénergique), ne permettant qu'une déplétion des fibres issues du Locus Coeruleus.

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7.0 ANNEXES

Article 1 :

Dopamine, depression and antidepressants

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REVIEW ARTICLE

Dopamine, depression and antidepressants

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ABSTRACT

The relationship between depression and dopamine deficiency in the mesolimbic pathway has been hypothesized for many years. The experimental studies with animal models of depression and the human studies implicate the role of the dopamine system in depression. Not only do dopaminergic receptor agonists, but also antagonists such as olanzapine exhibit antidepressant effects associated with standard antidepressants in patients with treatment-resistant depression. This paradoxical result suggests that further investigations are necessary to understand the role played by dopamine in depression.

INTRODUCTION

The monoamine hypothesis based on the deficiency of one or other monoamines is commonly evoked to explain the physiopathology of depression. This hypothesis, initially based on noradrenaline and serotonin deficiency, is extended to dopamine. The implication of dopamine was suggested earlier by clinical observations. Thus, depression is a common disturbance in schizophrenia and Parkinson's disease which are pathologies known to present with a dopamine central system dysfunction [1,2]. Moreover, there are similarities between symptoms of Parkinson's disease, schizophrenia and depression. Some symptoms of depression such as anhedonia (inability to experience pleasure) and decreased motor activity are also observed in schizophrenia [3,4]. The symptoms of Parkinson's disease such as psychomotor retardation and diminished motivation are common in depressed patients [5]. Biochemical evidence in patients with depression is derived from the study of homovanillic acid, a dopamine metabolite. Reduced venoarterial plasma concentration gradients of homovanillic acid were found in depressed patients [6]. This likely implication of dopamine in depressive illness was also proved by the technique of acute tyrosine depletion [7]. Tyrosine is the precursor of dopamine synthesis and results of neuropsychological tests of healthy volunteers with a reduction in tyrosine availability to the brain paralleled those reported in previous investigations of unipolar depression. All these results are consistent with the hypothesis of a role of dopamine in depression physiopathology. To understand this role, data concerning dopamine cerebral pathways and receptors are shortly reviewed. From these elements, the relationships between dopamine, depression and antidepressants are examined.

DOPAMINE PATHWAYS AND RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

Four main dopaminergic pathways were identified within the central nervous system. The ventral tegmental area is the place of origin of two projection pathways towards the cortex (the mesocortical pathway) and the limbic area (the mesolimbic pathway); the hypothalamus is the place of origin of a projection towards the pituitary gland which controls prolactin secretion (the tuberoinfundibular pathway) and a dopaminergic projection extends from the substantia nigra to the striatum (the nigrostriatal pathway) the degeneration of which is implicated in Parkinson's disease.

Using these pathways, dopamine receptors are located. Five genes encoding dopamine receptors were identified.

These receptors are divided in two subfamilies: the D_1-like receptor subtypes (D_1 and D_5) coupled with the Gs protein activate adenylyl cyclase and the D_2-like subfamily (D_2, D_3, and D_4) coupled with G proteins inhibit adenylate cyclase [8]. D_1 and D_2 dopamine receptors are the most abundant subtypes in the central nervous system, but D_1 dopamine receptor is the most widespread. D_1 mRNA was found in the striatum, nucleus accumbens, olfactory tubercule, hypothalamus and thalamus. In other areas such as substantia nigra pars reticula with numerous binding sites for the D_1 dopamine receptor, no mRNA was detected, suggesting that in these areas the D_1 dopamine receptor is present in projections only [9]. The D_5 dopamine receptor is expressed at much lower level than the D_1 dopamine receptor and its distribution is limited to the hippocampus and thalamus (the lateral mamillary nucleus and the parafascicular nucleus of the thalamus). The D_2 dopamine receptor is located mainly in the striatum, olfactory tubercule, nucleus accumbens, the substantia nigra pars compacta, the ventral tegmental area and the pituitary gland. D_2 dopamine receptors are pre- and post-synaptic receptors contrary to D_1-like receptors which are mainly post-synaptic receptors [9]. D_4 dopamine receptors were found with a low expression in the basal ganglia and a higher expression in the frontal cortex, medulla, amygdala, hypothalamus and mesencephalon. However, this high expression is weak in comparison with other dopamine receptors [9]. D_3 dopamine receptors are expressed in the limbic area (nucleus accumbens, olfactory tubercule and islands of Calleja) and at a lower level in the striatum [9]. The D_3 dopamine receptors exist as autoreceptors that inhibit neuronal dopamine synthesis and post-synaptic receptors. These receptors by negatively regulating dopamine neuronal activity and/or by post-synaptic action exhibit an inhibitory influence on locomotor activity [10].

The genetic techniques for negatively modulating dopamine receptor expression such as knockout animals and antisense technology showed that the disruption of D_3, D_4, D_5 dopamine receptor functions involved an increase or an improvement in the behavioural activity of animals contrary to the results observed with the disruption of D_1, D_2 dopamine receptor functions. Although these results have to be interpreted with caution as a compensatory mechanism could develop, these observations suggest that the most abundant dopamine receptors D_1 and D_2 are involved in positive regulation of behavioural activity whereas the D_3, D_4, D_5 receptors are inhibitory by likely negative modulation of D_1 and/or D_2 receptor function in some cases [10].

Numerous studies investigated the role of different 5-HT receptors in the control of brain dopamine transmission. This relationship between serotonin and dopamine systems was demonstrated by microdialysis studies. Thus, the serotoninergic stimulation of the prefrontal cortex [11], the striatum [12,13] or the nucleus accumbens [14,15] potently releases dopamine. The increase of dopamine release in the prefrontal and frontal cortex by action of 5-HT_{1A} agonists was shown by different authors [16,17]. Lejeune and Millan [18] demonstrated that the selective activation of 5-HT_{1A} receptors also elicits an increase in the ventral tegmental area dopaminergic output. In vitro, 5-HT_{1B} receptors have been shown to underlie the 5-HT-induced inhibition of $GABA_B$ receptor-mediated inhibitory post-synaptic potentials in dopamine neurones of the rat mid-brain [19]. In vivo, the selective activation of 5-HT_{1B} receptors does not cause a significant change in the basal activity of dopamine neurones in the ventral tegmental area, suggesting that 5-HT_{1B} receptors do not play a role in the control of the mesolimbic dopamine system [20]. However, the intrastriatal administration of a 5-HT_{1B} receptor agonist induces a significant increase of dopamine level [21]. Similarly, the 5-HT moduline which is a 5-$HT_{1B/1D}$ receptor endogenous modulator, increases the release of dopamine in the striatum after direct administration in this area [21]. An inhibitory action of 5-HT on dopamine neurones in the ventral tegmental area could be exerted through 5-HT_2 receptors and more precisely the 5-HT_{2C} receptors which play a prominent role in the control of mesocorticolimbic dopamine pathways: 5-HT_{2C} receptor agonists decrease, whereas 5-HT_{2C} receptor antagonists enhance mesocorticolimbic dopamine function [22]. This control is also dependent on adenosine. Thus, the adenosine–dopamine interactions modulate dopaminergic function in the basal ganglia where the mesolimbic, nigrostriatal dopamine pathways lead to. The interaction between adenosine A_{2A} and dopamine D_2 receptors and between adenosine A_1 and dopamine D_1 receptors regulate different GABAergic neurones in the basal ganglia [23].

DOPAMINE AND DEPRESSION

Increasing evidence from human and animal studies suggest a relationship between dopamine transmission in the central nervous system and depression. In depressed patients, a compensatory up-regulation of D_2 receptor density was observed in the basal ganglia/cerebellum in comparison with healthy subjects accord-

ing to the hypothesis of an association between depression and a deficiency of dopamine transmission [24]. Surprisingly, an up-regulation of dopamine transporter which results in a more effective re-uptake of dopamine into the pre-synaptic neurones was found in depressed patients [25]. The expected result was a down-regulation of dopamine transporter in depressed patients to compensate the deficiency of dopamine transmission. The authors explain this unexpected result by alteration of dopamine transporter which would be primary to the compensatory mechanism and would lead to low intrasynaptic dopamine concentration. Anhedonia is a frequent symptom of depression and it is commonly hypothesized that anhedonia is associated with a dysfunction of the dopaminergic reward system [26]. Indeed, this system is functionally and anatomically closely connected with the dopamine mesolimbic pathway. A dysfunction of both ascending dopaminergic pathways is therefore expected to cause anhedonia. However, this hypothesis is not supported by experiments showing that dopaminergic dysfunction is associated with a disorder of motivation rather than anhedonia [27].

The animal models of depression also suggest an implication of dopamine in the physiopathology of depression. The forced swimming test in an animal model was used to predict the antidepressant activity of drugs. With this model, Cervo et al. [28] showed that the mesolimbic dopaminergic system has a permissive role in the effect of desipramine, as the antidepressant-like effect of desipramine was reduced after the administration of sulpiride bilaterally into the nucleus accumbens. An indirect effect of dopaminergic system in the antidepressant-like activity of selective serotonin re-uptake inhibitors was also shown by Renard et al. [29] in the mice forced swimming test, as the antidepressant-like effect of selective serotonin re-uptake inhibitors was modulated by agonists and antagonists of dopamine receptors. The chronic mild stress-induced anhedonia is another animal model of depression. The behavioural and biochemical changes associated with the chronic mild stress are a decrease in D_2/D_3 receptor binding in the nucleus accumbens [30] and a functional subsensitivity to the rewarding and locomotor stimulant effects of the D_2/D_3 agonist quinpirole administered systemically or within the nucleus accumbens [31]. Other studies in rodents demonstrated that exposure to stress decreases levels of brain-derived neurotrophic factor in brain regions associated with depression [32]. Moreover, a link exists between dopamine system and brain-derived neurotrophic factor, as it appears that the brain-derived neurotrophic factor controls the expression of D_3 receptor gene [33]. These results suggest the neurotrophic hypothesis of depression implicates also the dopaminergic system.

DOPAMINE AND ANTIDEPRESSANTS

The relationship between dopamine and depression was confirmed by the fact that antidepressants act on the dopamine system. Studies on animal models suggest that antidepressants enhance neurotransmission in the dopamine mesolimbic system [34,35]. More precisely, chronic treatment with antidepressant drugs induce presynaptic dopamine receptor subsensitivity and/or postsynaptic dopamine receptor supersensitivity which could be mediated by the inhibition of melatonin-induced effects [36]. In the frontal cortex of rats, antidepressants such as desipramine, a potent inhibitor of the noradrenaline re-uptake carrier, increases extracellular concentrations of dopamine by preventing the dopamine re-uptake into noradrenergic neurones [37,38]. Fluoxetine, a selective serotonin re-uptake inhibitor also increases the extracellular dopamine concentration in the prefrontal cortex by a mechanism not dependent on serotonin [39]. However, depletion of serotonin prevented the antidepressant-like effect of fluoxetine but not desipramine in the rat forced swimming test [40]. Thus, this elevated extracellular concentration of dopamine in the frontal cortex plays a role in the antidepressant-like effect of desipramine but is insufficient to explain the antidepressant-like effect of fluoxetine in the forced swimming test.

Elements to understand the effect of antidepressants such as the selective serotonin re-uptake inhibitor is given by the study of the mechanism which links serotonin and dopamine systems. The indirect action of serotonin re-uptake inhibitors on dopamine transmission partly explains the delay before appearance of a therapeutic effect of serotonin re-uptake inhibitors. Indeed, Prisco and Esposito [41] showed that fluoxetine inhibits dopaminergic function in the ventral tegmental area by enhancing the synaptic levels of serotonin which possibly acts through $5\text{-HT}_{2C/2B}$ receptors. Chronic fluoxetine administration induce tolerance to its inhibitory effect on dopaminergic activity, possibly as a consequence of the down-regulation of $5\text{-HT}_{2C/2B}$ receptor. From this result, the delay before appearance of an antidepressant effect with fluoxetine could partly be attributed to the delay before emergence of a tolerance to the inhibition of

mesocorticolimbic transmission of dopamine because of the action of serotonin on the 5-HT_2 receptors. The adenosine receptors are other potential targets to modulate dopamine function and consequently to obtain the antidepressant effect. Thus, an antidepressant-like effect of adenosine A_{2A} receptor antagonist was found in the forced swimming test and was prevented by administration of haloperidol, a dopamine D_2 receptor antagonist, suggesting that the antidepressant-like effect of adenosine A_{2A} receptor antagonists was mediated by an increase in dopaminergic transmission [42].

A direct rather than an indirect action of drugs on the dopamine system could also constitute another strategy to obtain an antidepressant effect. Thus, the antidepressant-like activity of bupropion which was presented as a selective inhibitor of dopamine uptake by blocking the dopamine transporter was demonstrated in animal models of depression such as the forced swimming test [43]. However, this pharmacological mechanism remains questionable as the occupancy of dopamine transporter sites by bupropion is low during clinical treatment, suggesting that another mechanism could take part in the antidepressant effect of bupropion [44]. However, the antidepressant-like effect of a pure dopamine re-uptake inhibitor GBR 12783 in the forced swimming test seems to depend on the stimulation of dopaminergic system and particularly D_2 but not D_1 dopamine receptors [45]. Other antidepressants capable of increasing the dopamine extracellular concentrations were developed but with an unspecific action for the dopamine system. Thus, moclobemide, a reversible monoamine oxidase A inhibitor [46], and DOV 21-947, an inhibitor of serotonin noradrenaline and dopamine re-uptake [47] can induce an increase of extracellular dopamine concentrations. The development of this last antidepressant acting on serotonin, noradrenaline and dopamine systems was based on the hypothesis that dopamine has a pivotal role in depression and that a broad antidepressant spectrum will produce a more rapid onset of action and/or higher efficacy than agents inhibiting the re-uptake of serotonin and/or noradrenaline [48]. This hypothesis is consistent with results previously presented concerning the delay before appearance of the antidepressant effect induced with a selective serotonin re-uptake inhibitor.

According to the hypothesis of the dopamine system deficiency in depression, the correction of this deficiency by direct agonists of dopamine receptors could constitute a therapeutic strategy. Thus, pramipexole, a D_2/D_3 agonist, reversed the effects of chronic mild stress [49]. This strategy was evaluated in the mouse forced swimming test by adding dopamine agonists to selective serotonin reuptake inhibitors [29]. The antidepressant-like effect of citalopram, fluvoxamine and paroxetine is potentiated when pre-treated with a D_1-specific agonist (SKF 38393), a D_2 agonist (bromocriptine), a D_3 agonist (PD 128 907) and a D_1 antagonist (SCH 23390). This last result with a D_1 antagonist can be explained according to the hypothesis of a monoamine deficiency in depression by blockade of pre-synaptic receptor which prevents the inhibitory feedback mediated by this receptor. This hypothesis is substantiated by Cho et al. [50] who showed that SCH 23390 induces an increase of the firing rate of dopamine. This association of antidepressants and drugs acting on the dopamine system was also investigated in humans. In the treatment of refractory depression, a potential benefit of the addition of cabergoline, a dopamine agonist, with minalcipran, a serotonin noradrenaline re-uptake inhibitor was found [51]. However, this result concerns only two case reports. Similarly, a clinical improvement in the treatment of resistant depression was found by adding amantadine, which is known to exhibit effect at the level of dopaminergic system, with standard antidepressant treatments but in an open study [52]. Another case report also indicates that the addition of a dopamine agonist, bromocriptine, to a standard antidepressant, imipramine, improved depressive symptoms in a woman with refractory depression [53]. These results have to be confirmed by double-blind controlled trials. The antidepressant effect of pramipexole which exerted an antidepressant effect in bipolar and treatment-resistant depression in open trials [54,55] and case reports [56] was investigated in patients with treatment-resistant bipolar depression by a randomized, double-bind, placebo controlled trial [57]. The authors of this study concluded that pramipexole added to mood stabilizers was effective among patients with bipolar depression but larger randomized controlled trials are needed to affirm these initial observations. All these results indicate the potential benefit of the stimulation of the dopaminergic system in resistant depression.

According to these results, the blockade of the dopaminergic system by antipsychotic drugs contribute to the emergence of depressive symptoms. This hypothesis was confirmed by Bressan et al. [58] in schizophrenic patients treated by typical antipsychotic drugs. They found that the degree of striatal dopamine D_2 receptor blockade induced by typical antipsychotic treatment was directly correlated with the presence and severity of depressive symptoms in schizophrenia. However, olanza-

pine which is an atypical antipsychotic has a greater therapeutic activity in depressive symptoms accompanying schizophrenia than haloperidol [59]. Atypical antipsychotics have a 5-HT_{2C} antagonistic activity contrary to typical antipsychotic. This activity could explain that these compounds can opposite to the decrease of dopamine mesolimbic transmission and present a greater antidepressant activity than typical antipsychotics according to the hypothesis of monoamine deficiency in depression. Surprisingly, the combination of an atypical antipsychotic olanzapine, with a selective serotonin re-uptake inhibitor, fluoxetine demonstrated clinical efficacy in a double-blind clinical trial in patients presenting with treatment-resistant depression [60]. The antagonistic activity of olanzapine for dopamine receptor could appear inconsistent with the theory of monoamine deficiency. However, a large increase of extracellular dopamine concentration in rat prefrontal cortex was observed when olanzapine was administered in combination with fluoxetine [61]. The addition of 5-HT_{2C} antagonist to selective serotonin reuptake inhibitor also produced a potentiation of selective serotonin reuptake inhibitor-induced increases at the hippocampal serotonin level. Moreover, the antidepressant-like effect of fluoxetine is enhanced in the tail suspension test with a line of 5-HT_{2C} receptor-null mutant mice. All these results suggest the impact of a 5-HT_{2C} antagonist activity to reinforce the antidepressant effect of selective serotonin reuptake inhibitor [62]. Another explanation could be that a reduction of functional dopamine activity could have an antidepressant effect in certain subtypes of depression. This hypothesis is consistent with the antidepressant properties of roxindole, a dopamine autoreceptor agonist as such an agonist reduces the firing rate, as well as synthesis and release in dopaminergic neurones through a negative feedback mechanism via stimulation of autoreceptors [63]. Similarly, the efficacy of pramipexole, a dopamine agonist which demonstrated its antidepressant activity [57] cannot be only attributed to the stimulation of dopaminergic system by pramipexole as this drug stimulates D_2 post-synaptic receptors only at high doses, but stimulates the dopamine D_3 presynaptic autoreceptors which decrease dopamine transmission, at low doses [64].

CONCLUSION

This review indicates that the dopamine system plays a role in the physiopathology of depression and constitutes one of the potential targets of antidepressants. However, the therapeutic strategy which would consist to correct a dopamine deficiency by dopamine receptor agonist could not be efficient in all the forms of depression. A previous case report [53] illustrates this idea. The addition of bromocriptine 2.5–5 mg/day with imipramine improved the depressive symptoms of a patient with refractory depression but the clinical status returned to the original level when dose was increased to 15 mg/day. Thus, an overstimulation of the dopamine system could also be detrimental for depressive patients. This suggestion is only based on the results of a case report but emphasizes that further investigations are necessary to understand the exact role played by dopamine in depression.

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Article 2 :

5-HT_{1B} Receptor : A Target for Antidepressant Drugs ?

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DDR

Research Overview

5-HT$_{1B}$ Receptor: A Target for Antidepressant Drugs?

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Strategy, Management and Health Policy				
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ABSTRACT It is generally accepted that about two thirds of patients treated for depression respond only after several weeks (2 to 8 weeks) whilst a third do not respond at all. A depressed patient's response to a treatment is defined by at least 50% reduction of the symptoms evaluated on a standard instrument (i.e., Hamilton Depression Rating Scale). Thus, a response to an antidepressant treatment cannot be considered as a remission. Remission can take many months to occur. It is then crucial to find new targets for antidepressants development or co-administration strategies in order to reduce the long delay in onset of action and improve the efficiency of current treatments. According to their mechanism of action, current antidepressants induce an increase in serotonin and/or noradrenaline neurotransmission by increasing the monoamine extracellular level available in the synaptic cleft. It is then highly possible that the antidepressant effect depends on the synaptic receptor(s) activated. In the case of serotonergic compounds, 14 subtypes of receptors could be stimulated. In this short review, we focus on the impact of 5-HT$_{1B}$ receptor activation in the mediation of antidepressant-like effect. Drug Dev. Res. 65:141–146, 2005. © 2005 Wiley-Liss, Inc.

Key words: 5-HT$_{1B}$ receptors; antidepressant; augmentation strategy

INTRODUCTION

Many studies indicate that 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors are involved in the mechanism of action of antidepressants and in their long delay in onset of action; thus it is commonly suggested that time for antidepressant-like effect to occur is linked to desensitization of one or both 5-HT$_1$ autoreceptor subtypes [Dremencov et al., 2000; Hen, 1992; Pineyro and Blier, 1996; Sayer et al., 1999]. However, even if 5-HT$_{1A}$ receptor blockade has been largely investigated as a potent augmentatory strategy, especially by the use of pindolol, only a few studies are available about the implication of 5-HT$_{1B}$ receptors in mood disorders.

5-HT$_{1B}$ RECEPTORS: A SHORT REVIEW

5-HT$_{1B}$ Receptors: Human or Rodents?

5-HT$_{1B}$ receptor had first been claimed to only exist in rodents (mice, rats) [Pedigo et al., 1981] but more recent studies, using a genetic approach, have provided the evidence that the 5-HT$_{1B}$ receptor is

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homologuous to the human 5-$HT_{1D\beta}$ receptor [Adham et al., 1992; Boess and Martin, 1994; Hartig et al., 1996; Hoyer and Martin, 1997]. The human receptor contains 4 additional amino acid residues compared with the rodent one (390 vs. 386: [Adham et al., 1992; Demchyshyn et al., 1992; Hamblin et al., 1992; Jin et al., 1992; Maroteaux et al., 1992; Veldman and Bienkowski, 1992; Voigt et al., 1991; Weinshank et al., 1992]). Both receptors are coupled negatively to an adenylate cyclase via a G protein [Findlay and Eliopoulos, 1990; Hamblin and Metcalf, 1991; Hibert et al., 1991; Maroteaux et al., 1992; Seuwen et al., 1988; Trumpp-Kallmeyer et al., 1992]. Numerous studies have also demonstrated that pharmacological differences between these 2 receptors are essentially linked to the replacement of threonine (in 5-$HT_{1D\beta}$ receptor) by aspargine (5-HT_{1B} receptor) in the seventh transmembrane domain of the receptor. This modification is responsible for the affinity of 5-HT_{1B} receptor for β-blockers (i.e., propranolol: [Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993]).

5-HT_{1B} Receptors: Physiological Involvement

It has been demonstrated that 5-HT_{1B} receptors are implicated in the control of aggression [de Almeida and Miczek 2002; Dirks et al., 2001; Geyer 1996; Saudou et al., 1994], of sleepiness [Boutrel et al., 1999; Monaca et al., 2003]; motor behaviour [Geyer 1996; Millan et al., 2003; Skingle et al., 1996], appetite [De Vry and Schreiber 2000; Lee and Simansky 1997; Lucas et al., 1998; Simansky and Nicklous 2002]; anxiety [Frances et al., 1990a,b; Lin and Parsons 2002]; drug abuse [Przegalinski et al., 2003]; sexual behavior [Hillegaart and Ahlenius 1998]; and thermoregulation [Hagan et al., 1997].

5-HT_{1B} Receptors: Pharmacological Involvement

In the CNS, 5-HT_{1B} receptors are both presynaptic and postsynaptic. Presynaptic receptors are located on serotonergic neurons (autoreceptors), whereas postsynaptic ones are found on non-serotonergic neurons (heteroreceptors). 5-HT_{1B} autoreceptors are involved in the control of 5-HT release in various forebrain areas [de Groote et al., 2002a,b, 2003a,b; Hjorth and Tao, 1991; Knobelman et al., 2000; Martin et al., 1992; Roberts et al., 2000; Sharp et al., 1989] as well as in 5-HT synthesis [Hjorth et al., 1995]. When located on non-serotoninergic neurons, 5-HT_{1B} receptors act as terminal heteroreceptors controlling the release of other neurotransmitters such as dopamine [Benloucif et al., 1993; Galloway et al., 1993; Yan and Yan, 2001a], glutamate [Boeijinga and Boddeke 1996; Muramatsu et al., 1998], acetylcholine [Maura et al., 1989], and GABA [Feuerstein et al., 1996; Peruzzi and Dut 2004; Yan and Yan, 2001b].

Location and Gene Expression

In situ hybridization shows expression of mRNA encoding for 5-HT_{1B} receptor in cells of the dorsal and median raphe nuclei, consistent with data showing that the 5-HT_{1B} receptor acts as an autoreceptor on 5-HT terminals; expression of 5-HT_{1B} receptor encoding mRNA is also detected in various brain areas such as the CA1 region of the hippocampus, the striatum, the layer 4 of the cerebral cortex, and the cerebellum (Purkinje cells).

In situ hybridization histochemistry also demonstrates the presence of 5-HT_{1B} receptor mRNA in rat trigeminal and dorsal root ganglia, in line with the well-established existence of presynaptic 5-HT_{1B} receptors on trigeminal fibers in the spinal caudal nucleus of the trigeminal nerve and primary afferent fibers in the dorsal horn of the spinal cord, respectively.

5-HT_{1B} RECEPTORS: A POTENT TARGET FOR NEW ANTIDEPRESSANT DRUGS

The monoamine hypothesis of depression suggests that this disease is linked to a hypofunctionning of the central monoaminergic system: serotonin and noradrenaline [Coppen, 1967; Duman et al., 1997]. Depression treatments (physical: electroconvulsive shocks or chemical: antidepressant drugs) have been developed in order to enhance the monoaminergic transmission increasing monoamine release, decreasing monoamine reuptake or metabolism. However, even if these treatments induce amelioration of a depressed state, they are not sufficient, particularly since approximately 30% of patients are non-responders to the current treatments and there is a long delay of action necessary for AD effects to appear (2–8 weeks). In the last decade, many augmentation strategies have been developed to potentiate the activity of antidepressant drugs or to reduce their long onset of action by acting on different targets, such as co-administration with lithium, anti-epileptics, or NK1 receptor antagonists.

Several authors [Davidson and Stamford, 1998; de Montigny and Blier, 1991; Dremencov et al., 2000; Hen, 1992; Pineyro and Blier, 1996; Sayer et al., 1999] have demonstrated that the long delay of action of AD was linked to the time necessary for 5-HT_{1A} presynaptic receptor desensitization to occur. Thus, many augmentation strategies using 5-HT_{1A} receptor antagonists have been developed. According to the observation that activation of both 5-HT_{1A} and 5-HT_{1B} receptors induces a decrease in 5-HT neurotransmission (5-HT_{1A} receptor activation reduces neural firing and 5-HT_{1B} activation decreases 5-HT release), it

would have been highly presumable that 5-HT$_{1B}$ receptor blockade would increase therapeutic effects of SSRIs. Preclinical data clearly demonstrate that the absence, or blockade, of 5-HT$_{1B}$ receptors potentiates the increase in the 5-HT extracellular level (evaluated by microdialysis intracerebral in vivo in the medial prefrontal cortex and in the hippocampus) induced by a single i.p. administration of selective serotonin reuptake inhibitors (SSRIs) [de Groote et al., 2002a; Gobert et al., 1997; Malagie et al., 2001, 2002] whereas 5-HT$_{1B}$ receptors antagonists are devoid of effects when administered alone. It has been suggested that this lack of efficiency could be attributed to the involvement of 5-HT$_{1B}$ receptor in the raphe nucleus [Roberts et al., 1999], which would counteract pharmacological effects in forebrain areas. This last explanation is then not valuable since Hjorth et al. [2000] have demonstrated that NAS-181 (5-HT$_{1B}$ receptor antagonist) is devoid of effect on [5-HT]$_{EC}$ levels in rat frontal cortex when administered systemically or locally. This suggests that 5-HT$_{1B}$ receptors antagonists are devoid of effect on basal conditions in both hippocampus and cortex [Adell et al., 2001; de Groote et al., 2002a,b, 2003a; Gardier et al., 2001; Knobelman et al., 2000; Roberts et al., 2000]. Recent studies demonstrate that the activity of 5-HT$_{1B}$ receptors could be improved by an allosteric modulator (5-HT modulin); it is then possible that 5-HT modulin efficiency is dependent on the 5-HT release. These neurobiochemical data lead us to think that 5-HT$_{1B}$ receptors antagonists and compounds acting on the 5-HT modulin binding site could be of significant interest in the treatment of mood disorders.

However, this increase in extracellular 5-HT level obtained following a co-administration of a SSRI and a 5-HT$_{1B}$ receptor antagonist is associated with a loss of the AD-like properties of SSRI evaluated in mice forced swimming test (FST) [Gardier et al., 2001]. Although it has been demonstrated that in rats 5-HT$_{1B}$ receptors antagonists (SB 216641 and GR 127935) can be used to augment the behavioral effects of imipramine, desipramine, and moclobemide [Tatarczynska et al., 2002, 2004], the same team has also shown that 5-HT$_{1B}$ receptor antagonists fail to potentate the effects of citalopram [Tatarczynska et al., 2004]. It has been also demonstrated that 5-HT$_{1B}$ receptor agonists exert an AD-like effect in behavioural tests [O'Neill and Conway, 2001; Redrobe and Bourin, 1999] or can be used to augment the effect of an antidepressant [David et al., 2001; Redrobe et al., 1996] even if 5-HT$_{1B}$ receptors agonist treatment (general or local administration) induces a decrease in 5-HT outflow in mice striatum [de Groote et al., 2003a], rat hippocampus [Hjorth and Tao, 1991; Martin et al., 1992; Sharp et al., 1989], mice hippocampus [de Groote et al., 2002a], mice cortex [de Groote et al., 2002b; 2003a], mice striatum [Knobelman et al., 2000], and guinea pig cortex [Roberts et al., 2000]. Taken together, these results suggest that an AD-like effect can be obtained even if extracellular 5-HT levels are decreased whereas usually antidepressant drugs are effective because they increase serotonergic neurotransmission [Preskorn, 1994; Wong et al., 1995].

A confirmation of these results would indicate that 5-HT$_{1B}$ postsynaptic receptors are highly implicated in the mediation of an antidepressant-like effect of SSRIs.

CONCLUSION

It is suggested that 5-HT$_{1B}$ postsynaptic receptors (heteroreceptors) activation mediates the behavioural AD-like effects of SSRIs in animal models of depression (and probably in human), whereas 5-HT$_{1B}$ presynaptic receptors (autoreceptors) limit their neurobiochemical effects. Therefore, two different strategies could be developed. The first one would be the co-administration of a selective 5-HT$_{1B}$ presynaptic receptor antagonist together with an SSRI drug, in order to augment the 5-HT release (as already suggested by Moret and Briley [2000]). The second strategy would be to administrate a 5-HT$_{1B}$ agonist that could directly activate postsynaptic receptors and maybe decrease the long onset of action of SSRI antidepressants. As 5-HT$_{1B}$ postsynaptic receptors are located on non-serotonergic neurons, this would indicate that serotonin may not be the final common pathway of the mechanism of action of SSRIs. Indeed, antidepressant-like effects can only be triggered when other(s) monoaminergic system(s) (via 5-HT$_{1B}$ autoreceptor activation) are activated. Considering the location of 5-HT$_{1B}$ heteroreceptors (GABAergic, glutamatergic, cholinergic, and dopaminergic neurons) and based on the fact that among these, only dopamine has already been involved in depressive states [Dailly et al., 2004], we hypothesized that antidepressant-like effect of SSRIs might be mediated by the activation of 5-HT$_{1B}$ heteroreceptors located on dopaminergic neurons. If confirmed, this hypothesis would explain the contradictory results of the augmentation of AD effects of SSRIs by pindolol; since pindolol is a 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors antagonist, its co-administration with SSRIs induces a potentiation of extracellular 5-HT increase but the therapeutic benefit is not clear. Based on our hypothesis, this lack of efficiency could be linked to the 5-HT$_{1B}$ postsynaptic receptor blockade.

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Article 3 :

Potentiation of antidepressant-like activity with lithium: mechanism involved

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Potentiation of Antidepressant-Like Activity with Lithium: Mechanism Involved

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Abstract: In the last decade, many augmentation strategies have been developed to increase the activity of antidepressant drugs or to reduce their long onset of action by acting on different targets. One of the first augmentation strategy used in psychiatric disorders is coadministration of lithium and antidepressant drugs. However, the underlaying mechanism of action involved in the potentiatory effect of lithium is still unclear and many hypotheses have been suggested such as activity on BDNF, ACTH, thyroid hormones and serotonin neurotransmission. All these systems being embedded in each other, we focused on the 5-HT neurotransmission-increase induced by lithium treatment. Based on neurobiochemical and behavioral results we tried to better understand its mechanism of action and we concluded that effect of lithium on 5-HT neurotransmission could be linked to a partial agonist activity on 5-HT_{1B} autoreceptors, or to a modulatory activity on these receptors, located in the cortical area in the case of a short term treatment, or in the hippocampus in the case of a long term treatment. We also suggested that the anti-manic effect of lithium was linked to this activity on 5-HT_{1B} receptors, occurring this time on 5-HT_{1B} postsynaptic (heteroreceptors on dopaminergic pathways) receptors levels.

Key Words: Lithium, Antidepressant, FST, serotonin, 5-HT_{1B} receptor, mechanism of action.

INTRODUCTION

Lithium (Li) has been widely used alone or in combination with other antidepressants (AD) in order to treat unipolar and/or bipolar disorders and to prevent antidepressant resistance in patient. Its neurobiochemical mechanism of action is still unclear, many hypotheses are evoked and preclinical data exhibit various mechanisms of action.

Clinical and preclinical studies have shown that the effect of lithium on serotonin (5-HT) function may occur at multiple levels such as serotonin synthesis [1-4], variation on serotonin turnover [5, 6] and lithium has been involved in the appearance of the 5-HT syndrome [7, 8]. All these papers report an increase in 5-HT neurotransmission [3, 7, 9-15]. But this activity of lithium could not be linked to a decrease of 5-HT metabolism [16], nor to an activity on 5-HT2 receptors [17]. Yet, it seems to be possible that this increase in 5-HT neurotransmission could be related to an antagonist action of lithium on 5-HT_1 autoreceptors, and mainly at 5-HT_{1B} autoreceptor level [18-21] since it has been demonstrated that lithium did not alter pharmacological response to the administration of a 5-HT_{1A} receptor agonist [22]. Moreover, activity of postsynaptic 5-HT_{1A} receptors in limbic areas was proved to be enhanced [23]. The effect of lithium on 5-HT_{1B} presynaptic autoreceptors, would result in an increase in 5-HT release, autoreceptors being largely implicated as being responsible for the long delay of action of selective serotonin reuptake inhibitors (SSRIs), and as a limiting factor in their therapeutic effect, therapeutical effect appears only after their desensitization [11, 24].

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In behavioural psychopharmacology, numerous and various tests have been used to demonstrate the augmentation effect of lithium on the antidepressant-like (AD-like) effect of various drugs. However, it appears that the forced swimming test is the most commonly used in preclinical studies and it is a very easy suitable test to predict antidepressant activity as well as to explain the mechanism of action implicated in the AD-like effect; that is why we focus on this test to exhibit mechanism of action of lithium in serotoninergic pathways (for a review of activity of antidepressant on the FST, see [25]).

There is growing evidence to suggest that lithium acts at the serotoninergic presynaptic receptor level to increase the antidepressant activity of ADs. This activity of lithium results in an increase in 5-HT release. This effect could be linked to a decrease in the sensitivity of presynaptic 5-HT receptors resulting in a decrease of their inhibitory properties [11, 21, 24].

THE NEUROBIOCHEMICAL EFFECTS OF LITHIUM

It has been reported that a long term treatment (3 weeks) with lithium increased the basal level of tissue 5-HT concentration in the hippocampus, but the same treatment was devoid of effect on 5-HT concentrations in frontal cortex of rats [26]. The increase obtained in the hippocampus was similar to that obtained following a 3 week treatment with desipramine (10 mg/kg/day) or escitalopram (10 mg/kg/day) [26]. This suggests that hippocampus, but not cortex, was involved in long term lithium treatment effects. Moreover, an electrical stimulation of dorsal raphe nucleus increased the release of 5-HT (3-4 fold more) on the short treatment group, but was devoid of effect on the long term treatment one [14]. These results are in accordance with those of Baptista *et al.* [9], which demonstrated an enhancement of the

amphetamine effect on $[5\text{-HT}]_{EC}$ levels in the perifornical hypothalamus, but not in the hippocampus following a subchronic treatment with lithium.

Muraki *et al.* [22], investigated the effect of citalopram (SSRI) on median prefrontal cortex (mPFC) $[5\text{-HT}]_{EC}$ levels following a subchronic (1 week) lithium diet treatment. The subchronic lithium treatment group showed a significantly higher basal levels of extracellular 5-HT compared to control group; thus this indicates that lithium potentiates the neurobiochemical effect of an acute dose of SSRI on the mPFC. An acute dose of 5-HT_{1A} agonist (MKC-242) induced a decrease in 5-HT release in both groups; this decrease could not be counteracted by increasing lithium doses, suggesting that lithium and MKC-242 do not act on the same receptors. This was already suggested in 1999, when the same team [27] showed that the behavioural effects of MKC-242 were enhanced following a subchronic (1 week) lithium treatment. Then, we could conclude that lithium may exert its pharmacological effect *via* 5-HT_{1B} autoreceptors.

Cordeiro *et al.* [28] studied the impact of lithium on the modulation of the expression of mRNA coding for vesicular monoamine transporter 2 (VMAT2) in rat brain, their results showed an increase of mRNA of 50 to 100% in the raphe nuclei, the ventral tegmental area, and the substantia nigra, which are the brain areas containing the most of 5-HT_{1B} receptors [29-31] reinforcing the involvement of 5-HT_{1B} receptors in the mediation of lithium effect.

There was no effect of co-administration of citalopram (acute) and lithium (subchronic) on $[5\text{-HT}]_{EC}$ dialysate levels in the hippocampus [32], but the effect of a chronic administration of citalopram on $[5\text{-HT}]_{EC}$ levels could be potentiated by subchronic lithium (always in the hippocampus), suggesting that in the case of a chronic treatment, lithium effects were obtained *via* hippocampal 5-HT_{1B} autoreceptors (5-HT neurons from median raphe nucleus), whereas in the case of an acute treatment, cortical 5-HT_{1B} autoreceptors (dorsal raphe nucleus innervation) are involved [33]. In this last study, Muraki *et al.* showed that subchronic lithium increased basal extracellular 5-HT level, but the percentage of increase in mPFC [5-HT] obtained on acute injection of citalopram were the same in control and Li-treated rats, this is in line with the hypothesis that lithium effects were obtained following a 5-HT_{1B} receptor desensitization, because others studies demonstrates no statistically differences in 5-HT extracellular cortical levels following an acute administration of SSRI on wild-type and 5-HT_{1B} receptors knockout mice [34, 35].

Some effects of lithium appear immediately after its administration and are probably mediated *via* cellular mechanisms (activity directly on 5-HT_{1B} receptors), whereas others only appear, or disappear, after several days (weeks) of treatment, those are probably linked to a modulator activity of lithium on gene expression. Thus, long-term lithium treatment was also associated with an increase in 5-HT transporter protein in cortical regions [36, 37] which could explain the loss of lithium activity in these areas following a chronic administration by counteracting its neurobiochemical effects (the increase in 5-HT release being masked by an increase in its uptake). This effect of Li on 5-HT transporters does not appear in others brain areas, explaining why following a chronic treatment, only hippocampal (and not cortical) 5-HT levels were changed. At the opposite, arachidonic acid incorporation was unchanged in most of the cortical areas of rats but was changed in hippocampal tissues following 6 week of a LiCl diet in rats [38]. This indicates that these two brains areas are differentially regulated further lithium treatment. In the case of a subchronic treatment, both located receptors could be involved in lithium effects [22, 39].

Gambarana *et al.* [40] demonstrated that chronic treatment with lithium, induces a decrease in dopamine extracellular ($[DA]_{EC}$) levels evaluated in the nucleus accumbens, and that the increase in $[DA]_{EC}$ following a cocaine administration, was significantly lower in lithium treated animals. More recently, Kitaichi *et al.* [41] demonstrated that a subchronic treatment with lithium induced an increase in extracellular serotonin levels in mPFCx whereas NA and DA were (not statistically significantly) decreased. These results were consistent with the findings of Carli *et al.* [42] which demonstrated an increase in DA transporter uptake sites. According to the fact that 5-HT_{1B} heteroreceptor stimulation induces an increase in DA release [43–46], the activity of lithium on this receptor could explain the decrease in DA levels. Since it has been demonstrated that caudate putamen dopamine levels were unchanged further an acute treatment with lithium, it appears that the effect of lithium on DA levels, and so on 5-HT_{1B} heteroreceptors, were obtained only after a chronic treatment [47].

Carli *et al.* [36] also demonstrated that a chronic treatment with lithium induced an increase in 5-HTT binding sites in brain areas containing nerve terminals, whereas no changes were obtained in areas containing cell bodies. No variation in 5-HT_{1A} binding sites was obtained, even if an increase of adenylate cyclase activity was demonstrated. These two variations should be considered as compensatory mechanisms pursuant to the activity of lithium on 5-HT_{1B} receptors.

THE EFFECT OF LITHIUM IN THE FORCED SWIMMING TEST

The mouse forced swimming test is an acute behavioral test, which predicts the efficacy of AD treatment [48]; the model is sensitive to a wide range of current AD treatments, including tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, dopamine reuptake inhibitors and atypical antidepressant (for review, [25]). Moreover, FST has also been used to investigate the mechanism of action of antidepressant drugs. This is why we estimate that it is the best test to analyze the AD-like effect of lithium. Yet, this test is also a useful model, relatively simple to perform and reliable across laboratories. The test involves placing mice individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm water, maintained at 23-25°C, and remained there for 6 min. After an initial phase of vigorous activity, swimming attempts cease and the animal adopts a characteristic immobile posture. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. The time of immobility was recorded during the last 4-min of the 6-min testing period, thus after 2

min of habituation. The test must be performed by the same well trained experimenters, blind to the treatment administered. Antidepressant drugs decrease the duration of immobility time.

Indeed, it has been demonstrated that administration of lithium alone (30 minutes before testing) was capable of reducing immobility time at 2, 4 and 8 mEq/kg [49] but the same team demonstrated that lithium alone was devoid of effect when administered 45 min before testing [50, 51]. In this last study, it was also demonstrated that pre-treatment with a subactive dose of lithium (1 mEq/kg) potentiates the AD-like effect of numerous drugs, such as tricyclics antidepressants (imipramine), serotonin selective reuptake inhibitors (citalopram, paroxetine, fluoxetine and fluvoxamine), MAO inhibitors (moclobemide), and atypical antidepressants (iprindole, trazodone and mianserin) but was devoid of augmentation effect on drugs that do not act on serotoninergic pathways (NA uptake inhibitors: désipramine, maprotiline and viloxazine; or dopamine uptake inhibitor: bupropion). Redrobe *et al*., 1998 [51], also showed a potentiatory effect of acute lithium on an acute dose of venlafaxine (NA and 5-HT uptake inhibitor). Kitamura *et al*. [52] also demonstrated that a chronic (15 day) co-administration of imipramine (10 mg/kg i.p.) and lithium (100 mg/kg p.o.) induces an AD-like effect in the FST in rats, whereas chronic lithium and imipramine alone were devoid of effect (an acute dose of imipramine, 30 mg/kg significantly reduces immobility time in FST). These results suggest that the augmentation of AD-like effect in the mouse forced swimming test by lithium was *via* 5-HT mechanisms, especially *via* 5-HT_{1B} autoreceptors, which have already been involved in the potentiation mechanism.

It has been demonstrated that the absence of 5-HT_{1B} receptors in knockout mice, or their blockade by a selective 5-HT_{1B} receptor antagonist (GR 127935) potentiates the increase in the extracellular serotonin levels induced by a single intra-peritoneal (i.p.) administration of SSRIs in mice [34, 35, 53-55] using various SSRIs (paroxetine, fluoxetine and fluvoxamine) and 5-HT_{1B} receptor antagonists (GR 127935 and NAS-181) because 5-HT_{1B} receptors locally control 5-HT release [56-58]; however, these antagonists are devoid of effect under basal conditions in cortical and hippocampal areas [59-61] this is confirmed by the fact that basal extracellular 5-HT levels did not significantly differ between wild-type and knock-out mice [35]. However, it has been recently shown that an acute or a chronic treatment with a new 5-HT_{1B} receptor antagonist increases the extracellular levels of serotonin in guinea pig cortex [62]. Some authors suggest that 5-HT_{1B} receptor antagonists acts *via* MRN [59, 63] whereas DRN would be under 5-HT_{1D} regulation [64-67]. This explains the potentiation of the effect of SSRIs on hippocampal but not on cortical monoamine levels [34]. At the opposite 5-HT_{1B} receptor agonists are able to decrease 5-HT release in both raphe nuclei and are reversed by 5-HT_{1B} receptor antagonists [59, 64, 65, 68].

These data suggest that activation of 5-HT_{1B} autoreceptors limits the effects of SSRIs on dialysate 5-HT levels at serotonergic nerve terminals mainly located in the hippocampus, the cortex and in the striatum in various species [64, 69-73]. A chronic treatment using SSRI (i.e. paroxetine for 21 days) induces a desensitization of rat MRN, hippocampus, hypothalamus and cortical 5-HT_{1B} receptors [58].

Surprisingly, it was found that this increase in extracellular 5-HT levels measured by intra-hippocampal *in vivo* microdialysis technique in awake, freely moving mice was not correlated with an increase of the antidepressant-like (AD-like) activity, which was evaluated by using the forced swimming test (FST) in mice [60]. At the opposite, SSRI-induced decrease in immobility in the FST is absent in 5-HT_{1B} knockout mice and blocked by GR 127935 in wild-type suggesting therefore that activation of postsynaptic 5-HT_{1B} heteroreceptors mediates the antidepressant-like effects of SSRIs.

According to these results, it appears that even if the blockade of 5-HT_{1B} autoreceptors potentiates the neurobiochemical effects of SSRIs (in the microdialysis experiments), it also induces an inhibition of their therapeutical effects (in the FST), probably through the blockade of postsynaptic 5-HT_{1B} receptors. Activation of postsynaptic 5-HT_{1B} receptors has been involved in the control of various behaviors such as agressivity [74-76], sleepiness [77], motor behavior [74, 78] and appetite [79, 80]. It is also well established that 5-HT_{1B} receptor agonists could induce an antidepressant like effect in animals [81] and subactive doses of these agonists could be used to potentiate AD-like effect of subactive doses of antidepressant drugs [82, 83]. This AD-like effect should be linked to 5-HT_{1B} heteroreceptors, which are located on dopaminergic [84], glutamatergic [85], cholinergic [86, 87] and GABAergic neurons [88]. However, Tatarczynska *et al*. [89] showed that subactive doses of 5-HT_{1B} receptor antagonists could be used to potentiate subactive doses of "non SSRI" antidepressants in rats (imipramine, moclobemide and désipramine). These kind of differences between rats and mice have already been observed in behavioural potentiation studies; it was shown that pindolol enhanced the AD-like effect of various SSRI in mice [51, 83, 90], while it failed to potentiate SSRI in rats [91, 92].

In addition, other studies demonstrate that lithium was able to enhance the anti-immobility effects of the 5-HT_{1A} receptor agonist gepirone in the FST [49, 93] and of ipsaspirone in the TST [94], thus confirming the neurobiochemical results of Muraki *et al*. [22, 27] indicating that lithium does not act on 5-HT_{1A} receptors. Moreover, Muraki *et al*. results shown that lithium does not act on 5-HT_{1A} presynaptic receptors (changes in extracellular 5-HT levels as measured by microdialysis being considered due to changes in 5-HT autoreceptor activity) whereas behavioral studies [49, 93, 94] clearly demonstrate that lithium does not act on postsynaptic 5-HT_{1A} receptors (the AD-like effect of these substance being mediated *via* postsynaptic receptors). It has also been shown that co-administration of the non selective 5-$HT_{1A/1B}$ receptor agonist, RU 24969, and lithium induces a decrease in the immobility time in the FST in mice (-25%, [18]); the same results were obtained using a more selective 5-HT_{1B} receptor agonist, anpirtoline; the decrease in immobility time was potentiated by 45% [18]. It is highly possible, that the AD-like activity of 5-HT_{1B} receptor agonists could be linked to their activity on postsynaptic neurons, which is consistent with the fact that lithium could be used in an augmentation strategy because of its activity on 5-HT_{1B} autoreceptors.

Taken together, these results seem to indicate that lithium only blocks 5-HT_{1B} autoreceptors, but is devoid of effect on postsynaptic receptors (the antagonism on these receptors would have induced a decrease in the AD-like effect). This activity on autoreceptors is confirmed by the increase in [5-HT]$_{EC}$ levels obtained in microdialysis studies. However, neurobiochemical studies exhibit an activity of lithium on brain dopaminergic levels, this activity should be linked to an activity on postsynaptic 5-HT_{1B} receptors. Thus, it seems more valuable to conclude that lithium does not act as a 5-HT_{1B} receptor antagonist, but as a 5-HT_{1B} partial agonist, or as a modulatory agent of 5-HT_{1B} receptor activity. It would be really interesting to confirm this hypothesis by using microdialysis on mice following administration of an acute dose of a 5-HT_{1B} receptor antagonist, with co-administration of a range of doses of lithium.

CONCLUSION

We postulated that lithium treatment induces a desensitization of 5-HT_{1B} receptors, and that such desensitization following acute and chronic treatment does not exert effects on the same brain area. It appears that acute treatment with lithium induces a desensitization of 5-HT_{1B} autoreceptors, located in the cortex, indicating that desensitization affects serotoninergic afferents from dorsal raphe nucleus, whereas chronic lithium treatment induces a desensitization of 5-HT nerves from median raphe nucleus *via* hippocampal 5-HT_{1B} autoreceptors, the desensitization of dorsal raphe nucleus afferents being counteracted by an increase in 5-HT transporters binding sites. This explanation is in line with the findings of Szumlinski *et al.* [95] who demonstrated that the median raphe nucleus is involved in behavioral effects induced by a chronic treatment, whereas the dorsal raphe nucleus is involved in acute treatments, suggesting that DRN 5-HT_1 autoreceptors are a limiting factor on treatment initiation.

It appears that short-term treatment with lithium should be effective at the beginning of an antidepressant strategy (by potentiating the increase in 5-HT), whereas its effect in resistant depression was linked to the increase in the binding sites of antidepressant drugs (5-HT transporters). Its efficiency in bipolar disorders could be explained by the decrease of DA levels [40-42] resulting in an anti-manic effect.

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RÔLE DES RÉCEPTEURS 5-HT_{1B} ET DE LA DOPAMINE DANS L'ACTIVITÉ DE TYPE ANTIDÉPRESSEUR DES IRSSs DANS LE TEST DE LA NAGE FORCÉE CHEZ LA SOURIS

Les inhibiteurs de recapture sélectifs de la sérotonine (IRSSs) exercent leur activité antidépressive en augmentant la concentration extracellulaire de sérotonine dans la fente synaptique qui induit l'activation de 14 sous types de récepteurs. Parmi ces récepteurs, le sous type 1B possède un rôle fondamental car son activation (injection locale ou systémique d'anpirtoline) induit un effet antidépresseur alors que son blocage empêche l'apparition des effets des IRSSs. Le maintien de l'activité antidépressive de l'anpirtoline chez des animaux dont les autorécepteurs ont été détruits démontre que se sont les hétérorécepteurs 5-HT1B qui sont responsables des effets comportementaux. L'activité de type antidépresseur des IRSSs disparaissant chez des animaux dont le système dopaminergique a été préalablement lésé, nous avons donc suggéré que l'effet des IRSSs nécessite une augmentation de la neurotransmission dopaminergique consécutive à l'activation des récepteurs 5-HT1B.

Mots Clés : test de la nage forcée, récepteurs 5-HT1B, dopamine, IRSS, souris, déplétion, monoamine

ROLE OF 5-HT_{1B} RECEPTORS AND DOPAMINE IN THE ANTIDEPRESSANT-LIKE ACTIVITY OF SSRIs IN THE MICE FORCED SWIMMING TEST

SSRIs induce an increase in extracellular serotonin which is responsible of their antidepressant-like (AD-like) properties. Among all 5-HT receptors subtypes activated, 5-HT1B subtype appears to be strongly involved in the mediation of this anti-immobility effect. Indeed, 5-HT1B receptors activation (following local or systemic infusion of anpirtoline) induces an AD-like effect, whereas 5-HT1B receptor blockade antagonises the activity of SSRIs. Anpirtoline being still efficient in 5-HT1B autoreceptors of lesioned mice it suggests that AD-like effects of 5-HT1B receptors agonists are mediated by activation of 5-HT1B heteroreceptors. Since AD-like effect of SSRIs is absent on dopamine lesioned mice, we have suggested that SSRIs activity requires an enhancement of dopamine neurotransmission to occur, and that this enhancement appears further to the activation of 5-HT1B receptor.

Keywords :Forced swimming test, 5-HT1B receptors, dopamine, SSRI, mice, lesion, monoamine