CHARACTERIZATION OF THE SEROTONERGIC AND NORADRENERGIC SYSTEMS IN MICE LACKING THE 5-HT_{1A} RECEPTOR AND ITS RELEVANCE TO THE MECHANISM OF ACTION OF ANTIDEPRESSANT DRUGS

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November, 2001

A Thesis submitted to the Faculty of Graduate studies and Research in partial fulfillment of the requirements of the degree of Master of Science.

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RÉSUMÉ

L'implication des systèmes monoaminergiques centraux dans la régulation de l'humeur, particulièrement celle des systèmes sérotonergique (5-HT) et noradrénergique (NE), est chose connue depuis maintenant près de quarante années. Les différentes classes de molécules prescrites pour traiter les différents troubles affectifs influent d'ailleurs directement sur la transmission médiée par ces systèmes, que ce soit à l'étape de leur synthèse, de leur relâchement, de la stimulation de leurs récepteurs, de leur recapture ou encore de leur dégradation. La théorie prédominante veut que l'augmentation de la transmission sérotonergique soit la voie de convergence finale des traitements antidépresseurs existants.

Des études pharmacologiques et électrophysiologiques ont montré que les autorécepteurs somatodendritiques de type 5-HT_{1A} jouent un rôle essentiel dans le contrôle du taux de décharge et du relâchement de 5-HT par les neurones du noyau raphé dorsal, ce qui en font une cible extrêmement intéressante pour de nouvelles molécules adjuvantes dont le rôle serait de potentialiser, voire accélérer l'action des molécules antidépressantes déjà cliniquement utilisées. Car si l'augmentation des niveaux synaptiques de 5-HT semble être le but ultime des traitements antidépresseurs actuels, elle n'est pas observée suite à l'administration aigüe de ces composés et dépenderait de la désensibilisation des récepteurs 5-HT_{1A} du raphé dorsal, un phénomène d'ailleurs corrélé

temporellement avec le délai d'action clinique de deux à trois semaines associé à la prise chronique d'antidépresseurs et ce, quelque soit leur classe.

La caractérisation de l'impact, sur la fonction des systèmes 5-HT et NE, de la délétion du gène codant pour le récepteur 5-HT_{1A} chez des souris, est un moyen de choix pour évaluer l'importance du récepteur dans ces systèmes.

Des enregistrements électrophysiologiques extracellulaires unitaires ont donc été effectués dans des neurones 5-HT du raphé dorsal de souris mutantes dépourvues de récepteurs 5-HT_{1A} pour en déterminer le taux de décharge spontané, en parallèle avec des expériences de superfusion de tranches de cerveau préincubées dans une solution de 5-HT ou de NE marquée au tritium, paradigme servant à évaluer la sensibilité des autorécepteurs somatodendritiques 5-HT_{1D} mésencéphaliques ainsi que des autorécepteurs terminaux 5-HT_{1B} et α_2-adrénergiques corticaux et hippocampaux, afin de déterminer s'il y a sublimation de la fonction des récepteurs 5-HT_{1A} par ces autres récepteurs qui contrôlent aussi de manière importante les neurones 5-HT et NE.

Dans le cadre de nos études, il a été démontré que de la disparition de la boucle de rétroaction négative médiée par l'activation des autorécepteurs somatodendritiques 5-HT_{1A} résulte en une drastique augmentation (90%) du taux de décharge spontané des neurones 5-HT du raphé dorsal chez les souris

mutantes, sans pour autant que la sensibilité des autres autorécepteurs étudiés n'ait été altérée. Cette observation, additionnée à la perte de l'activité inhibitrice médiée par les récepteurs 5-HT_{1A} postsynaptiques, confirme l'état hypersérotonergique de ces souris, expliquerait le phénotype anxieux de ces souris et soutiendrait le rôle indispensable du récepteur 5-HT_{1A} dans le contrôle de l'activité neuronale sérotonergique.

ABSTRACT

The implication of central noradrenergic and serotonergic systems in the control of mood has been known for forty years. Indeed, the different classes of molecules prescribed in the treatment of major depression directly interfere with the transmission mediated by these amines, either at the level of the synthesis, the release, the stimulation of receptors, the reuptake or the degradation. According to the leading theory in the field, the increase in serotonergic transmission is the final convergence point of currently prescribed antidepressant drugs.

Pharmacological and electrophysiological studies have shown the essential role of the somatodendritic 5-HT_{1A} autoreceptor in the control of dorsal raphe 5-HT neuronal firing frequency and release; these characteristics make this receptor an extremely interesting target for adjuvant drugs that potentiate or even accelerate the effect of actual antidepressants molecules. In fact, augmentation of synaptic levels of 5-HT, thought to be necessary for the beneficial action of antidepressants in mood, does not immediately occur subsequent to acute administration of those molecules but depends on the desensitization of 5-HT_{1A} somatodendritic autoreceptors located in the dorsal raphe, a phenomenom temporally correlated with the onset of action of all antidepressant classes.

The characterization of the impact of 5-HT_{1A} receptor gene deletion on 5-HT and NE neuronal function is thus an interesting mean to further evaluate this receptor's importance in these circuitries.

Extracellular unitary electrophysiological recordings have been performed in DR-5-HT cells of mice lacking 5-HT_{1A} receptors to measure the spontaneous firing rate. At the same time, superfusion experiments of brain slices preincubated with tritiated 5-HT or NE have been conducted to evaluate the sensitivity of 5-HT_{1D} somatodendritic autoreceptors in the mesencephalon, of terminal 5-HT_{1B} and α_2-adrenergic autoreceptors in the prefrontal cortex and hippocampus and of α_2-adrenergic heteroreceptors in the prefrontal cortex and hippocampus to see if the function of the 5-HT_{1A} receptors has been sublimated by these receptors.

Within the context of our studies, it has been concluded that the removal of the negative feedback loop mediated by the activation of 5-HT_{1A} somatodendritic autoreceptors results in a drastic increase (90%) in the spontaneous firing rate of 5-HT-DR cells null mutant animals, without any concomitant modification in the sensitivity of the other autoreceptors examined. This observation, in addition with the loss of the postsynaptic 5-HT_{1A} receptors inhibitory action, confirms the hyperserotonergic state of these mice, fits well with their anxious behavior and supports the pivotal role of the 5-HT_{1A} receptor in this network.

PREFACE AND CONTRIBUTION OF THE AUTHORS

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The thesis must include the following:

a) a table of contents;

an abstract in English and French;

b) an introduction which clearly states the rational and objectives of the research;

c) a comprehensive review of the literature (in addition to that covered in the introduction to each paper);

d) a final conclusion and summary.

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In order to conform to the above-stated regulations, I would like to mention the contribution of the following people:

My supervisor Dr. Pierre Blier, for guiding the project and preparing the manuscript; Dr. René Hen's group at Columbia University for their work in creating the 5-HT_{1A} null mutant animals used in our experiments; Claude Bouchard and Normand Lavoie for their general technical assistance; Dr. Gabriella Gobbi in teaching me the rudiments of "in vivo" extracellular electrophysiology; and the following students and people in the lab for their kind help: Steven T. Szabo, Tarek Zghoul, Helene Cameron, Guillaume Lucas, Malika Robichaud and Jordanna Bermack.

ACKNOWLEDGEMENTS

J'aimerais naturellement témoigner de la reconnaissance à mes parents, Louis et Louise, pour l'intérêt qu'ils ont toujours eu à nous voir évoluer dans des domaines qui nous plaisent vraiment. Les efforts qu'ils ont consentis à notre réussite scolaire ont été et demeurent toujours des plus appréciés. Je remercie également Ai Thanh pour son support moral de tous les instants.

Je remercie le Dr. Pierre Blier qui a à la fois supervisé et activement contribué à ce travail de maîtrise. Par son style direct, efficace et enthousiaste, il a indéniablement et positivement influencé la façon dont j'aborde maintenant la neurotransmission et les systèmes monoaminergiques centraux.

Je désire également souligner l'apport de mes collègues et amis, Dr Guillaume Lucas et Philip de Guzman, pour les judicieux commentaires qu'il ont émis suite à la lecture du présent mémoire.

En dernier lieu, je m'en voudrais de ne pas saluer mes collègues de laboratoire, Dr Steven T. Szabo, Tarek Zghoul, Dr Jean-Claude Béique, Malika Robichaud, Jordanna Bermack, Danny Bélanger, Helen Cameron, Dr Gabriella Gobbi, Claude Bouchard et Normand Lavoie. Ils ont tous su, à leur façon, rendre ces moments passés à l'Unité de neurobiologie psychiatrique de l'Université McGill des plus agréables.

CHAPTER 1: INTRODUCTION

Since the first antidepressant drugs were discovered serendipitously, it took a few years to find out that they interfere with noradrenergic (NE) and serotonergic (5-HT) neurotransmissions (Schildkraut, 1965). In 1964, it was suggested that tricyclic antidepressants block NE reuptake, a discovery that converged with the already-known capacity of monoamine oxydase inhibitors to inhibit its catabolism. Consequently, the catecholamine deficit theory of affective disorders was born. The understanding of the antidepressants mechanism of action further broadened to include 5-HT neurotransmission as a critical variable in the equation, an idea that eventually lead to the development of selective serotonin reuptake inhibitors (SSRIs) and 5-HT_{1A} receptor agonists (Blier and deMontigny, 1994). The progress in molecular biology then allowed the cloning of a multitude of receptor subtypes for the two transmitters (7 for NE and 15 for 5-HT) and brought investigators to search for the receptors particularly involved in controlling the firing rate and the transmitter release. As an increase in both NE and 5-HT neurotransmission is thought to underlie the clinical effects of most antidepressant treatments, the selective activation of their receptors is one of the future steps in the quest for more efficient and tolerable drugs.

Pharmacological and electrophysiological studies supported the view of the 5-HT_{1A} receptor as one of these critical receptors (Blier et al., 1998) modulating 5-HT and NE systems. After reviewing the basic knowledge of 5-HT and NE systems from neuroanatomical, pharmacological, biochemical and electrophysiological perspectives, the issue of the interaction between the two

systems will be addressed, paving the way to the presentation of our study on the 5-HT_{1A} knockout animals.

1.1 THE SEROTONERGIC SYSTEM

1.1.1 NEUROANATOMY

Using histochemical fluorescence and autoradiography, 5-HT-containing cell somata have been found in high concentrations along the midline of the brain stem, clustered in the nine raphe nuclei (B1-B9). The pontine and medullary nuclei project caudally to the spinal cord which explains the involvement of 5-HT in motor control, pain perception and visceral regulation (Graeff, 1997). Rostrally lie the midbrain nuclei, the two more important being the dorsal (DRN) and medial raphe (MRN) nuclei. These groups of neurons massively innervate the forebrain (as no other classical transmission system does in terms of density) where they modulate the function of areas involved in affectivity, cognition and neuroendocrine control (Azmitia, 1978).

Six pathways stem from the DRN and the MRN with only two of them proceeding through the medial forebrain bundle (MFB). There is a certain level of topography in these projections as some brain areas are selectively innervated by one of these two nuclei while others receive mixed afferences (Graeff, 1997). For instance, the 5-HT terminals connecting the septum and dorsal hippocampus mostly emanate from MRN neuronal bodies while those reaching the amygdala and the ventral hippocampus mainly originate from the DRN. The hypothalamus receives projections from both nuclei which remain segregated, the DRN and

MRN respectively sending axons to the medial and lateral parts of this region. One of the fiber systems to come from the dorsal raphe is the periventricular tract (DRPT) which supplies the periventricular hypothalamus, the periventricular thalamus and the midbrain periaqueductal gray matter (PAG), three areas formed of undifferentiated gray matter and which encircle both the third ventricle and the acqueduct of Sylvius. The dorsal raphe-cortical tract (DRCT) and the dorsal raphe-forebrain tract (DRFT) are also formed of axons whose cell bodies are located in the DRN. They notably, but not restrictively, send projections to the basal ganglia, more specifically to the caudate-putamen and the globus pallidus, respectively. Besides, the DRCT massively innervates all the layers of the parietal and temporal neocortex in a highly branched manner. The piriform and temporal cortices receive DRFT fibers in parallel. The MRN-cortical projections pass through the MFB and extend to the frontal, cingulate and entorhinal cortices.

In addition of their different patterns of innervation, the 5-HT projections originating either from the DRN or the MRN also differ by the nature of their terminals, as shown by many electron microscopy studies (Molliver, 1987). Indeed, the axons whose cell bodies are located in the dorsal raphe (D fibers) are comparatively fine, profusely branched in their target areas and endowed with small pleomorphic varicosities which makes the occurence of synaptic contacts difficult to demonstrate. Oppositely, the fibers to emanate from the MRN (M fibers) are thicker and terminated in large beaded spherical varicosities which

make better-defined synapses on target cells. These anatomical differences are further strengthened by lesion studies done with halogenated amphetamines, such as para-chloramphetamine showing the selective destruction of D but not M fibers (Blier et al., 1990).

Although the relevance of this duality remains largely unexplored, the high number of non-specialized neuronal terminations, in addition to the existence of extrasynaptic receptors for this transmitter, supports the view of the 5-HT system as a modulatory system operating through "volume transmission" (Descarries and Mechawar, 2000), setting the tone of under way synaptic transmission. The specificity of action of such a system is lessened as compared to classical synaptic transmission but compensated by a broader range of action. It is also dependent on the diversity of receptors and their respective patterns of expression.

	5-HT$_{1A}$	**5-HT$_{1B}$**	**rodent 5-HT$_{1B}$**	**5-HT$_{1D}$**
Previous names	-	5-HT$_1$-like 5-HT$_{1X}$ 5-HT$_{1D\beta}$	5-HT$_{1B}$	5-HT$_1$-like 5-HT$_{1X}$ 5-HT$_{1D\alpha}$
Selective agonists	8-OH-DPAT Flesinoxan	Sumatriptan	CP93129	Sumatriptan PNU109291
Selective antagonists	WAY100635	GR127935 SB224289	GR127935 cyanopindolol	GR127935 BRL15572
Transductional properties	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase
	5-HT$_{2B}$	**5-HT$_{2C}$**	**5-HT$_3$**	**5-HT$_4$**
Previous names	5-HT$_{2F}$	5-HT$_{1C}$	M	-
Selective agonists	BW723C86	RO600175	SR57227 *m*-chlorophe-nylbiguanide	Cisapride BIMU8
Selective antagonists	SB204741	SB242084 RS102221	MDL72222 Ondansetron	GR113808 SB204070
Transductional properties	Induction Inositol phosphates	Induction Inositol phosphates	Cation channel opening	Stimulation adenylyl cyclase

	5-HT$_{1E}$	**5-HT$_{1F}$**	**5-HT$_{2A}$**	
Previous names	-	-	D 5-HT$_2$	
Selective agonists	-	LY344864 LY344370	-	
Selective antagonists	-	-	Ketanserin MDL100907	
Transductional properties	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Induction Inositol phosphates	
	5-HT$_{5A}$	**5-HT$_{5B}$**	**5-HT$_6$**	**5-HT$_7$**
Previous names	-	-	-	5-HT$_1$-like 5-HT$_{1Y}$ "orphan"
Selective agonists	-	-	-	-
Selective antagonists	-	-	RO046790 RO630563	SB258719
Transductional properties	Inhibition adenylyl cyclase??	-	Stimulation adenylyl cyclase	Stimulation adenylyl cyclase

TABLE 1. Classification of 5-HT receptors. Based on Saxena et al., 1998.

Antidepressant Treatment	Responsiveness of somatodendritic 5-HT_{1A} receptors	Function of terminal 5-HT autoreceptors	Function of terminal α_2-adrenergic receptors	Responsiveness of 5-HT_{1A} postsynaptic receptors	Net 5-HT transmission
Selective serotonin reuptake inhibitors	Decreased	Decreased	NC	NC	Increased
Monoamine oxidase inhibitors	Decreased	NC	Decreased	NC or decreased	Increased
5-HT_{1A} agonists	Decreased	NC	ND	NC	Increased
Tricyclic antidepressants	NC	NC	ND	Increased	Increased
Electroconvulsive shocks	NC	NC	NC	Increased	Increased

TABLE 2. Effects of long-term administration of antidepressant drugs on the serotonergic system. From the work of Blier and deMontigny 1994; NC = no change; ND = no data. Reproduced with permission.

1.1.2 RECEPTORS SUBTYPES

Progress in molecular biology has permitted the cloning of fifteen receptor subtypes activated by 5-HT in vertebrates (Murphy et al., 1998), each of them adding to the complexity of the system by their unique pattern of expression, cellular localization (pre- or postsynaptic) and regulation. Each of those subtypes are transmembrane G-protein coupled metabotropic receptors with the exception of the 5-HT_3 receptor, a pentameric ligand-gated ion channel (Marick, 1991). Those receptors are grouped in seven distinct families (5-HT_1-5-HT_7) based on their homology and second messenger machinery (Table 1).

The 5-HT_1 receptors are a relatively well-characterized family, on which a special emphasis will be placed since their role as presynaptic autoreceptors controlling the firing rate and the release of 5-HT is imperative to the relevance to the mechanism of action of antidepressants (Table 2). Five receptor subtypes belong to this family so far: the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}. Encoded by intronless genes (365-422 amino acids), they share an average sequence homology of 40%. They are predominantly coupled to G_i or G_o and their activation by ligand binding produces either an inhibition of AMP formation (G_i) or an opening of K^+ channels (G_o) (Stamford, 2000).

5-HT_{1A} RECEPTORS

The 5-HT_{1A} receptor is expressed throughout the CNS both pre- and postsynaptically (Hamon, 1997). Presynaptically, it is mainly found on the cell bodies of MR and DR-5-HT neurons where it assumes the role of an inhibitory somatodendritic autoreceptor controlling the firing rate (Blier et al. 1987) and the release of 5-HT from projection areas (Kennett et al. 1987; Bohmaker et al. 1993), as shown by extensive pharmacological studies with 5-HT_{1A} agonists such as 8-OH-DPAT. Conversely, in awake cats, the blockade of this receptor by the systemic administration of the 5-HT_{1A} antagonist WAY-100635 increases 5-HT neuronal activity (Fornal et al. 1996). Also of interest, presynaptic 5-HT_{1A}-R activation induces hyperphagia and anxiolytic-like effects in behavioural paradigms (De Vry, 1995).

Postsynaptically, high amounts are found in the hippocampus, septum and amygdala, structures that are all part of the limbic system. Their activation also produces a decrease in the firing rate of postsynaptic cells (Sprouse and Aghajanian, 1988). Stimulation of the postsynaptic 5-HT_{1A} receptors classically results in a so-called "5-HT syndrome" including a flat body posture, forepaw treading, headweaving hypothermia and ACTH release (Wilkinson and Dourish, 1991).

Partial 5-HT_{1A}R agonists, such as buspirone and gepirone, are clinically used in the treatment of anxiety disorders (Coplan et al. 1995).

5-HT_{1B} RECEPTORS

Although the 5-HT_{1B} receptors are less ubiquitous than the 5-HT_{1A}, they are still found in high concentrations in many different regions of the brain such as the basal ganglia, the striatum and the frontal cortex, where they act as terminal autoreceptors (Hervas, 1998). Negatively coupled to adenylate cyclase, they were originally thought to be present only in rodents, based on a different binding affinity to compounds such as the β-adrenoreceptor antagonist cyanopindolol. Following its sequencing, the murine 5-HT_{1B} receptor was discovered to share 93% of overall homology and 96% of identity in the transmembraine domains with the previously cloned human 5-$HT_{1D\beta}$ receptor, which is only found in higher species. Considering that the human 5-$HT_{1D\beta}$ receptor is distributed similarly to the rodent 5-HT_{1B} and that their different pharmacological profiles have been explained by a single mutation in the transmembrane spanning region, they were reclassified as species homologues of the same receptor and renamed h5-HT_{1B} (formerly 5-$HT_{1D\beta}$) and r5-HT_{1B}, with the h and r prefix referring to the human and rat species respectively (Hartig et al., 1996).

The 5-HT_{1B} receptor controls 5-HT release at the serotonergic terminals. For instance, in microdialysis experiments, i.v. administration of the specific 5-HT_{1B} receptor agonists CP93,129 decreases the amount of 5-HT in the hippocampus of rats (Hjorth and Tao, 1991). The protein is thus in an interesting position to attenuate any acute and transient extracellular increase in 5-HT to occur in

projection areas innervated by serotonergic afferents. Because many classes of antidepressants produce such an effect, the use of potent and selective 5-HT_{1B} antagonists as adjuvants could prevent such a negative feedback and facilitate serotonergic transmission (Hervas, 1998).

Interest in molecules with 5-HT_{1B} agonistic properties have also been amplified by the discovery of the antimigraine properties of the 5-HT_{1B}/5-HT_{1D} agonist sumatriptan, a compound which may act either through the 5-HT_{1B} receptors located on cerebral arteries (their activation mediates vasocontriction) or those located on trigeminovascular afferents (whose activation blocks neurogenic inflammation and nociceptive transmission) (Hamel, 1999). Additional evidence supports an action through 5-HT_{1B} autoreceptors located on 5-HT neurons themselves.

5-HT_{1D} Receptors

As aforementioned, this receptor was originally found to share a considerable degree of homology with the murine 5-HT_{1B} receptor (Hartig, 1996). However in humans, the story was complicated by the sequencing of not only one but two genes encoding for 5-HT_{1D} receptors: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. The issue was eventually resolved by the cloning of a rat 5-HT_{1D} receptor which shares a high homology and a similar pattern of expression (r5-HT_{1D} receptor mRNA has been found in the caudate-putamen, nucleus accumbens, hippocampus, cortex, dorsal raphe and locus coeruleus (Hoyer et al., 1991) with the h5-$HT_{1D\alpha}$. Given such

similarities, this rat receptor has been named r5-HT_{1D} and is now considered homologous to the human 5-$HT_{1D\alpha}$, which itself is now classified as h5-HT_{1D} (see above for the nomenclature concerning the 5-$HT_{1D\beta}$).

The presence of 5-HT_{1D} mRNA in the raphe suggests a role of somatodendritic autoreceptor for this protein, in complement with the 5-HT_{1A} receptors. Indeed, sumatriptan reduces the electrically-evoked release of tritiated 5-HT from preincubated mouse mesencephalic slices (Pineyro, 1995). The rationale behind this apparent redundancy is unsettled, but the control of 5-HT release mediated by the 5-HT_{1D}R distinguishes itself from the one exerted by the 5-HT_{1A}R by its firing rate independency (Stamford et al., 2000). This property, added to different expression patterns and gene regulations, likely contributes to the plasticity of the serotonergic system.

1.1.3 BIOCHEMISTRY

SYNTHESIS

The biochemical pathway responsible for 5-HT synthesis starts with the precursor, the amino acid L-tryptophan, whose availability is dependent upon the diet. Its transport from blood to brain is facilitated by a neutral amino acid carrier {the leucine-preferring (L) transport system according to Christensen's nomenclature (Bannai et al. 1984)}, like other amino acids phenylalanine, leucine and methionine. The concentration of the other amino acids competing for the carrier thus has a major influence on its transport through the blood-brain barrier. Indeed, a tryptophan depletion paradigm exploiting this characteristic has been developed and is used clinically to evaluate the relationship between brain 5-HT levels and the mechanism of action of psychotherapeutic drugs (Young, 1996).

The second step is the conversion of tryptophan to 5-hydroxytryptophan (5-HTP), a reaction catalyzed by the enzyme tryptophan hydroxylase, whose expression is specific to cells that synthetise 5-HT. The Km of this enzyme for tryptophan is 30-60 μM (Frazer and Hensler, 1999), a concentration similar to that of tryptophan in the brain. If the concentration of tryptophan in the whole brain truly represents its concentration in 5-HT neurons, the enzyme is not saturated under normal conditions: the synthesis of 5-HT in brain should consequently follow tryptophan levels. This is the case, even though the relationship is not completely straightforward and is yet to be fully understood (Frazer and Hensler, 1999)

The third step in the 5-HT biosynthesis pathway is the conversion of 5-HTP to 5-HT, a reaction catalyzed by the aromatic L-amino acid decarboxylase (AADC), a soluble pyridoxal-5'-phosphate-dependent enzyme. AADC is not specific to 5-HT neurons since it is also expressed in dopaminergic neurons, where it catalyses the transformation of 3,4-dihydroxyphenylalanine (DOPA) to dopamine. The Km of the enzyme for 5-HTP, 10 μM, makes it far from being saturated under normal conditions. A supplementation in 5-HTP thus leads to an increase in 5-HT synthesis, but also causes non-selective synthesis and release of 5-HT by dopaminergic cells.

STORAGE

5-HT is stored in typical synaptic vesicles via active transport from the cytoplasm as a result of an electrochemical gradient generated by a vesicular H^+-ATPase pump. In that process common to most of the classical neurotransmitters (Gasnier, 2000), the uptake of cytoplasmic amine is coupled to the extrusion of a proton. The effects of the monoamine-depleting drug reserpine on mood, which is one of the tenets of the monoamine hypothesis of depression, is due to the specific blockade of the particular isoform of this vesicular pump in monoaminergic cells, the vesicular membrane transporter 2 (VMAT2) (Weihe, 1994; Peter, 1995). Part of the mood-enhancing effects produced by the 5-HT releasers fenfluramine and MDMA is also mediated by their association to the substrate-binding site of VMAT2 (Bankson and Cunningham, 2001). This

interaction with the transporter would interfere with 5-HT uptake and cause the transmitter to be massively released by a nonexocytotic mechanism.

REUPTAKE

Following Ca^{2+}-dependent release of 5-HT and subsequent stimulation of pre- or postsynaptic receptors, 5-HT is pumped back into the cells through a specific 12 transmembrane domain membrane carrier, the serotonin transporter (SERT). In the CNS, SERT mRNA is only found in the 5-HT cells of the raphe nuclei, which makes it a highly specific way to identify those cells (Zhou, 1996). The transport role of this membranous protein is supported by lesion studies showing that the selective destruction of central 5-HT neurons is associated with a marked decrease in the high-affinity uptake of [^{3}H]5-HT in brain areas innervated by 5-HT afferences (Marcusson, 1988).

The uptake system has a K_m value for 5-HT of approximately 0.2-0.5 μM. The process is dependent on temperature and external Na^+ and Cl^- and is even blocked by Na^+/K^+ ATPase inhibitors. From this evidence, a model has been developed where the energy necessary to the 5-HT reuptake is used to maintain the Na^+ gradient across the membrane rather than to be directly used in the transport of 5-HT. According to this model, one Na^+, one Cl^- and one protonated 5-HT must bind to the transporter to generate a conformational change (a process that might involve the opening of a pore) and allow transmitter reuptake (Frazer and Hensler, 1999).

SERT shares about 50% of homology with the transporters for norepinephrine (NET) and dopamine (DAT), which is sufficient to provide pharmacological specificity since the more conserved regions are found in TMDs 1, 2, 4 and 8, regions not directly involved in ligand binding. Indeed, secondary amine tricyclic antidepressants, such as desipramine, are 25- to 150-fold more potent at blocking the reuptake of NE than 5-HT (Finn et al., 1998). Some drugs, like cocaine, can block all three transporters.

This transporter is the primary target of both tricyclic antidepressants and SSRIs, and its blockade would eventually lead to an increase of 5-HT in the synaptic terminals, a final effect common to all major classes of antidepressants and thought to be necessary to their clinical action (Table 2).

CATABOLISM

The intracellular breakdown of 5-HT is accomplished by the mitochondrial enzyme monoamine oxydase (MAO), a flavin-containing enzyme that converts 5-HT to 5-hydroxyindoleacetaldehyde, a product subsequently oxidized in the brain to yield 5-hydroxyindolacetic acid (5-HIAA) by an NAD^+-dependent aldehyde dehydrogenase (Weyler, 1990). Two different isoforms of MAO are known to exist in monoaminergic cells, MAO-A and MAO-B; they share 70% of homology (Bach et al., 1988; Grimsby, 1991). MAO-A preferentially degrades 5-HT and NE over DA while the opposite is true for MAO-B. However, this substrate preference

is not absolute, as each isoform can take over and degrade the transmitter it is not a priori suited for. Selective inhibitors exist for the two enzymes. For instance, MAOIs, like clorgyline, and the more selective RIMAs (reversible inhibitors of MAO-A), like moclobemide, target the type A and are clinically used in the treatment of mood disorders. When chronically administered, these molecules enhance 5-HT and NE transmission by increasing the levels of unmetabolized transmitter in the neurons and, consequently, the amount of action potential-dependent transmitter release (Blier et al. 1986). Deprenyl is a molecule which preferentially inhibits the type B and is used to counteract the dopamine deficit in Parkinson's disease.

Curiously, 5-HT neurons predominantly contains MAO-B (Jahng, 1997). A role in preventing the accumulation of a false transmitter would explain this observation. In any case, the observation of increased brain levels of 5-HT following the administration of the MAO-A inhibitor clorgyline proves the importance of MAO-A in 5-HT catabolism.

1.1.4 ELECTROPHYSIOLOGY

The serotonergic raphe neurons fire action potentials at slow and steady rate in different species (Jacobs, 1991; Vander Maelen and Aghajanian, 1983). This firing pattern is attributed to the pacemaker alternation of hyperpolarization and depolarization ramps involving a calcium-dependent K^+ outward current and a

low threshold T-type Ca^{2+} current (Aghajanian, 1982 and 1984). A voltage-dependent outward K^+ current is also implicated and simultaneously activated with the T current. Known as the I_A current, its opening slows down the rate of depolarization and thus the firing frequency of the cell.

Interestingly, the firing rate of these cells is more influenced by the behavioral state than the environmental stimulation, at least in cats (Jacobs and Fornal, 1993). Indeed, the DR 5-HT neurons pass from an average firing frequency of 1.5 Hz during slow wave sleep to an average of 4-7 Hz during behavioral activation (Jacobs, 1991). Furthermore, they are virtually silent during REM sleep, a observation which is temporally correlated with muscle atonia. However, pain, hot environment exposure, increase in blood pressure, insulin-induced hypoglycemia, painful stimuli and physical restraint were not found to affect their activity (Jacobs and Fornal, 1993). These observations, in addition with the increase in firing rate measured in 25% of DRN and MRN of cats involved in stereotyped oral-buccal movements in cats, unravels a strong relationship between motor output and 5-HT neuronal activity (Jacobs and Fornal, 1993). Such a link should be kept in mind when interpreting data gathered with common paradigms used in antidepressant research.

1.2 THE NORADRENERGIC SYSTEM

1.2.1 NEUROANATOMY

The Locus Coeruleus (LC), an irregular aggregate of pigmented cells located next to the periventricular gray matter of the superior portion of the fourth ventricle, is the major supplier of noradrenergic fibers in the brain. In rats, it corresponds to group A6 following Dahlström and Fuxe`s nomenclature (Dahlström and Fuxe, 1964). In humans, the nucleus is partially immixed with the mesencephalic nucleus of the trigeminal nerve, although the large globular neurons of the latter nucleus are also present dorsally and rostrally at the outskirts of the central gray matter (Pearson et al., 1990). LC neurons can be divided in two categories: medium-sized round cells with eccentric nuclei and important clumps of melanin granules, and small oval non-pigmented cells with barely sufficient cytoplasm (Olszewski et al., 1954; Russel, 1955). Besides, a more diffuse collection of those two types of cells can be found ventrolaterally and constitutes the nucleus subcoeruleus (NSC).

Unlike other noradrenergic groups of neurons dispersed in the pontine and medullar lateral tegmentum (A5 and A7), the LC-NE neurons form a compact entity. Their projections reach the telencephalon, the diencephalon, the midbrain, the cerebellum, the pons, the medulla, and the spinal cord. The LC thus appear to be a specific transmission system (Parent, 1996).

The LC and SCN-NE cells, as revealed by immunohystochemistry, are partly intermixed with neurons secreting many different neurotransmitters. The list includes acetylcholine, γ-aminobutyric acid (GABA), 5-HT and different neuropeptides (Fodor et al., 1992; Jones, 1991; Pioro et al., 1990). Furthermore, light and electron microscopic autoradiography have shown the presence μ-opioid receptors on noradrenergic neurons themselves (Beaudet, 1997).

ASCENDING PROJECTIONS

In humans, the LC ascending projections are found throughout the neuraxis (Fig. 2A). Apart from the midbrain 5-HT system, no other reticular formation cell group has been found to send as many projections to the forebrain (Parent, 1996). On their way, these projections pass through the midbrain (rostrally), next to the to the medial longitudinal fasciculus (laterally) and to the central gray matter (ventrolaterally). At the level of the caudal diecenphalon, the main ascending noradrenergic axons reach the medial forebrain bundle (MFB) by first entering the mammillary peduncle and the ventral tegmental area. These fibers are part of the MFB when they pass through the hypothalamus. The bundle continues further rostrally to divide at the level of the anterior commissure, where it separates in two bundles innervating the diencephalic and telencephalic structures. A stria medullaris component deviates caudally to reach midline regions of the thalamus, while the amygdala is innervated by the stria terminalis. The noradrenergic projections to the thalamus are among the more abundant of

their kind, innervating the intralaminar thalamic nuclei, the anterior nuclear group and the lateral geniculate body. They account for the involvement of noradrenaline in sleep processes (Jones, 1991). Other afferents fibers coming from the LC go to the hippocampus, the cingulate cortex and the subiculum via the fornix. The rostral, dorsal and lateral cortex of the frontal lobe noradrenergic innervation is provided by the more rostral LC-projecting fibers, which pass from the MFB to the external capsule. Some groups of axons, smaller in size, separate from the main bundle (also named "dorsal catecholamine pathway") and project into the third ventricle periaqueductal and periventricular gray matters.

An important contigent of LC neurons (10%) innervates the cerebral and cerebellar cortices. The superior cerebellar peduncle is the gateway of NE fibers reaching the cerebellum, where they connect in the Purkinje cell somata vicinity and the lower third of the molecular layer.

The brainstem structures such as the inferior and superior colliculi, the primary sensory, the association nuclei as well as a portion of the pontine nuclei are connected by noradrenergic projections. The spinal cord also receives LC and SCN fibers via the anterior and lateral funiculi, which innervate portions of the anterior and intermediate gray matter at all levels (Parent, 1996).

1.2.2 RECEPTOR SUBTYPES

Noradrenaline produces its effects through receptors belonging to three major families: α_1, α_2 and β. The distinction between α and β receptors was originally made upon their different affinities for synthetic derivatives of catecholamines. For instance, the agonist isoproterenol and the antagonist propanolol preferentially bind 2 receptors, while phentolamine is a specific blocker of K receptors (Bylund, 1992; Bylund, 1994; Hieble et al., 1995). The adrenergic receptors can further be classified as α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2 and β_3. They exert many important functions peripherically both on neural and non-neural tissue, as shown in table 1. In the brain, the main adrenergic receptors to be found are the α_2 and β_1 receptors.

α_2 RECEPTORS

Among diverse functions, α_2 receptors act as presynaptic autoreceptors throughout the peripherical and central nervous systems, not only on noradrenergic fibers themselves but also on non-adrenergic terminals. For instance, the application of the α_2-agonist clonidine to LC-NE cell bodies decreases their firing rate and the release of NE in terminal regions such as the cortex and the hippocampus (Mongeau et al., 1997). The application of the same compound to terminal regions of the serotonergic system also diminishes the

release of 5-HT (Tao and Hjörth, 1992). Conversely, the antidepressive properties of the clinically used α_2-antagonist mirtazapine are partly attributable to the augmented terminal release of 5-HT (de Boer, 1995; Haddjeri et al., 1997).

1.2.3 Biochemistry

Synthesis

The initial step in the synthesis of catecholamines dopamine and norepinephrine is the meta-hydroxylation of their amino acid precursor, L-tyrosine, into 3,4-dihydroxy-L-phenylalanine (L-DOPA) (figure 2B). This reaction is catalysed by the mixed-oxidase tyrosine hydroxylase (TH), an enzyme also requiring molecular oxygen, Fe^{++} and biopterin (Shiman, 1971). It is a complex formed 4 subunits each having a molecular weight of 60 000 which is found in all catecholamine-secreting cells. The K_m of TH for tyrosine is in the micromolar range ($0.4\text{-}2 \times 10^{-5}$ M) and is saturated by tyrosine under normal conditions. The enzyme is thus the limiting step in the pathway, a major difference when compared to the 5-HT biosynthesis pathway where tryptophan availability is the critical variable. Indeed, marked reductions in the levels of dopamine and norepinephrine can be achieved in the brain and in sympathetically-innervated tissues of the periphery by competitive inhibitors of TH such as the tyrosine analog α-methyl-p-tyrosine (AMPT) (Udenfriend et al., 1965; Engelman et al., 1968).

The second step is the decarboxylation of L-DOPA to dopamine, a reaction that requires the ubiquitously expressed DOPA decarboxylase (DDC) or aromatic amino acid decarboxylase (AADC), the same enzyme involved in serotonin synthesis (Christenson et al., 1970). DDC has a low K_m (4×10^{-4} M) and a fast V_{max} that allows it to readily transforms L-DOPA into dopamine.

The third step, the 2-hydroxylation of dopamine, which finally yields norepinephrine, occurs in noradrenergic and adrenergic but not in dopaminergic cells. Dopamine β-hydroxylase (DBH), a mixed-function oxidase and tetrameric glycoprotein composed of 77 and 73 kDa subunits, is the enzyme governing this reaction which takes place inside the vesicles (Craine et al., 1973; Lamouroux et al., 1987). It requires molecular oxygen and ascorbic acid as cofactors and contains Cu^{2+} which is implicated in electron transfer during the reaction. Most of DBH is bound to the interior of synaptic vesicles, a topological observation that would explain its release from the nerve terminals following the application of trains of action potentials (Weinshilboum et al., 1971).

STORAGE

As mentioned previously, norepinephrine enters the vesicles through 12 transmembrane domain transporters VMAT2, vesicular transporters common to all monoaminergic cells and that can be blocked by reserpine. Different from serotonergic vesicles, noradrenergic vesicles also contain ATP, which suggests a role of neuromodulation for the purine.

Reuptake

The reuptake of noradrenaline back into the nerve terminals is carried out by the norepinephrine transporter (NET), a 12 membrane-spanning domain protein of 617 amino acids which, as mentioned previously, shares 50% of homology with SERT and DAT (Fraser et al., 1999). Like its homologs, its action is Na^+-dependent and blocked by inhibitors of the Na^+/K^+-activated ATPase. Some clinically-used antidepressants, like desipramine and nisoxetine, exert their effect by blocking the normal function of this transporter.

Catabolism

Similarly to 5-HT, Norepinephrine is first metabolized to its corresponding aldehyde by the mitochondrial enzyme MAO_A. In the brain, this intermediate is rapidly oxidized to become an acid by the enzyme aldehyde dehydrogenase. Knockout mice for MAO_A exhibit high levels of NE and 5-HT and an aggressive behavior in behavior tests (Cases et al., 1995). This impulsive aggressivity was also observed in men from a Dutch family with a point mutation in the gene encoding MAO_A and resulting in its complete inactivation (Brunner et al., 1993).

1.3 INTERACTIONS BETWEEN THE 5-HT AND NE SYSTEMS

The idea of 5-HT and NE systems being reciprocally connected and exerting a mutual influence is supported by both anatomical and pharmacological evidences. Firstly, the LC receives dense 5-HT projections (which do not entirely originate from the dorsal raphe, however) while the DR is innervated by LC-NE projections. Secondly, as previously mentioned, the activation of α_2-adrenoreceptors by the i.v. administration acutely reduces 5-HT synthesis in both hippocampus and dorsal raphe nucleus (Yoshioka ,1992). The action of clonidine on α_2-adrenoreceptors develops along three different mechanisms: 1) it shuts down the firing rate of LC-NE through somatodendritic receptors, thus decreasing the tonic excitatory LC-NE input (exerted through α_1-adrenergic heteroreceptors) on 5-HT neurons 2) it decreases the release of transmitter by NE terminals through the terminal autoreceptors, also removing the LC-NE tonic input and 3) it decreases the release of 5-HT by 5-HT terminals through heteroreceptors.

The issue of the LC-NE neuronal modulation by the 5-HT system has also been addressed using a 5-HT fibers lesioning strategy. In fact, Haddjeri et al. (1997) have found the LC-NE neuronal activity to be elevated after the destruction of 5-HT fibers with the selective neurotoxin 5,7-dihydroxytryptamine, thus unravelling an inhibitory tone exerted by the 5-HT system on noradrenergic neurons.

In light of these complex interrelations, it is easy to conceive that a noradrenergic reuptake inhibitor, like reboxetine, or a selective serotonin reuptake inhibitor, like citalopram, may affect the activity of both systems, even if it is primarily targetting a neuronal element belonging only to one of the two transmission systems (Szabo et al., 2000)

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CHAPTER 2: "MODIFICATION OF SEROTONIN NEURON PROPERTIES IN MICE LACKING 5-HT_{1A} RECEPTORS"

MODIFICATION OF SEROTONIN NEURON PROPERTIES IN MICE LACKING 5-HT_{1A} RECEPTORS

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Submitted to: European Journal of Pharmacology

Date: June 14, 2001

2.1 ABSTRACT

Using null mutant mice for the serotonin $(5\text{-HT})_{1A}$ receptor (5-HT_{1A} -/-), extracellular electrophysiological recordings were first conducted to evaluate the impact of its genetic deletion on the firing rate of dorsal raphe 5-HT neurons. Experiments were also done using brain slices to assess whether any compensation phenomenon had taken place in key receptors known to control 5-HT and norepinephrine (NE) release. The mean firing rate of 5-HT neurons was nearly doubled in 5-HT_{1A} -/- mice, although 65% of the neurons were firing in their normal range. In preloaded brain slices, the $5\text{-HT}_{1D/B}$ agonist sumatriptan equally inhibited the electrically-evoked release of [^{3}H]5-HT in mesencephalic slices (containing the dorsal and median raphe) from wildtype and 5-HT_{1A} -/- mice. The 5-HT_{1B} agonist CP 93129 and the α_2-adrenergic agonist UK 14,304 produced the same inhibitory effect in both groups of mice in hippocampus and frontal cortex slices. No difference was observed on the UK14,304-mediated inhibition of [^{3}H]NE from preloaded slices of the two latter structures between the two groups of mice. In conclusion, the loss of control of the 5-HT_{1A} autoreceptor in 5-HT_{1A} -/- mice lead to a smaller enhancement of 5-HT neuronal firing than expected and it did not alter 5-HT or NE release in any of the brain structures examined. In addition, it was not associated with changes in the function of 5-HT_{1D} and 5-HT_{1B} autoreceptors and of α_2-adrenergic heteroreceptors on 5-HT neurons, nor of that of α_2-adrenergic autoreceptors on NE terminals.

KEY WORDS: 5-HT_{1A} mutant mice · Autoreceptors · Heteroreceptors · Frontal cortex · Hippocampus · Raphe

RUNNING TITLE: 5-HT and NE function in 5-HT_{1A} -/- mice

2.2 INTRODUCTION

The serotonin (5-hydroxytryptamine; 5-HT) 5-HT_{1A} receptors are $G_{i/o}$ protein-coupled receptors, located on the cell body and dendrites of 5-HT neurons and on postsynaptic neurons with a particularly high density in limbic structures. These receptors mediate an inhibitory effect on firing rate through K^+ channels. They can also be coupled to Ca^{2+} channels and second messenger systems (Jacobs and Azmitia, 1992).

The therapeutic response of several antidepressant drugs like selective serotonin reuptake inhibitors (SSRIs) relies in part on the desensitization of 5-HT_{1A} autoreceptors, *via* the partial loss of the negative feedback action normally exerted by this autoreceptor on 5-HT neuronal firing (Blier and de Montigny, 1994). Other antidepressant treatments, like tricyclic antidepressants and electroconvulsive shock treatment, increase the sensitivity of postsynaptic 5-HT_{1A} receptors. Nevertheless, a net effect of all major classes of antidepressant treatments on 5-HT transmission is an increase in the degree of activation of postsynaptic 5-HT_{1A} receptors (Haddjeri et al., 1998). Consistent with the proposed mechanism of action of SSRIs in major depression, the 5-HT_{1A} antagonist pindolol shortens the onset of action of SSRIs, by decreasing the inhibitory feedback on 5-HT neuron firing rate (Artigas et al. 1996; Blier and Bergeron, 1998). This leads rapidly to enhanced synaptic levels of 5-HT in the presence of 5-HT reuptake blockade by SSRIs. Moreover, 5-HT_{1A} agonists like buspirone and gepirone can be used, alone or in combination with

antidepressant drugs, to treat major depression and generalized anxiety disorder (Bouwers and Stein, 1997; Robinson et al. 1990; Wilcox et al. 1996).

The neurotransmitter norepinephrine (NE) can also play an important role in the mechanism of action of antidepressants (Anand and Charney, 2000). Moreover, the 5-HT and NE neurotransmitter systems are endowed with reciprocal interactions throughout the brain (Mongeau et al., 1997). For example, the α_2-adrenergic agonist clonidine decreases 5-HT neuronal firing and release as a result of its inhibitory effect on NE neuronal function (Tao and Hjörth, 1992). In contrast, the α_2-adrenergic antagonist mirtazapine would exert its antidepressant action by enhancing NE release, and indirectly that of 5-HT as well, after long-term administration (de Boer, 1995; Haddjeri et al., 1997a). Conversely, a three-week but not a subacute treatment with SSRIs attenuates the firing rate of NE neurons (Szabo et al., 1999, 2000).

Null mutants animals for the 5-HT_{1A} receptor (5-HT_{1A} -/-) have been produced and shown to be more anxious in different behavioral tests (Ramboz et al., 1998; Heisler et al., 1998; Parks et al., 1998). Given the prior demonstration that 5-HT_{1A} autoreceptor function is absent in 5-HT_{1A} -/- mice (Ramboz et al. 1998), this study was thus undertaken to evaluate the impact of this genetic deletion on 5-HT neuronal firing activity and to determine if any compensation phenomenon had taken place in presynaptic receptors controlling 5-HT and NE neuronal function in the mutants. To this end, extracellular electrophysiological recordings

were carried out on dorsal raphe 5-HT neurons. The sensitivity of 5-HT_{1B} autoreceptors and α_2-adrenergic heteroreceptors, located on 5-HT terminals, and of somatodendritic 5-HT_{1D} autoreceptors, as well as that of α_2-adrenoreceptors on NE terminals were also assessed.

2.3 MATERIALS AND METHODS

ANIMALS

Male 129/SvEvTac wild-type mice and null mutant male mice lacking 5-HT_{1A} receptors weighting 20-30 g on the day of the experiment were used (Columbia University, Center for Neurobiology and Behavior, New York, USA). These 5-HT_{1A} mutant mice were produced from 129/SvEvTac cells and the resulting chimeras were bred with 129/SvEvTac females (Ramboz et al., 1998). The animals were maintained on a 12:12 hours light:dark cycle with free access to food and water. Principles established by Canadian Committee on Animal Care were followed at all times and the procedures approved by the McGill University Ethics Committee.

EXTRACELLULAR UNITARY RECORDINGS

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p. using a 4% aqueous solution) and were mounted in a stereotaxic apparatus. Additional doses (100 mg/kg, i.p.) were given to maintain the anesthesia during the experiment. The recordings were performed with single-barrelled glass micropipettes filled with 2 M NaCl. The electrodes were positionned at 0.5-0.9 mm posterior to lambda and lowered in the dorsal raphe. The neurons were usually found at a depth of 2.5-3.5 mm below the surface of the brain. They were identified using the following criteria: a generally slow (0.5 to 2.5 Hz) and regular firing rate and long-duration (0.8 to 1.2 ms) positive potentials.

SUPERFUSION EXPERIMENTS

The animals were killed by decapitation and the brain immediately removed and then dissected on an ice-cold glass plate. Slices, 400 μm thick, from the mesencephalic raphe, hippocampus and frontal cortex were prepared with a McIlwain tissue chopper. The slices were then incubated for 30 min at 37°C in Krebs buffer containing 100 nmol/l [^{3}H]-5-HT creatinine sulphate (specific activity: 21.8 Ci/mmol); NEN Research Products, MA, USA) or 100 nmol/l [^{3}H]-NE hydrochloride (specific activity: 13.5 Ci/mmol); NEN Research Products, MA, USA) and bubbled with a mixture of 95% O_2/5% CO_2. The composition of the Krebs solution was the following (in mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, Na_2 EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, the slices were washed, transferred to glass chambers, and superfused at a rate of 0.5 ml min^{-1} with oxygenated Krebs solution maintained at 37°C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion for the three structures. Two periods of stimulation, S_1 and S_2, were carried out within the same experiment at 8 min and 52 min, respectively, after the end of the 90-min washing period. The electrical field (30 mA, 2 ms, 3 Hz for 2 min) was generated in the chambers between 2 platinum electrodes positioned 2 cm apart. The frequency of stimulation was chosen because it was within the range of the firing rate of 5-HT neurons recorded in freely moving animals (Jacobs, 1986). The first stimulation period (S_1) was used as a control and the drugs were added 20 min before S_2

and remained present throughout the rest of the experiment. At the end of the superfusion period, the slices were solubilized with 0.5 ml Soluene 350 (Packard Instruments, Downers grove, Il, U.S.A.) and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results are expressed as the fraction of tritium content present at the time of the onset of the respective collection periods. The fractional release evoked by electrical stimulation was calculated as the difference between the total amount of radioactivity released during stimulation and the basal outflow obtained in the sample preceding the onset of stimulation (sp_1 or sp_2). To assess the drug-induced changes of electrically-evoked release of tritium from the slices preloaded with [^{3}H]-5-HT or [^{3}H]-NE, the ratio of fractional release between the second and the first period of stimulation (S_2/S_1) were also calculated to determine whether the drugs altered the basal outflow of radioactivity. The amount of tritium released by electrical stimulation under these conditions provides a reliable estimate of the release of tritiated or endogenous 5-HT (Baumann et al., 1981; Blier and Bouchard, 1993).

DRUGS

The following drugs were used: UK14,304 (RBI, Natick, MA, USA), sumatriptan succinate (Glaxo, Middlesex, UK) and CP93129 (Tocris, Ballwin, MO, USA).

STATISTICAL ANALYSES

Results are expressed as means ± standard errors. Differences between the wildtype mice (5-HT_{1A} +/+) and the 5-HT_{1A} -/- mice were compared with the Student's two-tailed *t* test. The effects of the drugs on fractional release were assessed using a one-way ANOVA, with the mutant genotype as the main factor, followed when necessary by the post-hoc Tukey test. In order to detect treatment effects, the experiments were conducted by studying simultaneously in the same superfusion apparatus slices prepared from a wildtype and slices from a knockout mouse with the same drug solutions. This experimental design was deemed optimal to minimize the problem of inter-experimental variations. Probability values equal or smaller to 0.05 were considered as significant.

2.4 RESULTS

SPONTANEOUS FIRING ACTIVITY OF 5-HT NEURONS

Spontaneously active dorsal raphe 5-HT neurons were recorded from 18 5-HT_{1A} +/+ and 12 5-HT_{1A} -/- mice. Their mean spontaneous firing rate was however nearly doubled in the 5-HT_{1A} -/- mice as compared to the 5-HT_{1A} +/+ mice (wildtype mice: 1.1 $\pm$ 0.2 Hz, n = 59; knockout mice: 2.0 $\pm$ 0.1 Hz, n = 57; Fig. 1). Interestingly, in the 5-HT_{1A} +/+ mice only three neurons were firing at a rate above 2 Hz, whereas in the 5-HT_{1A} -/- mice 35% of the neurons were firing in that upper range (Fig 2).

ELECTRICALLY EVOKED RELEASE OF [^{3}H]5-HT FROM PRELOADED SLICES IN 5-HT -/- MICE

The spontaneous outflow of radioactivity (sp_1), which is mainly comprised of [^{3}H]5-hydroxyindole acetic acid, in contrast to electrically evoked tritium that is principally comprised of [^{3}H]5-HT (Blier and Bouchard 1993), was not different in cortical, hippocampal or mesencephalic slices when comparing wildtype and mutant mice. The fractional release of [^{3}H]-5-HT evoked by S_1 in the absence of any drug was also not significantly different in slices of any of these three structures from 5-HT_{1A} -/- animals as compared to the controls studied in parallel in the same experiments (Table 1). The radioactivity remaining in the slices at the end of the experiment was unchanged in the 5-HT_{1A} -/- when compared to the 5-HT_{1A} +/+ mice (data not shown).

Effect of the 5-HT_{1B} agonist CP93129 on the Electrically Evoked Release of Tritium from [^{3}H]5-HT Preloaded Slices

The addition of CP93129 to the superfusate, 20 minutes before S_2, did not alter the spontaneous outflow of tritium but resulted in a significant inhibition of [^{3}H]-5-HT in cortical slices [$F(2,14) = 18.9$, $p<0.001$ and $F(2,18) = 13.1$, $p<0.001$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 3A] at the 30 nmol/l concentration (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) that was equal in both strains of mice ($p=0.71$, $p=0.80$, $p=0.22$ for the 0,10 and 30 nmol/l concentrations, respectively). In hippocampal slices, a concentration-dependent inhibition was observed [$F(3,29) = 20.4$, $p<0.001$ and $F(3,27) = 12.6$, $p<0.001$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-,respectively; Fig. 3B] for 10 (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively), 30 (post-hoc test, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) and 100 nmol/l concentrations (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively). However, the degree of inhibition was equal in both strains of mice ($p=0.43$, $p=0.80$, $p=0.48$, $p=0.79$ for the 0, 10, 30 and 100 nmol/l concentrations, respectively).

Effect of the α_2-Adrenergic Agonist UK14,304 on the Electrically Evoked Release of Tritium from [^{3}H]5-HT Preloaded Slices

The fractional release of [^{3}H]-5-HT evoked in S_2 in the presence of the agonist of the α_2-adrenergic agonist was significantly diminished [$F(2,20) = 6.4$, $p<0.01$ and

$F(2,21) = 9.1$, $p<0.001$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 4A] by the addition of 100 (post-hoc test, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) and 300 nmol/l UK14,304 in the cortex (post-hoc tests, $p<0.05$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively), as previously reported for other species (Blier et al., 1990; Blier and Bouchard, 1994). However, no significant change was observed between 5-HT_{1A} +/+ and 5-HT_{1A} -/- animals ($p=0.71$, $p=0.66$ and 0.67 for the 0, 100 and 300 nmol/l concentrations, respectively). In the hippocampus, a significant inhibition was also observed [one-way ANOVAs, $F(3,30) = 6.5$, $p<0.002$ and $F(3,27) = 8.0$, $p<0.001$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 4B] after the addition of 100 (post-hoc tests, $p<0.01$ and $p<0.05$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively), 300 (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) and 1000 nmol/l (post-hoc tests, $p<0.05$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively), but no difference resulted between the two strains of mice ($p=0.43$, $p=0.34$, $p=0.37$ and $p=0.33$ for the 0, 100, 300 and 1000 nmol/l concentrations, respectively).

Effect of the 5-$HT_{1D/1B}$ Agonist Sumatriptan on the Electrically Evoked Release of Tritium from [^{3}H]5-HT Preloaded Slices

Sumatriptan produced a significant diminution in the release of [^{3}H]-5-HT by preloaded mesencephalic slices [$F(2,36) = 16.7$, $p<0.001$ and $F(2,27) = 3.8$, $p<0.03$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 5] at the 30 (post-hoc tests, $p<0.01$ and $p<0.05$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) and 100

nmol/l concentrations (post-hoc tests, $p<0.01$ and $p<0.05$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively), without altering the spontaneous outflow of tritium. No difference was observed in the 5-HT_{1A} -/- when compared to the 5-HT_{1A} +/+ mice ($p=0.16$, $p=0.72$ and $p=0.35$ for the 0, 30 and 100 nmol/l concentrations, respectively).

Effect of the α_2-Adrenergic Agonist UK 14,304 on the Electrically Evoked Release of Tritium from [^{3}H]NE Preloaded Slices

In control conditions, the spontaneous outflow of radioactivity (sp_1) was not significantly different, neither in cortical nor in hippocampal slices preloaded with [^{3}H]NE in 5-HT_{1A} -/- and 5-HT_{1A} +/+ mice (Table 2). The electrically evoked release of [^{3}H]NE in S_1 was also unaffected in mutant animals when compared to that of normal littermates in both structures (Table 2). The release of [^{3}H]NE was decreased in preloaded cortical slices from knockout and control animals following the addition of UK14,304 to the superfusate [$F(2,12) = 28.7$, $p<0.001$ and $F(2,11) = 57.3$, $p<0.001$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 6A] at the 10 (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively) and 100 nmol/l concentrations (post-hoc tests, $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively). However, these concentrations produced an equal inhibition in both groups of mice ($p=0.85$, $p=0.44$ and $p=0.53$ for the 0, 10 and 100 nmol/l concentrations, respectively). In hippocampal slices, UK14,304 inhibited the evoked release of [^{3}H]NE from both strains [$F(2,13) = 14.3$, $p<0.001$ and $F(2,13) = 7.9$, $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-,

respectively; post-hoc tests for 10 mmol/l: $p<0.01$ and $p<0.05$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; post-hoc tests for 100 mmol/l: $p<0.01$ and $p<0.01$ for 5-HT_{1A} +/+ and 5-HT_{1A} -/-, respectively; Fig. 6B]. These concentrations of the α_2-adrenergic agonist produced the same degree of inhibition in both groups of mice ($p=0.36$, $p=0.44$ and 0.57 for the 0, 10 and 100 nmol/l concentrations, respectively).

2.5 DISCUSSION

The results of the present study indicate that the firing rate of dorsal raphe 5-HT neurons was markedly increased in 5-HT_{1A} -/- mice because of the absence of the 5-HT_{1A} autoreceptors (Ramboz et al, 1998; see Figs. 1,2). In contrast, the electrically-evoked release of [^{3}H]5-HT from preloaded brain slices was unaltered in 5-HT_{1A} -/- mice, as well as its modulation by 5-HT_{1D}, 5-HT_{1B} and α_2-adrenergic receptors in various brain structures. In addition, the genetic deletion of the 5-HT_{1A} receptor did not result in a modification of the electrically evoked release of [^{3}H]NE from preloaded brain slices or in an altered capacity of α_2-adrenergic autoreceptors to diminish NE release in two forebrain structures.

Considering that 5-HT_{1A} receptors mediate an inhibitory effect on the firing activity of 5-HT neurons, an increase in firing rate in 5-HT_{1A} -/- mice was expected. Indeed, the mean spontaneous firing rate of dorsal raphe 5-HT neurons was increased by 90% in 5-HT_{1A} -/- animals as compared to their wildtype littermates (Figs. 1,2). Similarly, in neurokinin 1 null mutant mice, the spontaneous firing rate of 5-HT neurons is enhanced to the same degree as reported in the present study in 5-HT_{1A} -/- mice (Santarelli et al. 2001). The latter phenomenon was attributed to a marked desensitization of the 5-HT_{1A} autoreceptor in these mice lacking the receptor on which substance P acts. Taken together, these results indicate that when a loss of the inhibitory effect of the 5-HT_{1A} autoreceptor is achieved, without interfering with other inactivating mechanisms of 5-HT, the firing rate of 5-HT neurons is approximately doubled,

when these neurons are recorded under anesthesia. Consistent with the present observations, Ase et al. (2000) have recently observed an increased 5-HT turnover, as measured by the determination of the 5-HT/5-HIAA ratio, in the dorsal/median raphe. However, these authors concluded this alteration should result in an increased release of 5-HT at the cell body level of 5-HT neurons, which was actually not seen in the present experiments (Table 1). Nevertheless, it is important to mention that the rate of neuronal depolarization in the present study was driven at a fixed rate of 3 Hz. Consequently, only microdialysis experiments carried out under basal conditions will determine whether 5-HT release in the raphe area is enhanced in 5-HT_{1A} -/- mice. It is quite possible that this parameter would not be increased because about 65% of 5-HT neurons in 5-HT_{1A} -/- mice were still firing in the range of those in the 5-HT_{1A} +/+ mice (Fig. 2).

The brain slice superfusion paradigm used in the present study has been used in the past to document changes in receptor sensitivity following various pharmacological treatments. For instance, it has been shown by two groups of investigators that chronic SSRI treatment enhances 5-HT release and desensitizes terminal 5-HT_{1B} autoreceptors (Blier and Bouchard, 1994; El Mansari et al, 1995; Moret and Briley, 1990), consistent with results obtained *in vivo* electrophysiological studies (Chaput et al., 1986, 1991). Moreover, it has been reported that long-term administration of monoamine oxidase and of NE reuptake inhibitors diminish the function of α_2-adrenergic receptors controlling the release of 5-HT in the hippocampus, using both experimental approaches (see

Mongeau et al., 1997 for a review). Thus, given the similar release of 5-HT obtained herein in normal and mutant mice, it may be concluded that the absence of 5-HT_{1A} receptors did not lead to any alteration of the responsiveness of the presynaptic receptors that were studied. Preliminary data obtained using only the IC_{50} concentration of the 5-HT_{1B} agonist CP93129 to study the sensitivity of the 5-HT_{1B} autoreceptor in the hippocampus suggested that the latter receptor was sensitized in 5-HT_{1A} -/- mice (Ramboz et al., 1998). Clearly, upon studying more than one concentration of this 5-HT_{1B} agonist, the present results did not confirm this initial observation (Fig. 3B). Results from microdialysis experiments examining the decrease of extracellular levels of 5-HT by local perfusion of the same 5-HT_{1B} agonist are fully consistent with the results presented herein (Knobelman et al., 2001). Furthermore, it is important to mention that both the present superfusion paradigm and the microdialysis approach have revealed that 5-HT release is unaltered in 5-HT_{1B} -/- mutant mice, despite the loss of the negative autoregulatory influence of the terminal 5-HT_{1B} autoreceptor (Pineyro et al, 1995; Trillat et al., 1997; Knobelman et al., 2001).

There are now three microdialysis studies that examined the extracellular levels of 5-HT in postsynaptic structures of 5-HT_{1A} -/- mutant mice. Two showed unaltered levels of 5-HT in the striatum and/or the hippocampus (He et al., 2001; Knobelman et al., 2001) and one an increase in the frontal cortex and the hippocampus (Parsons et al., 2001). It is always possible to claim that such divergent results could be attributed to the use of different background strains of

mice, but the one striking difference of the latter work, showing an altered level of 5-HT, is that much older mice were used. It is important to emphasize here that the present experiments were carried out with mice originating from the same laboratory and were approximately of the same age as those used by Knobleman et al. (2001). Both studies yielded identical results with respect to 5-HT levels and 5-HT_{1B} autoreceptor sensitivity.

One possibility to envisage for the lack of alteration of 5-HT release in the 5-HT_{1A} -/- mice is that the 5-HT transporter could theoretically be up-regulated to compensate for the increased number 5-HT molecules released into synaptic cleft. Nevertheless, the present results would not support this contention as the amount of [^{3}H]5-HT remaining in the slices after the experiments, a reliable index of reuptake in the presence of unaltered release and presynaptic receptor function, was not different in the 5-HT_{1A} -/- mice when compared to the controls. This conclusion is also in line with previous immunohistochemical results showing a normal density and distribution of the 5-HT transporter in these animals (Heisler et al., 1998).

The NE and 5-HT systems are known to interact extensively (Mongeau et al., 1997). For instance, although acute injection of the SSRI paroxetine does not alter the firing rate of locus coeruleus NE neurons, a robust inhibitory effect gradually develops as the treatment is prolonged (Szabo et al., 1999). Moreover, many pharmacological compounds targeting primarily the NE system, like the

selective NE reuptake inhibitors desipramine and nisoxetine, also induce profound alterations of 5-HT neuronal elements after long-term administration (Mongeau et al., 1997; Yoshioka et al., 1996). It therefore remains possible that, even if no change in NE neuronal function have been identified in the present study, potential mutation-induced alterations in NE neuronal function could have been dampened by the 5-HT system. For instance, it will be interesting to examine the firing rate of NE neurons in 5-HT_{1A} -/- mice, given that 5-HT neurons exert a tonic inhibitory action on the latter parameter and that 5-HT turnover is increased in the locus coeruleus of 5-HT_{1A} -/- mice (Ase et al., 2000; Haddjeri et al., 1997b).

In conclusion, the present study documented a marked increase in the mean firing activity of 5-HT neurons. However, it did not put into evidence alterations of the electrically evoked release of [^{3}H]5-HT and [^{3}H]NE in 5-HT_{1A} -/- mice nor of the presence of adaptive changes of the responsiveness of presynaptic 5-HT and NE receptors controlling the release of these two neurotransmitters. Since the firing rate of 5-HT neurons was nearly doubled in these mice, it will be interesting to determine which 5-HT neuronal elements allow such an apparent homeostasis to be maintained. Finally, based on the present results, it is possible to tentatively conclude that the anxious phenotype of 5-HT_{1A} -/- mice is mainly due to the absence of the postsynaptic 5-HT_{1A} receptors because 5-HT release appears to be normal in the mutant mice. Therefore, there should not be any compensation

occurring by altered 5-HT release at 5-HT receptor subtypes other than the 5-HT_{1A} subtype in 5-HT_{1A} -/- mice.

ACKNOWLEDGMENTS. Funded in part by a Medical Research Council of Canada grant (MT-11014) and salary support from the University of Florida to P.B. M.R. was in receipt of a studentship from the Royal Victoria Hospital Research Foundation and the Fonds de la Recherche en Santé du Québec.

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APPENDICES

TABLE 1. FRACTION OF TOTAL TRITIUM RELEASED FROM HIPPOCAMPUS, FRONTAL CORTEX AND MIDBRAIN RAPHE SLICES PRELOADED WITH [^{3}H]5-HT IN 5-HT$_{1A}$ +/+ AND 5-HT$_{1A}$ -/- MICE.

	Spontaneous tritium outflow (sp_1)			Electrically evoked release of [^{3}H]5-HT (S_1)	
	5-HT$_{1A}$ +/+	5-HT$_{1A}$ -/-		5-HT$_{1A}$ +/+	5-HT$_{1A}$ -/-
Hippocampus	2.23 ± 0.09 (n=67)	2.31 ± 0.11 (n=64)		1.16± 0.09 (n=67)	1.22 ± 0.10 (n=64)
Frontal cortex	2.04 ± 0.08 (n=60)	2.05 ± 0.07 (n=63)		1.46 ± 0.1 (n=60)	1.38 ± 0.10 (n=63)
Midbrain raphe	1.90 ± 0.10 (n=30)	2.09 ± 0.11 (n=22)		1.02 ± 0.11 (n=30)	1.06 ± 0.17 (n=22)

Spontaneous outflow of radioactivity (sp_1) refers to the percentage of total tissue radioactivity released during the 4 min preceding the first stimulation. S_1 is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the perfusate. The data were generated from a total of 33, 29 and 41 mice for the three structures, respectively. None of the mean values in mutant mice were significantly different from those obtained in the wildtype mice ($p>0.05$) using the non-paired Student`s t test. In all experiments, slices prepared from 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$ -/- mice were processed simultaneously.

TABLE 2. FRACTION OF TOTAL TISSUE TRITIUM RELEASED FROM HIPPOCAMPUS AND FRONTAL CORTEX SLICES PRELOADED WITH [^{3}H]NE IN 5-HT$_{1A}$ +/+ AND 5-HT$_{1A}$ -/- MICE.

	Spontaneous tritium outflow(sp_1)			Electrically evoked release of [^{3}H]NE (S_1)	
	5-HT$_{1A}$ +/+	5-HT$_{1A}$ -/-		5-HT$_{1A}$ +/+	5-HT$_{1A}$ -/-
Hippocampus	0.80 ± 0.1 (n=19)	0.74 ± 0.09 (n=18)		1.93 ± 0.30 (n=19)	1.57 ± 0.22 (n=18)
Frontal cortex	0.80 ± 0.07 (n=17)	0.81 ± 0.07 (n=16)		1.55 ± 0.13 (n=17)	1.68 ± 0.14 (n=16)

Spontaneous outflow of radioactivity (sp_1) refers to the percentage of total tissue radioactivity released during the 4 min preceding the first stimulation. S_1 is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the perfusate. The data were generated from a total of 12 mice for each structure. None of the mean values in mutant mice were significantly different from those obtained in the wildtype mice ($p>0.05$) using the non-paired Student`s t test. In all experiments, slices prepared from 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$ -/- mice were processed simultaneously.

LEGENDS TO THE FIGURES

FIGURE 1. Examples of representative recordings of 5-HT neurons obtained from 5-HT_{1A} +/+ and 5-HT_{1A} -/- mice during systematic electrode descents through the dorsal raphe nucleus. The dots between each recording signify that the recordings were not continuous. The numbers above each tracing represent the distance between the surface of the brain and the site of recording within the dorsal raphe. The bar at the bottom of the figure applies to both traces.

FIGURE 2. Scattergrams depicting the firing frequency of all spontaneously active 5-HT neurons encountered during systematic electrode descents through the dorsal raphe of wildtype mice (**A**) and of 5-HT_{1A} null mutant mice (**B**).

FIGURE 3. Effects of the 5-HT_{1B} agonist CP93129 on the inhibition of electrically-evoked release of [^{3}H]5-HT from preloaded (**A**) frontal cortex and (**B**) hippocampus slices in wildtype (5-HT_{1A} +/+) and mutant (5-HT_{1A} -/-) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± s.e.m. for at least four experiments per group of animals. * $p < 0.05$; ** $p < 0.01$, versus control (Tukey`s test).

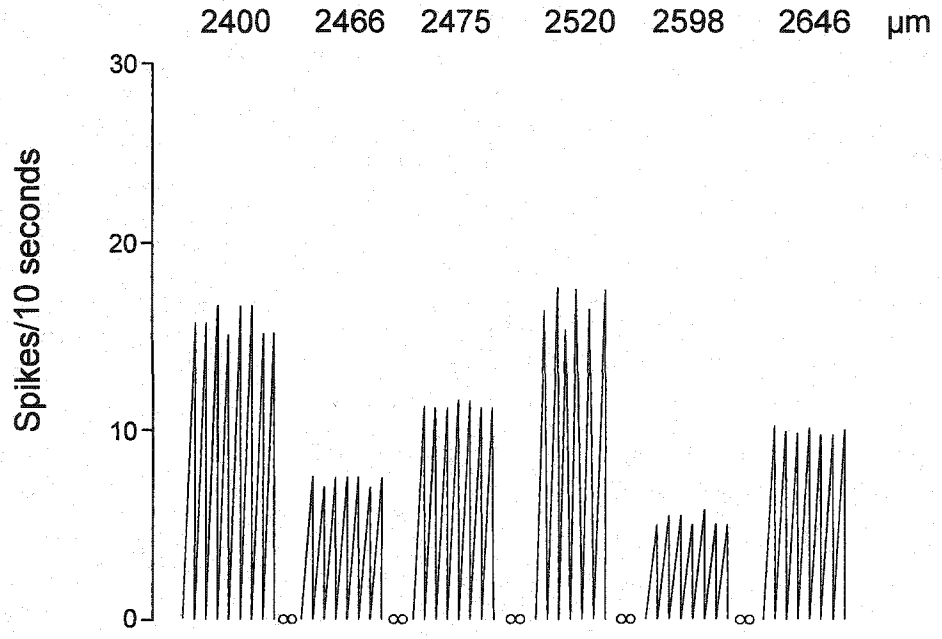
FIGURE 4. Effects of the α_2-adrenoreceptor agonist UK 14304 on the inhibition of electrically-evoked release of [^{3}H]5-HT from preloaded (**A**) frontal cortex and (**B**) hippocampus slices in wildtype (5-HT_{1A} +/+) and mutant (5-HT_{1A} -/-) mice. The agonist was introduced 20 min before S_2 and remained present until the end of

the experiment. Values are expressed as means ± s.e.m. for at least four experiments per group of animals. * $p < 0.05$; ** $p < 0.01$, versus control (Tukey`s test).

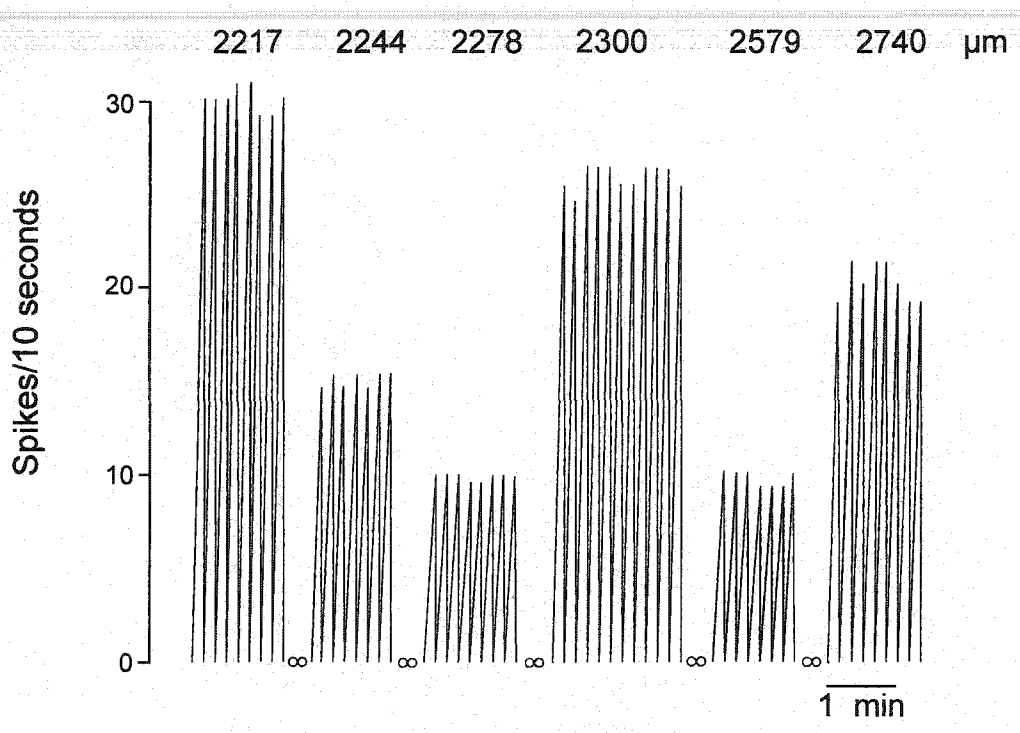
FIGURE 5. Effects of the 5-$HT_{1D/1B}$ agonist sumatriptan on the inhibition of electrically- evoked release of [^{3}H]5-HT from preloaded mesencephalic slices in wildtype (5-HT_{1A} +/+) and mutant (5-HT_{1A} -/-) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± s.e.m. for at least five experiments per group of animals. * $p < 0.05$; ** $p < 0.01$, versus control (Tukey`s test).

FIGURE 6. Effects of the α_2-adrenoceptor agonist UK14304 on the inhibition of electrically-evoked release of [^{3}H]NE from preloaded (**A**) frontal cortex and (**B**) hippocampus slices in wildtype (5-HT_{1A} +/+) and mutant (5-HT_{1A} -/-) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± s.e.m. for at least four experiments per group of animals. * $p < 0.05$; ** $p < 0.01$, versus control (Tukey`s test).

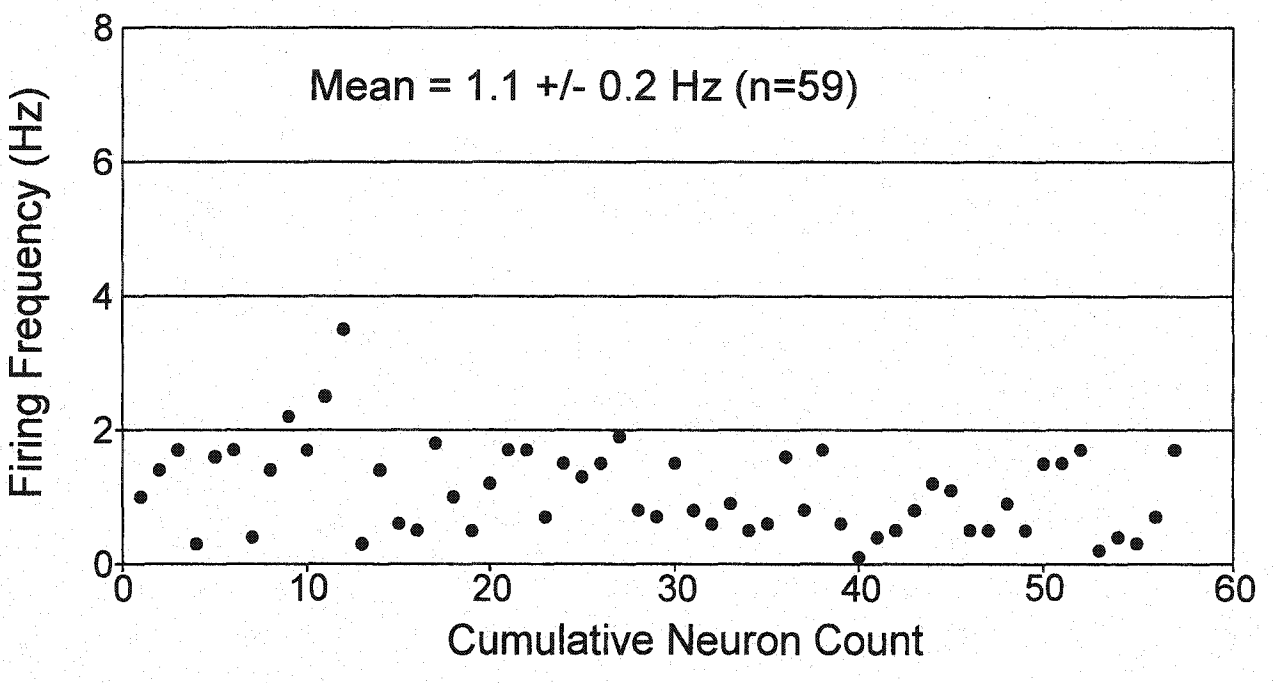
A- 5-HT$_{1A}$ +/+



B- 5-HT$_{1A}$ -/-



A - Wild Type



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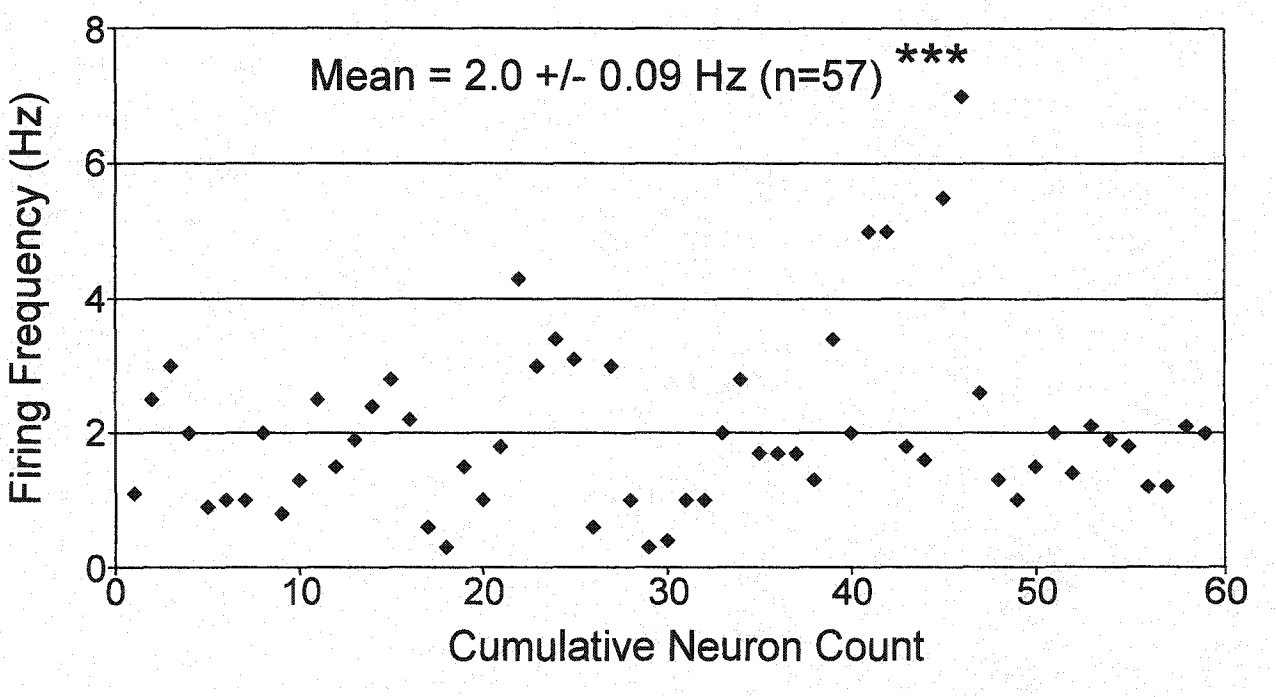
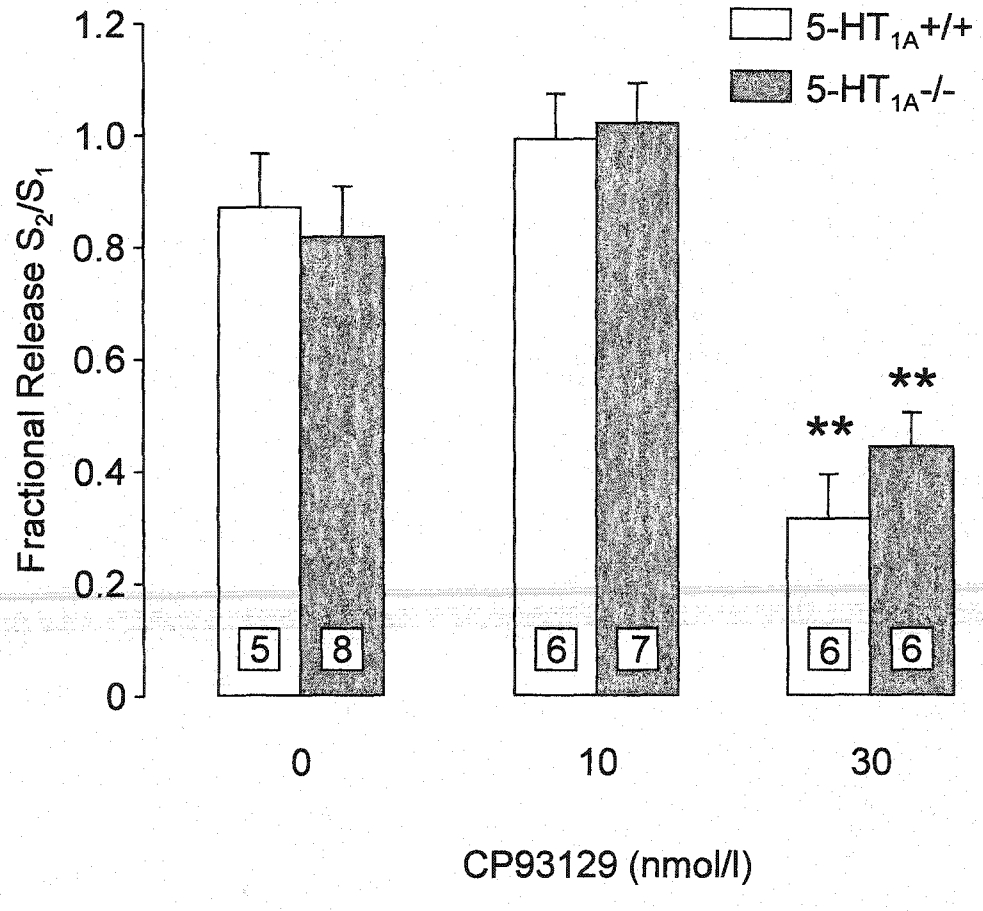
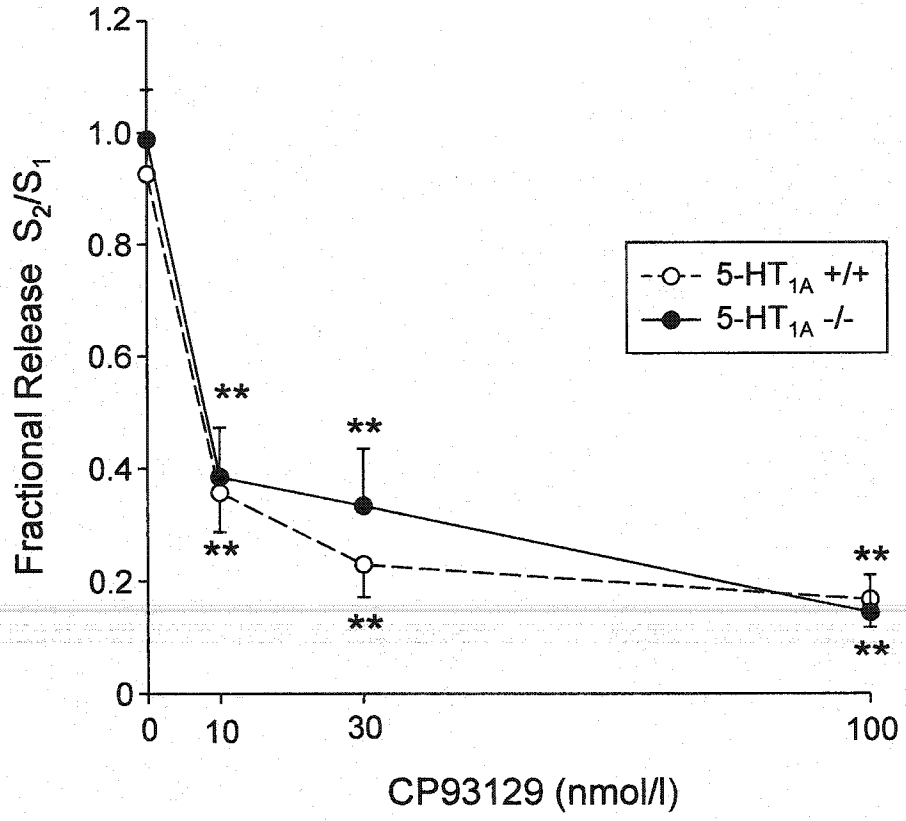
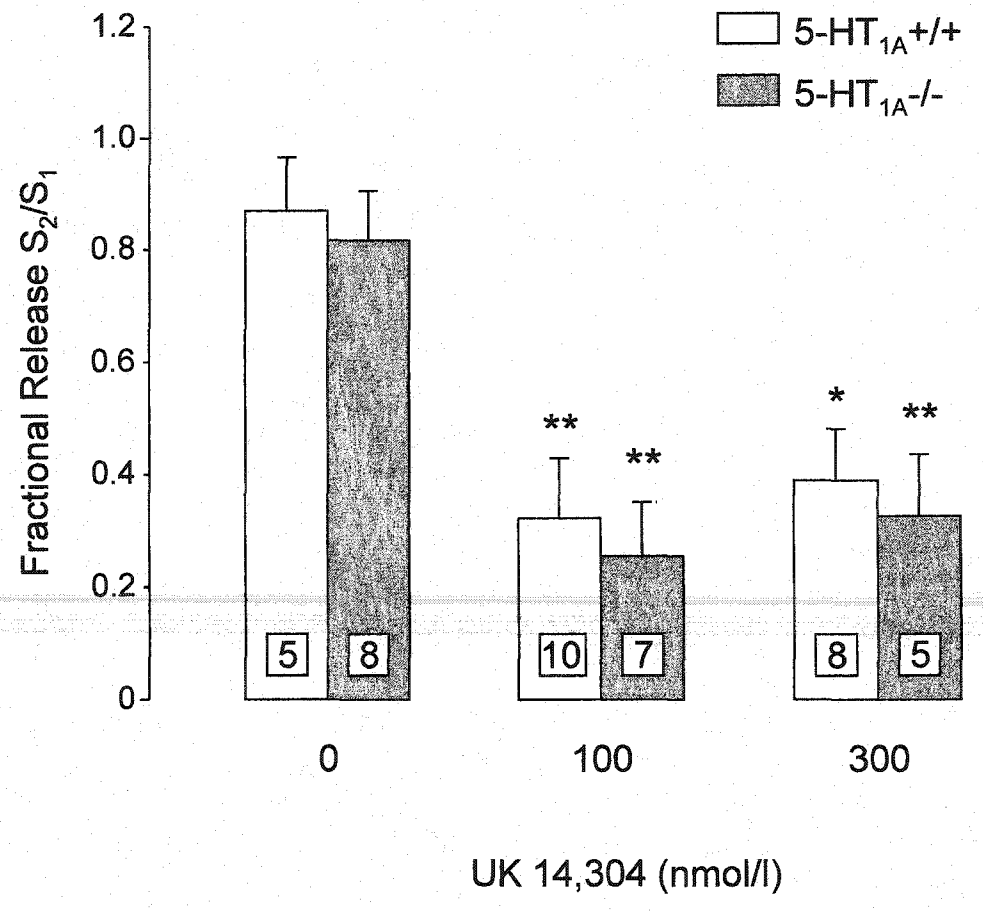
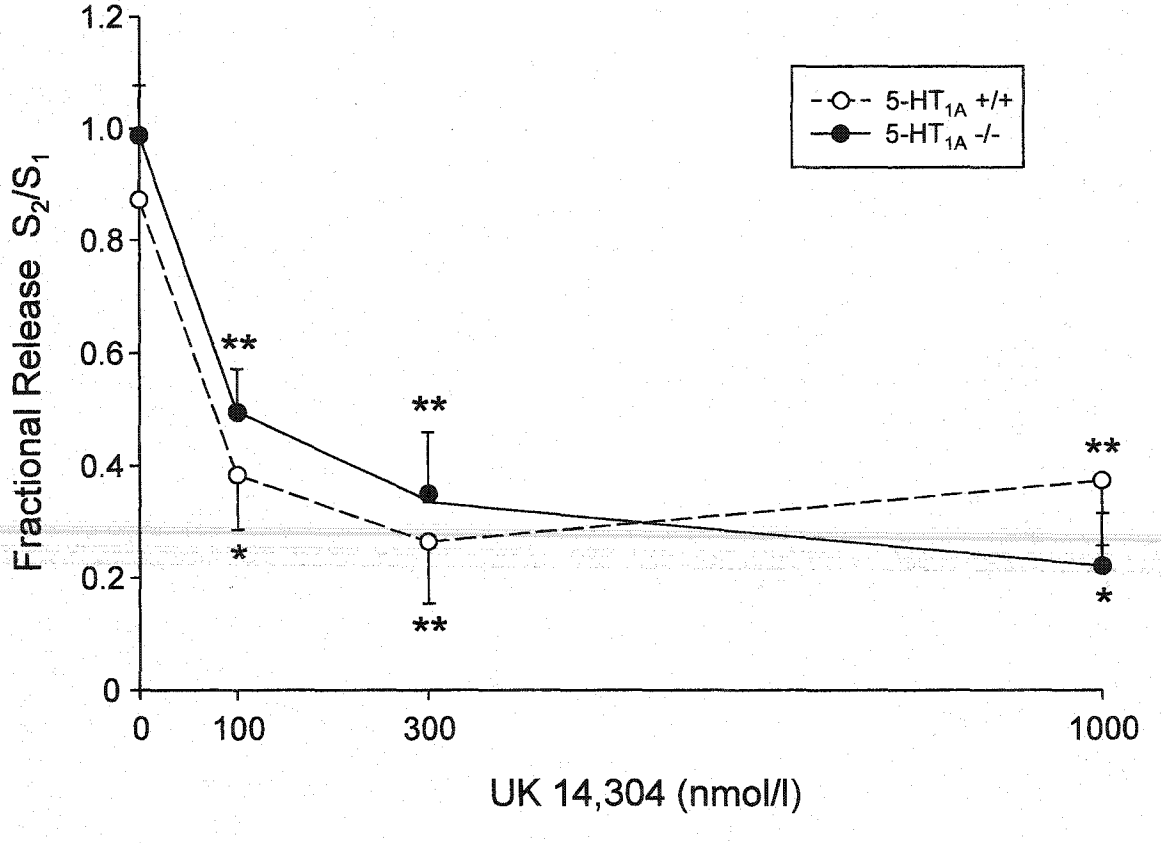



5-HT1A+/+
5-HT1A-/-
Fractional Release S2/S1
1.2
1.0
0.8
0.6
0.4
0.2
0
5
8
6
7
6
6
**
**
0
10
30
CP93129 (nmol/l)


1.2
1.0
0.8
0.6
0.4
0.2
0
Fractional Release S_2/S_1
5-HT$_{1A}$ +/+
5-HT$_{1A}$ -/-
**
**
**
**
**
**
0
10
30
100
CP93129 (nmol/l)


5-HT1A+/+
5-HT1A-/-
Fractional Release S2/S1
1.2
1.0
0.8
0.6
0.4
0.2
0
5
8
10
7
8
5
**
**
*
**
0
100
300
UK 14,304 (nmol/l)


1.2
1.0
0.8
0.6
0.4
0.2
0
Fractional Release S_2/S_1
0
100
300
1000
UK 14,304 (nmol/l)
5-HT$_{1A}$ +/+
5-HT$_{1A}$ -/-
**
**
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*
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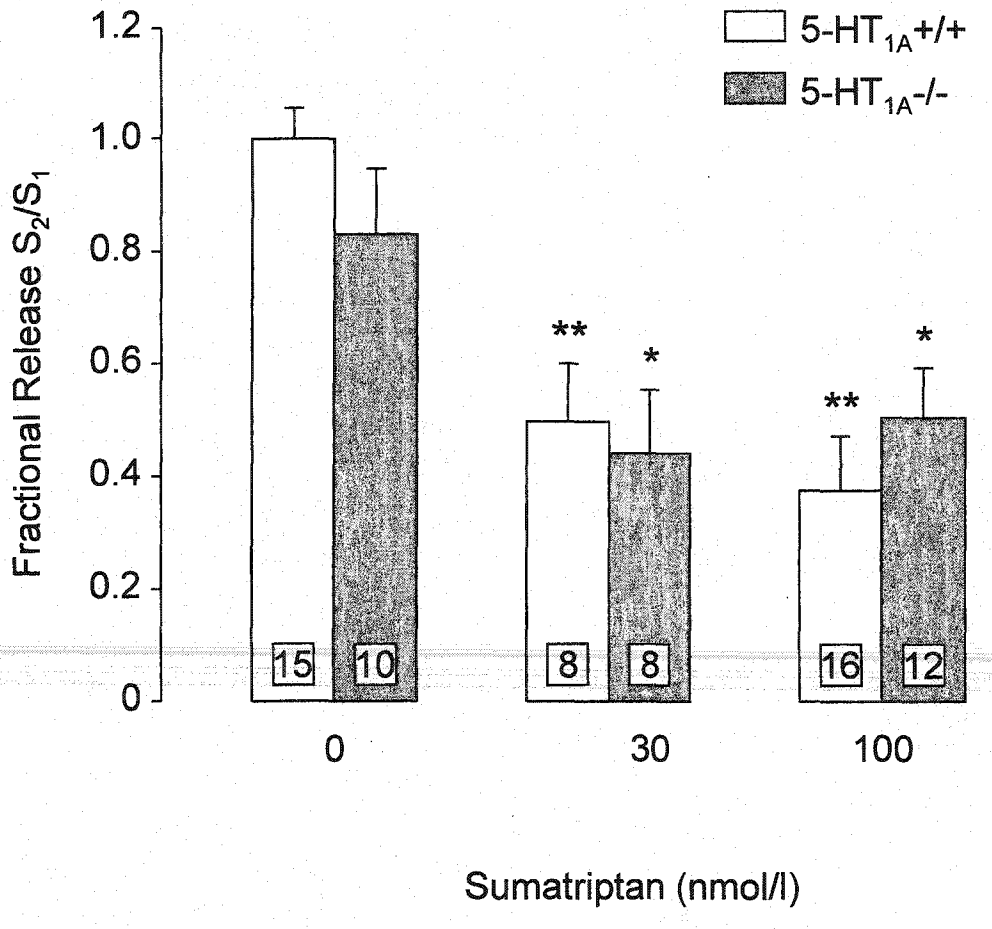
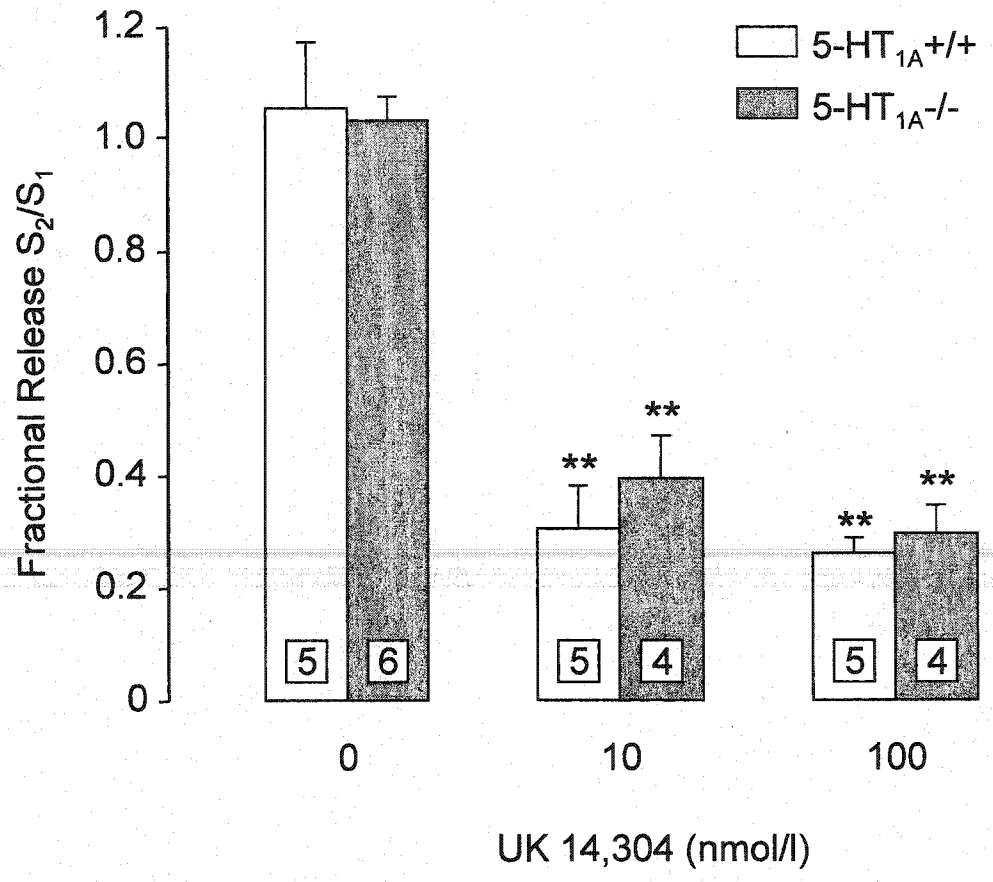
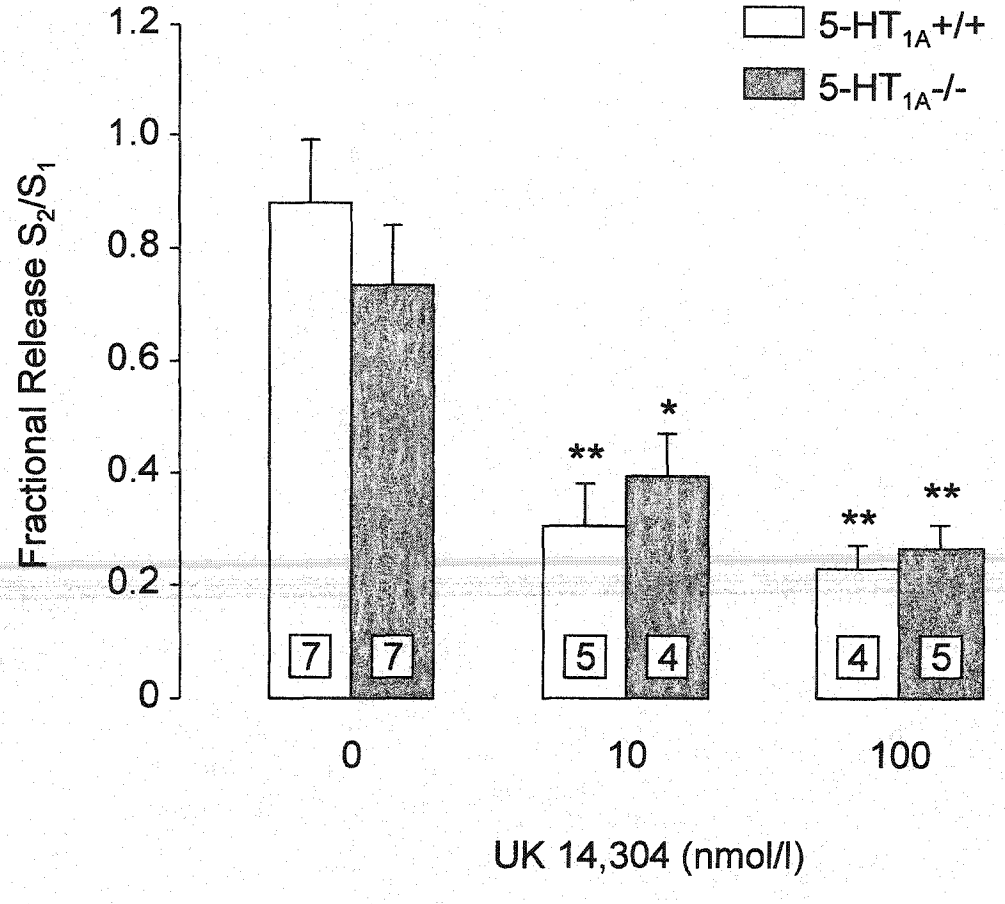

1.2
1.0
0.8
0.6
0.4
0.2
0
Fractional Release S_2/S_1
5-HT$_{1A}$+/+
5-HT$_{1A}$-/-
**
*
**
*
15
10
8
8
16
12
0
30
100
Sumatriptan (nmol/l)


1.2
1.0
0.8
0.6
0.4
0.2
0
Fractional Release S_2/S_1
5-HT$_{1A}$+/+
5-HT$_{1A}$-/-
**
**
**
**
5
6
5
4
5
4
0
10
100
UK 14,304 (nmol/l)


5-HT1A+/+
5-HT1A-/-
Fractional Release S2/S1
1.2
1.0
0.8
0.6
0.4
0.2
0
7
7
5
4
4
5
**
*
**
**
0
10
100
UK 14,304 (nmol/l)