5-HT$_{1B}$ RECEPTORS AND SEROTONIN FUNCTION

- microdialysis studies in rats and knockout mice -

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- microdialyse studies in ratten and knockout muizen -

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The fundamental importance of the central serotonergic system is reflected in the extensive innervation of brain areas by serotonin (5-HT) nerve fibers and the diversity of the serotonin receptor family. To date, seven 5-HT receptor families with 14 different receptor subtypes are identified. Detailed research on each of the different subtypes is necessary to understand the complexity of the serotonergic system and the functional role of the different receptor subtypes. Dysfunction of the central serotonergic system has been implicated in the pathophysiology of several psychiatric disorders. The serotonergic system is an important target in the treatment of psychiatric disorders like schizophrenia, depression and anxiety. Selective 5-HT reuptake inhibitors (SSRIs) are drugs that enhance 5-HT levels by preventing the reuptake of 5-HT. SSRIs were introduced in the 1980s and since then widely used in the treatment of depression and some anxiety disorders. A major clinical problem is, however, that it requires several weeks before a therapeutic effect is achieved. This delayed onset of action suggests that adaptive changes occur. Pre-clinical studies indicated a role of 5-HT autoreceptors in the delayed of action of SSRIs. $5-HT_{1B}$ receptors are present in the brain both as autoreceptors, controlling the release of 5-HT at serotonergic nerve terminals, and as heteroreceptors modulating other neurotransmitters, suggesting that this receptor is involved in a variety of functions. Therefore, $5-HT_{1B}$ receptors may be a potential target to augment treatment with SSRIs. Moreover, dysfunction of $5-HT_{1B}$ receptors has been associated with aggression, impulsivity, alcoholism and drug abuse. More insight in the functional role of $5-HT_{1B}$ receptors contributes to our understanding of this receptor subtype in 5-HT neurotransmission and in its role in psychiatric disorders.

Central serotonergic system

Serotonergic innervation of the forebrain
In the 1950s, the existence of 5-HT in the mammalian brain was first reported (Twarog and Page, 1953). Serotonin is a bioamine and is synthesized from tryptophan, an amino acid originating from the diet. Cell bodies containing 5-HT are located in nine discrete cell clusters, located near the midline of the brainstem (Dahlstrom and Fuxe, 1964). The most caudal raphe nuclei project mainly to the gray matter of the spinal cord, while the 5-HT neurons innervating the forebrain mainly originate in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN). The 5-HT cells in the raphe nuclei innervate virtually all forebrain areas (Steinbusch, 1981) as shown in Figure 1. The DRN is the most extensively studied and is the larger nucleus with about 11,500 serotonergic cells, and the

MRN contains about 1100 serotonergic cells. The serotonergic system appears similar in primates and non-primates in the distribution of 5-HT cell bodies and their projection network (Jacobs and Azmitia, 1992). In primates, however, the raphe nuclei are more laterally localised and the DRN is more highly organized. Both neuroanatomical and functional mapping studies have indicated that the projections from the DRN and MRN have distinct patterns of distribution, with some forebrain areas receiving mixed innervation of these nuclei and others receiving relatively selective DRN or MRN inputs. For example, the frontal cortex and striatum appear to be predominantly innervated by the DRN, while the dorsal part of the hippocampus is rather exclusively innervated by the MRN and the ventral hippocampus receives mixed input from both DRN and MRN (see Jacobs and Azmitia, 1992, McQuade and Sharp, 1997). Moreover, serotonergic connections between the raphe nuclei exist. The main source of 5-HT fibers reaching the DRN arises either from the DRN itself or from the MRN (Jacobs and Azmitia, 1992). Inputs to the DRN arise from many sources, from the locus coeruleus (noradrenergic), the substantia nigra (dopaminergic), periaqueductal gray (neuropeptides), the hypothalamus, and the lateral habenula (see Jacobs and Azmitia, 1992). The DRN also receives input from the medial prefrontal cortex (mPFC), other cortical areas and hypothalamic nuclei (Hajos et al., 1999, Peyron et al., 1998).
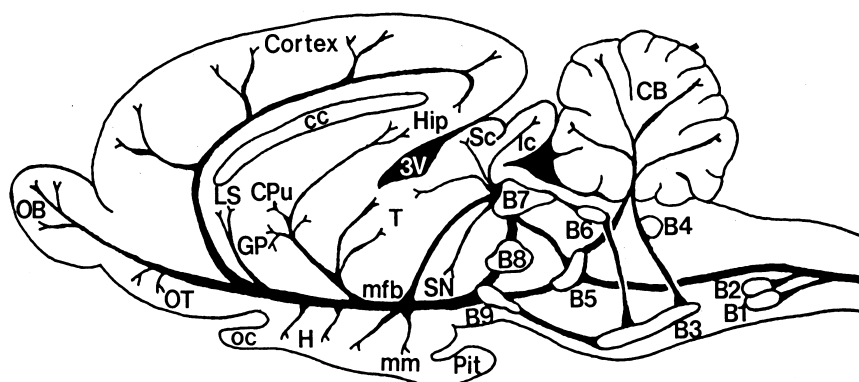
Figure 1. Schematic drawing of the location of the serotonergic nuclei (B1-B9) and their major projections in the brain. B7: dorsal raphe nucleus, B8: median raphe nucleus, 3V: third ventricle, CB: cerebellum, cc: corpus callosum, CPu: caudate putamen, GP: globus pallidus, H: hypothalamus, Hip: hippocampus, Ic: inferior colliculus, LS: lateral septum nuclei, mfb: medial forebrain bundle, mm: mammillary nucleus, OB: olfactory bulb, oc: optic chiasma, OT: olfactory tubercle, Pit: pituitary, Sc: superior colliculus, SN: substantia nigra, T: thalamus. *Adopted from H. Sijbesma (thesis 1991).*

5-HT receptors

5-HT exerts its function through activation of different receptor subtypes. To date, seven different 5-HT receptor families (5-HT$_{1-7}$) with 14 subtypes are identified. The 5-HT receptors are classified as 5-HT$_{1A,1B,1D,1E,1F}$, 5-HT$_{2A,2B,2C}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_{5A,5B}$, 5-HT$_6$ and 5-HT$_7$ (Hoyer et al., 1994, Barnes and Sharp, 1999). The 5-HT receptor subtypes display a distinct pattern of distribution within the CNS. 5-HT receptors are all G-protein coupled receptors, except 5-HT$_3$ receptors, which are ion channel coupled. In this chapter 5-HT$_1$ receptors will be discussed, details on other 5-HT receptor subtypes and their function can be found in recent review (Barnes and Sharp, 1999).

Regulation of 5-HT neurotransmission

5-HT$_{1A}$ autoreceptors

5-HT is released at nerve terminals through exocytosis, triggered by action potential induced depolarisation. Presynaptically located 5-HT transporters terminate the action of 5-HT (see box 1). After reuptake, extravesicular 5-HT is deaminated by mono-oxidase (MAO), and subsequently oxidated by aldehyde dehydrogenase to its metabolite 5-HIAA. Electrophysiological studies have demonstrated that the neuronal activity of raphe cells is inhibited by 5-HT sensitive receptors on the cell bodies and dendrites of serotonergic cells in the raphe nuclei (Sprouse and Aghajanian, 1987). Activation of these receptors results in inhibition of neuronal activity, and subsequent in diminished 5-HT release at the nerve terminals. It is well known that somatodendritic autoreceptors are of the 5-HT$_{1A}$ receptor subtype. Selective drugs for 5-HT$_{1A}$ receptors, like 8-OH-DPAT, have been found to inhibit raphe cell firing (Sprouse and Aghajanian, 1987, Arborelius et al., 1995). In vivo microdialysis studies have clearly shown that activation of 5-HT$_{1A}$ receptors decreases output of 5-HT in several brain areas, including the dorsal and median raphe nuclei, frontal cortex, hippocampus and striatum and amygdala (Hjorth and Sharp, 1991, Bosker et al., 1996, Bosker et al., 1997a). Moreover, raphe 5-HT neurons may be controlled through postsynaptic 5-HT$_{1A}$ receptors located in the mPFC (Hajos et al., 1999, Casanovas et al., 1999) and in the amygdala (Bosker et al., 1997b). This long feedback loop may be restricted to specific serotonergic projection areas, since activation of 5-HT$_{1A}$ receptors in the hippocampus, a brain structure rich in 5-HT$_{1A}$ receptors, has no effect on 5-HT release in this brain area (Kreiss and Lucki, 1994).

What is the source of 5-HT in the raphe nuclei? Ultrastructural studies reported that axon terminals are observed in the raphe nuclei, but only few terminals make synaptic contacts (Jacobs and Azmitia, 1992). Extracellular 5-HT levels are twice as high in the raphe nuclei as in 5-HT projection areas (Bel and Artigas, 1992). Therefore it is thought that the source of 5-HT in the raphe is mainly from somatodendritic origin, and differs from the 5-HT release at nerve terminals in the projection areas (see Pineyro and Blier, 1999). Nevertheless, stimulation of these receptors by locally applied 5-HT$_{1A}$ receptor agonists reduces 5-HT output in the raphe nuclei, indicating that extracellular 5-HT in the DRN and MRN is modulated by somatodendritic 5-HT$_{1A}$ autoreceptors (Adell et al., 1993, Bosker et al., 1994, Bosker et al., 1996).

5-HT$_{1B}$ autoreceptors

On serotonergic nerve terminals, the release of 5-HT is controlled by inhibitory 5-HT$_{1B}$ autoreceptors. In vitro release studies indicated the existence of a terminal autoreceptor of the 5-HT$_{1B}$ receptor subtype (Engel et al., 1986, Maura et al., 1986, Hoyer and Middlemiss, 1989). The negative feedback on 5-HT output by 5-HT$_{1B}$ autoreceptors was supported by in vivo microdialysis studies using local administration of the 5-HT$_{1B}$ receptor agonist CP93129, a compound that does not cross the blood-brain barrier, into different brain structures, including the hippocampus and the frontal cortex (Hjorth and Tao, 1991, Bosker et al., 1995, Adell et al., 2001). Interestingly, an in vivo electrophysiology study in mice demonstrated that stimulation of 5-HT$_{1B}$ receptors following intravenous applied CP94253, a centrally active compound, increased firing rate of dorsal raphe 5-HT neurons (Evrard et al., 1999). This was unexpected, as previous studies performed in rat found no evidence for such an excitatory control of DRN neuron firing activity through 5-HT$_{1B}$ receptors (Sprouse et al., 1997). Nevertheless, a microdialysis study in rats indicated that stimulation of 5-HT$_{1B}$ receptors by CP93129 reduced 5-HT release in the DRN and even more markedly in the MNR (Adell et al., 2001). These findings are supported by a voltammetry study showing that CP93129 reduced 5-HT release both in the DRN and MRN (Hopwood and Stamford, 2001). The intravenous administration of CP94253 to anaesthetised rats did not affect DRN 5-HT neurons, but showed a biphasic effect on the firing rate of MNR causing an increase after a high dose and decrease after lower doses of CP94253 (Adell et al., 2001). The localisation of 5-HT$_{1B}$ receptors in the raphe is not completely clear, but is presumably on 5-HT nerve terminals. Autoradiographic studies demonstrated binding of 5-HT$_{1B}$ receptors in the raphe nuclei (Boschert et al., 1994), while 5-HT$_{1B}$-like immunoreactivity could not be

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Box 1. Volume neurotransmission

Ultrastructural analysis of the serotonergic system demonstrated that 5-HT neurotransmission is predominantly non-synaptic (Bunin and Wightman, 1999). Neurotransmission through synaptic junctions, or wiring transmission, is the classic concept of a neurotransmitter, which is released by exocytosis into the synaptic cleft, activates postsynaptic receptors, and subsequent the neurotransmitter is removed from the synaptic cleft by reuptake sites. Volume neurotransmission, also referred to as paracrine or non-synaptic neurotransmission, can occur when receptors are located extrasynaptically and if released neurotransmitter reaches the extrasynaptic space at sufficiently high concentrations to activate its receptor (Zoli et al., 1999). It has been reported that 5-HT systems have a great spillover of 5-HT out of the synaptic junction (Bunin and Wightman, 1998). Consistent with the notion of volume neurotransmission, 5-HT transporters have been demonstrated along 5-HT cell axons and perisynaptic area, suggesting a broad range of 5-HT uptake sites beyond synaptic junctions in rat brain (Pickel and Chan, 1999, Tao-Cheng and Zhou, 1999). Furthermore, 5-HT_{1A} and 5-HT_{1B} receptors predominate at extrasynaptic and nonsynaptic sites (Boulenguez et al., 1996, Sari et al., 1999, Riad et al., 2000). In the DRN, some 5-HT varicosities are junctional, but most are non-junctional (Chazal and Ralston, 1987). In most 5-HT projections areas, for example in the frontal cortex, hippocampus and neostriatum, varicosities are also predominantly non-junctional, whereas, for example in the substantia nigra (SN) pars reticulata almost all varicosities are of the junctional type (Moukhles et al., 1997). The relative frequency of junctional and non-junctional synaptic contacts in different 5-HT projection areas indicates region specific actions of 5-HT. Moreover, the non-junctional network of most 5-HT projection areas is in line with the general view that 5-HT acts as a global modulatory system.

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detected in the raphe (Sari et al., 1999). Taken together, these findings suggest that 5-HT_{1B} receptors localised in the MRN may affect 5-HT release. In addition to 5-HT_{1A} and 5-HT_{1B} autoreceptors, there is substantial evidence that 5-HT_{1D} receptors localised in the DRN may act as autoreceptors controlling the release of 5-HT (Pineyro et al., 1995a;Pineyro et al., 1996, Starkey and Skingle, 1994, Davidson and Stamford, 2000, Moret and Briley, 1997). Why are multiple 5-HT_1 receptor subtypes needed for the autoregulation of 5-HT? It has been suggested that 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, each different located, may have different roles in controlling 5-HT release (Stamford et al., 2000).

5-HT$_{1B}$ receptors

Species differences

Literature on 5-HT$_{1B}$ receptors is sometimes confusing due to the use of old nomenclature. Originally, 5-HT$_{1B}$ receptors were found in species like rat, hamster, mouse, and opossum, while 5-HT$_{1Dß}$ receptors were found in human, guinea pig, cow and dog. Cloning of the mouse 5-HT$_{1B}$ receptor (Voigt et al., 1991, Adham et al., 1992 , Maroteaux et al., 1992) revealed a high homology of 96% in the transmembrane domain with the 5-HT$_{1Dß}$ receptor (Jin et al., 1992) . The receptors are considered as species homologues and are now classified as r5-HT$_{1B}$ and h5-HT$_{1B}$ receptors (Hartig et al., 1996). Despite the high homology, rodent 5-HT$_{1B}$ receptors bind certain ß-adrenoceptor antagonists, such as propranolol and pindolol, with a much higher affinity than human 5-HT$_{1B}$ receptors (Adham et al., 1994). This pharmacological difference is due to only one amino acid difference in the seventh transmembrane domain of the receptor (Oksenberg et al., 1992). For intracellular signal transduction, 5-HT$_{1B}$ receptors (like all 5-HT$_1$ receptors) are negatively coupled to adenylate cyclase through G$_{i/o}$ proteins (Adham et al., 1992).

Distribution

5-HT$_{1B}$ receptors are expressed throughout the brain, the highest binding densities are found in the basal ganglia, substantia nigra, globus pallidus, and moderate binding densities in the striatum, cortical areas, hippocampal areas, thalamic nuclei and deep cerebellar nuclei (Maroteaux et al., 1992, Bruinvels et al., 1993, Sari et al., 1999). In both the DRN and MRN, mRNA for 5-HT$_{1B}$ receptors was detected, and 5-HT$_{1B}$ receptor mRNA levels were markedly reduced following lesion of the serotonergic system, indicating that 5-HT$_{1B}$ receptors are synthesised in serotonergic neurons within the raphe nuclei (Doucet et al., 1995).

But not in all projection areas where binding sites for 5-HT$_{1B}$ receptors are demonstrated, mRNA for this receptor could be detected. This mismatch between binding sites and mRNA of 5-HT$_{1B}$ receptors, combined with lesion studies, indicated that 5-HT$_{1B}$ receptors are present as presynaptic receptors, both on serotonergic and non-serotonergic nerve terminals (Boschert et al., 1994). For example, in the substantia nigra high binding densities are found, but no mRNA for 5-HT$_{1B}$ receptors, while in the striatum, the projection area of the SN, both 5-HT$_{1B}$ receptors binding sites and mRNA was demonstrated. Taken together, this mismatch indicated that 5-HT$_{1B}$ receptors are present on projection neurons from to striatum to the substantia nigra.

Pharmacology
Research on $5\text{-}HT_{1B}$ receptors has been complicated because most available ligands are not selective (see Hoyer et al., 1994). The most potent agonists include CP93129, CP94253, RU24969, 5-CT and a potent antagonist is methiothepin, but all these compounds also have (some) affinity for other 5-HT receptor subtypes, particularly for $5\text{-}HT_{1A}$ receptors (Barnes and Sharp, 1999). For example, CP93129 is a potent agonist with a 200 times greater selectivity for $5\text{-}HT_{1B}$ than for $5\text{-}HT_{1A}$ receptors (Macor et al., 1990). A more selective $5\text{-}HT_{1B}$ receptor antagonist is GR127935, although this compound does not discriminate between $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors (Skingle et al., 1995). SB224289, is also a selective $5\text{-}HT_{1B}$ receptor antagonist (Gaster et al., 1998). Both GR127935 and SB224289 are selective antagonists for both rodent and human $5\text{-}HT_{1B}$ receptors, but these compounds have been found to display partial agonistic properties at $h5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors in vitro, although there is no in vivo evidence for such properties (Pauwels, 1997, Millan et al., 1999). As outlined above, there is a pharmacological difference between human and rodent $5\text{-}HT_{1B}$ receptors. Recently, a new selective rodent $5\text{-}HT_{1B}$ receptor antagonist, NAS-181, has come available that has been shown to enhance 5-HT synthesis and metabolism (Berg et al., 1998, Stenfors et al., 2000).
Interestingly, 5-HT moduline, an endogenous peptide has been discovered that was found to interact with $5\text{-}HT_{1B}$ receptors in a non-competitive inhibitory way (Massot et al., 1996, Rousselle et al., 1998). In mice lacking $5\text{-}HT_{1B}$ receptors binding of 5-HT moduline is absent, indicating that the $5\text{-}HT_{1B}$ receptor is the specific target for this peptide (Cloez-Tayarani et al., 1997). 5-HT moduline appears capable of altering the reactivity of $5\text{-}HT_{1B}$ receptors making it less sensitive to an agonist. Behavioural studies suggest that 5-HT moduline may be involved in conditions related to anxiety and stress (Grimaldi et al., 1999, Chennaoui et al., 2000).

$5\text{-}HT_{1B}$ heteroreceptors
Studies in hippocampal synaptosomes have indicated a modulatory role of $5\text{-}HT_{1B}$ receptors in acetylcholine release (Maura et al., 1986, Bolanos-Jimenez et al., 1995, Sarhan and Fillion, 1999). Furthermore, a microdialysis study has demonstrated an increase of acetylcholine levels following $5\text{-}HT_{1B}$ receptor stimulation with CP93129 in the frontal cortex (Consolo et al., 1996). In the dorsal subiculum, glutamate release has been reported to be inhibited through $5\text{-}HT_{1B}$ receptors localised in the CA1 area of the hippocampus (Boeijinga and Boddeke, 1993, Ait Amara et al., 2001). Microdialysis studies have shown that stimulation of $5\text{-}HT_{1B}$ receptors with locally applied CP93129 results in increased dopamine (DA)

levels in the striatum (Galloway et al., 1993, Benloucif et al., 1993), in the prefrontal cortex (Iyer and Bradberry, 1996), and in the nucleus accumbens (Yan and Yan, 2001). Effects mediated by 5-HT$_{1B}$ receptors are general inhibitory in nature, thus the increases of DA observed following 5-HT$_{1B}$ receptor stimulation are presumably indirectly mediated effects. Electrophysiological studies have reported that activation of 5-HT$_{1B}$ receptors inhibits the release of GABA from terminals that innervate DA neurons in the substantia nigra and the ventral tegmental area, resulting in disinhibition of DA neurons (Johnson et al., 1992, Cameron and Williams, 1994, Morikawa et al., 2000).

Behavioural pharmacology
Most studies on the behavioural effects of 5-HT$_{1B}$ receptor stimulation, compared the effects of 5-HT$_1$ receptor agonists like TFMPP and mCPP, or mixed 5-HT $_{1A/1B}$ receptor agonists like RU24969 and eltoprazine with selective 5-HT$_{1A}$ receptor agonists like 8-OH-DPAT, or in combination with 5-HT$_{1B}$ receptor antagonists, like cyanopindolol and propranolol. Stimulation of 5-HT$_{1B}$ receptors has been found to be involved in a variety of behaviours. Aggressive behaviour in rodents is reduced by so-called serenics, compounds with high affinity for 5-HT$_{1B}$ receptors such as eltoprazine, a 5-HT$_{1A/1B}$ receptor agonist (Olivier and Mos, 1992). Furthermore, 5-HT$_{1B}$ receptors have been implicated in locomotor activity (Geyer, 1996), thermoregulation, sexual behaviour, feeding behaviour, and anxiety (see for a review Chopin et al., 1994).

Psychiatric disorders and 5-HT$_{1B}$ receptors

5-HT and stress
It is well known that stress affects the serotonergic system (Chaouloff et al., 1999). For example, the stress-related neuropeptide corticotropin-releasing factor (CRF) modulates the neuronal firing activity of the DRN (Lowry et al., 2000, Kirby et al., 2000) and several in vivo microdialysis studies have shown that stress affects extracellular 5-HT in the hippocampus (Adell et al., 1997, Kirby et al., 1995, Linthorst et al., 2000). The functional responsiveness of 5-HT$_{1B}$ receptors, as measured on 5-HT and acetylcholine release in hippocampal synaptosomes, has been shown to be reduced after acute restraint stress, suggesting that 5-HT$_{1B}$ receptors desensitize after stress (Bolanos-Jimenez et al., 1995). Learned helplessness is a model in which some (but not all) animals that are exposed to inescapable foot shock stress, display learned helpless behaviour when tested 24 hours later in a foot shock avoidance paradigm. The learned helplessness

paradigm models some aspects of stress-related disorders including depression and post-traumatic stress disorder. Increases in 5-HT_{1B} receptor mRNA levels in the DRN have been observed in helpless rats, suggesting increased synthesis of 5-HT_{1B} autoreceptors (Neumaier et al., 1997). Furthermore, in the cortex of helpless rats, increased 5-HT_{1B} receptor binding sites were observed, while cortical levels of extracellular 5-HT were decreased, supporting the hypothesis that a deficit in 5-HT release is associated with learned helpless behaviour (see Moret and Briley, 2000).

5-HT autoreceptors and the action of SSRIs
SSRIs are widely used in the treatment of depression; however, it takes several weeks of chronic administration before a therapeutic effect is achieved. This delayed therapeutic onset of SSRIs and other antidepressants can be explained by adaptive changes in 5-HT autoreceptors (see for a extensive review Pineyro and Blier, 1999). At the beginning of treatment with a SSRI, extracellular 5-HT levels are increased. Subsequently, the increased 5-HT levels in the raphe nuclei results in inhibition of 5-HT neuronal firing (Sprouse and Aghajanian, 1987), 5-HT synthesis (Barton and Hutson, 1999) and release (Adell and Artigas, 1991, Hjorth and Auerbach, 1994). After sustained administration of a SSRI, the 5-HT_{1A} autoreceptor desensitizes and firing activity is restored in the presence of the SSRI (Blier et al., 1998, Hjorth et al., 2000). A limiting role of 5-HT autoreceptors in the acute effects of SSRIs is supported by several microdialysis studies (see box 2) that have used 5-HT_{1A} receptor antagonists (Invernizzi et al., 1997, Hjorth, 1993, Trillat et al., 1998) and 5-HT_{1B} receptor antagonists (Rollema et al., 1996, Cremers et al., 2000, Gobert et al., 2000). Moreover, administration of SSRIs combined with both 5-HT_{1A} and 5-HT_{1B} receptor blockade results in a synergistic increase of extracellular 5-HT (Gobert et al., 1997, Dawson and Nguyen, 2000, Sharp et al., 1997). Furthermore, following chronic SSRI treatment changes in 5-HT_{1B} receptors have been demonstrated, suggesting a role of 5-HT_{1B} receptors in the mechanism of action of SSRIs (O'Connor and Kruk, 1994, el Mansari et al., 1995, Newman et al., 2000). That adaptive changes of 5-HT autoreceptors are implicated in the delayed therapeutic effect of SSRIs in depression is supported by the acceleration of the antidepressant response of SSRIs when combined administration with the 5-HT_{1A} autoreceptor antagonist pindolol in patients (see box 3).

Box 2. In vivo brain microdialysis

Microdialysis studies have provided important information on the function of the 5-HT system, and particularly contributed to the understanding of the *in vivo* effects of antidepressants on extracellular 5-HT (and other neurotransmitters) in different brain structures. The in vivo brain microdialysis technique was developed in the 1980s, initially to study DA neurotransmission (Zetterstrom et al., 1983) and soon followed by studies on extracellular 5-HT (Kalen et al., 1988). In this technique, a small probe with a permeable membrane is inserted in a brain structure of interest while artificial cerebrospinal fluid is perfused through the probe. Neurochemicals that passively diffuse across the membrane, are collected in the perfusion fluid, and are subsequently measured by means of high-pressure liquid chromatography with electrochemical detection (HPLC-ECD). Studies with tetrodotoxin added or Ca^{2+} omitted from the perfusion fluid indicated that extracellular 5-HT is of neuronal origin. Moreover, autoreceptor induced changes in 5-HT output support that the sampled neurotransmitter is directly related to neurotransmission. Therefore, it is assumed that extracellular 5-HT, sampled by means of in vivo microdialysis reflects functional release of 5-HT (Westerink and Timmerman, 1999). The levels of 5-HT in dialysates are in general low (femtomole range), therefore usually samples of 10 to 20 minutes are collected to yield 5-HT levels above detection limit. The amount of 5-HT in dialysis samples is dependent on the surface (active tip) of the dialysis membrane and on the flow rate of the perfusion fluid. In many studies, a SSRI is added to the perfusion fluid to increase 5-HT levels. To study 5-HT$_{1B}$ autoreceptors, adding a SSRI to the perfusion fluid is not desirable, because this increases extracellular 5-HT and subsequently activates 5-HT$_{1B}$ autoreceptors. Microdialysis studies have been performed mostly in rat and guinea pig, but with the generation of mutant mice, the application of the microdialysis technique in mice became of interest. The relative small brain of mice restricts the method to larger brain structures like the frontal cortex, hippocampus or striatum.

Obsessive compulsive disorder

SSRIs are not only used as antidepressants, but are also effective in the treatment of obsessive compulsive disorder (OCD), an anxiety disorder (Hollander, 1998, Blier and de Montigny, 1999). In depression it requires several weeks before a therapeutic effect of SSRI is obtained, while in OCD patients treatment with SSRIs requires higher dosages with an even later therapeutic

onset to obtain an anti-obsessional effect. It was hypothesized that this later therapeutic onset in OCD (about 8 weeks) is necessary to desensitize terminal 5-HT receptors in the orbital frontal cortex (el Mansari et al., 1995). The orbital frontal cortex is thought to be involved in OCD, based on changes in brain activity (glucose metabolism) of OCD patients demonstrated by means of in vivo imaging studies, and moreover, these changes could be restored by chronic treatment with SSRIs (Saxena et al., 1999). Pharmacological studies in humans are limited due to restricted availability of drugs, but anti-migraine drugs such as sumatriptan and zolmitriptan, both $5\text{-HT}_{1B/1D}$ receptor agonists, can be used and have been shown to increase plasma growth hormone (Whale et al., 1999). A role of $5\text{-HT}_{1B/1D}$ receptors in OCD, was suggested by a study indicating that OCD symptoms were worsened in patients following a challenge with sumaptriptan (Dolberg et al., 1996), but this could not be confirmed by others using sumatriptan or zolmitriptan (Ho Pian et al., 1998, Boshuisen and den Boer, 2000). Interestingly, children with oppositional defiant disorder (ODD), a disorder associated with aggressive and impulsive behaviour, showed an increased response to sumatriptan on growth hormone (Snoek et al., 2002).

Variations in the 5-HT_{1B} receptor gene
The serotonergic system provides interesting candidate genes, including the 5-HT_{1B} receptor gene, for studies on genetic polymorphism in psychiatric disorders (see for a review Veenstra-VanderWeele et al., 2000). Polymorphisms, or variations, in the 5-HT_{1B} receptor gene may be important in the development of pathophysiology and may be important in determining the response to drugs of abuse. At least three polymorphisms (G861C, T-261G and T371G) in the human 5-HT_{1B} gene are known and in a preliminary family based study the presence of a linkage disequilibrium between a 5-HT_{1B} receptor gene polymorphism (G861C) and OCD was found (Mundo et al., 2000). In a Finnish population, an association between antisocial alcoholism and a 5-HT_{1B} receptor polymorphism (G861C) was suggested (Lappalainen et al., 1998), although such an association of 5-HT_{1B} receptors to antisocial substance abuse was not found by others (Kranzler et al., 2002). Also a postmortem study could not identify a relationship between suicide, major depression, alcoholism or pathological aggression with 5-HT_{1B} receptor binding or the G861C genotype (Huang et al., 1999). Nevertheless, studies on genetic polymorphism need examination of large populations, thus future studies may reveal or exclude involvement of 5-HT_{1B} receptor gene variations in different psychiatric disorders.

Box 3. Augmentation strategies for SSRI-treatment

There is some clinical evidence indicating that co-administration of pindolol, a ß-adrenoceptor and 5-HT$_{1A}$ receptor antagonist, has an accelerating effect on the treatment with SSRIs (Artigas et al., 2001), although larger controlled studies could not support this finding (McAskill et al., 1998). It is debated whether the effects of pindolol on SSRIs are mediated through blockade of 5-HT$_{1A}$ autoreceptors, as a PET study revealed that not more than 40% of 5-HT$_{1A}$ autoreceptors are occupied at the pindolol dosages used (Rabiner et al., 2001). Moreover, a preclinical study in guinea pigs suggested that the observed effects on 5-HT output by pindolol might be due through ß-adrenoceptor blockade (Cremers et al., 2001). Furthermore, pindolol has been shown to display partial agonistic properties at 5-HT$_{1A}$ receptors (Sprouse et al., 2000, Arborelius et al., 2000). Although the findings with pindolol seem conflicting, it indicates the clinical potential of blockade of 5-HT autoreceptors with more selective ligands at the start of treatment with a SSRI may have a beneficial effect in patients with major depression.

5-HT$_{1B}$ receptor knockout mice

Due to a lack a selective receptor antagonists, the generation of constitutive 5-HT$_{1B}$ receptor knockout mice yielded a new model to study the functional role of 5-HT$_{1B}$ receptors. By homologous recombination in embryonic stem cells, homozygous mutant mice lacking both copies of the gene encoding the 5-HT$_{1B}$ receptor were generated (see for details Saudou et al., 1994).

Behavioural phenotype
Locomotor activity in 5-HT$_{1B}$ receptor knockout mice is normal, but a role of the 5-HT$_{1B}$ receptors in locomotor activity is supported by the finding that the locomotor response to RU24969, a 5-HT$_{1A/1B}$ receptor agonist, is absent in the knockouts (Saudou et al., 1994, Ramboz et al., 1996). In a resident intruder paradigm, 5-HT$_{1B}$ receptor knockout mice are more aggressive relative to wildtype mice, which is in line with previous findings that activation of 5-HT$_{1B}$ receptors reduces aggressive behaviour in rodents (Saudou et al., 1994, Bouwknecht et al., 2001a). 5-HT$_{1B}$ knockout mice display increased exploratory behaviour (Malleret et al., 1999) and response to novelty (Zhuang et al., 1999). Increased impulsivity in 5-HT$_{1B}$ receptor knockout mice has been suggested (Brunner and Hen, 1997) and is

supported by findings that 5-HT_{1B} receptor knockouts show more impulsive behaviour in a operant paradigm of decision making and response inhibition (Pattij et al., 2002). The altered exploratory behaviour and the observed increased aggressive and impulsive behaviour, suggest that 5-HT_{1B} receptor knockout mice are more reactive to mild disturbances in their environment. This suggestion is further supported by the finding that physiological reactions to mild disturbances are increased in these knockouts (Bouwknecht et al., 2000a, 2001a). The 5-HT_{1B} receptor knockout mouse has been proposed as a model for substance abuse (Scearce-Levie et al., 1999). 5-HT_{1B} receptor knockout mice show an increased vulnerability to cocaine and an increased propensity to self-administer this drug (Rocha et al., 1998). Increased alcohol consumption is reported in 5-HT_{1B} receptor knockout mice (Crabbe et al., 1996), although this finding could not be replicated by others (Phillips et al., 1999, Risinger et al., 1999, Bouwknecht et al., 2000b, Gorwood et al., 2002).

Serotonergic system of 5-HT_{1B} KO mice

In the absence of inhibitory 5-HT_{1B} autoreceptors, increases in 5-HT levels may be expected. Serotonergic neurons in the DRN of 5-HT_{1B} receptor knockout mice displayed normal firing properties and a normal response to 5-HT_{1A} autoreceptor activation (Evrard et al., 1999). Electrically evoked release of 5-HT was increased in the raphe and hippocampus brain slice of 5-HT_{1B} receptor knockout mice (Pineyro et al., 1995b), but a microdialysis study showed that depolarisation evoked 5-HT release in the hippocampus and cortex was similar in wildtype and knockouts and furthermore, basal 5-HT levels were normal in these brain structures (Trillat et al., 1997). In brain tissue of 5-HT_{1B} receptor knockouts decreased 5-HT levels were found in some brain areas, such as the spinal cord and nucleus accumbens, but not in most other forebrain regions examined, suggesting normal biosynthesis of 5-HT in the main serotonergic projection areas including the cortex and hippocampus (Ase et al., 2000). These normal properties of the 5-HT system may be the result of compensatory changes in 5-HT_{1B} receptor knockout mice. Changes in 5-HT transporters have been observed in some brain structures, together with increased 5-HT innervation in the amygdalo-hippocampal nucleus and the ventral hippocampus of the knockouts, indicating region specific adaptive changes in the serotonergic system to compensate for the loss of 5-HT_{1B} receptors (Ase et al., 2001).

After acute administration of the SSRIs paroxetine and fluoxetine, augmented 5-HT levels were reported in the hippocampus, but not in the striatum of 5-HT_{1B} receptor knockout mice (Malagie et al., 2001, Knobelman et al., 2001a), and in the frontal cortex an augmented response was only found after a lower dose of

paroxetine (Malagie et al., 2001). Possibly, upon systemic administration of SSRIs, the contribution of terminal 5-HT$_{1B}$ receptors on 5-HT output in the striatum and frontal cortex was obliterated by the simultaneous activation of 5-HT autoreceptors in the raphe.

Aims and outline

The aim of this thesis is to explore the role of 5-HT$_{1B}$ receptors in 5-HT function, by comparing the neurochemical effects of different serotonergic drugs on extracellular 5-HT and DA levels in wildtype and 5-HT$_{1B}$ receptor knockout mice, and in rats using a selective 5-HT$_{1B}$ receptor antagonist.

In part I of this thesis, in vivo microdialysis studies in wildtype and 5-HT$_{1B}$ receptor knockout mice are described. To examine the functional role of the 5-HT$_{1B}$ autoreceptors in the regulation of 5-HT release, the effects of 5-HT$_{1B}$ receptors stimulation and administration of SSRIs in different brain structures were compared in the two genotypes. The SSRI fluvoxamine was locally administered into serotonergic projection areas to circumvent activation of 5-HT autoreceptor in the raphe nuclei that affect 5-HT output at nerve terminals. The effects of SSRIs, systemic paroxetine and local fluvoxamine, on hippocampal 5-HT levels are described in **chapter 2**. The role of 5-HT$_{1B}$ receptors in 5-HT output in the mPFC was assessed in **chapter 3** by local administration of fluvoxamine and by co-administration of the selective 5-HT$_{1B}$ receptor antagonist NAS-181. In **chapter 4**, the interaction between 5-HT and DA in the striatum was examined as previous studies in rat indicated that stimulation of 5-HT$_{1B}$ receptors increases DA outflow. To assess, both the role of 5-HT$_{1B}$ autoreceptors on 5-HT output and the role of 5-HT$_{1B}$ heteroreceptors on DA output, 5-HT$_{1B}$ receptors were stimulated directly by a 5-HT$_{1B}$ receptor agonist and indirectly by enhancing 5-HT levels through local administration of a SSRI and a 5-HT releaser.

In part II, the effect of NAS-181, a new selective rodent 5-HT$_{1B}$ receptor antagonist, on extracellular 5-HT was evaluated using microdialysis in rat frontal cortex. In **chapter 5**, the potency of NAS-181 to antagonize the decrease in 5-HT induced by a 5-HT$_{1B}$ receptor agonist was compared with two other 5-HT$_{1B}$ receptor antagonist, both in the presence and absence of a SSRI. In the study presented in **chapter 6**, tested the hypothesis whether the effect of a 5-HT$_{1B}$ receptor antagonist depends on extracellular 5-HT levels by using different strategies to increase cortical 5-HT levels combined with administration of NAS-181. In **chapter 7**, the main findings are discussed and concluded with future directions.

CHAPTER 2

The effects of selective serotonin reuptake inhibitors on extracellular 5-HT in the hippocampus of 5-HT$_{1B}$ receptor knockout mice

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Abstract

The effects of two selective serotonin reuptake inhibitors (SSRIs) on 5-hydroxy-tryptamine (5-HT) in the hippocampus were studied in wildtype and in 5-HT$_{1B}$ receptor knockout (KO) mice using in vivo microdialysis. Basal 5-HT levels were not different between the two genotypes. The functional absence of 5-HT$_{1B}$ receptors was examined in the knockout mice by local infusion of the 5-HT$_{1B}$ receptor agonist, CP93129 (1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one) into the hippocampus. CP93129 (1 μM) decreased 5-HT levels in wildtype mice, but not in 5-HT$_{1B}$ KO mice. Systemic administration of the SSRI paroxetine (5 mg/kg i.p.) increased extracellular 5-HT levels. The increase of 5-HT in 5-HT$_{1B}$ KO mice was almost twofold higher than in wildtype mice. Systemic administration of SSRIs stimulates both terminal 5-HT$_{1B}$ autoreceptors and somatodendritic 5-HT$_{1A}$ autoreceptors. Therefore, another

SSRI, fluvoxamine, was applied locally into the hippocampus in order to activate only terminal 5-HT$_{1B}$ autoreceptors. Local administration of 0.3 µM fluvoxamine resulted in comparable increases in extracellular 5-HT in both genotypes, whereas 1.0 µM fluvoxamine produced a twofold greater increase in 5-HT levels in 5-HT$_{1B}$ KO as compared to wildtype mice. In conclusion, the differences in hippocampal 5-HT output between wildtype and 5-HT$_{1B}$ KO mice after local or systemic SSRI administration show that 5-HT$_{1B}$ autoreceptors play a significant role in the inhibition of 5-HT release at serotonergic nerve terminals. In addition, the different dose-response to fluvoxamine between the two genotypes also suggest that 5-HT$_{1B}$ KO mice have possible adaptations of 5-HT transporters in order to compensate for the loss of the terminal 5-HT$_{1B}$ autoreceptor.

Introduction

Selective serotonin reuptake inhibitors (SSRIs) are widely used in the treatment of psychiatric conditions like depression and anxiety disorders. SSRIs exert their effects by blocking serotonin (5-HT) reuptake, thereby increasing extracellular 5-HT levels. Since the onset of clinical efficacy is delayed to 2-4 weeks, it is suggested that adaptive processes might be implicated. The release of 5-HT in the forebrain is controlled by autoreceptors on serotonergic cell bodies and on terminals in projection areas. The terminal 5-HT autoreceptor is of the 5-HT$_{1B}$ subtype and stimulation of this receptor results in decreased 5-HT release (Engel et al., 1986, Maura et al., 1986, Hoyer and Middlemiss, 1989). In vivo microdialysis studies have shown that local infusion of a 5-HT$_{1B}$ receptor agonist into the hippocampus results in a decrease of extracellular 5-HT in rat (Hjorth and Tao, 1991, Bosker et al., 1995) and mouse (Trillat et al., 1997). Chronic treatment with antidepressants alters 5-HT autoreceptors in rats (Haddjeri et al., 1999, Le Poul et al., 2000). Terminal 5-HT$_{1B}$ receptors in different brain areas including the hippocampus, hypothalamus and frontal cortex have been found to desensitize after sustained administration of SSRIs (Blier et al., 1984, Moret and Briley, 1990, O'Connor and Kruk, 1994, el Mansari et al., 1995, Newman et al., 2000). Several studies on the role of autoreceptors in the mechanism of action of SSRIs, have used selective 5-HT$_{1A}$ and non-selective 5-HT$_{1B/1D}$ receptor antagonists in combination with SSRIs. The combination of a SSRI with a 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor antagonists synergistically increases 5-HT release in rat frontal cortex (Gobert et al., 1997, Sharp et al., 1997, Dawson and Nguyen, 2000), guinea pig frontal cortex, hippocampus (Roberts et al., 1996) and hypothalamus (Rollema et al., 1996). Due to a lack of selective 5-HT$_{1B}$ receptor antagonists, there is

relatively little data on the role of the terminal 5-HT_{1B} autoreceptor in controlling 5-HT release. The selective 5-HT_{1B} receptor antagonist, SB-224289, potentiated the effect of fluoxetine in rat frontal cortex (Gobert et al., 2000) and produced an increase in extracellular 5-HT in the hippocampus, but not in the frontal cortex of guinea pigs (Roberts et al., 1998). 5-HT_{1B} receptors are located in serotonergic projections to forebrain regions as terminal autoreceptors and as heteroreceptors. Several studies indicate that 5-HT_{1B} receptors present in the dorsal raphe nucleus (DRN) are also involved in the release of 5-HT (Starkey and Skingle, 1994, Pineyro et al., 1995a, Moret and Briley, 1997, Davidson and Stamford, 1995). Thus, blockade of $5\text{-HT}_{1B/1D}$ receptors in the raphe nuclei after systemic administration of $5\text{-HT}_{1B/1D}$ receptor antagonists may also contribute to the effects on 5-HT levels in output areas.

The development of 5-HT_{1B} receptor knockout (KO) mice has generated a model to study the importance of the 5-HT_{1B} receptor (Saudou et al., 1994) and allows the investigation of SSRI actions in the absence of 5-HT_{1B} receptors. Recent studies have found increased 5-HT levels in hippocampus and frontal cortex after systemic SSRI administration in mice lacking 5HT_{1B} receptors (Malagie et al., 2001, Knobelman et al., 2001b).

In the present study we report on the effects of SSRIs on 5-HT release in the hippocampus of wild type and 5-HT_{1B} KO mice following systemic and local administration. In mice lacking the 5-HT_{1B} receptor, augmented 5-HT responses are expected following SSRI administration due to the absence of inhibitory terminal 5-HT_{1B} autoreceptors. Since, systemic administration of SSRIs also activates 5-HT autoreceptors in the raphe nuclei, effects of local administration of the SSRI fluvoxamine were studied to assess the role of the terminal 5-HT_{1B} autoreceptor.

Material & Methods

Animals

In this study, male wildtype and 5-HT_{1B} knockout mice with a 129/SV genetic background were tested. The mice were group-housed, eight per cage and kept on a 12-hour light-dark cycle (6 a.m. on, 6 p.m. off) at constant room temperature ($22 \pm 2^{\circ}C$) with freely available food and water. For the experiments, bodyweights of the mice were between 25 and 30 grams at an age between 12 and 16 weeks. The two genotypes differ in bodyweight, the 5-HT_{1B} KO mice being slightly heavier as compared to the wildtype mice (Bouwknecht et al., 2001b). Wildtype and 5-HT_{1B} knockout mice were bred in separate homozygous lines at the animal

facilities, GDL, Utrecht, The Netherlands. The original wildtype and 5-HT$_{1B}$ knockout mice were obtained from Dr. René Hen, Columbia University, New York, USA. See for details on the generation of the 5-HT$_{1B}$ KO mice the original publication by Saudou et al (1994). The ethical committee for animal research of the University Medical Center Utrecht, The Netherlands, approved the study.

Surgery
Mice were anaesthesized with chloralhydrate (400 mg/kg, i.p.) and lidocaine (2%) was applied on the skull. For surgery, the mice were placed in a stereotaxic frame using a mouse adaptor (Stoelting, Germany) with modified earbars. The mice were on a heating pad during surgery. A small hole was drilled in the skull for the implantation of the probe and two small holes for anchor screws. A self-constructed microdialysis probe with an AN filtral 69 membrane, outer diameter 310 μM (Hospal, Uden, The Netherlands), was placed in the right hippocampus. The coordinates were: AP -2.8 mm from bregma, ML -3.5 mm, DV -4.0 mm from dura, according to the stereotaxic atlas of the mouse brain (Franklin and Paxinos, 1997). The exposed membrane tip length was 2 mm. The probe was secured in place with dental cement and two anchor screws in the skull. After surgery, mice were injected with saline (0.5 ml i.p.) to prevent dehydratation and the mice were housed separately.

Microdialysis
Microdialysis experiments started the day after surgery. Mice were tested on two subsequent days to reduce the number of animals. The treatment groups were randomized over the two days. Mice were in their homecage during the experiments. The animals were connected to a high precision pump (Harvard PHD2000, Harvard Scientific, USA) using mouse swivels (Type 375/25, Instech Laboratories, Inc, USA) and PEEK-tubing (ID0.005", OD0.020") to allow free movements of the mice. Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM CaCl$_2$, 1.0 mM MgCl$_2$) was perfused through the microdialysis probe with a flow of 1.13 μl/min. The experiments were performed during the light phase and started after three hours equilibration. Samples were collected every 20 minutes in vials containing 7.5 μl acetic acid, using fraction collectors (type 142, CMA, Sweden). Samples were stored at –80°C until High Performance Liquid Chromatography (HPLC) analysis. At the end of the experiment the mice were decapitated, the brains were removed and fixed in 4% formaldehyde. The brains were cut in 50 μm slices on a vibratome to verify the position of the probe. In case of improper probe placement, data was excluded.

Drugs

Paroxetine (donated by GSK, Harlow, UK) was dissolved in sterile 0.9 % saline on the day of the experiment. Fluvoxamine (donated by Solvay Pharmaceuticals, Weesp, The Netherlands) and CP93129 dihydrochloride (1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one, obtained from Tocris, UK) were dissolved in Ringer solution.

HPLC-ECD analysis

5-HT was analyzed by HPLC with electrochemical detection. Samples (25 µl) were injected onto a LUNA 3 µm RP18 column (100x2 mm, Phenomenex, Bester, The Netherlands) using a Triathlon autosampler and a Gynkotek P580 pump (Separations, The Netherlands). Separation was performed at 40ºC. The electrochemical detector (Intro, ANTEC Leyden, The Netherlands) was set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 µl/l triethylamine, 30 µl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 µl sample (signal to noise ratio 2).

Data analysis and statistics

Values for the first four consecutive samples were averaged to calculate the basal levels of extracellular 5-HT. Student's t-tests were used to compare basal 5-HT values between the two genotypes. In the figures, all values are expressed as percentages of basal levels ± SEM. The data were statistically analyzed with SPSS (Version 8.0). Effects of 5-HT response to SSRI treatment were analyzed by multivariate analysis of variance (ANOVA) with time as 'within' and dose and genotype as 'between' factors. When appropriate, data were broken down and effects of drug dosages or genotype were analyzed by pairwise comparison. For the treatment period, the area under the curve (AUC) was calculated. AUC values were analyzed with ANOVA and post hoc analyses when appropriate. The significance level for all analyses was set at 5%. In the figures the point of injection or start of the local infusion (timepoint zero) is corrected for the lagtime of the microdialysis system.

Results

Basal 5-HT levels

Basal levels of extracellular 5-HT in the hippocampus were not different between the two genotypes (ANOVA; $F(1,68)=2.0$, $p=0.17$, NS). The mean basal levels were 5.66 ± 0.47 fmol/sample for wildtype (n=34) and 6.63 ± 0.54 fmol/sample for 5-HT$_{1B}$ KO mice (n=35).

Effects of the 5-HT$_{1B}$ receptor agonist CP93129

The 5-HT$_{1B}$ receptor agonist, CP93129 (1 µM), was infused by reversed microdialysis for a period of 60 minutes (Fig. 1). ANOVA revealed an effect of dose by genotype ($F(1,26)=6.8$, $p<0.05$). In wildtype mice, local administration of 1 µM CP93129 significantly decreased extracellular 5-HT to $58 \pm 11\%$ from baseline levels. Local infusion of CP93129 had no effect in 5-HT$_{1B}$ KO mice, demonstrating the functional absence of 5-HT$_{1B}$ receptors in the knockout mice.

Effects of systemic administration of paroxetine

Acute administration of paroxetine (5 mg/kg, i.p.) increased extracellular 5-HT in the hippocampus of wildtype and 5-HT$_{1B}$ KO mice (Fig. 2). ANOVA revealed significant effects of time ($F(8,186)=9.7$, $p<0.001$), dose ($F(1,21)=20.9$, $p<0.001$) and of genotype ($F(1,21)=7.8$, $p<0.05$). wildtype mice ($F(1,11)=21.9$, $p<0.01$) and in 5-HT$_{1B}$ KO mice ($F(1,10)=8.7$, $p<0.05$). Contrast analyses revealed a genotype effect for paroxetine treatment ($F(1,11)=9.0$, $p<0.05$). The paroxetine-induced
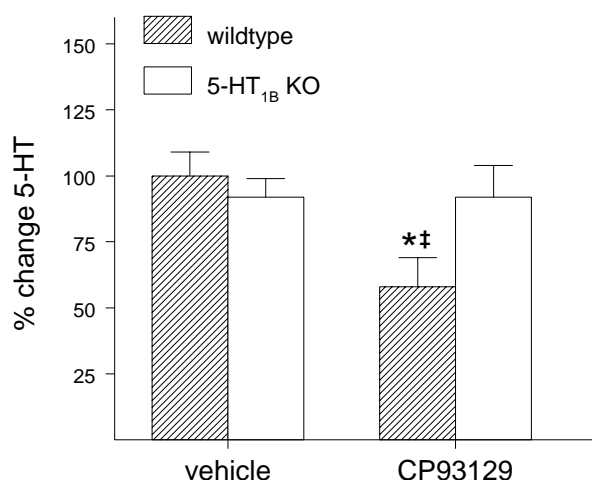

Fig 1. Local administration of the 5-HT$_{1B}$ receptor agonist CP93129 into the hippocampus. Mean percent change in 5-HT from basal levels is expressed as the AUC $\pm$ S.E.M. for a 120 min period. CP93129 (1 µM) was infused for 60 minutes. Symbols: $*$ indicates a significant drug effect ($p<0.05$) and $\ddagger$ indicates a significant genotype effect ($P<0.05$). For each group n=6-8 mice.
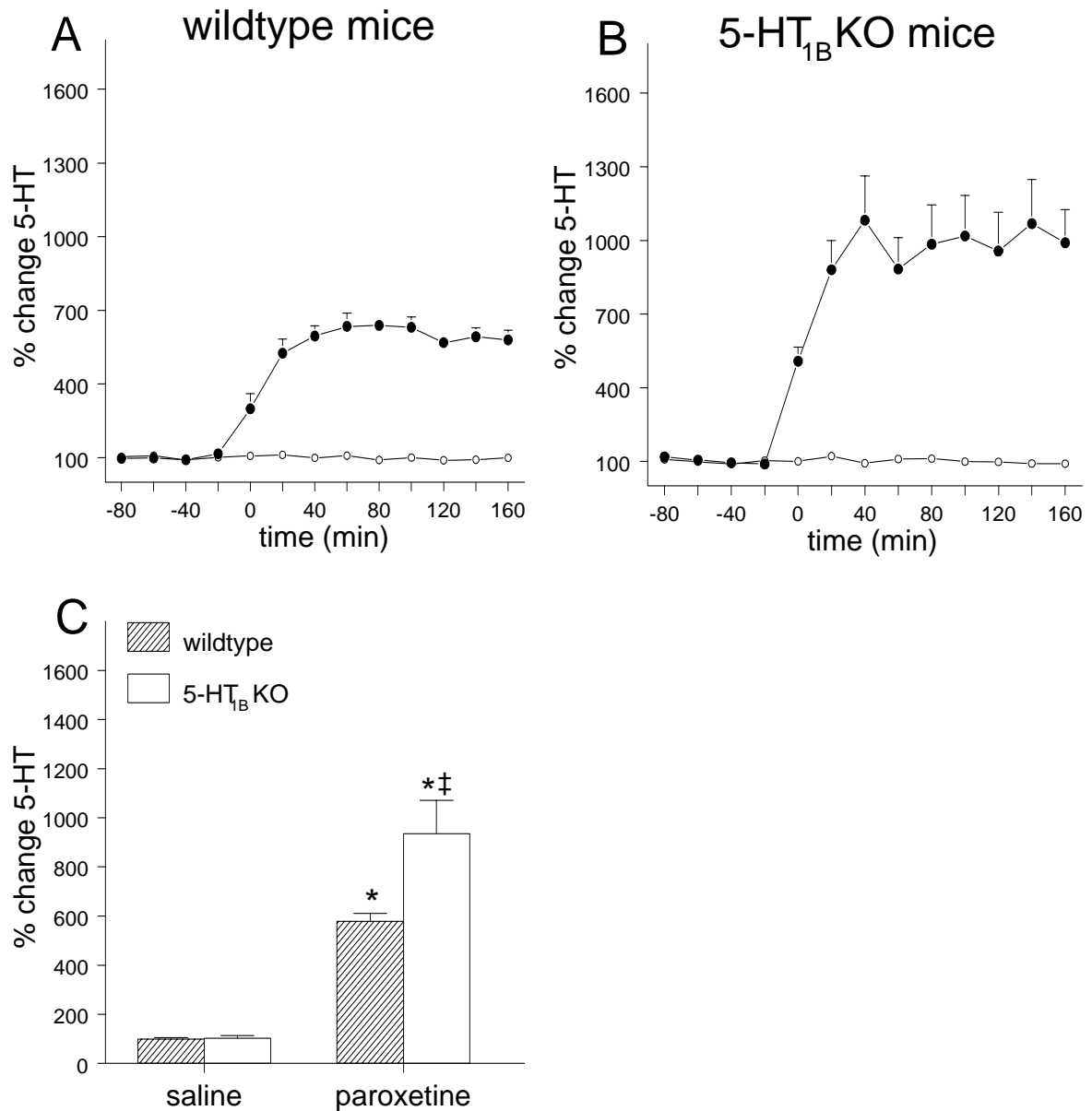
Fig 2. Systemic injection of paroxetine. Data are expressed as mean percent change in 5-HT from basal levels $\pm$ S.E.M.. Saline (O) or paroxetine (5mg/kg, i.p.) ($\bullet$) was injected at timepoint zero in wildtype mice (A) or 5-HT$_{1B}$ knockout mice (B). (C) Mean percent change in 5-HT from basal levels is expressed as the AUC $\pm$ S.E.M. for the treatment period after i.p. injection of saline or paroxetine (5 mg/kg) injection. Symbols: $*$ indicates a significant drug effect P <0.05) and $\ddagger$ indicates a significant genotype effect (P<0.05). For each group n=6-8 mice.

increase in extracellular 5-HT was almost twofold higher in 5-HT$_{1B}$ KO mice as compared to the wildtype mice. Taking the AUC as outcome variable similar results were obtained (Fig. 2C).

Effects of local administration of fluvoxamine

The local administration of fluvoxamine (0.3 and 1.0 µM) induced an increase in extracellular 5-HT in both genotypes (Fig. 3). ANOVA revealed an effect of time ($F_{(12,408)}=10.6$, $p<0.001$), of dose ($F_{(2,34)}=57.8$, $p<0.001$) and of dose by genotype ($F_{(2,34)}=10.4$, $p<0.001$). In wildtype mice, administration of 0.3 and 1.0 µM fluvoxamine induced a significant increase in 5-HT as compared to vehicle ($F_{(2,17)}=39.0$, $p<0.001$), but no difference between the two fluvoxamine dosages. In 5-HT$_{1B}$ KO mice, both dosages of fluvoxamine increased 5-HT levels as compared to vehicle ($F_{(2,17)}=29.6$, $p<0.001$), the difference being greater with the highest dosage. Statistical analyses revealed a dose-dependent effect ($F_{(1,11)}=14.6$, $p<0.05$). When data were broken down on dosage, a significant difference was found for the highest dosage of fluvoxamine between the genotypes ($F_{(1,12)}=9.1$, $p<0.05$). The highest dosage of fluvoxamine induced an almost twofold greater increase in extracellular 5-HT in the knockout as compared to the wildtype mice. No differences in 5-HT response were found between the genotypes for the lower dosage of fluvoxamine or vehicle. Taking the AUC as outcome variable, similar results were obtained. The highest dosage of fluvoxamine (1.0 µM) induced in an almost twofold increase in 5-HT in the knockouts as compared to the wildtype mice (Fig. 3C).

Discussion

The main finding of the present study is that extracellular levels of 5-HT are regulated differently in mice lacking the 5-HT$_{1B}$ receptors as compared to wildtype mice. Basal extracellular 5-HT levels in 5-HT$_{1B}$ KO and wildtype mice were similar, but blockade of 5-HT reuptake sites by systemic paroxetine administration resulted in a greater increase in hippocampal 5-HT in the 5-HT$_{1B}$ KO than in wildtype mice. Inhibition of the uptake sites in the hippocampus by local administration of fluvoxamine resulted also in augmented 5-HT levels in the 5-HT$_{1B}$ KO mice, but the effect appeared to be dose-related in that it was only apparent at the higher SSRI concentration. In addition, local administration of the 5-HT$_{1B}$ receptor agonist CP93129 into the hippocampus decreased absence of the 5-HT$_{1B}$ autoreceptor in the latter strain.
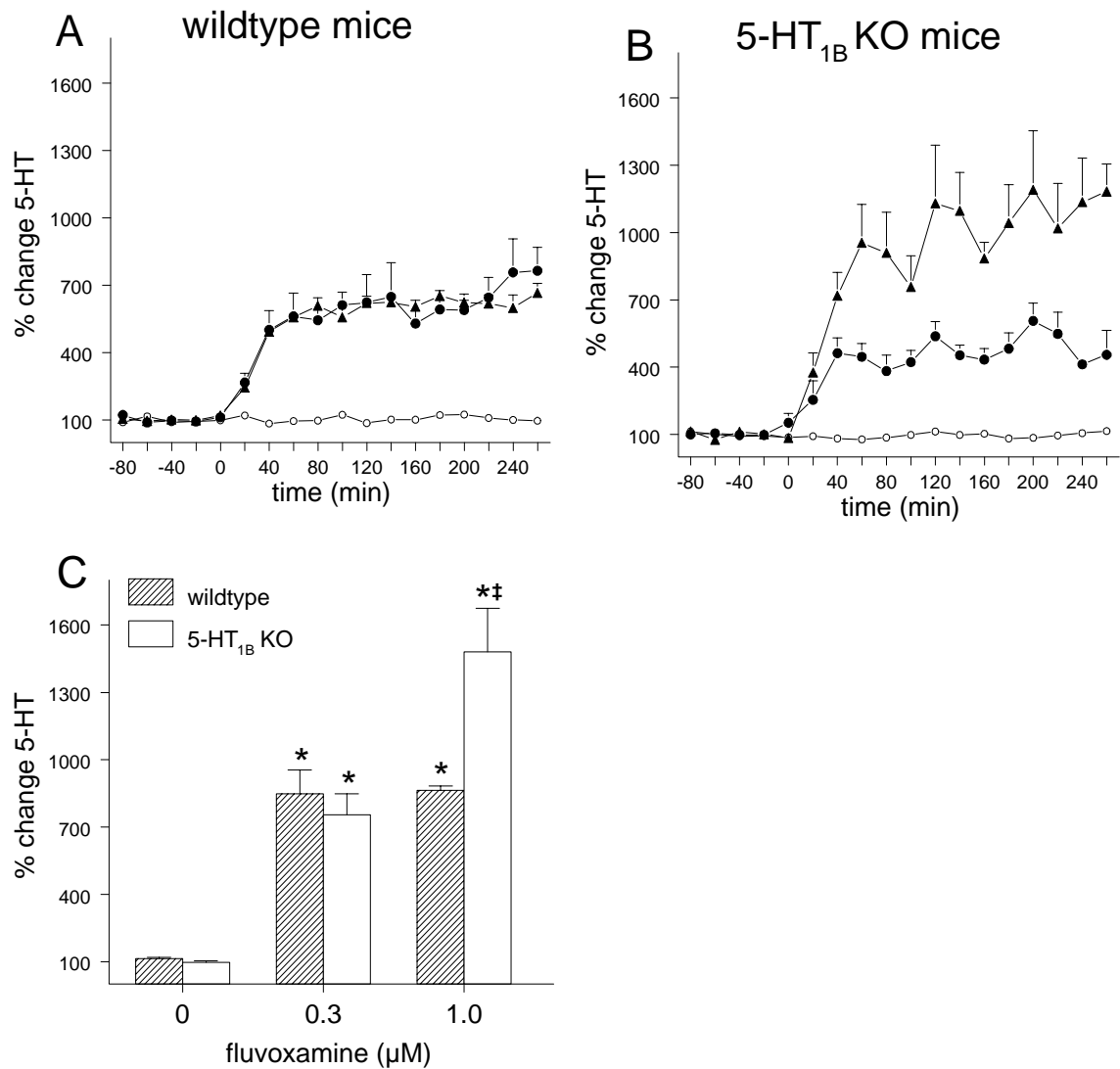
Fig 3. Local administration of fluvoxamine into the hippocampus. Data are expressed as mean percent change in 5-HT from basal levels ± S.E.M.. Ringer solution (○), 0.3 μM (●) or 1.0 μM fluvoxamine (▲) was infused for 240 minutes starting at timepoint zero in wildtype mice (A) or 5-HT$_{1B}$ knockout mice (B). (C) Mean percent change in 5-HT from is expressed basal levels as the AUC ± S.E.M. after infusion of Ringer solution (0 μM), 0.3 μM or 1.0 μM fluvoxamine. Symbols: ∗ indicates a significant drug effect (P<0.05) and ‡ indicates a significant genotype effect (P<0.05). For each group n=6-8 mice.

These data confirm and extend previous findings using systemic administration of SSRIs, indicating that 5-HT$_{1B}$ autoreceptor stimulation limit the effects of acute SSRI administration (Malagie et al., 2001, Knobelman et al., 2001b). A confounding factor of studies using systemic administration of SSRIs is that an effect of somatodendritic 5-HT$_{1A}$ autoreceptors on 5-HT output cannot be excluded. There is circumstantial evidence that stimulation of 5-HT$_{1B}$ receptors in the raphe nuclei may affect 5-HT levels in projection areas (Pineyro and Blier, 1996, Davidson and Stamford, 1995). Studies with mixed 5-HT$_{1B/1D}$ receptor antagonists have revealed differences between the dorsal raphe and medial raphe nucleus innervated brain areas (Roberts et al., 1997, Roberts et al., 1998). The hippocampus receives innervation from both the dorsal raphe and medial raphe nucleus, although there is a preferential input from the medial raphe nucleus to the dorsal part of the hippocampus (Steinbusch, 1981, Mokler et al., 1998). Moreover, systemic administration of SSRIs may also affect other 5-HT modulating receptors in the raphe nuclei, such as the 5-HT$_{1A}$ autoreceptor and the 5-HT$_{1D}$ receptor. To exclude these effects, we applied the SSRI fluvoxamine and the 5-HT$_{1B}$ receptor agonist, CP93129, locally into the hippocampus by reversed microdialysis. Although the microdialysis probe was placed mainly in the ventral part of the hippocampus, we refer to the hippocampus rather than to the ventral hippocampus, due to the relative small size of the mouse brain. We used fluvoxamine, rather than paroxetine, because the former SSRI was found to have better properties to cross the dialysis membrane used in this study. After local administration of fluvoxamine 5-HT levels were stable after one hour of infusion, whereas after local infusion of paroxetine (1 μM) 5-HT levels increased slowly and were not stable after three hours of infusion (data not shown). Our results confirm the view that release of 5-HT in the hippocampus is regulated by terminal 5-HT$_{1B}$ autoreceptors. The data are also in line with studies in other species using 5-HT$_{1B}$ receptor antagonists. Thus, GR127935, a mixed 5-HT$_{1B/1D}$ receptor antagonist, potentiated the effects of SSRIs in rat frontal cortex, presumably by blocking terminal 5-HT$_{1B}$ autoreceptors, after systemic (Gobert et al., 1997, Roberts et al., 1997 or local administration (Hertel et al., 1999). SB224289, a more selective 5-HT$_{1B}$ receptor antagonist, also augmented the SSRI-induced increase of extracellular 5-HT levels in rat frontal cortex, and guinea pig frontal cortex and hippocampus (Gobert and Millan, 1999, Roberts et al., 1999). Finally, local administration of a new selective 5-HT$_{1B}$ receptor antagonist, NAS-181, in the presence of a SSRI, resulted in increased extracellular 5-HT levels in rat hippocampus and prefrontal cortex (Hjorth et al., 2000). Taken together, these findings support the notion that terminal 5-HT$_{1B}$ autoreceptors negatively regulate 5-HT release and thereby restrain the effects of acute SSRI administration. Thus,

increased 5-HT levels were expected in mice lacking 5-HT$_{1B}$ receptors. In line with previous studies, we found that basal 5-HT levels in 5-HT$_{1B}$ KO mice were not different from those in the wildtype mice (Malagie et al., 2001, Knobelman et al., 2001a). This would suggest that either 5-HT$_{1B}$ receptors do not display endogenous activity under basal conditions or that compensatory changes have taken place during neurodevelopment. Interestingly, we found that two concentrations of fluvoxamine (0.3 and 1.0 µM) resulted in similar increases in extracellular 5-HT in the wildtype mice, suggesting that a maximum blockade of 5-HT reuptake was already achieved after the lower concentration of fluvoxamine. In 5-HT$_{1B}$ KO mice, on the other hand, the increase in extracellular 5-HT was not different from the wildtypes at the lower concentration of fluvoxamine, but augmented at the higher concentration, indicating a different dose-response relationship. A likely explanation could be an adaptation of the 5-HT transporter in the 5-HT$_{1B}$ KO mice during development, resulting in a different functional capacity. A higher uptake capacity could compensate for the lack of terminal 5-HT autoinhibition and may account for the normal basal 5-HT levels in the 5-HT$_{1B}$ KO mice as well as the higher concentration of the SRRI necessary to completely block the uptake of 5-HT. Developmental alterations have been previously described for the 5-HT transporter KO mice. These 5-HT transporter KO mice have upregulated 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in some brain areas to compensate for the lack of 5-HT transporters (Fabre et al., 2000). There is evidence for adaptation of the 5-HT$_{1A}$ receptors based on the finding that 5-HT$_{1B}$ KO mice show a reduced 5-HT response in the hippocampus after systemic injection of a 5-HT$_{1A}$ receptor agonist (Knobelman et al., 2001a). The authors propose based on this finding that 5-HT$_{1B}$ KO mice have desensitized 5-HT$_{1A}$ receptors in the MRN to compensate for the loss terminal 5-HT$_{1B}$ autoreceptors. These finding are not supported by other studies, since densities of 5-HT$_{1A}$ receptors (Malleret et al., 1999, Evrard et al., 1999) and raphe neuronal firing after stimulation of 5-HT$_{1A}$ receptors are normal in 5-HT$_{1B}$ KO mice (Evrard et al., 1999). Furthermore, postsynaptic 5-HT$_{1A}$ receptor function as measured by corticosterone release was normal in 5-HT$_{1B}$ KO Bouwknecht et al., 2001c. In the present study, SSRIs were administered locally into the hippocampus and therefore it is unlikely that somatodendritic 5-HT autoreceptor changes account for the present findings. Recently, regional changes in 5-HT transporters have been reported for 5-HT$_{1B}$ KO mice. Binding densities of 5-HT transporters were increased in the ventral hippocampus and amygdalo-hippocampal nucleus of 5-HT$_{1B}$ KO mice (Ase et al., 2001). A higher density of 5-HT reuptake sites has also been demonstrated for the raphe nuclei of 5-HT$_{1B}$ KO mice (Evrard et al., 1999). In support of the explanation that 5-HT$_{1B}$ KO mice have changes in 5-HT

transporters, an interesting interaction between the 5-HT transporter and the 5-HT_{1B} receptor has been reported. The clearance of 5-HT by the 5-HT transporter was prolonged after blockade of 5-HT_{1B} receptors in the hippocampus (Daws et al., 2000). This finding suggests plasticity between the 5-HT transporter and the 5-HT_{1B} autoreceptor. Taken together, these findings support compensatory changes of 5-HT transporters in mice lacking 5-HT_{1B} receptors.

In conclusion, the differences in hippocampal 5-HT output between wildtype and 5-HT_{1B} KO mice after local or systemic SSRI administration show that 5-HT_{1B} autoreceptors play a significant role in the inhibition of 5-HT release at serotonergic nerve terminals. In addition, the results also suggest that 5-HT_{1B} KO mice have possible adaptations of 5-HT transporters in order to compensate for the loss of the terminal 5-HT_{1B} autoreceptor.

CHAPTER 3

Extracellular serotonin in the prefrontal cortex is limited through terminal 5-HT$_{1B}$ autoreceptors

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Abstract

Rationale: 5-HT autoreceptors regulate extracellular 5-HT levels and have been suggested to limit the effects of acute treatment with selective serotonin reuptake inhibitors (SSRI). *Objectives*: The role of terminal 5-HT$_{1B}$ autoreceptors was assessed by comparing the effects of a SSRI on extracellular 5-HT in wildtype and 5-HT$_{1B}$ receptor knockout mice, and by using a 5-HT$_{1B}$ receptor antagonist. Since systemic SSRI-administration also activates somatodendritic 5-HT$_{1A}$ autoreceptors, a SSRI was administered locally to study the role of terminal 5-HT$_{1B}$ autoreceptors. *Methods*: In vivo microdialysis in wildtype and 5-HT$_{1B}$ receptor knockout (KO) mice was used to study the effects of the 5-HT$_{1B}$ receptor agonist CP93129 (1 μM), the SSRI fluvoxamine (0.3 and 1.0 μM) and the 5-HT$_{1B}$ receptor antagonist NAS-181 (1 μM) on extracellular 5-HT in the medial prefrontal cortex (PFC). *Results*: The 5-HT increase induced by local SSRI-administration was augmented in 5-HT$_{1B}$ KO mice as compared to wildtype mice and was augmented by simultaneous administration of a 5-HT$_{1B}$ receptor antagonist in the

latter genotype. Basal 5-HT levels did not differ between the two genotypes. Activation of $5\text{-}HT_{1B}$ receptors by CP93129 decreased extracellular 5-HT, whereas, 5-HT levels in wildtype mice were not affected by the $5\text{-}HT_{1B}$ receptor antagonist NAS-181. In $5\text{-}HT_{1B}$ KO mice, NAS-181 did not affect extracellular 5-HT and did not further increase the effect of fluvoxamine, showing that NAS-181 is a selective $5\text{-}HT_{1B}$ receptor antagonist. The greater increase in 5-HT levels following combined administration of a SSRI with NAS-181 in wildtype mice, as compared to $5\text{-}HT_{1B}$ KO mice, suggests possible adaptive changes in the knockout mice. *Conclusions*: The present study shows that terminal $5\text{-}HT_{1B}$ autoreceptors play a significant role in the regulation of 5-HT release in the PFC.

Introduction

The prefrontal cortex (PFC) plays a key role in cognitive and emotional processes, functions affected in depression, anxiety and other psychiatric disorders. An important target in the treatment of psychiatric disorders is the 5-HT transporter. Selective serotonin reuptake inhibitors (SSRIs) are widely used in the treatment of depression and anxiety disorders. SSRIs exert their effects by blocking 5-HT reuptake, thereby increasing extracellular 5-HT levels. Under normal conditions, 5-HT is rapidly cleared from the synaptic cleft by 5-HT transporters. The release of 5-HT is controlled by $5\text{-}HT_{1A}$ autoreceptors on serotonergic cell bodies and by $5\text{-}HT_{1B}$ autoreceptors on nerve terminals. Thus, activation of terminal $5\text{-}HT_{1B}$ autoreceptors attenuates 5-HT release (Engel et al., 1986, Maura et al., 1986, Hoyer and Middlemiss, 1989). In vivo microdialysis studies in rats and mice have shown that local administration of a $5\text{-}HT_{1B}$ receptor agonist into serotonergic projection areas decreases extracellular 5-HT (Hjorth and Tao, 1991, Bosker et al., 1995, Trillat et al., 1997, Hertel et al., 1999). Since the onset of clinical efficacy of SSRIs is achieved only after several weeks of treatment, it is suggested that adaptive processes are implicated. Chronic treatment with antidepressants alters 5-HT autoreceptors in rats (Haddjeri et al., 1998, Le Poul et al., 2000). Terminal $5\text{-}HT_{1B}$ receptors in different brain areas including the PFC have been found to desensitize after sustained administration of SSRIs (Blier et al., 1984, Moret and Briley, 1990, O'Connor and Kruk, 1994, el Mansari et al., 1995, Newman et al., 2000). To accelerate the efficacy of SSRIs, augmentation strategies with 5-HT autoreceptor antagonists have been suggested (Artigas, 1993, Hjorth, 1993). Several studies have shown that concurrent administration of SSRIs with $5\text{-}HT_{1A}$ or $5\text{-}HT_{1B}$ antagonists results in augmented 5-HT levels in different forebrain areas (Hjorth, 1993, Sharp et al., 1997, Gobert et al., 1997, Roberts et al., 1999, Gobert

et al., 2000, Cremers et al., 2000). After systemic SSRI administration, the output of 5-HT is affected by somatodendritic 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors. There is evidence to suggest that 5-HT$_{1B/1D}$ receptors located in the dorsal raphe nucleus are also involved in the release of 5-HT in projection areas (Starkey and Skingle, 1994, Pineyro and Blier, 1996, Pineyro et al., 1995b, Davidson and Stamford, 2000). Research on the role of terminal 5-HT$_{1B}$ receptors has been impeded by the lack of selective 5-HT$_{1B}$ receptor antagonists. NAS-181 is a new selective 5-HT$_{1B}$ receptor antagonist that has shown to enhance 5-HT metabolism and synthesis in rat brain (Berg et al., 1998, Stenfors et al., 2000). The development of 5-HT$_{1B}$ receptor knockout (KO) mice has generated another strategy to study the role of the 5-HT$_{1B}$ receptor (Saudou et al., 1994). Recent microdialysis studies in 5-HT$_{1B}$ receptor KO mice have shown increased 5-HT levels in the hippocampus or the PFC after systemic SSRI administration of this genotype as compared to wildtype mice (Malagie et al., 2001, Knobelman et al., 2001b, de Groote et al., 2002a).

In the present study, we report on the local effects of the SSRI fluvoxamine on extracellular 5-HT in the PFC of mice. Since systemic administration of SSRIs also activates 5-HT autoreceptors in the raphe nuclei, the effects of local fluvoxamine administration were studied to assess the role of the terminal 5-HT$_{1B}$ receptor. The release limiting effects of 5-HT$_{1B}$ autoreceptors on SSRI-induced 5-HT increases were studied in wildtype mice by blocking the 5-HT$_{1B}$ receptor and in mice lacking 5-HT$_{1B}$ receptors.

Material and Methods

Animals

In this study, male wildtype and 5-HT$_{1B}$ knockout mice on a 129/SV genetic background were tested (Saudou et al. 1994). The mice were kept eight per cage on a 12-hour light-dark cycle (6 a.m. on, 6 p.m. off) at constant room temperature (22 $\pm$ 2$°$C) and controlled humidity. The mice had free access to food and water. During the experiments, the mice were between 25-30 grams at an age between 12-16 weeks. The mice were bred in separate homozygous lines in the animal facilities, GDL, Utrecht, The Netherlands. The original wildtype and knockout mice were obtained from Dr. René Hen, Columbia University, New York, USA. The ethical committee for animal research of the University Medical Center Utrecht, The Netherlands, approved the study.

Surgery

Microdialysis probes were implanted in the medial PFC under chloralhydrate anaesthesia (400 mg/kg, i.p.) and lidocaine (2%) applied on the skull. For surgery, the mice were placed in a stereotaxic frame using a mouse adaptor (Stoelting, Germany) with modified earbars. During surgery mice were kept warm on a heating pad. A concentric self-constructed microdialysis probe with an AN filtral 69 membrane, outer diameter 310 μM (Hospal, Uden, The Netherlands), was placed in the medial PFC. The coordinates were: AP + 1.95 mm from bregma, ML − 0.7 mm at an angle of 8°, DV − 3.3 mm from the dura, according to the stereotaxic atlas of the mouse brain Franklin and Paxinos, 1997. The active dialysis surface length of the membrane was 2 mm. The probe was secured in place with dental cement and two anchor screws in the skull. After surgery mice were injected with saline (0.5 ml i.p.) to prevent dehydration and the mice were housed separately.

Microdialysis

Microdialysis experiments started 16-20 hours after surgery. Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) was perfused through the microdialysis probe with a flow of 1.13 μl/min using a high precision pump (Harvard PHD2000, Harvard Scientific, USA). Mouse swivels (Type 375/25, Instech Laboratories, Inc, USA) connected to PEEK-tubing (ID 0.005", OD 0.020") were used to allow unrestrained movements of the mice. The total dead volume of the dialysis system was 45 μl. After the start of the dialysis perfusion mice were left undisturbed for three hours. Experiments took place in the light period and the mice were in their homecage. Samples were collected every 20 minutes in vials containing 7.5 μl acetic acid and stored at −80°C until HPLC analysis. At the end of the experiment the mice were killed by cervical dislocation, the brains were removed and fixed in 4% formaldehyde solution. To verify the position of the probe the brains were cut in 50 μm slices on a vibratome. In case of improper probe placement, data was excluded. In total, four mice had had to be excluded from the analysis.

Drugs

The following drugs were used; CP93129 dihydrochloride (1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one, obtained from Tocris, UK), fluvoxamine maleate (donated by Solvay Pharmaceuticals, The Netherlands) and NAS-181 ((R)-(+)-2-[[[3-(Morpholinomethyl)-2H-chromen-8-yl]oxy]methyl] morpholine methane sulfonate), kindly donated by Dr. C. Stenfors (Astra Zeneca, Sweden). All drugs were dissolved in distilled water and further diluted in Ringer solution on the day of the experiment.

HPLC-ECD analysis

5-HT was analyzed by HPLC with electrochemical detection. Samples were injected onto an Inertsil ODS-3 column (3 µM, 2.1x100mm, Aurora Borealis,The Netherlands) using a Gilson pump and autosampler (Separations, The Netherlands). Separation was performed at 40ºC with the electrochemical detector (Intro, ANTEC Leyden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 µl/l triethylamine, 30 µl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 µl sample (signal-to-noise ratio 2).

Data analysis and statistics

Values for the first four consecutive samples were averaged to calculate the basal levels of extracellular 5-HT. Student's t-tests were used to compare basal 5-HT values between the two genotypes. In the figures, all 5-HT values are expressed as percentages of basal levels ± SEM. Effects of 5-HT response to drug treatment were analyzed by multivariate analysis of variance (ANOVA) with time as 'within' and dose and genotype as 'between' factors. To test the effects of fluvoxamine dosages, area under the curve (AUC) values were calculated for the infusion period of vehicle (Ringer solution) and the two dosages of fluvoxamine. AUC values were analyzed with ANOVA with dose as 'within' and genotype as 'between' subject factors. When appropriate, data were broken down on dose or genotype and comparisons were made by simple contrasts. The significance level for all analyses was set at 5%. In the figures the start of local infusion (time point zero) of drugs is corrected for the lag-time of the microdialysis system.

Results

Basal 5-HT levels

Basal levels of extracellular 5-HT in the PFC of the two genotypes were not different ($F_{(1,66)}= 0.73$, NS). The mean basal levels were 5.3 ± 0.32 fmol/25µl for wildtype (n = 36) and 5.1 ± 0.46 fmol/25µl for 5-HT$_{1B}$ KO mice (n = 31).

Local administration of the 5-HT$_{1B}$ receptor agonist CP93129

The selective 5-HT$_{1B}$ receptor agonist CP93129 (1 µM) was administered by reversed microdialysis for 60 minutes. Extracellular 5-HT levels were reduced

after CP93129 in wildtype mice, but not in 5-HT$_{1B}$ KO mice as shown in Fig 1. In wildtype mice CP93129 reduced extracellular 5-HT to 58 $\pm$ 7% as compared to vehicle (Ringer solution) (F(1,13)=15.3, p < 0.01).

Local administration of fluvoxamine

Local administration of the SSRI fluvoxamine increased extracellular 5-HT as shown in Fig. 2. The two dosages of fluvoxamine were administered cumulative by reversed microdialysis. After four baseline samples, 0.3 μM fluvoxamine was administered for 120 minutes, followed by 1.0 μM fluvoxamine for another 120 minutes. Multivariate ANOVA indicated a time effect (F(14,336)=26.9, p <0.001), a time x treatment interaction (F(14,336)=26.4, p < 0.001), a treatment effect (F(1,24)=180.9, p < 0.001), and a genotype effect (F(1,24)=31.8, p < 0.001). In wildtype mice, the maximum 5-HT increase was 239 $\pm$ 49 % and 352 $\pm$ 57 % following 0.3 μM or 1.0 μM fluvoxamine, respectively. In 5-HT$_{1B}$ KO mice, the maximum 5-HT increase was 347$\pm$ 53 % and 710 $\pm$ 40 % following 0.3 μM or 1.0 μM fluvoxamine, respectively. The AUC values in Fig. 2B show the effects of the two concentrations of fluvoxamine on 5-HT levels. ANOVA indicated an effect of dose for fluvoxamine in wildtype (F(2,12)=21.6, p < 0.001) and in 5-HT$_{1B}$ KO mice (F(2,14)=171.6, p < 0.001). In both genotypes, fluvoxamine dose-dependently increased extracellular 5-HT. The augmentation of 5-HT levels by fluvoxamine was significantly greater in the 5-HT$_{1B}$ KO mice than in wildtype mice (F(1,13)=22.9, p < 0.001) for both concentrations of fluvoxamine.
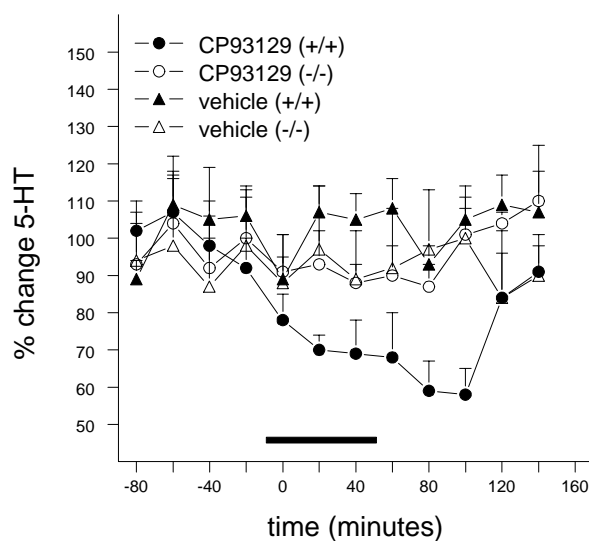

Fig 1. Local administration of the 5-HT$_{1B}$ receptor agonist CP93129 into the PFC. Data are expressed as the mean percent of basal 5-HT levels $\pm$ SEM. In wildtype (+/+) and 5-HT$_{1B}$ KO mice (-/-), 1 μM CP93129 was administered for 60 minutes as indicated by the bar. For vehicle groups n=7-9 and for CP93129 groups n=5-6 mice.
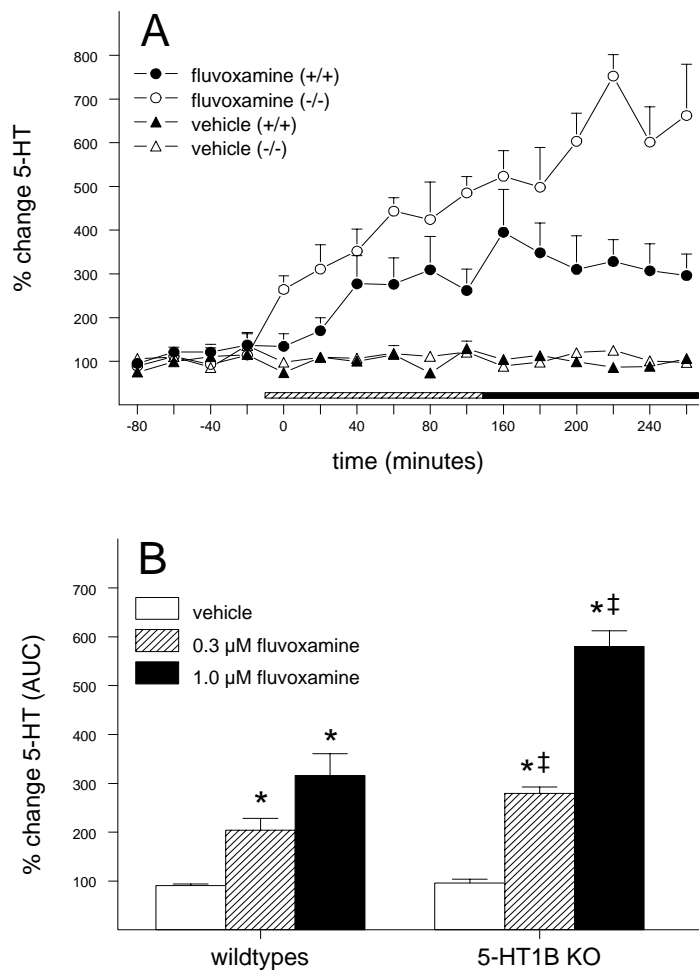
Fig 2. Local administration of fluvoxamine into the PFC. Data is expressed as mean percent of basal 5-HT levels $\pm$ SEM. A) Time course of the effects of fluvoxamine on extracellular 5-HT in wildtype (+/+) and 5-HT$_{1B}$ KO mice (-/-). At time point zero fluvoxamine was administered in two cumulative dosages, first 0.3 μM followed by 1.0 μM as indicated by the striped and black bar, respectively. B) Effects of fluvoxamine on extracellular 5-HT expressed as the AUC $\pm$ SEM for treatment periods of 0, 0.3 and 1.0 μM fluvoxamine in wildtype and 5-HT$_{1B}$ KO mice. Symbols: * indicates a significant dose effect ($p<0.05$) and $\ddagger$ indicates a significant genotype effect ($p<0.05$). For vehicle groups n=7-9 and for fluvoxamine groups n=7-8 mice.

Combined administration of fluvoxamine and the selective 5-HT$_{1B}$ receptor antagonist NAS-181

The effects of concurrent administration of fluvoxamine (1 µM) and the 5-HT$_{1B}$ receptor antagonist NAS-181 (1 µM) into the PFC are shown in Fig. 3. Mice received either fluvoxamine for 40 minutes followed by co-perfusion of fluvoxamine with NAS-181 for another 180 minutes, or perfusion of NAS-181 alone. Multivariate ANOVA revealed a time effect ($F(11,374)=24.5$, $p < 0.001$), a time x treatment interaction ($F(22,374)=23.0$, $p < 0.001$), a treatment effect ($F(1,34)=99.4$, $p < 0.001$) and a treatment x genotype interaction ($F(1,34)=4.6$, $p < 0.05$).

Combined administration of fluvoxamine and NAS-181 caused a significantly greater increase in extracellular 5-HT in wildtype mice than in 5-HT$_{1B}$ KO mice ($F(1,12)=4.9$, $p < 0.05$). The maximum increase of extracellular 5-HT after combined administration of fluvoxamine and NAS-181 was 1080 ± 125 % in wildtype mice and 777 ± 116 % in 5-HT$_{1B}$ KO mice. The 5-HT levels following NAS-181 were not different from baseline and were not different between wildtype and 5-HT$_{1B}$ KO mice.

Discussion

The main finding of the present study is that terminal 5-HT$_{1B}$ autoreceptors in the PFC limit the 5-HT elevating effects of SSRIs. Local administration of the SSRI fluvoxamine evoked a greater 5-HT increase in 5-HT$_{1B}$ KO than in wildtype mice. Addition of NAS-181, a 5-HT$_{1B}$ receptor antagonist, augmented the effects of fluvoxamine on 5-HT release in the PFC of wildtype mice, but not of 5-HT$_{1B}$ KO mice. Administration of NAS-181 alone did not affect 5-HT levels in either genotype. Basal 5-HT levels were not different between the two genotypes. Local administration of the 5-HT$_{1B}$ receptors agonist CP93129, reduced extracellular 5-HT in wildtype but not in 5-HT$_{1B}$ KO mice, confirming the absence of terminal 5-HT$_{1B}$ autoreceptors in the latter genotype.

The present data on the effects of SSRIs in 5-HT$_{1B}$ KO mice and in wildtype mice combined with a 5-HT$_{1B}$ receptor antagonist are in line with microdialysis studies in other species using 5-HT$_{1B/1D}$ receptor antagonists. Thus, GR127935, a mixed 5-HT$_{1B/1D}$ receptor antagonist augmented the effects of SSRIs on 5-HT levels in the frontal cortex of guinea pigs and rats, after systemic (Gobert et al., 1997;Roberts et al., 1998) and local administration (Hertel et al., 1999). Systemic administration of SB-224289, a more selective 5- HT$_{1B}$ receptor antagonist, also
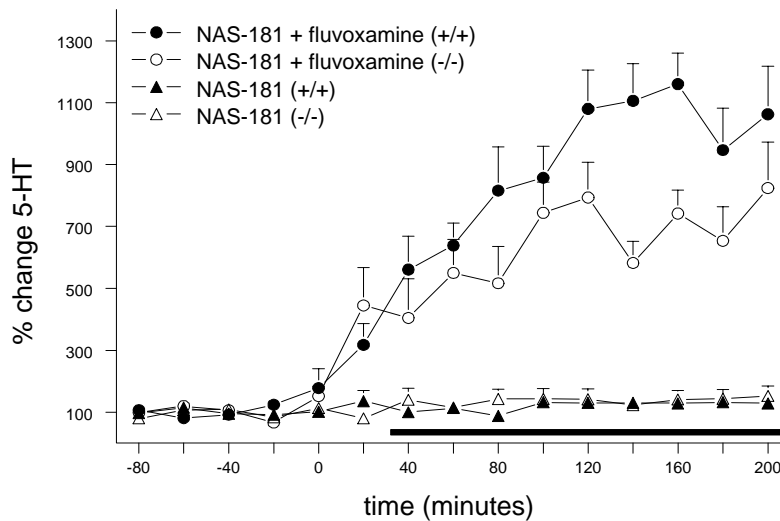
Fig 3. Combined administration of fluvoxamine and the 5-HT$_{1B}$ receptor antagonist NAS-181 into the PFC. Data is expressed as mean percent of basal 5-HT levels $\pm$ SEM. At time point zero 1.0 μM fluvoxamine was administered for 40 minutes in wildtype (+/+) or 5-HT$_{1B}$ KO mice (-/-) and followed by another 160 minutes combined with 1.0 μM NAS-181 as indicated by the bar. For wildtypes n=6-8 and for 5-HT$_{1B}$ KO mice n=5-6.

augmented the systemically induced SSRI- increase of extracellular 5-HT in rat PFC (Gobert et al., 2000). The selective 5-HT$_{1B}$ receptor antagonist, NAS-181, augmented the effects on 5-HT release after combined administration of the SSRI citalopram by reversed microdialysis into rat PFC (Hjorth et al., 2000). In contrast, NAS-181 applied systemically or locally did not augment 5-HT levels when citalopram was given systemically (Hjorth et al., 2000). Thus, blockade of terminal 5-HT$_{1B}$ autoreceptors does not seem to be sufficient to augment the effects of systemically administered SSRIs, when neuronal firing is diminished due to simultaneous 5-HT$_{1A}$ autoreceptor stimulation. The finding in the present study that NAS-181 alone did not affect extracellular 5-HT in wildtype mice, whereas it augmented the effect of locally applied fluvoxamine, supports the idea that the effect of 5-HT$_{1B}$ receptor antagonists is dependent on a endogenous tone which is low under basal conditions. This low endogenous tone at 5-HT$_{1B}$ autoreceptors in nerve terminals under basal conditions could also explain the finding that basal 5-HT levels were not different in 5-HT$_{1B}$ KO mice from those in wildtypes. In contrast to this explanation, it has been shown that systemically administered NAS-181

increased 5-HT metabolism (5-HIAA/5-HT ratio) in rat frontal cortex and other brain areas, suggesting that terminal 5-HT$_{1B}$ receptors are tonicallly active under these conditions (Stenfors et al. 2000). Methodological differences, however, make it difficult to compare effects on 5-HT metabolism with in vivo microdialysis studies measuring 5-HT output. In wildtype mice, the regulation of 5-HT release by 5-HT$_{1B}$ receptors was shown by local administration of CP93129, resulting in decreased extracellular 5-HT levels.

The present study shows that local administration of fluvoxamine resulted in a dose-dependent 5-HT increase that was greater in 5-HT$_{1B}$ KO mice than in the wildtype mice. This finding indicates that 5-HT$_{1B}$ autoreceptors play a significant role in the regulation of 5-HT release in the PFC. The effect on 5-HT release in the PFC following locally applied fluvoxamine differs from data reported for systemically administered SSRIs in wildtype and 5-HT$_{1B}$ KO mice. In a study by Malagie et al (2001), systemic injection of the SSRI paroxetine of 5 mg/kg, but not 1 mg/kg, resulted in a similar 5-HT increase in 5-HT$_{1B}$ KO and wildtype mice and the effect of paroxetine was not augmented by the 5-HT$_{1B/1D}$ receptor antagonist GR127935 in the wildtypes. A possibly explanation might be that the simultaneous activation of somatodendritic 5-HT$_{1A}$ autoreceptors could have limited the effect of 5-HT$_{1B}$ autoreceptors on 5-HT release following the high dosage of paroxetine. It should be noted that in our study, extracellular 5-HT was measured in the medial prefrontal cortex, whereas in the study by Malagie et al the frontal cortex was chosen, it can not be ruled out that 5-HT release is differentially regulated in these cortical areas. Regional differences of SSRI effects on extracellular 5-HT levels are well known (see for a review Hjorth et al. 2000) and are supported by recent studies in mice lacking 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors (Malagie et al. 2001, Knobelman et al. 2001b). In keeping with this notion, a differential role of postsynaptic 5-HT$_{1A}$ receptors in the medial and lateral prefrontal cortex was recently suggested (Celeda et al. 2001). Interestingly, administration of fluvoxamine combined with a selective 5-HT$_{1B}$ receptor antagonist, resulted in a greater 5-HT increase in wildtype than in 5-HT$_{1B}$ KO mice. NAS-181 did not augment the effect of fluvoxamine in 5-HT$_{1B}$ KO mice, indicating that NAS-181 is a selective 5-HT$_{1B}$ receptor antagonist in wildtype mice. The differential 5-HT response after fluvoxamine combined with NAS-181 between the two genotypes suggests that possible adaptive changes in 5-HT$_{1B}$ KO mice might compensate for the loss of terminal 5-HT autoreceptors. Since the knockout mice lack 5-HT$_{1B}$ receptors throughout life, adaptive changes during neurodevelopment might have taken place that can explain these differences (Scearce-Levie et al., 1999, Castanon et al., 2000). Indeed, in some brain areas of 5-HT$_{1B}$ KO mice, alterations in 5-HT metabolism and regional changes in 5-HT transporters have

been demonstrated, but so far not in the frontal cortex (Ase et al., 2000, Ase et al., 2001). In a previous study in wildtype and 5-HT$_{1B}$ KO mice, we found a different dose-response for fluvoxamine in the hippocampus after locally applied fluvoxamine, supporting compensatory adaptations in 5-HT$_{1B}$ KO mice at the level of the 5-HT reuptake site in this brain structure (de Groote et al., 2002a). Postsynaptic 5-HT$_{1A}$ receptors may also affect extracellular 5-HT levels (Bosker et al., 1997b, Hajos et al., 1999, Casanovas et al., 1999). Recently, it has been shown that neuronal pathways from the medial PFC endowed with 5-HT$_{1A}$ receptors projecting back to the dorsal raphe can inhibit 5-HT neuronal firing (Celada et al. 2001). Although binding studies show no evidence for possible adaptive changes (Evrard et al., 1999, Ase et al., 2001), functional changes in postsynaptic 5-HT$_{1A}$ receptors or 5-HT transporters might exist in the PFC of 5-HT$_{1B}$ KO mice.

In conclusion, terminal 5-HT$_{1B}$ autoreceptors in the PFC play a significant role in the regulation of 5-HT release. The 5-HT increase induced by local SSRI-administration was augmented in 5-HT$_{1B}$ KO mice as compared to wildtype mice and was augmented by simultaneous administration of a 5-HT$_{1B}$ receptor antagonist in the latter genotype. In 5-HT$_{1B}$ KO mice, the 5-HT$_{1B}$ receptor antagonist NAS-181 did not affect extracellular 5-HT and did not further increase the effect of fluvoxamine, showing that NAS-181 is a selective 5-HT$_{1B}$ receptor antagonist. Finally, the greater increase in 5-HT levels following combined administration of a SSRI with NAS-181 in wildtype mice, as compared to 5-HT$_{1B}$ KO mice, suggests possible adaptive changes in the knockout mice.

CHAPTER 4

Interactions between serotonin and dopamine in the striatum are not mediated by striatal 5-HT$_{1B}$ receptors

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submitted

Abstract

In vivo microdialysis was performed in wildtype and 5-HT$_{1B}$ receptor knockout mice to explore the role of 5-HT$_{1B}$ receptors in striatal serotonin (5-HT) and dopamine (DA) outflow. Local administration of the selective 5-HT reuptake inhibitor fluvoxamine (0.1 – 10 μM) dose-dependently increased 5-HT to the same extent in wildtype and 5-HT$_{1B}$ receptor knockout mice. The 5-HT$_{1B}$ receptor agonist CP93129 (0.5 μM) reduced 5-HT levels wildtype mice, but did not affect DA outflow. Striatal DA outflow was increased 5-fold by 50 μM CP93129, in wildtypes and surprisingly, 5-HT$_{1B}$ receptor knockout mice showed an identical response. The CP93129-induced DA increase was not attenuated by ritanserin, a 5-HT$_{2A/2C}$ receptor antagonist, but was completely blocked by tetrodotoxin, demonstrating that the DA release was from a neuronal origin. Increased striatal 5-HT levels should indirectly activate 5-HT receptors that facilitate DA release. The 5-HT releaser fenfluramine (50 μM) and the SSRI fluvoxamine (10 μM) both increased 5-HT and DA levels, but their effect was not different between the genotypes.

Concluding, striatal 5-HT$_{1B}$ autoreceptors are functionally present, but do not play a significant role in the effects of SSRIs on extracellular 5-HT. The results in 5-HT$_{1B}$ knockout mice do not support a role of striatal 5-HT$_{1B}$ heteroreceptors in DA outflow in the striatum.

Introduction

It has been well established that serotonin (5-HT) modulates dopamine (DA) neurotransmission (Soubrie et al., 1984, Bonhomme et al., 1995). Thus, the striatonigral pathway is innervated by serotonergic afferents both at dopaminergic nerve terminals in the striatum and at dopaminergic cell bodies in the substantia nigra (Steinbusch, 1981). Anatomical data suggest a role for 5-HT$_{1B}$ receptors in the interactions between 5-HT and DA pathways, because this receptor is positioned at sites where it can modulate both 5-HT and DA release in the striatum (Bruinvels et al., 1994, Sari et al., 1999). Anatomical studies have also shown that 5-HT$_{1B}$ receptors are abundantly present in the caudate putamen and in the substantia nigra, whereas mRNA for 5-HT$_{1B}$ receptors was not detected in the latter brain structure (Boschert et al., 1994). The mismatch between binding sites and mRNA has led to the idea that 5-HT$_{1B}$ heteroreceptors are present on GABA-ergic afferents projecting from the striatum to the substantia nigra. This is supported by electrophysiological studies showing that stimulation of 5-HT$_{1B}$ receptors inhibits GABA release from terminals that innervate DA neurons in the substantia nigra, resulting in disinhibition of DA neurons (Johnson et al., 1992, Cameron and Williams, 1994, Morikawa et al., 2000). Furthermore, there is neurochemical evidence that striatal DA release is modulated by 5-HT. In vivo microdialysis studies have shown increased DA outflow when striatal 5-HT levels are enhanced by exogenous applied 5-HT (Benloucif and Galloway, 1991, Yadid et al., 1994, Bonhomme et al., 1995) or by the 5-HT releaser fenfluramine (De Deurwaerdere et al., 1995, Baumann et al., 2001). Studies in rats using 5-HT$_{1B}$ receptor agonists indicated that 5-HT$_{1B}$ receptors facilitate DA release in the dorsal striatum (Benloucif et al., 1993, Galloway et al., 1993, Bentue-Ferrer et al., 1998, Ng et al., 1999). Furthermore, in striatal synaptosomes, activation of 5-HT$_{1B}$ receptor with CP93129, a 5-HT$_{1B}$ receptor agonist, modulates DA release in wildtype mice, whereas this effect was absent in mice lacking 5-HT$_{1B}$ receptors, supporting a role of 5-HT$_{1B}$ receptors in striatal DA release (Sarhan et al., 2000).

In the CNS, 5-HT$_{1B}$ receptors are also present as inhibitory autoreceptors on serotonergic nerve terminals. Activation of 5-HT$_{1B}$ autoreceptors reduces striatal 5-HT levels in rat (Abellan et al., 2000) and mouse (Knobelman et al., 2000). The 5-HT$_{1B}$ receptor knockout mouse is an interesting model to further explore the role of 5-HT$_{1B}$ receptors in the modulation of striatal 5-HT and DA release. In the absence of terminal 5-HT$_{1B}$ autoreceptors, increased 5-HT levels might be expected following administration of selective 5-HT reuptake inhibitors (SSRIs). In mice lacking 5-HT$_{1B}$ receptors, however, no difference in striatal 5-HT was found after systemic administration of the SSRI fluoxetine as compared to wildtype mice

(Knobelman et al., 2001a). Involvement of 5-HT$_{1B}$ receptors in an interaction between 5-HT and DA pathways is supported by studies in 5-HT$_{1B}$ KO mice showing that DA neurotransmission is altered in 5-HT$_{1B}$ KO mice (Scearce-Levie et al., 1999, Ase et al., 2000). 5-HT$_{1B}$ KO mice are more vulnerable to dopaminergic drugs like cocaine (Lucas et al., 1997, Rocha et al., 1998), although, cocaine-evoked DA levels in the striatum were not different between wildtype and 5-HT$_{1B}$ KO mice (Shippenberg et al., 2000). There are thus, no in vivo studies that have examined the effects of serotonergic intervention on DA outflow in mice lacking 5-HT$_{1B}$ receptors.

In the present study, in vivo microdialysis was used to examine the role of striatal 5-HT$_{1B}$ receptors in 5-HT and DA outflow by comparing wildtype with 5-HT$_{1B}$ KO mice. The effects on striatal 5-HT and DA were measured following local administration of different serotonergic drugs. CP93129, a selective 5-HT$_{1B}$ receptor agonist, was used to stimulate 5-HT$_{1B}$ receptors and the SSRI fluvoxamine and the 5-HT releaser fenfluramine were used to enhance 5-HT levels.

Material and Methods

Animals
In this study, male wildtype and 5-HT$_{1B}$ knockout mice on a 129/SV genetic background were tested (Saudou et al., 1994). The mice were housed eight animals per cage, kept on a 12-hour light-dark cycle (6 a.m. on, 6 p.m. off) at constant room temperature ($22 \pm 2°C$), controlled humidity 40 - 60 %) and free access to food and water. At the time of the experiments, the mice were at an age between 12-16 weeks and 25-35 grams. The mice were bred in separate homozygous lines in the animal facilities, GDL, Utrecht, The Netherlands. Offspring from the two breeding lines were regularly screened to confirm their genotype. Due to the unexpected findings with 50 µM CP93129, genotypes of mice from these groups were screened after the experiments. The original wildtype and knockout mice were obtained from Dr. René Hen, Columbia University, New York, USA. The ethical committee for animal research of the University Medical Center Utrecht, The Netherlands, approved all studies.

Microdialysis procedure
Microdialysis probes were implanted in the dorsal striatum under chloralhydrate anaesthesia (400 mg/kg, i.p.) and lidocaine (2%) applied on the skull. For surgery, the mice were placed in a stereotaxic frame using a mouse adaptor (Kopf,

Germany) with modified earbars. During surgery mice were kept warm on a heating pad. A concentric self-constructed microdialysis probe with an AN filtral 69 membrane, outer diameter 310 µM (Hospal, Uden, The Netherlands), was placed in the dorsal striatum according to the stereotaxic atlas of the mouse brain Franklin and Paxinos, 1997. The coordinates were: AP +0.80, ML –1.7 mm from bregma, DV –4.0 mm from the dura, with the toothbar set at 0 mm. The active dialysis surface length of the membrane was 2 mm. The probe was secured in place with dental cement and two anchor screws in the skull. After surgery mice were injected with saline (0.5 ml i.p.) to prevent dehydration and the mice were housed separately. Microdialysis experiments started 16-20 hours after surgery. Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) was perfused through the microdialysis probe with a flow at 1.13 µl/min using a high precision pump (Harvard PHD2000, Harvard Scientific, USA). Mouse swivels (Type 375/25, Instech Laboratories, Inc, USA) connected to PEEK-tubing (ID 0.005", OD 0.020") were used to allow unrestrained movements of the mice. After the start of the dialysis probe perfusion, mice were left undisturbed for three hours. Mice were tested in their homecage during the light period. Samples were collected every 20 minutes in vials containing 7.5 µl acetic acid and stored at –80°C until HPLC analysis. At the end of the experiment the mice were killed by cervical dislocation, the brains were removed and fixed in 4% formaldehyde solution. To verify the position of the probe the brains were cut in 50 µm slices on a vibratome. In case of improper probe placement, data was excluded.

Drugs
The following drugs were used; CP93129 dihydrochloride (1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one, obtained from Tocris, UK), fluvoxamine maleate (donated by Solvay Pharmaceuticals, The Netherlands), ritanserin (Janssen Life Sciences Products, Belgium), S(+)-fenfluramine hydrochloride (RBI, Natick, M.A, USA) and tetrodotoxin (Sigma, St. Louis, USA). All drugs, except ritanserin, were dissolved in distilled water and further diluted in Ringer solution to the final concentration on the day of the experiment. Ritanserin was first dissolved in a drop of acetic acid, further diluted to the final concentration and the pH was adjusted to that of Ringer solution.

HPLC-ECD analysis
5-HT, DA and their metabolites were analyzed by HPLC with electrochemical detection. Samples (25 µl) were injected onto an Inertsil ODS-3 column (3 µM, 2.1x100mm, Aurora Borealis, The Netherlands) using a Gilson pump and autosampler (Separations, The Netherlands). Separation was performed at 40ºC

with an electrochemical detector (Intro, ANTEC Leyden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 µl/l triethylamine, 30 µl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 µl sample (signal to noise ratio 2).

Data analysis and statistics
Values for the first four consecutive samples were averaged to calculate the basal levels of extracellular 5-HT and DA, uncorrected for probe recovery. Student's t-tests were used to compare basal 5-HT and DA values between the two genotypes. In the figures, all 5-HT or DA levels are expressed as percentages of basal levels ± SEM. Effects of drug treatment were analyzed by a repeated multivariate analysis of variance (ANOVA) with time as 'within' and treatment (or dose) and genotype as 'between' factors. When appropriate, data were broken down and pairwise comparisons were made. To test the concentration effects of fluvoxamine, area under the curve (AUC) values were calculated for the 200 min infusion period of vehicle (Ringer solution) and the different fluvoxamine concentrations. AUC values were analyzed with ANOVA with dose and genotype as 'between' subject factors. When appropriate, data was broken down on genotype and post-hoc comparisons with Bonferroni corrections were made. The significance level for all analyses was set at 5%. In the figures the start of local infusion (time point zero) of drugs is corrected for the dead volume of the microdialysis system.

Results

Basal levels
Basal levels of extracellular 5-HT and DA levels in the dorsal striatum were not different between wildtype and 5-HT$_{1B}$ KO mice. Basal 5-HT levels were 4.0 ± 0.3 fmol/sample in wildtype (n = 51) and 5.0 ± 0.6 fmol/sample in 5-HT$_{1B}$ KO mice (n = 45). Basal DA levels were 181.3 ± 14.6 fmol/sample in wildtype (n = 51) and 183.1 ± 15.9 fmol/sample in 5-HT$_{1B}$ KO mice (n = 45).

Effects of fluvoxamine and CP93129 on 5-HT outflow
Local administration of the SSRI fluvoxamine into the striatum increased 5-HT levels dose-dependently as shown in Fig.1. Three concentrations of fluvoxamine
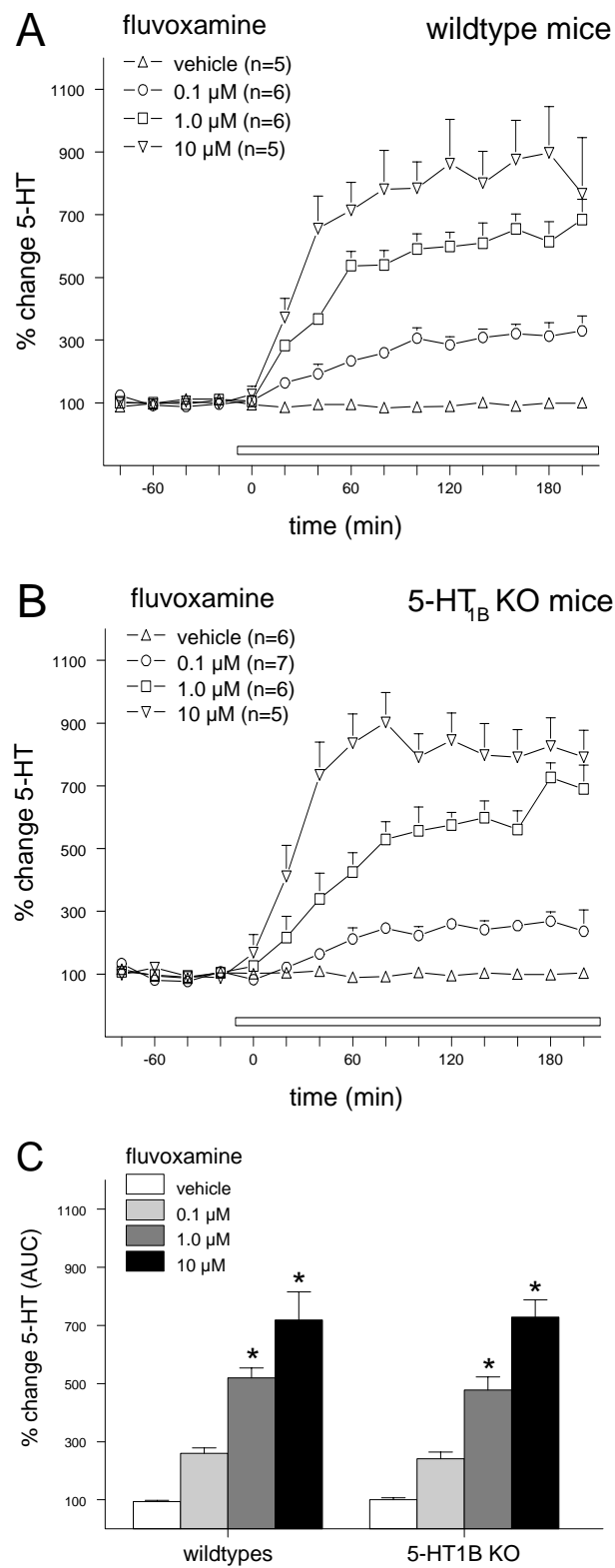
Fig.1. Effects of local administration of fluvoxamine into the dorsal striatum on extracellular 5-HT. Data are expressed as the mean percent change from basal 5-HT levels ± SEM. Time course of the effects of fluvoxamine at concentrations of 0.1, 1.0 and 10 μM in wildtype mice (A) and 5-HT$_{1B}$ KO mice (B). Effects of fluvoxamine expressed as AUC ± SEM (C). Symbol: * indicates a significant dose effect compared to vehicle within genotype ($P < 0.05$).

were administered by reversed microdialysis. A repeated measures ANOVA indicated an effect of time x dose ($F_{(27,333)}= 18.4$, $P < 0.001$), of dose ($F_{(1,37)}= 72.6$, $P < 0.001$), but not of genotype ($F_{(1,37)}= 0.14$, $P = 0.9$). Fluvoxamine at concentrations of 0.1, 1.0 and 10 μM increased 5-HT to about 3, 6 and 8-fold, respectively. The dose-response to fluvoxamine was similar in wildtype and 5-HT$_{1B}$ KO mice. Taking the AUC as outcome variable similar results were obtained (Fig.1C). Post-hoc comparison of the dose effects of fluvoxamine within genotype indicated significant effects between 1.0 and 10 μM fluvoxamine as compared to vehicle ($P < 0.001$), but not between vehicle and 0.1 μM fluvoxamine ($P = 0.29$ and $P = 0.18$ for wildtype and 5-HT$_{1B}$ KO mice, respectively). Furthermore, significant effects were found between the concentrations of 0.1, 1.0 and 10 μM fluvoxamine ($P < 0.01$), except for the 1.0 μM versus the 10 μM group in 5-HT$_{1B}$ KO mice ($P = 0.062$).

Local administration of the 5-HT$_{1B}$ receptor agonist, CP93129, decreased 5-HT levels in wildtype, but not in 5-HT$_{1B}$ KO mice as shown in Fig.2. A repeated measures ANOVA indicated an effect of treatment ($F_{(1,17)}= 6.2$, $P < 0.05$) and of genotype ($F_{(1,17)}= 13.5$, $P < 0.01$). In wildtype mice, CP93129 (0.5 μM) reduced 5-HT to 51 ± 9 % as compared to vehicle ($F_{(1,10)}= 11.2$, $P < 0.01$).

Effects of CP93129 on striatal DA outflow

Local administration of 50 μM CP93129 into the striatum increased DA levels, whereas 0.5 μM did not affect DA outflow as shown in Fig.3A. A repeated measures ANOVA indicated an effect time x dose ($F_{(16, 256)}= 7.9$, $P < 0.001$), of dose ($F_{(2,32)}= 55.6$, $P < 0.001$), but not of genotype ($F_{(2,32)}= 0.5$, $P = 0.98$).

Fig.2. Effects of local administration of the 5-HT$_{1B}$ receptor agonist CP93129 into the dorsal striatum on 5-HT outflow in wildtype (WT) and 5-HT$_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ± SEM. Time course of 0.5 μM CP93129 (black bar) administered for 120 minutes. For clarity reasons vehicle groups are not shown.
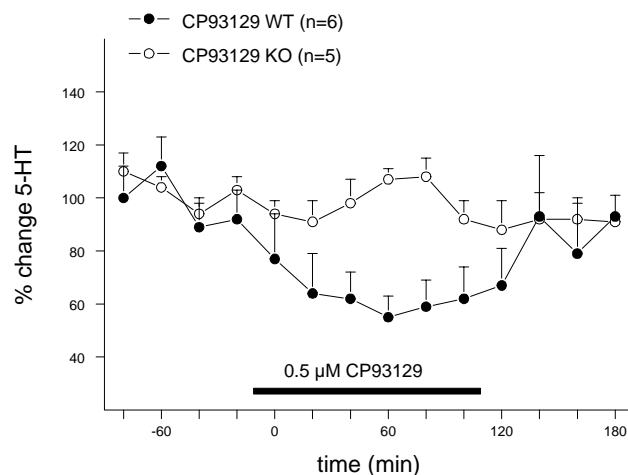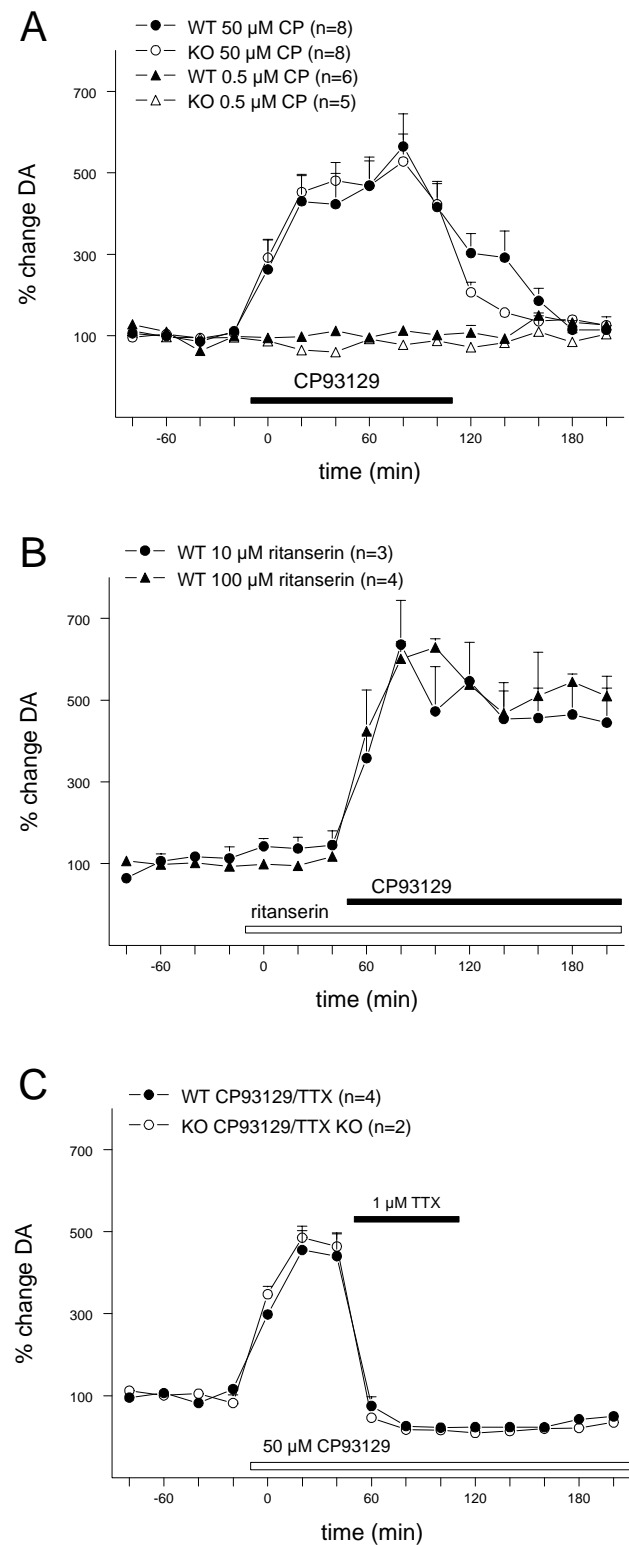
Fig.3. Effects of local administration of the 5-HT_{1B} receptor agonist CP93129 into the dorsal striatum on DA outflow in wildtype (WT) and 5-HT_{1B} KO mice (KO). Data are expressed as the mean percent change from basal level ± SEM. Time course of 0.5 μM and 50 μM CP93129 (black bar) administered for 120 minutes in wildtype and 5-HT_{1B} KO mice (A). Time course of local infusion of 10 and 100 μM of the 5-$HT_{2A/2C}$ receptor antagonist ritanserin (open bar) for 60 minutes followed by co-perfusion with 50 μM CP93129 (black bar) in wildtype mice (B). Time course of local infusion of 50 μM CP93129 minutes and co-perfusion with tetrodotoxin (TTX, black bar) for 60 minutes (C).

CP93129 (50 µM) increased DA levels in 5-HT$_{1B}$ KO mice to the same extent as in wildtypes when compared to vehicle ($P < 0.001$). The CP93129-induced increases were 525 ± 79 % and 527 ± 67 % in wildtype and 5-HT$_{1B}$ KO, respectively.

To test the involvement of 5-HT$_2$ receptors in the effect of CP93129, the 5-HT$_{2A/2C}$ receptor antagonist ritanserin was administered starting 60 minutes before CP93129 was co-perfused. In wildtype mice, the increase in striatal DA induced by 50 µM CP93129 was not affected by ritanserin (10 or 100 µM) as shown in Fig.3B. To test the neuronal origin of the CP93129-induced DA release, the sodium potassium channel blocker tetrodotoxin was co-perfused with CP93129.
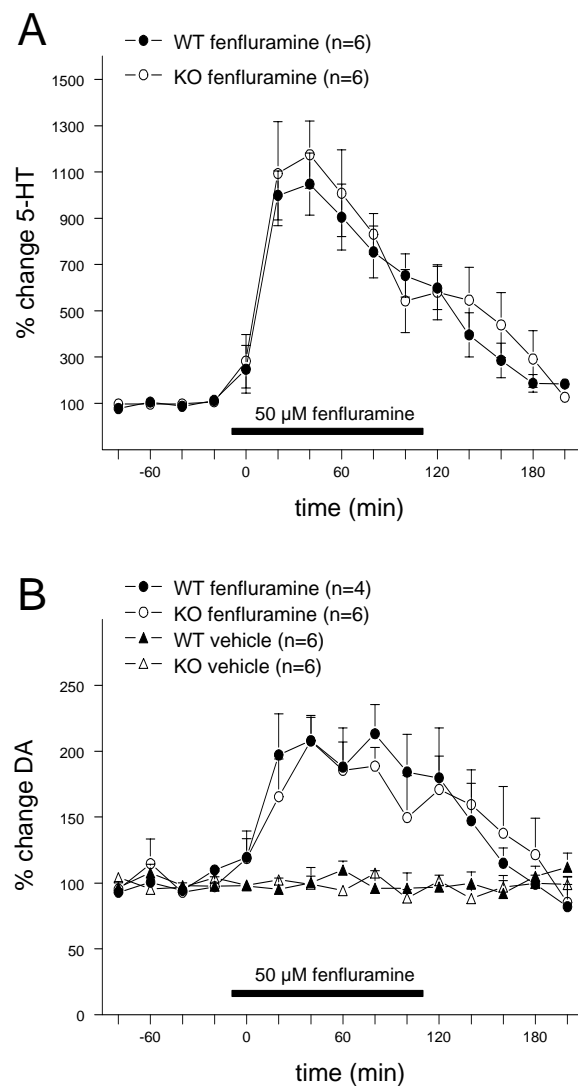
Fig.4. Effects of the 5-HT releaser fenfluramine on striatal 5-HT and DA outflow in wildtype (WT) and 5-HT$_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ± SEM. Time course of the effects 50 µM fenfluramine locally applied into the striatum for 120 minutes (black bar) on 5-HT outflow (A) and DA outflow (B).

Effects of 5-HT on DA outflow

Local administration of the 5-HT releaser fenfluramine increased 5-HT and DA levels as shown in Fig.4. For the effects of fenfluramine on 5-HT outflow, a repeated measures ANOVA indicated an effect of treatment ($F_{(1,18)}= 62.6$, $P < 0.001$), but not of genotype ($F_{(1,18)}= 0.01$, $P = 0.9$). Fenfluramine (50 μM) as compared to vehicle, significantly increased 5-HT levels to the same extent in both genotypes ($P < 0.001$). The mean maximum increases in 5-HT following fenfluramine were 1047 $\pm$ 134 % and 1122 $\pm$ 167 % in wildtype and 5-HT$_{1B}$ KO mice, respectively. For the effects of fenfluramine on DA outflow, a repeated measures ANOVA indicated an effect of treatment ($F_{(1,17)}= 19.5$, $P < 0.001$), but not of genotype ($F_{(1,17)}= 0.04$, $P = 0.8$). Fenfluramine (50 μM) as compared to vehicle, significantly increased 5-HT levels to the same extend in wildtype ($P < 0.01$) and 5-HT$_{1B}$ KO mice ($P < 0.5$). After 80 minutes infusion, the DA outflow in response to fenfluramine was increased to a mean maximum of 213 $\pm$ 15% and 188 $\pm$ 14 % in wildtype and 5-HT$_{1B}$ KO mice, respectively.

As shown in Fig.1, 10 μM fluvoxamine increased 5-HT levels to ~ 800 % of baseline. Fig.5. shows the effect of 10 μM fluvoxamine on DA outflow. A repeated measures ANOVA indicated an effect of treatment ($F_{(1,18)}= 31.0$, $P < 0.001$), but not of genotype ($F_{(1,18)}= 0.5$, $P = 0.5$). Fluvoxamine (10 μM) significantly increased DA outflow as compared to vehicle to the same extent in both genotypes, to a mean maximum of 148 $\pm$ 13 % in wildtype ($P < 0.001$) and to 139 $\pm$ 18 % in 5-HT$_{1B}$ KO mice ($P < 0.01$).
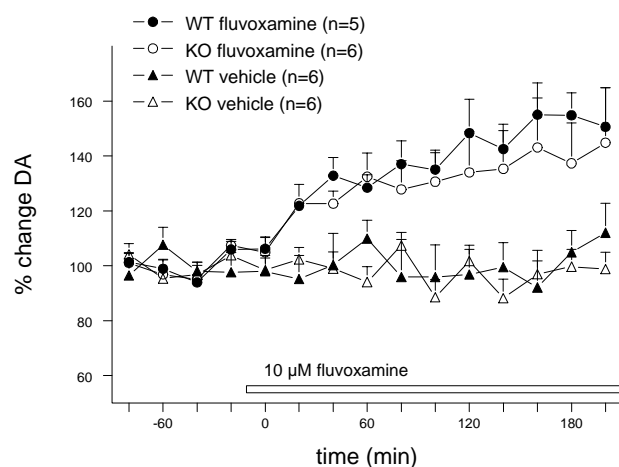

Fig.5. Effects of the SSRI fluvoxamine on DA outflow in wildtype (WT) and 5-HT$_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level $\pm$ SEM. Time course of the effects of 10 μM fluvoxamine (open bar) on striatal DA outflow.

Discussion

The present study confirms the presence of 5-HT$_{1B}$ autoreceptors in the dorsal striatum of mice, but also shows that these 5-HT autoreceptors do not play a prominent role in the effects of a locally applied SSRI on 5-HT release. The previous reported role of 5-HT$_{1B}$ heteroreceptors on striatal DA outflow could not be confirmed in this study. The putative selective 5-HT$_{1B}$ receptor antagonist CP93129 increased striatal DA levels to the same extent in wildtype and in 5-HT$_{1B}$ KO mice, indicating that the effect of CP93129 was not mediated by 5-HT$_{1B}$ receptors. Furthermore, the 5-HT induced extracellular DA increase by fenfluramine and fluvoxamine administration was not different between the two genotypes.

In line with other studies, we found that basal extracellular levels of 5-HT and DA in the dorsal striatum were not different between wildtype and 5-HT$_{1B}$ KO mice (Knobelman et al., 2001, Shippenberg et al., 2000). This suggests that, either 5-HT$_{1B}$ receptors do not affect 5-HT and DA release in the striatum under basal conditions, or that compensatory effects may have taken place during neurodevelopment. Moreover, no genotype difference was found in the effects on 5-HT levels following local administration of a SSRI into the striatum, suggesting that 5-HT$_{1B}$ autoreceptors do not limit these SSRI effects. This is in line with a previous study that showed a similar striatal 5-HT increase in wildtype and 5-HT$_{1B}$ KO mice upon systemic SSRI administration, although involvement of somatodendritic 5-HT$_{1A}$ autoreceptors may account for this systemic effect (Knobelman et al., 2001). In contrast to the effects in the striatum, we previously reported augmented 5-HT responses after locally applied fluvoxamine (1 μM) into the hippocampus and prefrontal cortex of 5-HT$_{1B}$ KO mice (de Groote et al., 2002a, 2002b). Densities of 5-HT transporters are about two times higher in the striatum as compared to hippocampal or cortical areas of mice (Bengel et al., 1997). Possibly, extracellular 5-HT is more efficiently removed by 5-HT transporters in the striatum relative to other brain areas. This might also explain why basal levels of 5-HT were not different between 5-HT$_{1B}$ KO and wildtype mice. Interestingly, in a study by Tao et al (2000) local administration of a high concentration of the SSRI citalopram (100 μM) into the nucleus accumbens was augmented by (-)-penbutolol, a non-selective 5-HT$_{1B}$ receptor antagonist. This might suggest that terminal 5-HT$_{1B}$ autoreceptors affect 5-HT release only when 5-HT reuptake sites are maximally blocked in striatal areas, although non-selective effects on 5-HT of (-)-penbutolol can not be excluded. Whether 10 μM fluvoxamine in our study maximally blocked striatal 5-HT reuptake sites is unclear, but in view of the observed 8-fold 5-HT increase it is unlikely that the absence of an

augmented 5-HT response in 5-HT$_{1B}$ KO mice is accounted by insufficient reuptake blockade. Nevertheless, infusion of CP93129 (0.5 µM) reduced 5-HT levels in wildtype mice, indicating that 5-HT$_{1B}$ autoreceptors are functionally present in the striatum in this genotype.

The 5-HT$_{1B}$ receptor agonist CP93129, in a concentration range of 10-500 µM, has been reported to increase DA outflow in rat striatum (Benloucif et al., 1993, Galloway et al., 1993, Bentue-Ferrer et al., 1998) and other brain structures (Iyer and Bradberry, 1996, Yan and Yan, 2001). This effect of CP93129 on DA outflow has been attributed by stimulation of 5-HT$_{1B}$ heteroreceptors. In the present study, however, DA outflow increased to the same extent in wildtype and 5-HT$_{1B}$ KO mice following administration of 50 µM CP93129. The present results, therefore, demonstrate that the DA increase induced by CP93129 is not mediated through 5-HT$_{1B}$ receptors. Thus, rat studies in which the effects of CP93129 on striatal DA outflow are attributed to stimulation of 5-HT$_{1B}$ heteroreceptors should be reconsidered (Benloucif et al., 1993, Galloway et al., 1993, Bentue-Ferrer et al., 1998). CP93129 also has affinity for 5-HT$_{1D}$, 5-HT$_{1A}$ and 5-HT$_{2C}$ receptor subtypes (Macor et al., 1990). Based on the affinity profile of CP93129 and the expression of 5-HT$_{2A/2C}$ receptors in the striatum, we choose ritanserin, a 5-HT$_{2A/2C}$ receptor antagonist, to investigate whether the effects of CP93129 may be accounted by stimulation of these receptor subtypes. Co-perfusion with ritanserin did not attenuate the effect of CP93129 on DA outflow, suggesting that 5-HT$_{2A/2C}$ receptor subtypes are not implicated. The effect of CP93129 on DA outflow was blocked by co-perfusion with tetrodotoxin, indicating that the DA release induced by CP93129 was from neuronal origin. There is circumstantial evidence that 5-HT$_3$ and 5-HT$_4$ receptor subtypes can also affect DA outflow (Bonhomme et al., 1995, Lucas et al., 2001). Possibly, CP93129 exerts its effect on DA outflow through stimulation of 5-HT$_3$ or 5-HT$_4$ receptors, although to our knowledge, affinity of CP93129 for these receptor subtypes has not been reported.

A locally applied 5-HT releaser or a SSRI into the striatum increased both 5-HT and DA levels. These findings are consistent with the idea that 5-HT modulates striatal DA outflow. Nevertheless, it can not be excluded that the effects of fenfluramine or fluvoxamine on DA outflow involve other effects of these compounds. It has been suggested that fenfluramine is not a very useful tool to study 5-HT and DA interactions since its effect on DA outflow in the striatum was still present after lesioning the serotonergic system in rats (De Deurwaerdere et al., 1995).

In the present study, no evidence for a role of 5-HT$_{1B}$ receptors in striatal DA outflow was found. This does not exclude modulation of striatal DA release by 5-HT$_{1B}$ receptors. Enriched binding densities for 5-HT$_{1B}$ receptors are found in the

substantia nigra, and these receptors are thought to play a role in the regulation of DA neuronal activity. Interestingly, a microdialysis study in the substantia nigra reported an increase of DA outflow to 4770% following 1 μM CP93129, suggesting that low doses of CP93129 may activate 5-HT_{1B} heteroreceptors in this brain structure (Thorre et al., 1998). Another possible explanation for the lack of effect of striatal 5-HT_{1B} heteroreceptors on DA outflow in the striatum, could be that interactions between 5-HT and DA neurotransmission are not elicited by activation of striatal 5-HT_{1B} receptors under basal conditions. There is evidence to suggest that stimulation of striatal 5-HT induced by low concentrations of SSRIs, increase DA outflow only when the striatonigral pathway is activated (Lucas et al., 2000).
Concluding, striatal 5-HT_{1B} autoreceptors are functionally present in the striatum, but do not play a significant role in the effects of a locally applied SSRI on 5-HT levels. The results support an interaction between 5-HT and DA neurotransmisson in the striatum, but this interaction is not mediated by striatal 5-HT_{1B} heteroreceptors.

CHAPTER 5

An evaluation of the effect of NAS-181, a new selective 5-HT$_{1B}$ receptor antagonist, on extracellular 5-HT levels in rat frontal cortex

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submitted

Abstract

In the mammalian brain 5-HT$_{1B}$ receptors are present as autoreceptors regulating the release of serotonin (5-HT) by inhibitory feedback. The antagonistic properties of NAS-181, a new selective antagonist for the rodent 5-HT$_{1B}$ receptor, was determined by using an agonist-induced decrease of extracellular 5-HT. The 5-HT$_{1B}$ receptor agonist CP93129 (0.03–0.3 µM) applied by reversed microdialysis, dose-dependently reduced 5-HT levels in rat frontal cortex. The suppressant effect of CP91239 (0.1 µM) was smaller in the presence of fluvoxamine (3-10 µM), a 5-HT reuptake inhibitor. The effects of NAS-181 on CP93129 were compared with GR127935, a mixed 5-HT$_{1B/1D}$ receptor antagonist, and SB224289, a 5-HT$_{1B}$ receptor antagonist. Both in the presence and absence of fluvoxamine, the suppressant effect of CP93129 on extracellular 5-HT was attenuated by NAS-181 (1 µM) and GR127935 (10 µm), but not by SB224289 (1 µM). In the absence of fluvoxamine, GR127935, SB224289 and NAS-181 all reduced 5-HT levels, suggesting partial agonistic properties of these compounds. In conclusion, the results show that NAS-181 is a potent 5-HT$_{1B}$ receptor antagonist.

Introduction

5-HT$_{1B}$ receptors are expressed throughout the mammalian brain, either as autoreceptors or heteroreceptors, with high densities in the basal ganglia, limbic and cortical areas (Boschert et al., 1994, Bruinvels et al., 1993). The release of 5-HT is regulated by 5-HT$_{1B}$ autoreceptors on serotonergic nerve terminals (Engel et al., 1986, Maura et al., 1986). The rodent and human 5-HT$_{1B}$ receptor are considered as species homologues based on their similar function and amino acid homology (Adham et al., 1992, Hoyer and Middlemiss, 1989). Rodent 5-HT$_{1B}$ autoreceptors are pharmacologically different from the human and guinea pig 5-HT$_{1B}$ receptor (formerly known as 5-HT$_{1Dß}$) (Hartig et al., 1992). This species difference in pharmacology is mainly based on the affinity of rodent 5-HT$_{1B}$ receptor for ß-adrenergic compounds and is due to a single amino acid difference (Oksenberg et al., 1992). Research on 5-HT$_{1B}$ receptors has been complicated due to a lack of selective antagonists. Most compounds that have been used to study 5-HT$_{1B}$ receptors so far are not selective for 5-HT$_1$ receptor subtypes or had additional affinity for other bi
nding sites. Recently, more selective 5-HT$_{1B}$ receptor antagonists have become available. GR127935 is a selective antagonist for both rodent and human 5-HT$_{1B}$ receptors, although it does not discriminate between 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes (Hutson et al., 1995). SB224289 is a more selective 5-HT$_{1B}$ receptor antagonist (Gobert et al., 2000). Despite their selectivity, GR127935 and SB224289 have been reported to display partial or inverse agonist properties at human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors, although there is as yet no in vivo evidence for such properties (Pauwels, 1997, Millan et al., 1999). NAS-181 is a new selective 5-HT$_{1B}$ receptor antagonist for rodent 5-HT$_{1B}$ receptors (Berg et al., 1998) and has been shown to increase 5-HT metabolism and synthesis (Stenfors et al., 2000). Microdialysis studies on the effects of 5-HT$_{1B}$ receptor antagonists on extracellular 5-HT have resulted in some conflicting findings. Whereas stimulation of 5-HT$_{1B}$ receptors decreases extracellular 5-HT levels (Hjorth and Tao, 1991, Bosker et al., 1995, Adell et al., 2001), administration of 5-HT$_{1B}$ receptor antagonists does not seem to affect 5-HT levels (Roberts et al., 1998). Systemically administered GR127935 has been found to decrease 5-HT levels in guinea pig frontal cortex, but this effect was explained by activation of 5-HT$_{1B/1D}$ receptors in the raphe nuclei (Roberts et al., 1998). Blockade of 5-HT$_{1B}$ receptors has been reported to augment the effect of systemic SSRIs on 5-HT levels in the frontal cortex (Gobert et al., 1997 Dawson and Nguyen, 2000, Hervas et al., 2000, Gobert et al., 2000), but not in all studies (Sharp et al., 1997). Following systemic administration of SSRIs, cell body 5-HT$_{1A}$ autoreceptors may limit the effects of 5-

HT$_{1B}$ receptors in serotonergic projection areas. To exclude effects of 5-HT receptors in the raphe on 5-HT output, antagonists can be applied locally into serotonergic projection areas by reversed microdialysis. Local infusion of GR127935 or NAS-181 has been reported to enhance 5-HT levels in the presence of a SSRI in the perfusion fluid (Hertel et al., 1999, Hjorth et al., 2000). It has been hypothesized that terminal 5-HT$_{1B}$ receptors have a low basal endogenous activity and that effects of a 5-HT$_{1B}$ receptor antagonist are only elicited when 5-HT levels are enhanced, i.e. in the presence of a SSRI (Hjorth, 1993).

In the present study, NAS-181 was compared with GR127935 and SB224289, two other selective 5-HT$_{1B}$ receptor antagonists. The antagonists were locally applied into rat frontal cortex and their properties to block the suppressant effect of the 5-HT$_{1B}$ receptor agonist CP93129 on extracellular 5-HT were assessed. The effects of the three antagonists co-perfused with CP93129 were studied both in the absence and presence of the SSRI fluvoxamine in the perfusion fluid.

Material and methods

Animals

Male Wistar rats (GDL, Utrecht, The Netherlands) weighing 250-300 gram were housed three per cage at standard conditions (22-24 °C, food and water ad lib) and at a 12-hour light/dark cycle (7 a.m. on, 7 p.m. off). The Ethical Committee for Animal Research of the University Medical Center Utrecht, The Netherlands, approved the study.

Microdialysis procedure

Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.). A self-constructed concentric microdialysis probe with an AN 69 Filtral membrane (Hospal, Uden, the Netherlands) was stereotaxically implanted in the frontal cortex. The probe was positioned in the frontal cortex using the following coordinates: AP + 3.7, ML –2.6 and DV –5.5 mm from bregma (Paxinos and Watson, 1982). The active dialysis surface length of the probes was 2 mm (outer diameter 310 µM). The probes were secured in place with dental cement. Microdialysis experiments started 16-20 hours after surgery. Probes were perfused with Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM CaCl$_2$, 1.0 mM MgCl$_2$) at a constant flow of 1.5 µl/min with a high precision pump (Harvard Scientific, USA). Probes were connected with PE-tubing to swivels (Instech Laboratories, USA) to allow unrestrained movements of the rats. After the start of the dialysis perfusion animals were left undisturbed for two hours before collection of samples started. Samples were collected every 15

min in vials containing 7.5 µl acetic acid. Until HPLC analysis, samples were stored at –80°C. At the end of the experiment the rats were deeply anaesthetized with chloral hydrate. After decapitation brains were removed and fixed in 4% formaldehyde solution. To verify the position of the probe the brains were cut in 50 µm slices on a vibratome. In case of improper probe placement, data was excluded.

HPLC-ECD analysis
5-HT was analyzed by HPLC with electrochemical detection. Samples were injected onto an Inertsil ODS-3 column (3 µM, 2.1x100mm, Aurora Borealis, The Netherlands) using a Gilson pump and autosampler (Separations, The Netherlands). Separation was performed at 40ºC with an electrochemical detector (Intro, ANTEC Leyden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 µl/l triethylamine, 30 µl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 µl sample (signal to noise ratio 2).

Drugs and experimental design
The following drugs were used; CP93129 dihydrochloride (1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one, obtained from Tocris, UK), fluvoxamine maleate (donated by Solvay Pharmaceuticals, The Netherlands) and NAS-181 ((R)-(+)-2-[[[3-(Morpholinomethyl)-2H-chromen-8-yl]oxy]methyl] morpholine methane sulfonate, obtained from AstraZeneca, Sweden), GR127935 (2'-methyl-4'-(5-methyl[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]amide, obtained from Solvay Pharmaceuticals, The Netherlands) and SB224289 (1'-methyl-5-(2'-methyl-4'-(5-methyl-1,2,4-oxadiazole-3-yl) biphenyl-4-carbonyl)-2,3,6,7 tetrahydrospiro [furo[2,3-f]indole-3-spiro-4'-piperidine, obtained from GlaxoSmithkline, UK). All drugs were first dissolved in distilled water and further diluted in Ringer solution on the day of the experiment. CP93129 was applied for 45 minutes. The administration of the receptor antagonists started 15 min before CP93129 was co-perfused and lasted until the end of the sampling period. For the fluvoxamine groups, the SSRI was present in the Ringer solution from the start of dialysis.

Data analysis and statistics
Values for the first four consecutive samples were averaged to calculate the basal levels of extracellular 5-HT, uncorrected for probe recovery. In the figures, all 5-

HT values are expressed as percentages of basal levels $\pm$ SEM. Effects of 5-HT response to drug treatment were analyzed by multivariate analysis of variance (ANOVA) for repeated measurements with time as 'within' and treatment (or dose) as 'between' factors. When appropriate, data were broken down on treatment (or dose) and pairwise comparisons were made of the time profiles between the treatment groups (or dose effects). The significance level for all analyses was set at 5%. In the figures showing time course, the start of local infusion (time point zero) of drugs is corrected for the dead volume of the microdialysis system.

Results

Activation of 5-HT$_{1B}$ receptors reduces cortical 5-HT levels

Local administration of the 5-HT$_{1B}$ receptor agonist CP93129 into the frontal cortex dose-dependently reduced 5-HT levels (Fig.1A). A repeated measures ANOVA indicated an effect of time ($F_{(9,234)}$= 11.8, P < 0.001), a time x dose interaction ($F_{(3,234)}$= 9.6, P < 0.001) and a dose effect ($F_{(3,26)}$= 11.8, P < 0.001). The levels of 5-HT were significantly reduced as compared to vehicle at CP93129 concentrations of 0.1 μM ($F_{(1,16)}$= 23.7, P < 0.001) and 0.3 μM ($F_{(1,15)}$= 19.6, P < 0.001), but not at 0.03 μM ($F_{(1,15)}$= 3.1, P = 0.10). Cortical 5-HT levels were reduced by CP93129 to a minimum of 45 $\pm$ 4.3 % and 50 $\pm$ 7.9 % of basal levels, at concentrations of 0.1 and 0.3 μM, respectively.

In the presence of the SSRI fluvoxamine, 0.1 μM CP93129 reduced 5-HT levels at concentrations of 3 and 10 μM, but not at 30 μM fluvoxamine (Fig.1B). A repeated measures ANOVA indicated an effect of time ($F_{(9,351)}$= 5.6, P < 0.001), a time x dose interaction ($F_{(36,251)}$= 1.8, P < 0.01) and an effect of dose ($F_{(4,39)}$= 13.7, P < 0.001). Cortical 5-HT levels were reduced by CP93129 in the presence of fluvoxamine at concentrations of 3 μM and 10 μM to a minimum of 72 $\pm$ 6.3 % and 59 $\pm$ 3.8% of basal levels, respectively. The effect of CP93129 (0.1 μM) was significantly greater in the absence of fluvoxamine as compared to the effects of CP9319 in the presence of 3 μM or 10 μM fluvoxamine ($F_{(1,14)}$= 9.4, P < 0.01 and $F_{(1,10)}$= 6.0, P < 0.05, respectively). There was no difference between the effects of CP93129 at 3 or 10 μM fluvoxamine. At a higher concentration of fluvoxamine (30 μM), CP93129 did not affect cortical 5-HT levels.

Effects of 5-HT$_{1B}$ receptor antagonists on extracellular 5-HT

In the absence of fluvoxamine, local administration of all three 5-HT$_{1B}$ receptor antagonist alone reduced 5-HT levels (Fig.2A). When compared to vehicle, 5-HT
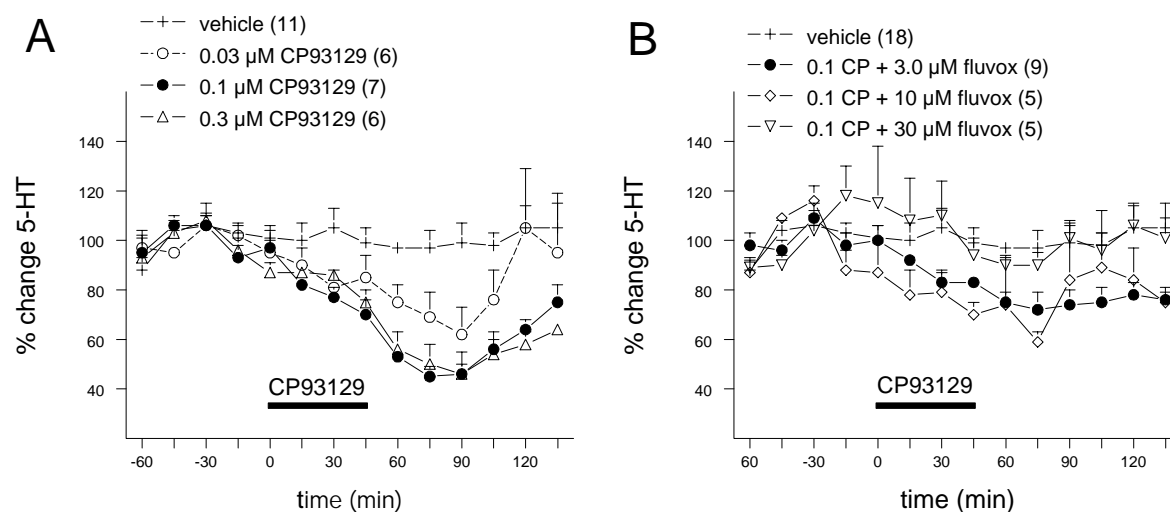
Fig.1. Time course of the effects of the 5-HT$_{1B}$ receptor agonist CP93129 on extracellular 5-HT in the frontal cortex. Data are expressed as mean percentage change from basal 5-HT levels ± SEM. CP93129 was administered locally into the frontal cortex for 45 minutes as indicated by the bar. CP93129 was administered at concentrations of 0.03, 0.1 and 0.3 µM (A) in the absence of a SSRI. CP93129 (0.1 µM) at concentrations of 3, 10 or 30 µM fluvoxamine (B). See Results for details on statistical analysis.

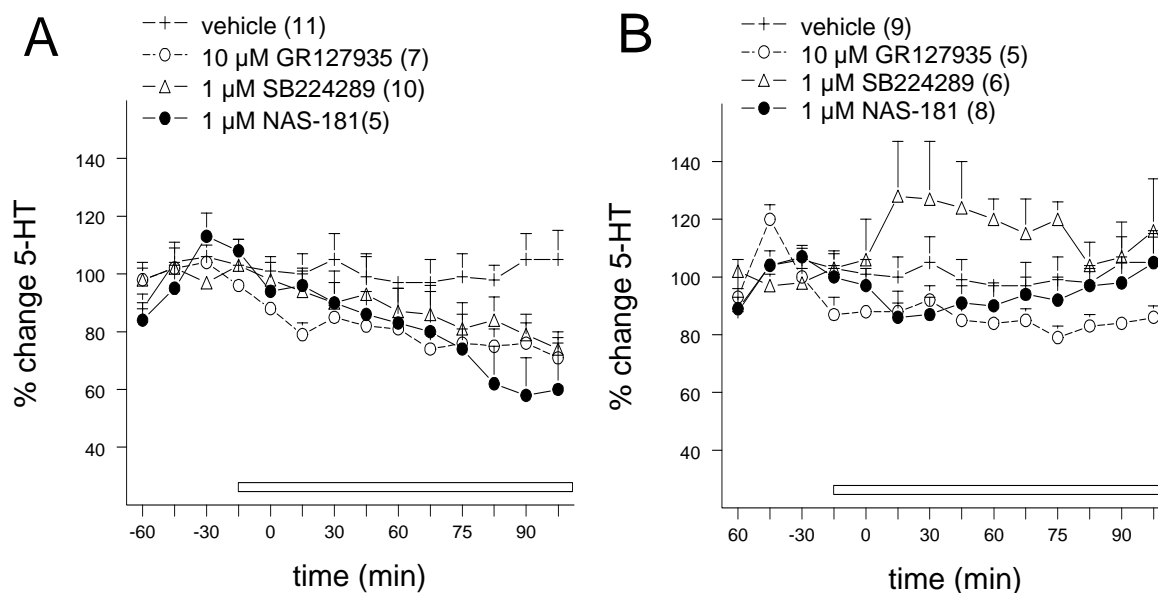**Fig.2.** Time course of the effects 5-HT$_{1B}$ receptor antagonists on extracellular 5-HT in the frontal cortex. Data are expressed as mean percentage change from basal 5-HT levels ± SEM. Locally administration of 10 µM GR127935, 1 µM SB224289 or 1 µM NAS-181, as indicated by the bar, either in the absence (A) or presence of 3 µM fluvoxamine (B). See Results for details on statistical analysis.

levels were significantly reduced by perfusion with 10 μM GR127935 (F(1,15)= 11.9, P < 0.05), 1 μM SB22428 (F(1,18)= 4.8, P < 0.05) and 1 μM NAS-181 (F(1,13)= 6.9 , P < 0.05).

In the presence of 3 μM fluvoxamine, the suppressant effects of NAS-181 and SB224289 were absent, although GR217935 slightly reduced 5-HT levels (Fig.2B). Administration of GR127935 alone in the presence of fluvoxamine slightly, but significantly reduced 5-HT levels as compared to vehicle (F(1,12)= 7.9, P < 0.05). Administration of SB224289 alone, slightly increased 5-HT levels, but this effect was not significantly different from vehicle (F(1,13)= 2.5, P = 0.14). Administration of NAS-181 alone did not affect 5-HT levels in the presence of fluvoxamine.

Effects of 5-HT$_{1B}$ receptor antagonists on the effect of CP93129

In the absence of fluvoxamine, the suppressant effect of CP93129 (0.1 μM) on 5-HT output was attenuated by co-perfusion with NAS-181 and GR127935, but not with SB224289 (Fig.3A,C,E). For the effects of GR127935, an effect of time (F(9,252)= 8.6, P < 0.001), a time x treatment interaction (F(27,252) 2.7, P < 0.001) and an effect of treatment (F(3,28)= 15.1, P < 0.001) was found. As shown in Fig.3A, co-perfusion with GR127935 attenuated the effect of CP93129 as indicated by a significant time x treatment interaction (F(9,108)= 7.3, P < 0.001). For the effects of SB224289, an effect of time (F(9,252)= 8.6, P < 0.001), a time x treatment interaction (F(27,252)= 2.7, P < 0.001) and an effect of treatment (F(3,28)= 15.1, P < 0.001) was found. As shown in Fig.3C, co-perfusion with SB224289 did not attenuate the effect of CP93129 (F(1,11)= 2.2, P = 0.16). For the effects of NAS-181, an effect of time (F(9,207)= 9.3, P < 0.001), a time x treatment interaction (F(27,207)= 3.0, P < 0.001) and an effect of treatment (F(3,23)= 11.5, P < 0.001) was found. As shown in Fig.3E, co-perfusion with NAS-181 significantly attenuated the effect of CP93129 (F(1,10)= 9.0, P < 0.05).

In the presence of 3 μM fluvoxamine, the suppressant effect of CP93129 on 5-HT levelswas attenuated by co-perfusion with NAS-181 and GR127935, but not with SB224289 (Fig.3B,D,F). For the effects of GR127935, an effect of a time x treatment interaction (F(27,207)= 3.1, P < 0.001) and of treatment (F(3,23)= 6.7, P < 0.05) was seen. As shown in Fig.3A, co-perfusion with 10 μM GR127935 blocked the effect of CP93129 as indicated by a significant time x treatment interaction (F(9,99)= 2.0, P < 0.001). For the effects of SB224289, an effect of time (F(9,225)= 2.2, P < 0.05), a time x treatment interaction (F(27,225)= 2.0, P < 0.01) and of treatment (F(3,25)= 10.3, P < 0.001) was found. Co-perfusion with 1 μM SB224289 in the presence of fluvoxamine did not attenuate the suppressant

effect of CP93129 on 5-HT levels (Fig.3D). For the effects of NAS-181, an effect of time $(F_{(9,252)}= 2.9, P < 0.01)$, a time x treatment interaction $(F_{(27,252)}= 2.8, P < 0.001)$ and an effect of treatment $(F_{(3,28)}= 4.4, P < 0.05)$ was found. As shown in Fig.3F, co-perfusion with 1 μM NAS-181 significantly blocked the effect of CP93129 $(F_{(1,13)}= 9.3, P < 0.01)$.

Discussion

The main finding of the present study is that NAS-181, a new selective 5-HT$_{1B}$ receptor antagonist, blocked the suppressant effect of CP93129 on cortical 5-HT levels. Stimulation of 5-HT$_{1B}$ receptors with CP93129 dose-dependently reduced 5-HT levels in the frontal cortex. The maximum suppressant effect of CP93129 (0.1 μM) on 5-HT levels was reduced in the presence of a SSRI in the perfusion fluid. Both in the presence and absence of fluvoxamine, the suppressant effect of CP93129 on 5-HT was attenuated by NAS-181 (1 μM) and GR127935 (10 μM), but not by SB224289 (1 μM). In the absence of fluvoxamine, all three antagonists showed agonistic properties and significantly reduced cortical 5-HT levels.

The effects of CP93129 confirm findings from previous microdialysis studies demonstrating that activation of 5-HT$_{1B}$ autoreceptors reduce 5-HT levels in rat frontal cortex (Hertel et al., 1999, Adell et al., 2001). The suppressant effect of CP93129 (0.1 μM) on 5-HT levels was reduced when fluvoxamine (3 or 10 μM) was added to the perfusion fluid. At higher concentrations of fluvoxamine (30 μM) the effect of CP3129 on 5-HT levels was absent. A possible explanation for this finding may be that the effect of CP93129 is limited due to competition at 5-HT$_{1B}$ autoreceptor sites by increasing endogenous 5-HT levels dose-dependently induced by fluvoxamine. Previous studies have shown that locally applied SSRIs

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Fig.3. (next page). Time course of the effects of 5-HT$_{1B}$ receptor antagonists on the suppressant effect of CP93129 on 5-HT levels. Data are expressed as mean percentage change from basal 5-HT levels ± SEM. The 5-HT$_{1B}$ receptor agonist CP93129 (0.1 μM) was locally administered into the frontal ortex for 45 minutes as indicated by the black bar either in the absence (left panel) or presence of 3 μM fluvoxamine (right panel). Administration of the 5-HT$_{1B}$ receptor antagonists 10 μM GR127935 (A-B), 1 μM SB224289 (C-D) and 1 μM NAS-181 (E-F), indicated by the white bars, started 15 minutes before the agonist was co-perfused and lasted until the end of the sampling period. See Results for details on statistical analysis.
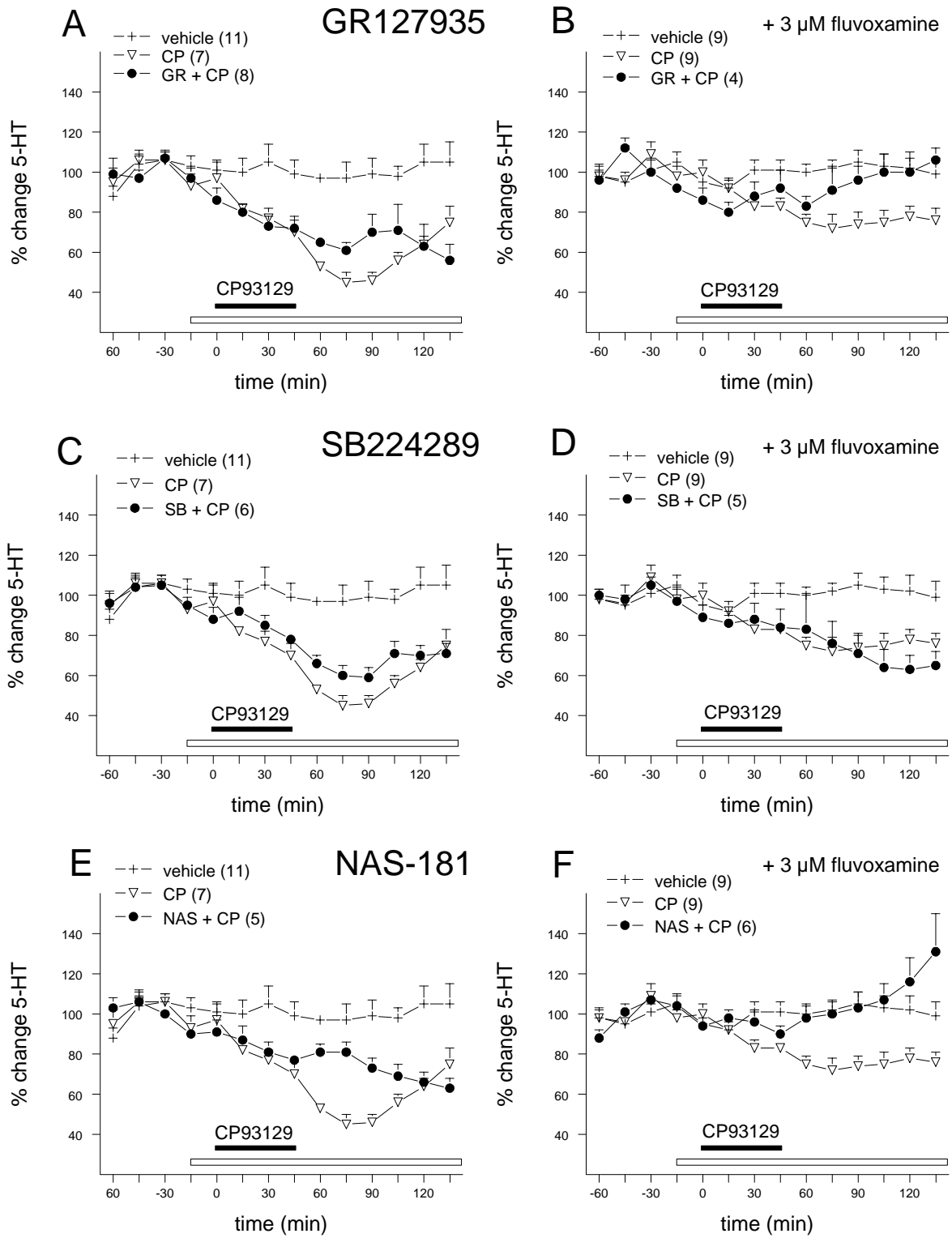
into the frontal cortex dose-dependently increase 5-HT levels up to 5-10 fold following high concentrations of the SSRI citalopram (Hervas et al., 2000, Tao et al., 2000), while in the present study 3 μM fluvoxamine increased cortical 5-HT levels about two-fold. At this concentration 5-HT reuptake sites are presumably not maximally blocked.

Surprisingly, in the absence of a SSRI in the perfusion fluid, 10 μM GR127935, 1 μM SB224289 and 1 μM NAS-181 reduced cortical 5-HT levels, suggesting partial agonistic properties of these receptor antagonists. GR127935 and SB224289 are compounds that have been used in many studies as $5-HT_{1B}$ receptor antagonists, although there is some evidence to suggest that these compounds are not silent receptor antagonists. GR127935 has been found to display partial and inverse agonistic properties at human $5-HT_{1B}$ receptors in vitro (Walsh et al., 1995, Pauwels, 1997). Moreover, GR127935 has been found to behave as a partial agonist at rodent $5-HT_{1B}$ receptors expressed in an opossum kidney cell line (Zgombick and Branchek, 1998). Also SB22429 has been found to display inverse agonist properties at human $5-HT_{1B}$ receptors, although there is thus far no evidence for such properties in vivo (Millan et al., 1999). Systemic administration of GR127935, but not of SB224289, has been reported to decrease 5-HT levels in the frontal cortex of guinea pigs, but this effect was attributed to the involvement of $5-HT_{1B/1D}$ receptors in the raphe nucleus (Roberts et al., 1999). Locally applied GR127935 (10 μM) into guinea pig cortex has been reported to slightly increase 5-HT levels, but this effect was not significantly different from vehicle (Hutson et al., 1995). In a previous study, NAS-181 administered systemically or locally (10 μM) had no effect on 5-HT levels in rat frontal cortex (Hjorth et al., 2000). In contrast, our findings show that 1 μM NAS-181 may act as a partial agonist at $5-HT_{1B}$ autoreceptors. Although we used a lower concentration of NAS-181, it is unlikely that this discrepancy can be attributed to the difference in concentration.

In the present study we chose a sub-maximal concentration of fluvoxamine to determine the potency of the three $5-HT_{1B}$ receptor antagonists to block the suppressant effect of CP93129 on cortical 5-HT levels. Co-perfusion of 3 μM fluvoxamine with 1 μM NAS-181 or 1 μM SB224289 had no effect on cortical 5-HT levels, whereas 10 μM GR127935 slightly reduced 5-HT levels. In previous studies, it was reported that 10 μM GR127935 did not affect 5-HT levels, whereas a tenfold higher concentration increased cortical 5-HT in the presence of the SSRI citalopram (1 μM) (Hervas et al., 1998, Hertel et al., 1999). In the presence of citalopram, SB224289 (1-100 μM) locally applied into the raphe nuclei had no effect on 5-HT levels (Adell et al., 2001). NAS-181 at a concentration of 10 μM was found to increase 5-HT levels in rat frontal cortex in the presence of

citalopram (Hjorth et al., 2000). In the present study, we choose a 10-fold lower concentration of NAS-181 because this concentration had no effect on 5-HT levels when co-perfused with fluvoxamine.

It has been suggested that the effect of an antagonist at the 5-HT$_{1B}$ autoreceptor is dependent on the endogenous tone at 5-HT$_{1B}$ binding sites, which is low at basal conditions (Hjorth, 1993). In the present study NAS-181 and GR127935 attenuated the effect of CP93129 both in the absence and presence of a sub-maximal concentration of locally applied fluvoxamine. The present finding that these receptor antagonists act as partial agonists when administered alone, but display antagonistic properties when the endogenous tone is enhanced by local fluvoxamine would fit with the notion that the effect of a 5-HT$_{1B}$ receptor antagonist depends the endogenous tone of this receptor. Also following stimulation of 5-HT$_{1B}$ receptors by an exogenous applied agonist, the endogenous tone at 5-HT$_{1B}$ sites will be raised. Therefore, in combination with a full agonist, a partial agonist may act as an antagonist. Indeed, co-perfusion of GR127935 and NAS-181 significantly attenuated the effect of the full 5-HT$_{1B}$ receptor agonist CP93129 in the absence of fluvoxamine. The effect of CP93129 was not attenuated by SB224289, possibly, the concentration used of this receptor antagonist was not sufficient to block 5-HT$_{1B}$ sites.

In conclusion, the antagonistic properties of NAS-181 in blocking the suppressant effect of locally applied CP93129 on 5-HT levels show that NAS-181 is a potent 5-HT$_{1B}$ receptor antagonist. Co-perfusion of NAS-181 or GR127935, but not of SB224289, attenuated the suppressant effect CP93129, both in the presence and absence of fluvoxamine. The reduced 5-HT levels following local administration of GR127935, SB224289 or NAS-181 suggests that these compounds may have partial agonistic properties.

CHAPTER 6

Role of extracellular serotonin levels in the effect of 5-HT$_{1B}$ receptor blockade

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submitted

Abstract

The release of serotonin (5-HT) at serotonergic nerve terminals is regulated by 5-HT$_{1B}$ autoreceptors. Several studies have reported that the effects of selective 5-HT reuptake inhibitors (SSRIs) on extracellular 5-HT are augmented by 5-HT$_{1B}$ receptor antagonists, whereas administration of these antagonists alone do not enhance 5-HT levels. It has been suggested that 5-HT$_{1B}$ receptors have low basal endogenous activity and therefore elevated endogenous 5-HT levels are needed to elicit an effect of 5-HT$_{1B}$ receptor antagonists. To test this hypothesis, different strategies were used to enhance 5-HT levels in rat frontal cortex to assess the effects of locally applied NAS-181, a new selective 5-HT$_{1B}$ receptor antagonist. Blockade of 5-HT$_{1B}$ receptors with NAS-181 dose-dependently augmented 5-HT levels when 5-HT levels were enhanced by a SSRI. No additional effect of NAS-181 on 5-HT output was found when 5-HT levels were enhanced by KCl depolarization induced release or by preventing degradation of 5-HT with the mono-oxidase inhibitor pargyline. In the presence of fluvoxamine, the increased 5-HT release evoked by KCl depolarization was augmented by NAS-181, supporting

the idea that blockade of 5-HT transporters is necessary to measure an effect of 5-HT$_{1B}$ receptor blockade. In conclusion, the results provide circumstantial evidence that the effect of a 5-HT$_{1B}$ receptor antagonist depends on extracellular 5-HT levels, but strongly suggest that additional 5-HT reuptake inhibition is required to detect any effect of 5-HT$_{1B}$ receptor antagonist on 5-HT levels by in vivo microdialysis.

Introduction

Selective 5-HT reuptake inhibitors (SSRIs) are widely used in the treatment of depression and anxiety disorders. However, several weeks of SSRI-treatment are needed before a therapeutic effect is achieved, suggesting that adaptive changes might be implicated. SSRIs inhibit the reuptake of 5-HT, thereby enhancing 5-HT levels. The release of 5-HT in the CNS is controlled by 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors. At serotonergic nerve terminals 5-HT release is regulated by 5-HT$_{1B}$ autoreceptors (Engel et al., 1986, Maura et al., 1986). In vivo microdialysis studies have shown that stimulation of 5-HT$_{1B}$ receptors inhibits the release of 5-HT in different brain areas (Hjorth and Tao, 1991, Bosker et al., 1995, Adell et al., 2001). Terminal 5-HT$_{1B}$ receptors have been found to desensitize after chronic administration of antidepressants in different brain areas, including the frontal cortex (Blier et al., 1984, Moret and Briley, 1990, O'Connor and Kruk, 1994, el Mansari et al., 1995, Newman et al., 2000). It has been suggested, therefore, that the therapeutic effect of SSRIs could be accelerated by concurrent administration of a 5-HT autoreceptor antagonist. There is some clinical evidence that pindolol, a ß-adrenoceptor and 5-HT$_{1A}$ receptor antagonist, may have a beneficial effect on SSRI-treatment (Artigas et al., 2001). In this view, also the 5-HT$_{1B}$ autoreceptor may be a target for augmentation strategies of SSRI-treatment. Whereas stimulation of 5-HT$_{1B}$ receptors results in reduced 5-HT levels, blockade of 5-HT$_{1B}$ receptors in general has no effect on 5-HT levels (Roberts et al., 1998, Hjorth et al., 2000, Gobert et al., 2000). The effect of systemic SSRIs on 5-HT levels are augmented by 5-HT$_{1B}$ receptor antagonists in the frontal cortex (Gobert et al., 1997 Dawson and Nguyen, 2000, Gobert et al., 2000) and other brain areas (Rollema et al., 1996, Cremers et al., 2000). Compounds that act both as a SSRI and 5-HT$_{1B}$ receptor antagonist have been found to potently enhance 5-HT levels (Matzen et al., 2000, Mitchell 2001). Furthermore, a limiting role of 5-HT$_{1B}$ autoreceptors in the effect of SSRIs on cortical 5-HT levels is supported by recent findings in mice lacking 5-HT$_{1B}$ receptors (Malagie et al., 2001, de Groote et al., 2002b). It has been suggested that 5-HT$_{1B}$ receptors have low basal endogenous

activity. This notion is based on findings that the effect of 5-HT$_{1B}$ receptor blockade was not measurable when a SSRI was omitted from the perfusion fluid (Hjorth, 1993), suggesting that stimulation of 5-HT$_{1B}$ receptors, i.e. through elevated 5-HT levels resulting from 5-HT reuptake inhibition, is required for 5-HT$_{1B}$ receptor antagonists to affect extracellular 5-HT levels.

In the present study, NAS-181, a new selective rodent 5-HT$_{1B}$ receptor antagonist was used to block 5-HT$_{1B}$ receptors (Berg et al., 1998, Stenfors et al., 2000). The aim of the present study was to investigate whether the effects of terminal 5-HT autoreceptor blockade depends on the endogenous tone at 5-HT$_{1B}$ autoreceptors. To test this hypothesis, different strategies were used to enhance 5-HT levels in rat frontal cortex to assess the effects of locally applied NAS-181. First, 5-HT levels were enhanced by the 5-HT reuptake inhibitor fluvoxamine administered locally by reversed microdialysis. Second, 5-HT levels were enhanced by local potassium chloride stimulation and third by local administration of pargyline, a mono-oxidase inhibitor through the microdialysis probe, to block 5-HT degradation.

Material and methods

Animals
Male Wistar rats (GDL, Utrecht, The Netherlands) weighing 250-300 gram were housed three per cage under standard conditions (22-24 ºC), food and water ad lib) and a 12-hour light/dark cycle (7 a.m. on, 7 p.m. off). The Ethical Committee for Animal Research of the University Medical Center Utrecht, The Netherlands, approved the study.

Microdialysis procedure
Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.). A self-constructed concentric microdialysis probe with an AN 69 Filtral membrane (Hospal, Uden, the Netherlands) was stereotaxically implanted in the frontal cortex. The probe was positioned in the frontal cortex using the following coordinates: AP + 3.7, ML –2.6 and DV –5.5 mm from bregma (Paxinos and Watson, 1982). The active dialysis surface length of the probes was 2 mm (outer diameter 310 µM). The probes were secured in place with dental cement. Microdialysis experiments started 16-20 hours after surgery, about two hours after lights were on. Probes were perfused with Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM CaCl$_2$, 1.0 mM MgCl$_2$) at a constant flow of 1.5 μl/min with a high precision pump (Harvard Scientific, USA). Probes were connected with PE-tubing to swivels (Instech Laboratories,

USA) to allow unrestrained movements of the rats. After the start of the dialysis perfusion, animals were left undisturbed for two hours before collection of samples started. Samples were collected every 15 minutes in vials containing 7.5 µl acetic acid. Until HPLC analysis, samples were stored at −80°C. At the end of the experiment the rats were deeply anaesthetized with chloral hydrate. After decapitation, brains were removed and fixed in 4% formaldehyde solution. To verify the position of the probe the brains were cut in 50 µm slices on a vibratome. In case of improper probe placement, data was excluded.

HPLC-ECD analysis

5-HT was analyzed by HPLC with electrochemical detection. Samples were injected onto an Inertsil ODS-3 column (3 µM, 2.1x100mm, Aurora Borealis,The Netherlands) using a Gilson pump and autosampler (Separations, The Netherlands). Separation was performed at 40ºC with the electrochemical detector (Intro, ANTEC Leyden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analyzed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 µl/l triethylamine, 30 µl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 µl sample (signal to noise ratio 2).

Drugs

The following drugs were used; fluvoxamine maleate (donated by Solvay Pharmaceuticals, The Netherlands), NAS-181 ((R)-(+)-2-[[[3-(Morpholinomethyl)-2H-chromen-8-yl]oxy]methyl] morpholine methane sulfonate) (donated by AstraZeneca, Sweden) and pargyline (Sigma, USA). All drugs were applied locally through the probe by reversed microdialysis. Drugs were first dissolved in distilled water and further diluted in Ringer solution on the day of the experiment. For the fluvoxamine groups, the SSRI was present in the Ringer solution from the start of dialysis. All other chemicals and reagents were of the purest commercially available.

Data analysis and statistics

Values for the first four consecutive samples were averaged to calculate the basal levels of extracellular 5-HT, uncorrected for probe recovery. In the figures, all 5-HT values are expressed as percentages of basal levels ± SEM. Effects of 5-HT response to drug treatment were analyzed by multivariate analysis of variance (ANOVA) for repeated measurements with time as 'within' and treatment (or dose) as 'between' factors. When appropriate, data were broken down on treatment (or

dose) and pairwise comparisons were made of the time profiles between the treatment groups (or dose effects). The significance level for all analyses was set at 5%. In the figures showing time course, the start of local infusion (time point zero) of drugs is corrected for the dead volume of the microdialysis system.

Results

Effects of NAS-181 in the presence of a SSRI
As shown in Fig.1, cortical 5-HT levels were enhanced by NAS-181 in the presence of the SSRI fluvoxamine. The effects of two concentrations NAS-181 were assessed in the absence and presence of fluvoxamine at two concentrations.

In the absence of fluvoxamine, an effect of time ($F_{(9,153)}$= 3.3, $p < 0.001$) and a time x dose interaction ($F_{(18,171)}$= 4.2, $p = 0.01$), but no dose effect ($F_{(2,17)}$= 2.0, $p = 0.078$) was found. Cortical 5-HT levels were reduced by 1 μM NAS-181 as compared to baseline ($F_{(1,14)}$= 5.4, $p < 0.05$). A tenfold higher concentration of NAS-181 did not affect 5-HT levels.

In the presence of 3 μM fluvoxamine, an effect of time ($F_{(9,207)}$= 2.3, $p < 0.001$) and a dose effect ($F_{(1,23)}$= 19.2, $p < 0.001$) was seen. Cortical 5-HT levels were not augmented by 1 μM NAS-181, whereas 10 μM NAS-181 increased 5-HT levels of baseline ($F_{(1,16)}$= 24.9, $p < 0.001$). One hour after the co-perfusion with NAS-181 started the mean maximum increase of 5-HT amounted to 139 ± 12 %.

In the presence of 10 μM fluvoxamine, a repeated measures ANOVA indicated an effect of time ($F_{(9,171)}$= 7.1, $p < 0.001$), a time x dose interaction ($F_{(18,171)}$= 4.2, $p <0.001$) and a dose effect ($F_{(2,19)}$= 9.6, $p < 0.001$). Cortical 5-HT levels were not augmented by 1 μM NAS-181, whereas 10 μM NAS-181 increased 5-HT levels from baseline ($F_{(1,14)}$= 14.0, $p < 0.01$). The mean maximum increase of 5-HT amounted to 184 ± 34 % of baseline levels after one hour of co-perfusion with NAS-181. No statistical difference was found between the effect of NAS-181 at 3 or 10 μM fluvoxamine ($F_{(1,13)}$= 0.99, $p = 0.34$).

Effects of NAS-181 in the absence of a SSRI
Fig.2. shows the effects of NAS-181 using two other strategies to enhance 5-HT levels without blocking 5-HT reuptake. Cortical 5-HT levels were increased by stimulation of 5-HT release with potassium chloride (KCl) (Fig.2A). A repeated measures ANOVA indicated an effect of time ($F_{(9,189)}$= 17.5, $p < 0.001$), a time x treatment interaction ($F_{(27,189)}$= 7.3, $p <0.001$) and a treatment effect ($F_{(3,21)}$=
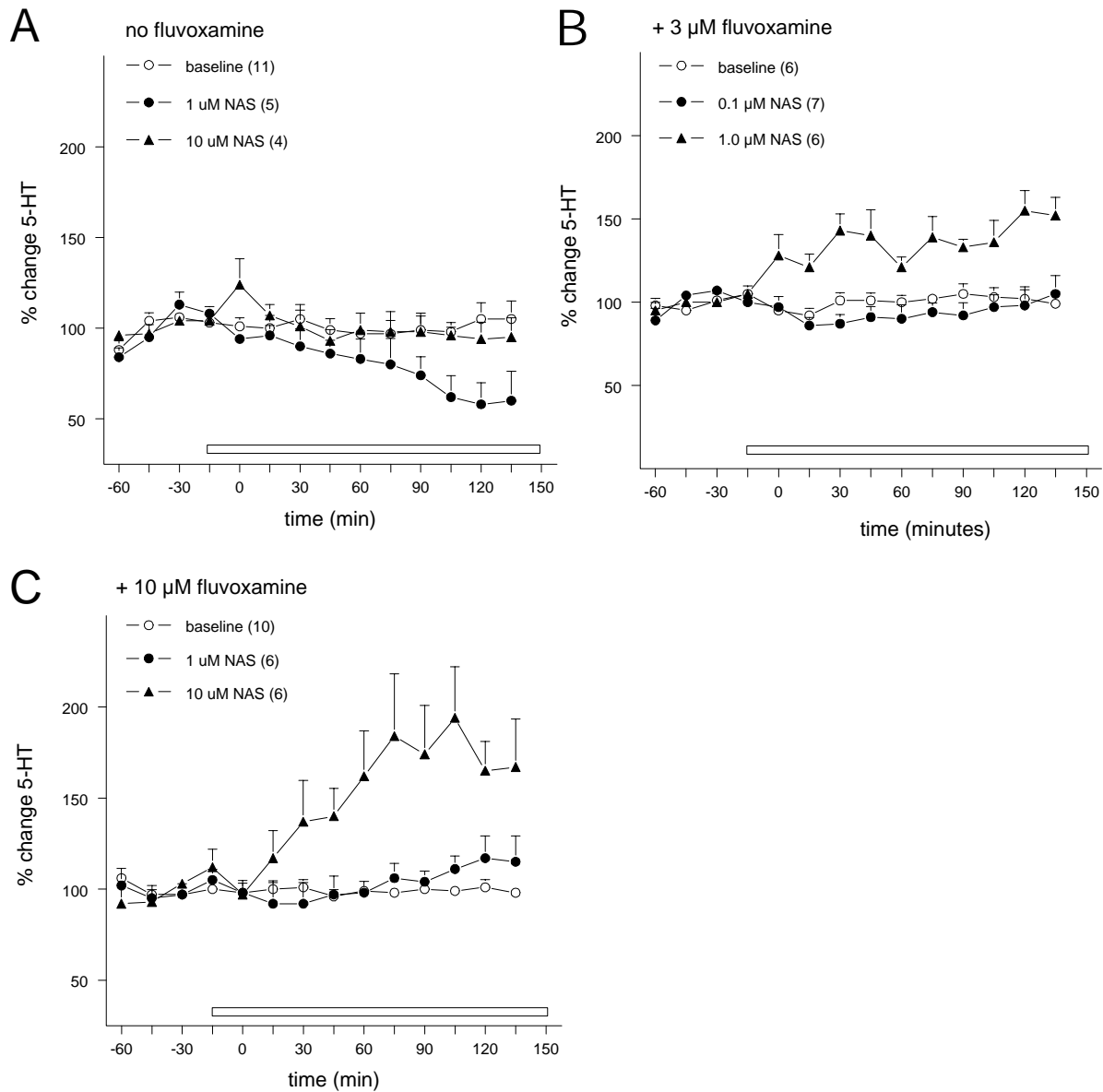
Fig.1. Effects extracellular 5-HT of the 5-HT$_{1B}$ receptor antagonist NAS-181 in the absence and presence of a SSRI. Data are expressed as mean change in 5-HT levels from basal ± SEM. Perfusion of NAS-181 (1 μM) started at time point zero indicated by the open bar, either in the absence of fluvoxamine (A), at 3 μM (B) or at 10 μM fluvoxamine (C). Fluvoxamine was present in the perfusion fluid from the start of the dialysis. See text Results for details on statistical analysis.

4.4, p < 0.05). Following 15 minutes of 120 mM KCl, cortical 5-HT levels were increased to a mean maximum increase to 334 ± 103 % from baseline (F(1,14)= 8.3, p < 0.05). Co-perfusion with 10 μM NAS-181 did not affect the 5-HT release evoked by KCl depolarization (F(1,8)= 0.5, p = 0.5). Local administration of pargyline, a mono-oxidase inhibitor, increased 5-HT levels significantly (Fig.2B). Statistical analysis revealed an effect of time (F(9,198)= 27.3, p < 0.001), a time x treatment interaction (F(27,198)= 10.9, p <0.001) and a treatment effect (F(3,22)= 11.2, p < 0.001).

Local perfusion of 500 μM pargyline increased cortical extracellular 5-HT levels to 237 ± 31 % of baseline following 2 hours of perfusion (F (1,14)= 18.1, p < 0.001). Co-perfusion with 10 μM NAS-181 had no additional effect on the pargyline-induced 5-HT increase (F(1,10)= 0.09, p = 0.77).
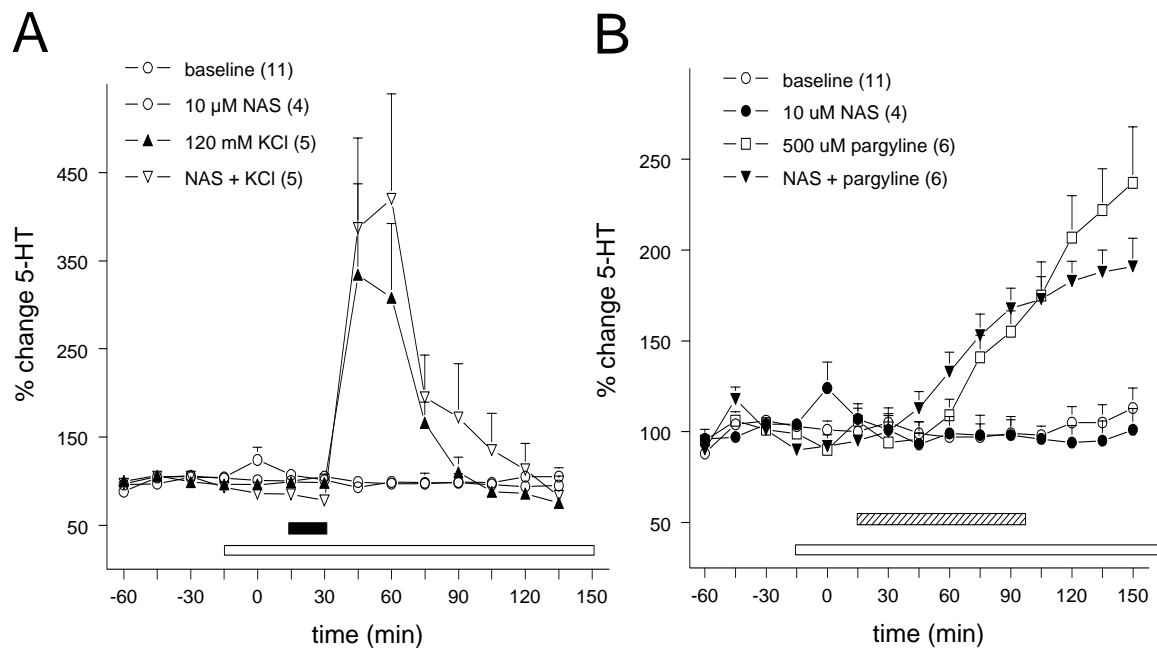
Fig.2. Effects of NAS-181 with enhanced 5-HT levels in the absence of a SSRI. Data are expressed as mean change in 5-HT levels from basal ± SEM. Local administration of 10 μM NAS-181 started at timepoint zero as indicated by the white bar. Stimulation of 5-HT release by 120 mM KCl (black bar) for 15 minutes (A). Local administration of 500 μM pargyline (hatched bar) started 30 minutes after perfusion with NAS-181 (B). See text Results for details on statistical analysis.
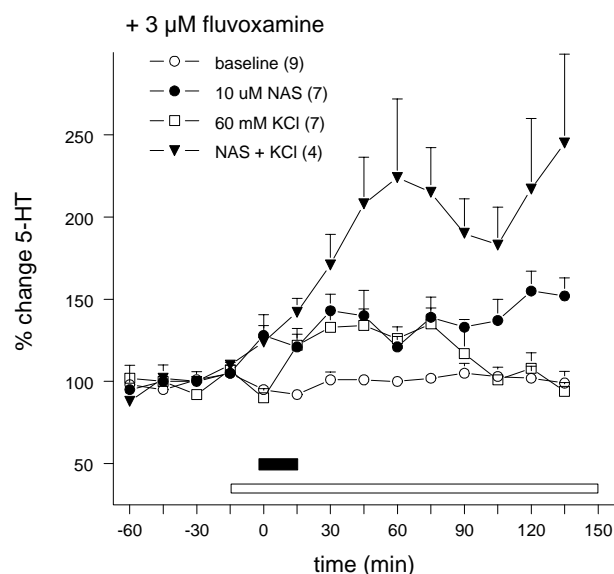
Fig.3. Effects of NAS-181 on KCl depolarization induced 5-HT release in the presence of a SSRI. Data are expressed as mean change in 5-HT levels from basal ± SEM. Stimulation of 5-HT release by 60 mM KCl for 15 minutes (black bar) in the presence of 3 µM fluvoxamine. Administration or co-administration of 10 µM NAS-181 (white bar) started 15 minutes before KCl stimulation. See text Results for details on statistical analysis.

Effects of NAS-181 on KCl-evoked 5-HT release in the presence of fluvoxamine

Fig.3 shows the effects of NAS-181 on KCl depolarization induced 5-HT release in the presence of 3 µM fluvoxamine. A repeated measures ANOVA indicated an effect of time ($F_{(9,225)}$= 7.2, $p < 0.001$), a time x treatment interaction ($F_{(27,225)}$= 3.4, $p <0.001$) and a treatment effect ($F_{(3,25)}$= 16.9, $p < 0.001$). Stimulation with 60 mM KCl in the presence of 3 µM fluvoxamine increased 5-HT to 134 ± 10 % of baseline ($F_{(1,14)}$= 5.0, $p < 0.05$). Co-perfusion of 10 µM NAS-181 with 3 µM fluvoxamine enhanced 5-HT levels to the same extent. Co-administration with 10 µM NAS-181 augmented the effect of KCl-evoked 5-HT release to a mean maximum of 208 ± 28 % of baseline ($F_{(1,9)}$= 15.6, $p < 0.01$).

Discussion

The main finding of the present study is that blockade of 5-HT$_{1B}$ receptors with NAS-181 augmented dose-dependently 5-HT levels in the presence of a SSRI. Local KCl depolarization or pargyline administration enhanced cortical 5-HT levels, but no effect of NAS-181 was found. Whereas, in the presence of fluvoxamine the effect of KCl depolarization induced release of 5-HT was augmented by NAS-181.

It has been suggested that the basal endogenous tone at 5-HT$_{1B}$ receptors is low (Hjorth, 1993). This may explain why extracellular 5-HT levels are not affected by local administration of 5-HT$_{1B}$ receptor antagonists alone, whereas at elevated extracellular 5-HT levels induced by co-perfusion with a SSRI, blockade of 5-HT$_{1B}$ receptors augments extracellular 5-HT. The present study confirms that 5-HT$_{1B}$ receptor antagonists enhance 5-HT levels, as NAS-181 at a concentration of 10 μM augmented 5-HT levels at two different concentrations of fluvoxamine. These findings are in line with previous studies in rat frontal cortex using locally applied 5-HT$_{1B}$ receptor antagonist in the presence of a SSRI in the perfusion fluid (Hertel et al., 1999, Hjorth et al., 2000). A lower concentration of NAS-181 (1μM) did not affect extracellular 5-HT levels in the presence of fluvoxamine, and reduced extracellular 5-HT levels in the absence of fluvoxamine, suggesting partial agonistic properties of this compound. In combination with a full agonist, a combined partial agonist may act as an antagonist. Indeed, co-perfusion of 1 μM NAS-181 attenuated the effect of the full 5-HT$_{1B}$ receptor agonist CP93129 (chapter 5). The discrepancy in the present findings that NAS-181 displayed partial agonistic properties at a concentration of 1 μM, but not at the 10-fold higher concentration is unclear.

To study whether the role of SSRIs in the effect of 5-HT$_{1B}$ receptor antagonists was due to the extracellular 5-HT concentrations or related to the properties of SSRIs, the effects of NAS-181 were studied using two other strategies aimed to enhance extracellular 5-HT levels. Local administration of pargyline gradually increased 5-HT levels, but co-perfusion with NAS-181 did not augment the effect of pargyline. Stimulation with 120 mM KCl evoked a fast increase in 5-HT levels, but co-perfusion with NAS-181 had no additional effect. Both pargyline and KCl depolarization are non-selective in that they increase extracellular levels of other neurotransmitters as well. Moreover, no steady-state 5-HT levels were obtained with either approach. The effects on extracellular 5-HT following KCl were only transient and 5-HT levels following pargyline were still rising 2 h after the administration started, which may have obliterated any effect of NAS-181 on 5-HT release. A study in mice, however, showed that the 5-HT$_{1B}$ receptor agonist CP93129 was able to attenuate the KCl-evoked release of 5-HT in the frontal cortex of wildtype mice, and this effect was absent in 5-HT$_{1B}$ receptor knockout mice (Trillat et al., 1997). Interestingly, in the presence of fluvoxamine, the evoked 5-HT increase induced by 60 mM KCl could be augmented by NAS-181. This may suggest that the effect of NAS-181 depends on the presence of a SSRI. There is circumstantial evidence to suggest that a functional interaction exist between the 5-HT$_{1B}$ autoreceptor and the 5-HT transporter. Daws et al (1999) have hypothesized that the activation of 5-HT$_{1B}$ receptors may enhance the activity of 5-

HT transporters resulting in a greater clearance of 5-HT from the synaptic cleft. This was supported by findings showing that the clearance of 5-HT in the hippocampus, as measured with in vivo voltammetry, was inhibited by different 5-HT_{1B} receptor antagonists (Daws et al., 1999). Moreover, the inhibitory effect on 5-HT clearance of a 5-HT_{1B} receptor antagonist co-administered with a SSRI appeared to be additive (Daws et al., 2000). Nevertheless, there may be a important difference in methodology between in vivo microdialysis and voltammetry. With the latter technique, 5-HT_{1B} receptors antagonists are found to inhibit the clearance of 5-HT, which is expected to result in enhanced extracellular 5-HT levels, but in most microdialysis studies no effects on extracellular 5-HT levels are found. An explanation for these discrepant findings for 5-HT_{1B} receptor antagonists on extracellular 5-HT levels in the absence of a SSRI might be that by 5-HT levels, as measured by *in vivo* microdialysis, do not detect actual release of 5-HT at the site of release. When the release of 5-HT is increased by blockade of inhibitory 5-HT_{1B} receptors, this increase in 5-HT may be not detected at the site of the probe as it is rapidly removed from the extracellular space before it can reach the microdialysis probe. The re-uptake of 5-HT is prevented by a SSRI, resulting in measurable increases in 5-HT levels in the sampled dialysates.

In conclusion, blockade of 5-HT_{1B} receptors with the selective 5-HT_{1B} receptor antagonist NAS-181 dose-dependently augmented 5-HT levels when 5-HT levels were enhanced by a SSRI. No additional effect of NAS-181 on 5-HT output was found when 5-HT levels were enhanced by KCl depolarization induced release or by blockade of 5-HT degradation with the mono-oxidase inhibitor pargyline in the absence of a SSRI. The increase in 5-HT release evoked by KCl depolarization was augmented by NAS-181 in the presence of fluvoxamine, supporting the idea that the effect of 5-HT_{1B} receptors blockade is dependent on the extracellular 5-HT levels. The results provide circumstantial evidence that the effect of a 5-HT_{1B} receptor antagonist depends on extracellular 5-HT levels, but also strongly suggest that 5-HT reuptake inhibition is necessary to measure the effects by means of in vivo microdialysis.

CHAPTER 7

General discussion

Functional role of 5-HT$_{1B}$ receptors
    5-HT$_{1B}$ autoreceptors
    5-HT$_{1B}$ heteroreceptors

Knockout mouse versus pharmacological tools
    5-HT$_{1B}$ receptor ligands
    Compensatory changes in 5-HT$_{1B}$ knockout mice
    The 5-HT$_{1B}$ knockout mouse as model to study 5-HT$_{1B}$ receptors

Methodological considerations on microdialysis
    Extracellular 5-HT
    Regulation of 5-HT in mouse and rat

Future directions

The aim of this thesis was to explore the role of 5-HT$_{1B}$ receptors in 5-HT function, which was studied by in vivo microdialysis in mice and rats. The first part of the thesis describes the role of 5-HT$_{1B}$ receptors on extracellular 5-HT levels by comparing the effects of SSRIs between wildtype and 5-HT$_{1B}$ receptor knockout mice in different brain areas. Furthermore a study was undertaken to assess the role of 5-HT$_{1B}$ heteroreceptors on DA outflow in the striatum. In the second part of this thesis, the effect of a new selective receptor antagonist on extracellular 5-HT was evaluated in the frontal cortex of rats. This chapter discusses the functional role of 5-HT$_{1B}$ auto- and heteroreceptors. The chapter is concluded with comments on the use of knockout mice and pharmacological tools to study 5-HT$_{1B}$ receptor function in rodents and suggestions for future research directions are given.

Functional role of 5-HT$_{1B}$ receptors

5-HT$_{1B}$ autoreceptors

To validate the microdialysis procedure in mice, we first aimed to replicate previously reported findings. Therefore, a SSRI was administered systemically and extracellular 5-HT levels were assessed in the hippocampus of wildtype and 5-HT$_{1B}$ knockout mice (chapter 2). The 5-HT response to the SSRI paroxetine was augmented in 5-HT$_{1B}$ KO mice and this finding is in line with previously reported results (Malagie et al., 2001). Following systemic SSRI-administration, also 5-HT$_{1A}$, 5-HT$_{1D}$, and 5-HT$_{1B}$ autoreceptors localised in the raphe nuclei may affect the output of 5-HT in serotonergic projection areas. Therefore, the role of 5-HT$_{1B}$ autoreceptors in the effects of SSRIs on extracellular 5-HT was assessed by local administration of the SSRI fluvoxamine into different brain areas. In 5-HT$_{1B}$ knockout mice relative to wildtypes, 5-HT levels were increased following locally applied fluvoxamine into the hippocampus and the medial prefrontal cortex, but not in the striatum (chapters 2,3,4). In the frontal cortex, the 5-HT$_{1B}$ receptor antagonist NAS-181 augmented the effect of locally applied fluvoxamine in mouse (chapter 3) and rat (chapter 6). The effects of a locally applied SSRI indicate that terminal 5-HT$_{1B}$ receptors are functionally involved in the regulation of 5-HT when 5-HT levels are enhanced. In the striatum of wildtype mice, stimulation of 5-HT$_{1B}$ receptors reduced extracellular 5-HT, indicating that 5-HT$_{1B}$ receptors are functionally present in this brain structure. In line with our findings, a previous study using systemic injection of a SSRI, found no differences between the two genotypes in the striatum (Knobelman et al., 2001a). Interestingly, after chronic SSRI-treatment increased 5-HT$_{1B}$ mRNA levels were observed in the striatum, suggesting that adaptive changes in 5-HT$_{1B}$ heteroreceptors in this brain area may be implicated in the chronic effects of antidepressants (Le Poul et al., 2000). Taken together, the present findings in rat, wildtype and 5-HT$_{1B}$ knockout mice, clearly indicate that 5-HT$_{1B}$ autoreceptors at serotonergic nerve terminals limit the acute effects of SSRIs in the hippocampus and prefrontal cortex, but not in the striatum.

5-HT$_{1B}$ heteroreceptors

In contrast to the well-established inhibitory role of 5-HT$_{1B}$ autoreceptors in 5-HT release, relatively little is known about the functional role of 5-HT$_{1B}$ heteroreceptors. The previously reported role of striatal 5-HT$_{1B}$ heteroreceptors in DA outflow in the striatum could not be confirmed in our study (chapter 4), since the effect of the putative selective 5-HT$_{1B}$ receptor agonist CP93129 on DA outflow was similar in the two genotypes, demonstrating that 5-HT$_{1B}$ receptors

were not involved. This finding does not exclude an effect of 5-HT$_{1B}$ heteroreceptors on DA outflow in the striatum. Enriched 5-HT$_{1B}$ binding sites are found in the basal ganglia and particularly in the substantia nigra (SN). 5-HT$_{1B}$ receptors are present as presynaptic heteroreceptors on GABA-ergic neurons projecting from the striatum to the SN (Boschert et al., 1994, Stanford and Lacey, 1996). Ultrastructural analysis of the SN pars reticulata (SNr) demonstrated that almost all 5-HT varicosities are of the junctional type, while in other brain areas predominantly non-junctional varicosities are found (Moukhles et al., 1997). The precisely organised 5-HT input to the SNr suggests that 5-HT can finetune the motor output of the basal ganglia by acting on the GABA projections either directly or through modulation of local release of dopamine by dopaminergic dendrites. Moreover, a role of 5-HT$_{1B}$ receptors in locomotor behaviour is supported by findings in 5-HT$_{1B}$ knockout mice showing that the hyperlocomotor effect of the 5-HT $_{1A/1B}$ agonist RU24969 is absent (Saudou et al., 1994). Furthermore, local administration of a 5-HT $_{1B/1D}$ receptor agonist into the SN induced contralateral turning behaviour in guinea pig and rat (see Barnes and Sharp, 1999). Interestingly, reduced 5-HT$_{1B}$ bindings sites were observed in post-mortem brains of patients with Huntington Disease, a severe movement disorder characterised by degeneration of striatal output neurons (Castro et al., 1998).

Furthermore, 5-HT$_{1B}$ receptors are also present on neurons projecting from the ventral tegmental area to the nucleus accumbens, a dopaminergic pathway implicated in the action of cocaine abuse (Cameron and Williams, 1994). In the nucleus accumbens, but not in the striatum, increased basal DA levels were found, suggesting a role of 5-HT$_{1B}$ receptors in the mesoaccumbal pathway (Shippenberg et al., 2000). A role of 5-HT$_{1B}$ receptors in this pathway is supported by findings in 5-HT$_{1B}$ receptor knockout mice showing increased propensity to self-administer this drug (Rocha et al., 1998) and an enhanced locomotor response to cocaine and other dopaminergic drugs (Rocha et al., 1998, Scearce-Levie et al., 1999).

The putative selective 5-HT$_{1B}$ receptor CP93129 has been reported to increase DA outflow in the PFC (Iyer and Bradberry, 1996), nucleus accumbens (Yan and Yan, 2001) and SN (Thorre et al., 1998). In keeping with the effects of CP93129 on striatal DA outflow of 5-HT$_{1B}$ receptor knockout mice, it would be of interest to study the effects of CP93129 in these brain areas of 5-HT$_{1B}$ receptor knockout mice. In our study in the mPFC (chapter 3), the initial aim was also to study the role of 5-HT$_{1B}$ receptors in DA outflow; however, extracellular DA levels are low in this brain area and could not be reliably analyzed in all mice. Extracellular DA levels in the PFC are estimated to be about 40 times lower than in the striatum of mice (Ihalainen et al., 1999). In the literature, it has been suggested that 5-HT$_{1B}$

receptors modulate hippocampal acetylcholine release in hippocampal synaptosomes (Maura et al., 1986, Sarhan and Fillion, 1999, Bolanos-Jimenez et al., 1994) and in the frontal cortex as measured by microdialysis (Consolo et al., 1996). Most of these studies also used CP93129 to stimulate 5-HT_{1B} receptors, therefore it would be also of interest to confirm in 5-HT_{1B} receptor knockout mice whether CP93129-induced acetylcholine release in forebrain regions is mediated by 5-HT_{1B} receptors.

Knockout mouse model versus pharmacological tools

5-HT_{1B} receptor ligands
In spite of the development of receptor subtype selective compounds, the main advantage of a knockout mouse model over pharmacological approaches is the selective deletion of one specific receptor. The putative selective 5-HT_{1B} receptor agonist CP93129 induced DA outflow to the same extent in knockout and wildtype mice (chapter 4). Thus, the 5-HT_{1B} receptor knockout mice revealed that CP93129, at a concentration of 50 μM, has affinity for other receptors. A previous study in synaptosomes suggested a differential sensitivity of 5-HT_{1B} auto- and heteroreceptors for CP93129, and using this technique, no effect of CP93129 on evoked striatal DA outflow was found in 5-HT_{1B} receptor knockout (Sarhan and Fillion, 1999, Sarhan et al., 2000). Methodological differences undoubtedly exist between in vitro synaptosomes and in vivo microdialysis, but our results obtained in 5-HT_{1B} receptor knockout mice clearly indicated that the effects of CP93129 on DA outflow are not mediated by 5-HT_{1B} receptors.

The new 5-HT_{1B} receptor antagonist NAS-181 potently augmented the effect of fluvoxamine on cortical 5-HT in wildtype, but not in 5-HT_{1B} receptor knockout mice, indicating that NAS-181 is a selective 5-HT_{1B} receptor antagonist in mice (chapter 3). Furthermore, the potency of NAS-181 was shown as it attenuated the decrease in extracellular 5-HT induced by CP93129 (chapter 5) and augmented the effect of fluvoxamine in the frontal cortex of rats (chapter 6). Rodent and human 5-HT_{1B} receptors are pharmacologically different (see chapter 1) and therefore are rodents not the first choice for testing 5-HT_{1B} receptors aimed for therapeutic applications in humans. Nevertheless, for fundamental research on 5-HT_{1B} receptors a selective rodent 5-HT_{1B} receptor antagonist is required as a pharmacological tool to discriminate between other 5-HT receptor subtypes and to gain more insight in the functional role of 5-HT_{1B} receptors in brain function. It is thought that 5-HT_{1B} autoreceptor blockade depends on the prevailing levels of 5-HT, since administration of a 5-HT_{1B} receptor antagonist alone does not increase

5-HT levels. This hypothesis was tested in chapter 6 and the results show that there was no additional effect of the 5-HT$_{1B}$ receptor blockade when 5-HT levels were enhanced, while only in the presence of a SSRI cortical 5-HT levels were augmented by NAS-181. The need of a SSRI to elicit an effect of 5-HT$_{1B}$ autoreceptor blockade may be a limitation of the microdialysis technique (see below). Nevertheless, a low basal endogenous tone at 5-HT$_{1B}$ sites would fit with the finding that basal 5-HT levels are normal in 5-HT$_{1B}$ receptor knockout mice.

Compensatory changes in 5-HT$_{1B}$ receptor knockout mice

A disadvantage of constitutive knockout mice is the possibility of compensatory changes that may have taken place during neurodevelopment. 5-HT$_{1A}$ and 5HT$_{1B}$ receptors are functionally mature during the early period of neurodevelopment (Hery et al., 1999). It is well known that serotonin is important during neurodevelopment, and particularly 5-HT$_{1A}$ receptors are thought to be involved during development as a neurotrophic factor (Jacobs and Azmitia, 1992, Azmitia, 1999). Adaptive changes to compensate for the lack of 5-HT autoregulation at nerve terminals may occur at the level of 5-HT release, firing rate, synthesis, re-uptake, 5-HT$_{1A}$ autoreceptors, 5-HT$_{1D}$ or other (postsynaptic) 5-HT receptors. Microdialysis studies have reported normal basal 5-HT levels in 5-HT$_{1B}$ receptor knockout mice in the hippocampus, frontal cortex and striatum (this thesis, Trillat et al., 1997, Knobelman et al., 2001a). This may indicate that adaptive changes have taken place, or that 5-HT$_{1B}$ receptors do not control 5-HT release under basal conditions. The dorsal raphe nucleus in 5-HT$_{1B}$ receptor knockout mice displays normal activity and firing properties with an equal response to somatodendritic 5-HT$_{1A}$ autoreceptor activation as wildtype mice, but was less sensitive to citalopram (Evrard et al., 1999). This suggests higher densities of 5-HT reuptake sites in 5-HT$_{1B}$ knockout mice that was supported by increased autoradiographic citalopram labelling in the DRN of these knockouts (Evrard et al., 1999), although this finding was not confirmed by others (Ase et al., 2001). No differences between 5-HT$_{1A}$ receptor binding sites were found in brains of wildtype and 5-HT$_{1B}$ receptor knockout mice (Malleret et al., 1999). Following stimulation of 5-HT$_{1A}$ receptors, no differences were found in corticosterone response or stress-induced hyperthermia between the two genotypes (Bouwknecht et al., 2002, Bouwknecht et al., 2001c). Interestingly, a decrease in G-protein coupling of 5-HT$_{1A}$ receptors was found in 5-HT$_{1B}$ knockout mice that may indicate a decrease in efficiency of 5-HT$_{1A}$ receptors (Ase et al., 2002). Moreover, the 5-HT$_{1B}$ receptor knockout mice, however, showed a blunted response to the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in the hippocampus, but not in the striatum (Knobelman et al., 2001b). The authors proposed a desensitisation of 5-HT$_{1A}$ autoreceptors in the

MRN of the knockouts. Interestingly, following locally applied fluvoxamine into the hippocampus, a differential dose response was found between the two genotypes, suggesting adaptive changes in 5-HT reuptake capacity in 5-HT$_{1B}$ receptor knockout mice (chapter 2). Indeed, increased 5-HT transporter densities in the hippocampus of 5-HT$_{1B}$ receptor knockout mice are reported (Ase et al., 2001). Taken together, the adaptive changes in the regulation of 5-HT release in the hippocampus of 5-HT$_{1B}$ receptor knockout mice, support the notion that 5-HT$_{1B}$ autoreceptors play a prominent role in 5-HT release in the hippocampus, a brain structure mainly innervated by the MRN. Thus, region specific compensatory changes are present in the 5-HT$_{1B}$ receptor knockout mice.

There is substantial evidence that 5-HT$_{1D}$ receptors act as 5-HT autoreceptors in the DRN (see for a review Stamford et al., 2000). Relatively little is known about the functional role of 5-HT$_{1D}$ receptors localised outside the raphe, but this receptor has a distribution similar to 5-HT$_{1B}$ receptors in the forebrain (Bruinvels et al., 1993). In 5-HT$_{1B}$ KO mice, 5-HT$_{1D}$ binding sites are unchanged (Malleret et al., 1999), thus there is as yet no evidence that 5-HT$_{1D}$ receptors compensate for the loss of terminal 5-HT$_{1B}$ autoreceptors.

The 5-HT$_{1B}$ receptor knockout mouse as a model to study 5-HT$_{1B}$ receptors
Despite compensatory changes, the studies in this thesis show that mice lacking 5-HT$_{1B}$ receptors display increased responses to SSRIs on extracellular 5-HT in the hippocampus and prefrontal cortex. The genetic deletion of the 5-HT$_{1B}$ receptor has no significant effect on basal 5-HT levels or on behaviour under basal conditions. Also, in mice lacking 5-HT$_{1A}$ receptors increased responses to SSRIs are found (Knobelman et al., 2001b, He et al., 2001, Parsons et al., 2001) and these mice display an anxious behavioural phenotype (Heisler et al., 1998, Parks et al., 1998, Olivier et al., 2001, Ramboz et al., 1998). One may expect severe changes in 5-HT neurotransmission in the absence of 5-HT autoreceptors, however, the phenotype of mice with a genetic deletion of 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors is rather mild. Interestingly, a recent study demonstrated an abnormal segregation of axons in somatosensory thalamocortical systems of 5-HT transporter knockouts and MAOA/5-HT transporter double knockouts. This segregation was normalized in MAOA/5-HT$_{1B}$ double and in MAOA/5-HT$_{1B}$/5-HT transporter triple knockouts, indicating that excessive activation of 5-HT$_{1B}$ receptors disrupts these projections (Salichon et al., 2001) and these projections are impaired in 5-HT$_{1B}$ receptor knockout mice (Upton et al., 2002). Besides a role of 5-HT$_{1B}$ receptors in the formation of thalamocortical projections from retinal ganglion cells to the superior collicus, it also shows that the genetic deletion of 5-HT re-uptake sites, MAOA and 5-HT$_{1B}$ receptors results in a viable phenotype.

Taken together, these findings suggest a large capacity of the serotonergic system for neuroplasticity.

Methodological considerations on microdialysis

Extracellular 5-HT
In vivo microdialysis detects extracellular levels of 5-HT, but this does not necessarily reflect the release of 5-HT within the synaptic cleft. A great spill-over of 5-HT diffuses out of the synaptic cleft into the extracellular fluid surrounding the dialysis probe. As outlined in Chapter 1, 5-HT may act predominantly as a volume transmitter. Consistent with the large spill-over of 5-HT outside the synaptic cleft, 5-HT re-uptake sites and 5-HT_{1B} receptors are mainly found extra-synaptically. The amount of extrasynaptic 5-HT in the extracellular space will be diluted as a result of re-uptake and enzymatic degradation. Moreover, 5-HT can arise from non-neuronal sources such as glial cells, disrupted nerve terminals due to probe implantation, blood platelets and blood circulation. Neuronal release of 5-HT levels sampled in dialysates from most forebrain areas has been well established using the classic criteria of tetrodotoxin and Ca^{2+} dependency. Changes in extracellular 5-HT can readily be detected after stimulation of terminal autoreceptors (decrease of 5-HT) or after administration of a SSRI (increase of 5-HT). By means of microdialysis, however, actual release of 5-HT may not be detected. The study presented in chapter 6 suggests that the effect of 5-HT_{1B} receptor blockade depend on the presence of a SSRI. When the release of 5-HT is increased by blockade of inhibitory 5-HT_{1B} autoreceptors, this increase in 5-HT may not be detected as it is rapidly removed from the extracellular space by 5-HT reuptake sites before it can reach the microdialysis probe. If the reuptake of 5-HT is blocked by a SSRI, the released amount of 5-HT will remain in the extracellular fluid and can be detected in the sampled dialysates. Decreases in 5-HT release can be detected, as re-uptake does not affect this process.

5-HT in mouse versus rat
Are there differences in the regulation of 5-HT release between rats and mice? In the microdialysis studies presented in this thesis, the sample time was 15 minutes in rats and 20 minutes in mice. At the start of the microdialysis studies in mice, the 5-HT levels detected in 15 minutes samples were too low and therefore the sample time was increased to 20 minutes. This may be due to a species difference in 5-HT regulation, or this difference may be caused by the small size of the mouse brain relative to that of a rat. In rats, a microdialysis probe can be

placed either in the ventral or in the dorsal part of the hippocampus, whereas in mice the probe is mainly placed in the ventral part, but also in the dorsal part. The local administration of 3 and 10 µM fluvoxamine dose-dependently increased extracellular 5-HT in rat (dorsal) hippocampus to about 2 and 3-fold of baseline, respectively (unpublished observations), whereas in wildtype mice both 0.3 and 1.0 µM fluvoxamine increased hippocampal 5-HT to 6-fold of baseline (chapter 2). These findings support a differential regulation of 5-HT output in the two species. As species difference between rat and mice exist, also strain differences in mice are known. For the studies in this thesis, 5-HT_{1B} receptor knockout mice on a 129/Sv background were tested. The genetic background of the knockout mouse has been found to be important for the outcome of behavioural studies (Crawley et al., 1997, Phillips et al., 1999 , Olivier et al., 2001). There is as yet no evidence for such an influence of the genetic background on basal 5-HT levels or for pharmacological effects on extracellular 5-HT in 5-HT_{1B} receptor knockout mice. Mouse strain-related differences, however, have been reported for basal DA dynamics, in the 129/Sv strain, relative to three other mouse strains the basal DA uptake was reduced (He and Shippenberg, 2000).

Future directions

The studies presented in this thesis demonstrate an inhibitory role of 5-HT_{1B} autoreceptors on extracellular 5-HT following acute locally applied SSRIs, in the hippocampus and prefrontal cortex, but not in the dorsal striatum. These findings support the notion that 5-HT_{1B} autoreceptors may be an interesting target for augmentation strategies of treatment with SSRIs. Further studies are needed to elucidate the contribution of region specific effects in the mechanism of action of SSRIs. Moreover, detailed studies on the regulation of 5-HT in different brain structures may provide more insight in the pathophysiology of different psychiatric disorders. Our study could not confirm the previously reported role of striatal 5-HT_{1B} heteroreceptors on DA outflow in the striatum, because the putative selective 5-HT_{1B} receptor CP93129 affected DA outflow in the knockouts to the same extent as in the wildtype mice. In contrast to 5-HT_{1B} autoreceptors, relatively little is known about the functional role of 5-HT_{1B} receptors. Due to restricted selectivity of pharmacological tools, the 5-HT_{1B} receptor knockout mouse is an interesting model to further explore the functional role of 5-HT_{1B} heteroreceptors. In addition, the selective 5-HT_{1B} receptor antagonist NAS-181 may be a valuable pharmacological tool to study 5-HT_{1B} receptor in rodents.

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Summary

The studies described in this thesis explore the role of 5-HT$_{1B}$ receptors in serotonin (5-HT) function in rats and in genetically modified mice lacking 5-HT$_{1B}$ receptors.

In **chapter 1** relevant literature is reviewed. The serotonergic system is complex as 5-HT exerts its function through 14 different receptor subtypes. Serotonergic neurons are located in the raphe nuclei and project to brain structures throughout the mammalian forebrain, including the hippocampus, frontal cortex and striatum. In the mammalian brain, 5-HT$_{1B}$ receptors are expressed either as an inhibitory 5-HT autoreceptor or as a 5-HT$_{1B}$ heteroreceptor. 5-HT$_{1B}$ autoreceptors are localised on serotonergic nerve terminals controlling 5-HT release. 5-HT$_{1B}$ heteroreceptors are present on non-serotonergic neurons, suggesting that this receptor subtype modulates other neurotransmitters and is involved in a variety of functions.

The serotonergic system is an important target in the treatment of psychiatric disorders. Selective serotonin reuptake inhibitors (SSRIs) are widely used in the treatment of depression and anxiety disorders, but a clinical problem is the delayed therapeutic effect. This delayed onset of action suggests that adaptive changes may occur. Previous preclinical studies have indicated a role of both cell body 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors in the effects of SSRIs. Moreover, dysfunction of 5-HT$_{1B}$ receptors has been associated with aggression, impulsivity, alcoholism and drug abuse. More insight in the functional role of 5-HT$_{1B}$ receptors contributes to our understanding of this receptor in psychiatric disorders. A limitation in research on 5-HT$_{1B}$ receptors has been the relative restricted selectivity of 5-HT$_{1B}$ receptor antagonists. Therefore, the generation of 5-HT$_{1B}$ KO mouse has provided an interesting model to study 5-HT$_{1B}$ receptors.

In the first part of this thesis, the role of 5-HT$_{1B}$ receptors in the effects of SSRIs on 5-HT levels was studied by comparing these effects in wildtype mice with mice lacking the 5-HT$_{1B}$ receptor by means of in vivo microdialysis. Activation of 5-HT$_{1B}$ autoreceptors reduces the release of 5-HT. Thus in the absence of 5-HT$_{1B}$ autoreceptors, increased extracellular 5-HT levels and an increased response to SSRIs might be expected. Previous studies have indicated regional effects of SSRIs and therefore the effects of SSRI on 5-HT output were examined in different brain structures. Upon systemic administration of a SSRI, both cell body and terminal 5-HT autoreceptors are activated. To examine the contribution of terminal autoreceptors on 5-HT levels, and to circumvent the effects of raphe 5-HT autoreceptors, a SSRI was locally administered into the brain structures of interest by reversed microdialysis.

Chapter 2 describes a study on the role of 5-HT$_{1B}$ autoreceptors in the effects of SSRIs in the hippocampus. Both systemic and local administration of a SSRI

resulted in an enhanced 5-HT response in 5-HT$_{1B}$ knockout mice relative to wildtypes, indicating that terminal 5-HT$_{1B}$ autoreceptors in the hippocampus limit the acute effects of SSRIs.

No difference was observed between the two genotyes in response to a lower concentration of the locally applied SSRI. This finding indicates that 5-HT reuptake sites might be increased in the hippocampus of 5-HT$_{1B}$ KO mice to compensate for the loss of 5-HT$_{1B}$ autoreceptors.

In **chapter 3** the effects on 5-HT output of locally administered fluvoxamine into the medial PFC are described. A locally applied SSRI resulted in an augmented response in 5-HT$_{1B}$ KO mice relative to wildtype mice. Blockade of 5-HT$_{1B}$ receptors with a selective 5-HT$_{1B}$ receptor antagonist augmented the effect of a SSRI on 5-HT output in wildtype mice, further supporting that 5-HT$_{1B}$ autoreceptors limit the effects of (local) SSRIs in this brain structure.

Chapter 4 describes a study on the role of 5-HT$_{1B}$ receptors in the interaction between 5-HT and dopamine in the dorsal striatum. The two genotypes showed an identical dose-response to local administration of fluvoxamine, indicating that 5-HT$_{1B}$ receptors do not play a prominent role in the regulation of striatal 5-HT release. In contrast, stimulation of 5-HT$_{1B}$ receptors reduced 5-HT levels in wildtype mice, indicating that 5-HT$_{1B}$ autoreceptors are functionally present in the striatum. A role of striatal 5-HT$_{1B}$ receptors in dopamine outflow could not be confirmed in 5-HT$_{1B}$ receptor knockout mice. The putative selective 5-HT$_{1B}$ receptor agonist CP93129 increased dopamine outflow in 5-HT$_{1B}$ receptor knockout mice to the same extend as in wildtype mice. Therefore, this effect of CP93129 cannot be mediated by striatal 5-HT$_{1B}$ heteroreceptors. Possibly the effect of CP93129 on dopamine involves 5-HT$_2$, 5-HT$_3$ or 5-HT$_4$ receptors. The effect of CP93129 on dopamine outflow was from a neuronal origin (tetrodotoxin dependent), but was not attenuated by 5-HT$_2$ receptor blockade. The 5-HT releaser fenfluramine and the SSRI fluvoxamine increased 5-HT and dopamine levels, but no difference was observed between the genotypes, further supporting that 5-HT$_{1B}$ receptors localised in the striatum do not modulate dopamine outflow in this brain structure.

In the second part of this thesis, studies are presented on the effects of NAS-181, a new selective 5-HT$_{1B}$ receptor antagonist, in rat frontal cortex. In **chapter 5**, the effect on 5-HT levels of NAS-181 was compared with two other 5-HT$_{1B}$ receptor antagonists. NAS-181 and GR127935, but not SB224289, attenuated the suppressant effect of a 5-HT$_{1B}$ receptor agonist on 5-HT levels. The three antagonists reduced 5-HT levels when given alone, but these effects were absent in the presence of a SSRI, suggesting some partial agonistic properties of these compounds. NAS-181 was found to be a selective 5-HT$_{1B}$ receptor antagonist.

In **chapter 6**, the hypothesis was tested whether the effect of a 5-HT$_{1B}$ receptor antagonist depends on extracellular 5-HT levels. Previous studies have shown

that administration of a 5-HT$_{1B}$ receptor antagonist alone has no effect on 5-HT levels. Different strategies were used to enhance extracellular 5-HT levels. NAS-181 augmented the effect of a locally applied SSRI. Whereas no additional effect of NAS-181 was observed when 5-HT levels were increased by depolarization induced 5-HT release (with potassium chloride) or by preventing degradation of 5-HT (with a mono-oxidase inhibitor). Interestingly, in the presence of a SSRI, the depolarization induced release of 5-HT was augmented by NAS-181. The data provide some evidence that the effect of a 5-HT$_{1B}$ receptor antagonist depends on extracellular 5-HT levels, but strongly suggest that additional 5-HT reuptake inhibition is required to detect any effect of 5-HT$_{1B}$ receptor antagonists on 5-HT levels by means of in vivo microdialysis.

The main results are discussed in **chapter 7**. The main advantage of a knockout model is its selectivity. In addition, the 5-HT$_{1B}$ receptor antagonist NAS-181 can be a valuable pharmacological tool to study 5-HT$_{1B}$ receptors in rodents. The studies in mice and rats described in this thesis clearly show that 5-HT$_{1B}$ autoreceptors limit the acute effects of local administration of a SSRI in the hippocampus and frontal cortex, but not in the striatum. These findings support the idea that blockade of both 5-HT reuptake sites and 5-HT$_{1B}$ autoreceptors might be a potential interesting augmentation strategy for treatment with SSRIs. The mice data indicate some evidence for compensatory changes in 5-HT$_{1B}$ KO mice, supporting the importance of 5-HT$_{1B}$ receptors in 5-HT neurotransmission. In contrast to the well-established role of 5-HT$_{1B}$ autoreceptors, relative little is known about 5-HT$_{1B}$ heteroreceptors. The previous reported role of 5-HT$_{1B}$ receptors in striatal dopamine release could not be confirmed in the 5-HT$_{1B}$ KO mice.

Samenvatting

De studies in dit proefschrift onderzoeken de rol van 5-HT_{1B} receptoren in serotonine (5-HT) functie in ratten en in genetisch gemodificeerde muizen waarin de 5-HT_{1B} receptor uitgeschakeld is.

In **hoofdstuk 1** wordt een overzicht gegeven van de relevante literatuur. Het serotonerge systeem is complex, omdat het zijn werking heeft via 14 verschillende receptor subtypen. Serotonerge neuronen zijn gelokaliseerd in de raphe kernen en projecteren naar hersenstructuren door de gehele voorhersenen, waaronder de hippocampus, frontale cortex en striatum. In de hersenen van zoogdieren is de 5-HT_{1B} receptor aanwezig of als 5-HT autoreceptor of als heteroreceptor. Op zenuwuiteinden van serotonerge neuronen zijn 5-HT_{1B} autoreceptoren aanwezig die de afgifte van 5-HT reguleren. 5-HT_{1B} heteroreceptoren zijn aanwezig op non-serotonerge neuronen, wat suggereert dat deze receptoren andere neurotransmitters kunnen moduleren en betrokken zijn bij een verscheidenheid aan hersenfuncties.

Het serotonerge systeem is een belangrijk doel in de behandeling van psychiatrische stoornissen. Selectieve serotonine heropname remmers (SSRIs) worden vaak gebruikt in de behandeling van depressie en angststoornissen, maar een klinisch probleem is de vertraagde therapeutische werking. Deze vertraagde werking kan mogelijk verklaard worden door het optreden van adaptieve veranderingen. Eerdere preklinische studies hebben laten zien dat zowel 5-HT_{1A} autoreceptoren op cellichamen als 5-HT_{1B} autoreceptoren op zenuwuiteinden een rol spelen in de effecten van SSRIs. Bovendien zijn er aanwijzingen voor dysfunctie van 5-HT_{1B} receptoren in agressief gedrag, impulsiviteit, alcoholisme en drug misbruik. Onderzoek aan de functie van 5-HT_{1B} receptoren geeft ons meer inzicht in de rol van deze receptor in psychiatrische stoornissen. Een beperking in het onderzoek aan 5-HT_{1B} receptoren is de relatieve geringe selectiviteit van 5-HT_{1B} receptor liganden. Daarom is generatie van genetisch gemodificeerde muizen waarin 5-HT_{1B} receptoren zijn uitgeschakeld een interessant model om 5-HT_{1B} receptoren te bestuderen.

Het eerste deel van dit proefschrift beschrijft studies waarin in vivo microdialyse is toegepast om de rol van 5-HT_{1B} receptoren in de effecten van SSRIs op 5-HT afgifte te onderzoeken, door deze effecten in wildtype muizen te vergelijken met 5-HT_{1B} receptor knockout (KO) muizen. Stimulatie van 5-HT_{1B} autoreceptoren resulteert in een afname van de extracellulaire hoeveelheden 5-HT. Dus in de afwezigheid van 5-HT_{1B} autoreceptoren kunnen toegenomen 5-HT concentraties en een toegenomen respons na toediening van een SSRI verwacht worden. Eerdere studies hebben regionale verschillen in de effecten van SSRIs aangetoond, daarom zijn verschillende hersenstructuren onderzocht. Na systemische toediening van een SSRI worden zowel 5-HT autoreceptoren op

cellichamen in de raphe kerne, als 5-HT autoreceptoren op zenuwuiteinden gestimuleerd. Om de bijdrage van 5-HT$_{1B}$ receptoren op zenuwuiteinden in SSRI-effecten op 5-HT te bepalen, en om de effecten van raphe 5-HT$_{1A}$ autoreceptoren te omzeilen, werd een SSRI lokaal toegediend in het hersengebied van interesse via omgekeerde microdialyse.

In **hoofdstuk 2** wordt de rol van 5-HT$_{1B}$ receptoren in the effecten van SSRIs in de hippocampus beschreven. Zowel systemische als lokale toediening van een SSRI resulteerden in een verhoogde 5-HT respons in 5-HT$_{1B}$ KO muizen in vergelijking met wildtype muizen, wat aantoont dat 5-HT$_{1B}$ autoreceptoren de effecten van SSRI op 5-HT afgifte verminderen. Echter, er werd geen verschil gevonden tussen wildtype en 5-HT$_{1B}$ KO muizen in de 5-HT respons na een lagere concentratie van de lokaal toegediende SSRI. Dit is mogelijk het gevolg van een toegenomen aantal 5-HT transporters in de hippocampus van 5-HT$_{1B}$ KO muizen ter compensatie van de afwezigheid van de 5-HT$_{1B}$ autoreceptor.

In **hoofdstuk 3** worden de effecten beschreven van een SSRI op 5-HT concentraties in de mediale prefrontale cortex. In 5-HT$_{1B}$ KO muizen resulteerde de lokale toediening van de SSRI fluvoxamine in een verhoogde 5-HT respons in de prefrontale cortex. Blokkade van 5-HT$_{1B}$ receptoren met een selectieve 5-HT$_{1B}$ receptor antagonist gaf een versterking van het SSRI effect op 5-HT concentraties in wildtype muizen, wat er ook op wijst dat 5-HT$_{1B}$ autoreceptoren de lokale effecten van een SSRI in deze hersenstructuur verminderen.

Hoofdstuk 4 beschrijft een studie naar de rol van 5-HT$_{1B}$ receptoren in de interactie tussen 5-HT en dopamine in het dorsale striatum. De dosis respons curve na lokale toediening van fluvoxamine was identiek in de twee genotypen, wat aangeeft dat 5-HT$_{1B}$ autoreceptoren geen prominente rol spelen in de afgifte van 5-HT in het striatum. In tegenstelling, stimulatie van 5-HT$_{1B}$ receptoren met CP93129 resulteerde in een verlaging 5-HT concentraties in wildtype muizen, maar niet in 5-HT$_{1B}$ KO muizen, wat aangeeft dat 5-HT$_{1B}$ autoreceptoren functioneel aanwezig zijn in het striatum. Een rol voor 5-HT$_{1B}$ receptoren in de afgifte van DA kon niet worden bevestigd in 5-HT$_{1B}$ KO muizen. De veronderstelde selectieve 5-HT$_{1B}$ receptor agonist CP93129 verhoogde dopamine concentraties in 5-HT$_{1B}$ KO muizen in dezelfde mate als in wildtype muizen. Daarom kan dit effect van CP93129 niet verklaard worden door betrokkenheid van 5-HT$_{1B}$ heteroreceptoren in het striatum. Mogelijk kan het effect van CP93129 op dopamine toegeschreven worden aan betrokkenheid van 5-HT$_2$, 5-HT$_3$ of 5-HT$_4$ receptoren. Het effect van CP93129 op dopamine was van neuronale origine (tetrodotoxin afhankelijk), maar werd niet verminderd door blokkade van 5-HT$_2$ receptoren. Lokale toediening van de 5-HT releaser fenfluramine of de SSRI fluvoxamine verhoogden zowel 5-HT als dopamine concentraties. Er was echter geen verschil in deze effecten tussen de twee genotypen, wat er ook op duidt dat 5-HT$_{1B}$ receptoren in het striatum de afgifte van dopamine in deze hersenstructuur niet beïnvloeden.

Het tweede deel van dit proefschrift omvat twee studies waarin de effecten van NAS-181, een nieuwe selectieve 5-HT$_{1B}$ receptor antagonist, op 5-HT afgifte in de frontale cortex van ratten zijn onderzocht. In **hoofdstuk 5** wordt een studie beschreven waarin het effect van NAS-181 is vergeleken met twee andere 5-HT$_{1B}$ receptor antagonisten. De 5-HT$_{1B}$ receptor antagonisten NAS-181 en GR127935, maar niet SB224289, konden de een receptor agonist geïnduceerde afname in 5-HT concentraties gedeeltelijk opheffen. Toediening van de drie receptor antagonisten afzonderlijk, in afwezigheid van een SSRI, resulteerde in afname van 5-HT concentraties, wat duidt op mogelijke partiële agonistische eigenschappen van deze liganden. De data laten zien dat NAS-181 een selectieve 5-HT$_{1B}$ receptor antagonist is.

In **hoofdstuk 6** is de hypothese getest of het effect van een 5-HT$_{1B}$ receptor antagonist afhankelijk is van de extracellulaire 5-HT concentraties. Eerdere studies hebben laten zien dat toediening van alleen een 5-HT$_{1B}$ receptor antagonist geen effect heeft op 5-HT concentraties. Daarom zijn in deze studie verschillende strategieën gebruikt om de extracellulaire 5-HT concentraties te verhogen. NAS-181 gaf een extra 5-HT toename als de 5-HT concentratie werd verhoogd door een SSRI. Er werd echter geen effect van NAS-181 gevonden als 5-HT concentraties werden verhoogd door depolarisatie geïnduceerde 5-HT afgifte (met kalium chloride) of door de afbraak van 5-HT te blokkeren (met een mono-oxidase remmer). Een interessante bevinding was dat het effect van depolarisatie geïnduceerde 5-HT afgifte in aanwezigheid van een SSRI wel door NAS-181 kon worden versterkt. De resultaten geven aan dat het effect van een 5-HT$_{1B}$ receptor antagonist afhankelijk lijkt te zijn van de extracellulaire hoeveelheid 5-HT, maar duiden er meer op dat 5-HT heropname geblokkeerd moet zijn om een effect van 5-HT$_{1B}$ receptor blokkade op 5-HT concentraties te meten met in vivo microdialyse.

Hoofdstuk 7 beschrijft de discussie van de voornaamste resultaten. Een belangrijk voordeel van een knockout muis model is de selectiviteit. Daarnaast kan de selectieve 5-HT$_{1B}$ receptor antagonist NAS-181 een waardevol farmacologische middel zijn in onderzoek aan 5-HT$_{1B}$ receptoren in ratten en muizen. De studies in ratten en muizen beschreven in dit proefschrift laten duidelijk zien dat de acute effecten van een lokaal toegediende SSRI worden verminderd door 5-HT$_{1B}$ autoreceptoren in de hippocampus en frontale cortex, maar niet in het dorsale striatum. Mogelijk kan blokkade van zowel 5-HT heropname en 5-HT$_{1B}$ autoreceptoren een interessante strategie kan zijn om de therapeutische werking van SSRI te versnellen. De bevindingen in muizen laten ook zien dat er mogelijke aanpassingen zijn in 5-HT$_{1B}$ KO muizen ter compensatie van het verlies van 5-HT$_{1B}$ receptoren, wat wijst op het functionele belang van deze receptor in 5-HT signaaloverdracht. In tegenstelling tot de algemene

aangenomen rol van 5-HT$_{1B}$ autoreceptoren is er relatief minder bekend over 5-HT$_{1B}$ heteroreceptoren. Een rol voor 5-HT$_{1B}$ heteroreceptoren in het striatum in de afgifte van dopamine kon niet worden bevestigd in de 5-HT$_{1B}$ KO muizen.

About the author

Lotte de Groote was born on March 30, 1972 in Winschoten, the Netherlands. She attended secondary education (VWO) at the Dr. Nassau College in Assen. From 1990 untill 1996 she studied Biology at the Rijksuniversiteit Groningen. During her specialisation in Medical Biology she participated in research projects on neuroanatomy and behaviour in rats at the Department of Animal Physiology (supervised by Dr. B. Buwalda and Prof. Dr. P.G.M. Luiten) and on molecular biology in zebrafish at the department of Developmental Genetics (supervised by Dr. M. Tempelaar). After graduation she participated in research on a rat model for neurodevelopmental disorder at the Department of Biological Psychiatry, University Hospital Groningen.

The research resulting in this thesis started in May 1998 at the Department of Psychiatry, University Medical Center Utrecht, supervised by Prof. Dr. H.G.M. Westenberg and by Prof. Dr. B. Olivier at the Department of Psycho-pharmacology, Utrecht University, The Netherlands.

In November 2002 she will start as a post-doc researcher at the Max Planck Institute of Psychiatry in Munich, Germany.

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