Pharmacological Reports
2010, 62, 564–577
ISSN 1734-1140

Review

Potential role of the 5-HT_6 receptor in depression and anxiety: an overview of preclinical data

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Abstract:
Mental disorders, such as depression and anxiety, pose both medical and social challenges. The clinical efficacy of current antidepressant/anxiolytic therapies is unsatisfactory; both antidepressant and anxiolytic drugs induce a variety of unwanted effects and have delayed onsets of action. Thus, a search for better and safer agents is continuously in progress. Preclinical results published so far have brought new insights into the possible role of recently discovered serotonin 5-HT_6 receptors in these disorders. This review surveys the current state of knowledge regarding potential antidepressant and anxiolytic activities of selective 5-HT_6 receptor ligands, namely, full agonists and antagonists, in animal models commonly used to predict such activity. Evidence indicates that both 5-HT_6 agonists and antagonists may evoke identical responses in animal models of depression and anxiety; however, the possible mechanisms of these effects seem to be diverse and are not clearly understood. Especially interesting are the augmented effects achieved by combining antidepressants or diazepam with a selective 5-HT_6 receptor antagonist.

Key words:
serotonin, 5-HT_6 receptor, 5-HT_6 agonist, 5-HT_6 antagonist, depression, anxiety, hippocampus, mice, rats

Introduction

Medicinal therapies for mood disorders, such as depression and anxiety, neither fully serve the efficacy demands of patients, nor are they free of side effects. Despite advances in pharmacotherapy, there continue to be many unmet clinical needs, ranging from efficacy in treatment-resistant patients to improved onset of action and to reductions in unwanted effects. Numerous combination therapies and novel targets have been identified that may demonstrate improvements in one or more of these desired areas. One of these approaches is directly targeting monoamine receptors, such as the recently discovered serotonin receptor 5-HT_6.

The 5-HT_6 receptor was first identified and sequenced in 1993 by separate groups [68, 78, 87]. It is one of three serotonin receptors positively coupled to the G_s-sensitive AC5 isoform [2], which induces 3'5'-cyclic adenosine monophosphate (cAMP) production [68, 78, 87]. The human and mouse 5-HT_6 receptors are glycoproteins consisting of 440 amino acids; in rats, the protein have 438 amino acids. All known homologues have 7 transmembrane domains that form 3 cytoplasmic and 3 extracellular loops [51, 52, 68].

In adult animals, *in situ* hybridization histochemistry assays analyzing 5-HT_6 receptor mRNA expression, as well as immunohistochemical and autoradiographic studies, have exhibited high concordance and have demonstrated the highest 5-HT_6 receptor expres-

sion in the striatum, nucleus accumbens, olfactory tubercle, and cortex, with moderate density in the amygdala, hippocampus, hypothalamus, thalamus, and cerebellum [8, 13, 25, 28, 32, 33, 35, 41, 42, 52, 68, 84, 87, 106, 118]. There appears to be a negligible density of 5-HT$_6$ receptors outside the central nervous system; faint expression is also detectable in the rat stomach, spleen, thymus, and peripheral blood lymphocytes [87, 100] as well as sympathetic ganglia [77]. The postsynaptic localization of the 5-HT$_6$ receptor is supported by the following: (I) electron microscopic analysis of 5-HT$_6$ immunohistochemistry revealed receptor staining primarily on dendritic and cilia processes, with little expression on cell bodies [32, 35] and high expression of 5-HT$_6$ receptor mRNA in serotonin projection fields [106]; (II) the presence of 5-HT$_6$ receptors is confined to the dendritic compartment [35]; and (III) no depletion of serotoninergic innervation *via* 5,7-dihydroxytryptamine on 5-HT$_6$ receptor expression in the rat nucleus accumbens, striatum and hippocampus has been observed [33]. All these data suggest that the 5-HT$_6$ receptor is located outside serotonin neurons and does not function as an autoreceptor.

During the last several years, a large amount of information has been collected about the 5-HT$_6$ receptor. A physiological role for this receptor within the central nervous system has been clearly established in studies focusing on learning and memory [36, 62, 67, 85, 113]. Strong evidence also supports the involvement of the 5-HT$_6$ receptor in centrally-regulated feeding behavior [16, 37]. Currently, 5-HT$_6$ receptor antagonists are being developed in clinical trials for treatment of Alzheimer's type dementia, cognitive impairment associated with schizophrenia, and obesity [47, 48, 55].

Preclinical efforts to evaluate a possible link between the 5-HT$_6$ receptor and affective disorders have generally been inconclusive. These studies have mainly used pharmacological tools, such as 5-HT$_6$ receptor antisense oligonucleotides and/or mice lacking functional 5-HT$_6$ receptors, in animal behavioral models designed to mimic, at least in part, human diseases. Very recently, selective antagonists and ago-

Tab. 1. The binding profile of selective 5-HT$_6$ receptor antagonists [15, 41, 42, 44, some data concerning the affinity of SB-399885 were received from GlaxoSmithKline company]

Receptor	pK$_i$		
	SB-271046	SB-399885	SB-258585
5-HT$_6$	8.9	9.1	8.6
5-HT$_{1A}$	6.4	5.7	6.2
5-HT$_{1B}$	6.6	5.4	6.4
5-HT$_{1D}$	6.6	5.8	6.4
5-HT$_{1E}$	< 5.0	< 5.0	5.6
5-HT$_{1F}$	< 6.0	< 5.1	6.2
5-HT$_{2A}$	< 5.6	6.0	6.0
5-HT$_{2B}$	< 5.4	6.4	5.5
5-HT$_{2C}$	5.7	6.7	5.9
5-HT$_4$	5.4	5.0	< 5.0
5-HT$_7$	5.4	< 5.1	5.5
D$_2$	5.6	5.3	5.4
D$_3$	6.3	5.9	6.1
D$_4$		< 5.0	
α_{1B}	5.7	5.6	5.5

Tab. 2. The binding profile of selective 5-HT$_6$ receptor agonists ([a] % inhibition at 1 μM drug concentration or K$_i$ (nM) determination at respective receptor according to Schechter et al. [93] and Cole et al. [18]), [b] data taken from [34]

Receptor	WAY-181187[a]	WAY-208466[a]	11q[a]	EMDT[b] K$_i$ (nM)
5-HT$_6$	2.2	4.8	2.0	16
5-HT$_{1A}$	0%	0%		170
5-HT$_{1B}$	36%	30%	36%	
5-HT$_{1D}$	21%	16%	21%	290
5-HT$_{1E}$				520
5-HT$_{1F}$	40%	22%	40%	
5-HT$_{2A}$	25%	351 nM	25%	> 10000
5-HT$_{2B}$	459 nM	313 nM	458 nM	
5-HT$_{2C}$ (Ant)	51%	217 nM	51%	1810
5-HT$_{2C}$ (Ago)	124 nM	644 nM	124 nM	
5-HT$_7$	1579 nM	4764 nM		
D$_2$	1%	0%		
D$_3$	1%	0%		
D$_4$	1%	0%		
α_1	1334 nM	0%		300

nists have become useful tools for studying the function of the 5-HT_6 receptor in mood control. Among them, SB-271046 (5-chloro-*N*-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methyl-benzothiophene-2-sulfonamide), SB-399885 (*N*-(3,5-dichloro-2-methoxyphenyl)-4-methoxy-3-(1-piperazinyl)benzenesulfonamide), and SB-258585 (4-iodo-*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzenesulfonamide) are the most widely used, selective, and potent 5-HT_6 receptor antagonists (Tab. 1) [15, 41, 42, 44]. Three other compounds, i.e., WAY-181187 (2-{1-[(6-chloroimidazo[2,1-b][1,3]thiazol-5-yl)sulfonyl]-1H-indol-3-yl}-*N*,*N*-dimethylethanamine), WAY-208466 [93], and LY-586713 [30], have recently been described as novel, selective, and full 5-HT_6 receptor agonists (Tab. 2); however, some chemical structures and receptor affinities for these compounds are not available. Another compound, **11q** (N_1-(6-chloroimidazo[2,1-*b*][1,3]-thiazole-5-sulfonyl)tryptamine), was shown to belong to a group of high affinity, potent, and full 5-HT_6 receptor agonists (Tab. 2) [18]. EMDT (2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine) is a successive 5-HT_6 receptor agonist (Tab. 2) [34] used in animal studies to examine the role of 5-HT_6 receptor in depression [101], but there is some uncertainty about the selectivity of EMDT [34]. The most relevant findings concerning the effects of 5-HT_6 receptor ligands in animal models of depression and anxiety are summarized in Tables 3 and 4, respectively. The experiments, listed in tables together with additional supporting and sometimes contradicting evidence, are discussed below.

Possible role of the 5-HT_6 receptor in depression

Monsma et al. [68], Kohen et al. [51], and Sebben et al. [94] showed that several tricyclic antidepressant drugs, such as amitriptyline, and atypical antidepressants, such as mianserin, display high affinity and antagonistic activity at the 5-HT_6 receptor. This finding together with the localization of the receptor in limbic and cortical brain areas [7, 8] has led to the implication of a role for the 5-HT_6 receptor in the pathogenesis and/or treatment of affective disorders. Furthermore, 5-HT_6 receptor expression appears to be regulated by glucocorticoids. Both adrenalectomy and blockade of glucocorticoid synthesis by metyrapone or aminoglutethimidine treatment selectively up-

Tab. 3. Effects of selective 5-HT_6 receptor antagonists and agonists in animal behavioral models of depression

Compound	Animal model	Effect (active doses)	Tested doses	Reference
Antagonists:				
SB-399885	Tail suspension test in mice	Antidepressant-like (10–30 mg/kg)	3–30 mg/kg	[109]
	Forced swim test in mice	Antidepressant-like (20 and 30 mg/kg)	10–30 mg/kg	[109]
	Forced swim test in rats	Antidepressant-like (1 × 10 mg/kg)	3–20 mg/kg	[107, 109]
		Antidepressant-like (3 × 3 or 10 mg/kg)	3 × 0.3–10 mg/kg	[39]
SB-399885 + antidepressants[a]	Forced swim test in rats	Antidepressant-like	3 mg/kg + antidepressant	[110]
SB-399885 + citalopram[b]	Forced swim test in rats	No change	3 mg/kg + 20 mg/kg	[110]
SB-271046	Tail suspension test in mice	No change	1–10 mg/kg	[101]
	Forced swim test in rats	Antidepressant-like (3 × 10 or 30 mg/kg)	3 × 3–30 mg/kg	[39]
SB-258585[c]	Forced swim test in rats	Antidepressant like (3 μg)	1–10 μg	[111]
Agonist:				
EMDT	Tail suspension test in mice	Antidepressant like (2.5–15 mg/kg)	1–15 mg/kg	[101]

[a] Synergistic enhancement when using a combination of inactive doses of SB-399885 together with imipramine, desipramine, bupropion, or moclobemide. [b] Combination of non-active doses of SB-399885 and citalopram. [c] SB-258585 administered into the CA1 region of the hippocampus

Tab. 4. Effects of selective 5-HT$_6$ receptor antagonists and agonists in animal behavioral models of anxiety

Compound	Animal model	Effect (active doses)	Tested doses	Reference
Antagonists:				
SB-399885	Four plate test in mice	Anxiolytic-like (3–20 mg/kg)	1–20 mg/kg	[109]
	Elevated plus maze in rats	Anxiolytic-like (0.3–3 mg/kg)	0.1–3 mg/kg	[109]
	Conflict drinking test in rats	Anxiolytic-like (1 and 3 mg/kg)	0.3–3 mg/kg	[108, 109]
SB-399885 + diazepam[a]	Conflict drinking test in rats	Anxiolytic-like	0.3 mg/kg + 2.5 mg/kg	[108]
SB-258585[c]	Conflict drinking test in rats	Anxiolytic-like (1 μg)	0.3–3 μg	[111]
Agonists:				
WAY-181187	Schedule-induced polydipsia test in rats	Anxiolytic-like (178 mg/kg)	56–178 mg/kg	[93]
11q	Schedule-induced polydipsia test in rats	Anxiolytic-like (178 mg/kg)	56–178 mg/kg	[18]

[a] Synergistic enhancement when using a combination of inactive doses of SB-399885 together with diazepam. [b] SB-258585 administered into the CA1 region of the hippocampus

regulated the 5-HT$_6$ receptor mRNA in the CA1 and CA3 pyramidal cells of the rat hippocampus [116]. As metyrapone and aminoglutethimidine treatments have been used in the clinic to treat resistant depression [54, 69], the authors speculated that this effect might have involved the 5-HT$_6$ receptor. Moreover, preliminary genetic studies revealed that bipolar affective disorder may be associated with variation in the 5-HT$_6$ gene [103].

Selective 5-HT$_6$ receptor antagonists

These results have urged scientists to conduct experiments with selective 5-HT$_6$ receptor ligands, administered acutely in animal models commonly used for evaluating the antidepressant potential of a drug. It has been shown that one of the antagonists, compound SB-399885, exerted an antidepressant-like effect in the forced swim and tail suspension tests in mice. These effects seem to be specific because SB-399885, administered at antidepressant doses, did not stimulate locomotor activity in mice [109]. In the forced swim test in mice, an anti-immobility effect of SB-399885 was dose-dependent and comparable to that of imipramine, which was used as a reference antidepressant in that study, whereas in the tail suspension test, the potential antidepressant activity of the 5-HT$_6$ receptor antagonist used was weaker than the effect observed after treatment with imipramine and was not dose-dependent [109].

It is well established that acute administration of antidepressants reduces immobility in behavioral tests of despair (i.e., the forced swim and the tail suspension tests) and increases expression of the immediate early gene c-*fos* mRNA in the brain [4, 20, 46]. Compound SB-271046, another 5-HT$_6$ receptor antagonist, did not change immobility behavior of mice in the tail suspension test as fluoxetine did. In agreement with behavioral data, treatment with SB-271046 alone had no effect on c-*fos* mRNA expression in certain limbic regions of the frontal cerebral cortex, including the cingulated cortex and the endopiriform cortex [101]. The reason for the discrepancies in the action of two selective 5-HT$_6$ receptor antagonists and chemical analogues in the mouse tail suspension test is unclear. It should be noted that both experiments were performed on C57BL/6J mice. Although the breeding institutions providing male mice were different [101], the behavioral outcome should have been similar, even though mice are not an ideal species in which to conduct behavioral or physiological experiments pertaining to 5-HT$_6$ receptor function. As described by Hirst et al. [40], the mouse is unique compared with the rat, pig, and human, all of which express relatively high levels of 5-HT$_6$ receptors. Hence, Svenningsson et al. [101] confirmed in their study that SB-271046 has a high affinity for 5-HT$_6$ receptors in the mouse forebrain by showing that it displaced specific [^{125}I]-

SB-258585 binding at nanomolar concentrations (EC_{50} = 4 nM). Nevertheless, it is noteworthy that doses used for both compounds and experimental protocols were inconsistent. SB-399885 produced a weak, but similar, anti-immobility effect at all doses used (i.e., 10, 20 and 30 mg/kg) [109], whereas SB-271046 was administered up to a dose of 10 mg/kg only; higher doses were not tested [101]. Hirano et al. [39] and Hirst et al. [44] demonstrated in *ex vivo* binding assays that administration of SB-399885 significantly inhibited specific [^{125}I]-SB258585 binding in the rat striatum with lower doses than those required for SB-271046 to significantly occupy brain 5-HT_6 receptors. The above results prove the higher efficacy of SB-399885 in comparison to that of SB-271046, at least in rats. The improved potency of SB-399885 seems to be due to an increase in brain penetration when compared to SB-271046, where it shows a 3-fold improvement in the brain: plasma ratio of 5% to 15% [44, 86]. Moreover, the duration of the tail suspension tests also varied: 6 min for SB-399885 [109] and 5 min for SB-271046 [101].

In the forced swim test in rats, the findings for both 5-HT_6 receptor antagonists are consistent. Treatment with an acute dose of 10 mg/kg of SB-399885 produced specific antidepressant-like activity by shortening the immobility time without a stimulatory effect on exploratory activity observed in the open field [107, 109]. The absence of a potential antidepressant action after administration of a higher dose of SB-399885 (i.e., 20 mg/kg) may be due to a sedative effect, as was evident in the open field test in rats [109]. In line with that study, Hirano et al. [39] demonstrated antidepressant-like activity for both SB-399885 and SB-271046 administered three times in rats. In agreement with brain 5-HT_6 receptor occupancy (62% and 96% for 3 and 10 mg/kg of SB-399885, respectively; 56% and 84% for 10 and 30 mg/kg of SB-271046, respectively) [39] and better brain penetration (15% for SB-399885 *vs.* 5% for SB-271046) [44], SB-399885 was approximately 3 times more potent than SB-271046 in the forced swim test in rats. Both SB-399885 and SB-271046 significantly suppressed immobility behavior of rats with doses of 3 and 10 mg/kg, respectively; their effects were slightly weaker than those of imipramine [39].

The antidepressant-like activity of SB-399885 in mice and rats and such activity of SB-271046 in rats are most probably connected with these compounds' 5-HT_6 receptor antagonistic properties because both compounds are selective ligands and blockers of 5-HT_6 sites [15, 44, 86]. Hence, direct involvement of other receptors in their effect ought to be excluded.

The shortening of immobility time, induced by antidepressants in behavioral tests of despair, depends on the enhancement of the central serotonin and catecholamine neurotransmission [10, 12, 20, 80]. Unfortunately, no information is available on the effect of SB-399885 on the basal levels of serotonin. Yet, a microdialysis study has shown that SB-271046 had no influence on the basal release of serotonin in the rat prefrontal cortex, frontal cortex, striatum and hippocampus [21, 22, 56, 63]. Moreover, 5-HT_6 receptor antagonists, SB-399885 and/or SB-271046 significantly increase cortical and hippocampal extracellular concentrations of dopamine, norepinephrine and acetylcholine in freely moving rats [43, 44, 56, 59, 63]. They also can potentiate the behavioral effects of amphetamine [23, 31, 82]. Furthermore, high levels of 5-HT_6 receptor-like immunoreactivity have been found in dopaminergic areas [32]. The microdialysis findings have been extended by a study described by Wesołowska et al. [107], which demonstrated that administration of *p*-chloramphetamine under their laboratory conditions reduced cortical and hippocampal concentrations of serotonin and its metabolite and did not modify the antidepressant-like effect of SB-399885 in the forced swim test in rats. However, its anti-immobility action in that test was abolished by the preferential D_1- and D_2-like receptor antagonists SCH-23390 and sulpiride, respectively, and by the α_2-adrenoceptor antagonist idazoxan, but not by prazosin, a blocker of α_1-adrenoceptors [107]. On the basis of microdialysis and behavioral studies, it could be proposed that the anti-immobility effect of 5-HT_6 receptor antagonists does not actually require any integrity of serotonin neurons and seems to be connected with activation of dopamine and norepinephrine systems *via* D_1- and D_2-like receptors and α_2-adrenoceptors.

It is well documented that 5-HT_6 receptor antagonists, including SB-399885, increase extracellular levels of acetylcholine in the hippocampus and prefrontal cortex in freely moving rats [44, 83]. Acetylcholine is of great importance in cognitive function and diseases, including Alzheimer's disease [91]; however, its influence on emotional regulation is less known. Janowsky et al. [49] and Shytle et al. [99] postulated that there is relative or actual cholinergic

hyperactivity in depression. Moreover, in animal models anticholinergic drugs enhance the potential antidepressant activity of imipramine [79] and suppress immobility behavior of mice in the forced swim and the tail suspension tests [11, 20]. Thus, these findings indirectly suggest that it is unlikely that the antidepressant-like effect of 5-HT$_6$ receptor antagonists observed in animal models develops as a consequence of enhanced acetylcholine release.

The behavioral evidence indicates that the selective blockade of 5-HT$_6$ receptors evoked by SB-399885 may facilitate the anti-immobility effects of antidepressants in which the mechanism of action is connected with the inhibition of norepinephrine/dopamine uptake and monoamine oxidase-A [110]. Combining a sub-efficacious dose of SB-399885 with ineffective doses of imipramine, desipramine, bupropion or moclobemide results in a pronounced decrease of immobility time in the forced swim test in rats [110]. Only citalopram injected in a non-active dose jointly with SB-399885 did not induce any effects characteristic of antidepressants in that test. However, no data are available so far on the effect of SB-399885 on antidepressants' blood-brain barrier penetration and pharmacokinetics parameters; thus, pharmacokinetic interaction between a selective 5-HT$_6$ antagonist and the antidepressants studied cannot be ruled out. All in all, the above cited behavioral and neurochemical results seem to point to an involvement of enhanced monoaminergic, namely, dopaminergic and/or noradrenergic, neurotransmission in the antidepressant-like activity of a 5-HT$_6$ antagonist [110].

The hippocampus is a limbic region that plays an important role in emotional states [38]. In recent years, clinical and laboratory experiments have strengthened the evidence for the role of the hippocampus in the pathophysiology of depression. Multiple imaging studies have revealed hippocampal volume reductions in depressed patients, which are changes that correlate well with disease duration and executive dysfunction [57, 71, 72, 92, 102, 112]. Furthermore, it seems that the hippocampus may play an important role in the action of antidepressants as well. It has been demonstrated that treatment patients with major depressive disorder using these drugs significantly improved memory and depressive symptoms without altering hippocampal volume, which could suggest that antidepressants improve hippocampal function in the absence of detectable structural changes [104]. An involvement of the hippocampus in the action of antidepressant drugs has also been demonstrated in animal studies. For example, imipramine [81] or desipramine [53] injected into the rat hippocampus was able to reduce immobility in the forced swim test. Moreover, electrolytic lesion of that structure completely abolished the effect of peripherally administered desipramine [53]. In addition, Sherman and Allers [95] found a strong correlation between the imipramine concentration in the rat hippocampus and the positive effect of the drug in the behavioral learned helplessness test, an animal model predictive of antidepressant potential. Finally, the finding that antidepressants increase hippocampal brain-derived neurotrophic factor (BDNF) expression seems to support the idea that hippocampal neurogenesis is involved in antidepressant action [64].

As has been mentioned above, 5-HT$_6$ receptors are present in the hippocampus, including its CA1 region [32, 33, 68, 106, 118], and the hippocampus has been proposed as one of the neuroanatomical structures involved in antidepressant-like activity of selective 5-HT$_6$ receptor antagonists [111]. In fact, recent behavioral results demonstrated that SB-258585, a selective 5-HT$_6$ receptor blocker, when injected into the CA1 region of the rat hippocampus, produced an antidepressant-like effect in the forced swim test. Its potential antidepressant activity cannot be attributed to changes in general activity because this drug, given at a dose producing an anti-immobility effect, did not change exploratory locomotor activity measured in the open field test in rats. It is noteworthy that the antidepressant-like effect of SB-258585 was comparable with the effect of imipramine [111].

Selective 5-HT$_6$ receptor agonists

5-HT$_6$ receptor agonists may also play a role in depression. Svenningsson et al. [101] showed that fluoxetine exerts a stimulatory action on cortical c-*fos* mRNA and phospho-Ser^{845}-GluR1 and reduces the immobility time of mice in the tail suspension test. All its actions were partially blocked by a selective 5-HT$_6$ receptor antagonist, SB-271046, administered at a dose that by itself produced no effect in those assays. Because fluoxetine has only low-to-moderate affinity for 5-HT$_6$ receptors [68], the authors concluded

that the inhibitory action of SB-271046 on fluoxetine-mediated effects involves blocking 5-HT_6 receptor activation that had been elicited by the fluoxetine-induced elevations of extracellular levels of serotonin and does not involve the direct competition of either compound at 5-HT_6 sites [101]. Additionally, they assessed the ability of the 5-HT_6 receptor agonist EMDT to mimic some of the antidepressant-like biochemical and behavioral effects of fluoxetine [101]. EMDT, like fluoxetine, increased the phosphorylation state of Thr^{34}-DARPP-32 both in brain slices and in the intact brain. It also increased phospho-Ser^{845}-GluR1 and the expression of c-*fos* mRNA throughout the striatum and cerebral cortex. Moreover, in a similar range of doses, EMDT significantly reduced the immobility time of mice in the tail suspension test. SB-271046 completely blocked the biochemical and behavioral antidepressant-like effects produced by EMDT [101].

It is well established that the shortening of immobility time, induced by antidepressant drugs and observed in behavioral tests of despair, depends on the enhancement of the central serotonin and catecholamine neurotransmission [10, 12, 20, 80]. Unfortunately, no information is available on the effect of EMDT on the levels of serotonin and catecholamines. A microdialysis study has only shown that a selective 5-HT_6 receptor agonist, WAY-181187, decreased the basal release of serotonin, dopamine and norepinephrine in the frontal cortex, striatum and amygdala of freely moving rats [93]. The latter effects were found to be blocked by local infusion of a $GABA_A$ receptor antagonist, bicuculline, which confirms a relationship between the 5-HT_6 receptor and GABAergic system and is entirely consistent with a study showing dense colocalization of the 5-HT_6 receptor with the GABA-synthesizing enzyme glutamic acid decarboxylase in the rat cortex, hippocampus and striatum [105, 114]. Additionally, acute administration of WAY-181187 increased the extracellular levels of GABA in the rat frontal cortex, dorsal hippocampus, striatum and amygdala without altering basal levels of glutamate. However, in hippocampal slices, pretreatment with WAY-181187 significantly and dose-dependently attenuated sodium azide-stimulated increases in glutamate concentrations [93]. Impairment of GABAergic neurotransmission has been described in patients with a variety of depressive illness [58, 76, 90]. Moreover, Sanacora et al. [89] observed higher glutamate levels in depressed patients compared with healthy age-matched controls. The preclinical studies and preliminary clinical trials with ketamine, an NMDA receptor antagonist [74, 117, 119], demonstrated its potential antidepressant activity, which supports the notion that attenuating glutamatergic neurotransmission can be beneficial. Thus, improvement of GABAergic neurotransmission in connection with dampening stimulated glutamatergic transmission could be proposed as a mechanism of the antidepressant-like activity of 5-HT_6 receptor agonists.

The above findings may suggest a difference in the mechanisms of the anti-immobility action of 5-HT_6 receptor agonists and antagonists, although each of them seems to be primarily connected with stimulating or blocking, respectively, 5-HT_6 receptors, given that the studied compounds are selective 5-HT_6 receptor ligands [15, 34, 43, 44, 86]. The limited available data do not, however, permit us to provide a definite explanation.

Several independent studies have demonstrated that repeated, but not acute, administration of antidepressants increases the expression of genes whose corresponding proteins regulate synaptic plasticity in the brain [19, 29, 70, 88]. As a result, BDNF and the effector immediate early gene activity-regulated cytoskeletal associated protein (*Arc*) have been identified as possible targets for antidepressant action [75, 96, 97]. Experiments conducted by de Foubert et al. [30] provided the first evidence for the involvement of the 5-HT_6 receptors in regulating BDNF and *Arc* mRNA expression. They showed that a selective 5-HT_6 receptor agonist, compound LY-586713, when administered acutely, caused marked increases of BDNF and *Arc* mRNA levels in hippocampal and cortical regions. These increases were attenuated by SB-271046 in all regions of the hippocampus and the parietal cortex. In conclusion, the above results may suggest that direct 5-HT_6 receptor activation results in a more rapid rise in BDNF and *Arc* mRNA expression, which does not require repeated administration, as is the case for some antidepressant drugs [19, 29, 70, 88]. Because LY-586713 is described as a selective 5-HT_6 receptor agonist, it is plausible that the 5-HT_6 receptor-mediated onset of BDNF gene expression following a single dose of LY-586713 is directly linked to the activation of 5-HT_6 receptors. Subsequently, increases in cAMP levels lead to activation of CREB, a known transcription factor for the BDNF gene [30].

From the above description, it is clear that 5-HT_6 receptors are involved in the antidepressant-like activity observed in animal models. An acute and selective

blockade of 5-HT$_6$ receptors may evoke an antidepressant-like effect that seems to be predominantly due to the enhancement of brain noradrenergic and/or dopaminergic neurotransmission, and the hippocampus seems to be one of neuroanatomical sites involved in that effect. Moreover, the selective blockade of 5-HT$_6$ receptors induced by SB-399885 may facilitate the anti-immobility activity of antidepressants whose mechanism of action is connected with norepinephrine/dopamine uptake inhibition or with the inhibition of monoamine oxidase-A. However, in this case, one cannot exclude pharmacokinetic interaction yet. In contrast, the stimulation of 5-HT$_6$ receptors may also evoke antidepressant-like activity, and 5-HT$_6$ receptors seem to play at least a partial role in the potential antidepressant effect of fluoxetine. The ability of a 5-HT$_6$ receptor agonist to enhance extracellular GABA levels and decrease stimulated glutamatergic neurotransmission supports the hypothesis that 5-HT$_6$ receptor agonists may be effective agents for the treatment of depression, particularly when glutamate levels are enhanced under pathologic circumstances.

Possible role of the 5-HT$_6$ receptor in anxiety

Few studies using 5-HT$_6$ receptor antisense oligonucleotides have explored the involvement of 5-HT$_6$ receptors in rodent models of anxiety, and the reported results have been inconsistent. In the Yoshioka et al. [118] study, seven days of 5-HT$_6$ receptor-directed antisense oligonucleotide treatment caused a 30% reduction in [^{3}H]-LSD binding sites, accompanied by a reduction in conditioned fear stress-induced serotonin release in the rat prefrontal cortex, which is suggestive of an anxiolytic-like response. Conversely, Hamon et al. [35] and Otano et al. [73] demonstrated anxiogenic-like activity following chronic administration of the same oligonucleotide sequence in two alternative rat models of anxiety: the elevated plus-maze and the social interaction tests, respectively. 5-HT$_6$ receptor-knockout mice, however, displayed few phenotypic abnormalities, except for differences in open field anxiety-related behaviors and in an elevated zero maze; i.e., there were no differences in total open quadrant dwell time, number of transitions between open and sheltered maze quadrants or head dips [9]. A major concern with data arising from this approach is that different developmental compensations may mask the true function of the receptor deleted, as is observed, for example, with 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor knockouts [1]. Additionally, the very low expression of 5-HT$_6$ receptors in the mouse brain [40] should call into question the value of using this species to examine basic 5-HT$_6$ receptor function.

Selective 5-HT$_6$ receptor antagonists

With the development of selective ligands, useful tools for further understanding the functions of 5-HT$_6$ receptors have been available. Recently, Wesołowska and Nikiforuk [109] have observed that the potent and selective 5-HT$_6$ receptor antagonist SB-399885 produced specific anxiolytic-like activity in animal models of anxiety. It dose-dependently and significantly increased the number of shocks accepted in the conflict drinking (Vogel) test in rats, a model that is widely used and considered to be one of the most specific methods for the detection of potential anxiolytic activity [66], without an effect on either the shock threshold or unpunished water consumption. This finding is supported by results obtained in an elevated plus maze test in rats, a procedure based on rodents' natural aversion to heights and open space. In this model, SB-399885 dose-dependently increased the percentage of time spent in and the number of entries into the open arms of the maze, without stimulating the general exploratory activity of rats detected in the open field test. Moreover, SB-399885 also had anti-anxiety-like activity in the four-plate test in mice [109]. Quantitatively, the potential anxiolytic effect of the 5-HT$_6$ receptor antagonist tested in all three models employed was approximately equivalent to that of diazepam, particularly in rats.

The potential anxiolytic-like effect of SB-399885 was not modified in rats whose serotonin neurons were destroyed by prior administration of *p*-chloramphetamine [108], which suggests that the above activity is not conditioned by the integrity of serotonin neurons. Such a concept is in line with neuroanatomical results that show that 5-HT$_6$ receptors are located outside serotonin neurons [32, 106]. Thus, the anti-conflict activity of SB-399885 observed in the Vogel test in rats does not depend on a serotonergic mecha-

nism. Wesołowska [108] has also demonstrated that the selective 5-HT_6 receptor antagonist tested helped to reveal the anticonflict action of diazepam; both were administered in a non-active dose. The additive effect of SB-399885 and diazepam may be regarded as a result of pharmacodynamic and/or pharmacokinetic interaction. Because the levels of SB-399885 and diazepam administered alone or jointly have not been analyzed, one cannot rule out a pharmacokinetic interaction at this stage of experimentation. Furthermore, the anticonflict activity of SB-399885 was reduced by the benzodiazepine receptor antagonist flumazenil, which was used at a dose reported to antagonize diverse effects of diazepam, including its anxiolytic-like effect [6, 26, 61, 108]. Because SB-399885 exhibits no affinity for GABA and benzodiazepine receptors [44], its anti-anxiety-like effect stems from a functional interaction between 5-HT_6 receptors and the GABA/benzodiazepine system. Such a conclusion is supported by neuroanatomical findings concerning the expression of 5-HT_6 receptor mRNA on GABAergic neurons [33, 105]. Moreover, Benes et al. [5] demonstrated an increase in 5-HT_6 receptor mRNA in the rat hippocampus after GABAergic transmission had been interrupted by a local infusion of picrotoxin. It has also been shown that another 5-HT_6 receptor antagonist, SB-357134, induced a concentration-dependent increase in the K^+-evoked GABA efflux in rat striatal slices [65].

Unfortunately, there is no information about the action of SB-399885 on the release of GABA in animal brain areas. However, it has been established that 5-HT_6 receptor antagonists, including SB-399885, increase the activity of the acetylcholine system [27, 44, 50, 60, 83, 98]. As has been presented in an excellent review written by Millan [66], cholinergic pathways do not play a pivotal role in the control of anxiety; however, some experimental evidence indicates that stimulation of cholinergic transmission is accompanied by anxiolytic-like activity in animals [3, 14, 24]. Thus, involvement of the cholinergic system in the anxiolytic-like activity of SB-399885 and in the synergistic effect of the 5-HT_6 receptor antagonist tested and diazepam cannot be excluded. On the other hand, Marcos et al. [65] demonstrated that the $GABA_A$ receptor antagonist bicuculline did not alter the acetylcholine release produced by SB-357134, another 5-HT_6 receptor antagonist. Further studies are necessary to explain this interaction.

The hippocampus seems to be an important site of action of anxiolytic compounds with diverse mechanisms. For example, 5-HT_{1A} receptor partial agonists, benzodiazepine receptor agonists, and ligands of ionotropic or metabotropic glutamatergic receptors exhibit anxiolytic-like effects in various models of anxiety after administration into the hippocampus [17, 66]. Similarly, the 5-HT_6 receptor blocker SB-258585, when injected into the CA1 region of the rat hippocampus, produced anxiolytic-like activity in the conflict drinking test [111]. Its effect seems to be specific because this agent, when administered at an anxiolytic dose, affected neither the shock threshold nor unpunished water consumption. However, its effect observed in the Vogel test in rats was weaker than that of diazepam.

Selective 5-HT_6 receptor agonists

As suggested by Schechter and his co-investigators [93], selective 5-HT_6 receptor agonists may play a potential therapeutic role in the treatment of some types of anxiety-related disorders. Thus, WAY-181187 and WAY-208466 [93], as well as compound **11q** [18], when administered acutely, effectively decreased water intake by rats that had not been water-deprived in the schedule-induced polydipsia test, a model considered to be predictive for efficacy in obsessive compulsive disorder [45]. Moreover, pharmacological studies have demonstrated that selective serotonin re-uptake inhibitors (SSRI) can also decrease adjunctive drinking in this test [115]; however, they have to be administered chronically to correspond with their clinical efficacy.

In addition to behavioral data, neurochemical results have revealed that selective 5-HT_6 receptor agonists increased extracellular levels of GABA in several areas of the rat brain associated with affective disorders [93]. Thus, an acute administration of WAY-181187 increased extracellular GABA concentrations in the rat frontal cortex, dorsal hippocampus, striatum, and amygdala; all effects were blocked by a selective 5-HT_6 receptor antagonist SB-271046. In addition to the acute effects, a fourteen-day treatment with another 5-HT_6 receptor agonist, WAY-208466, resulted in robust elevations in the extracellular levels of GABA in the rat dorsolateral frontal cortex, an ef-

fect similar in terms of magnitude and duration to that produced by WAY-181187 in the same brain region. These findings highlight the fact that chronic activation of 5-HT$_6$ receptors does not evoke desensitization. Moreover, employing *in vivo* microdialysis techniques, Schechter et al. [93] have also shown that WAY-181187 did not alter basal glutamate levels, but in hippocampal slices, this 5-HT$_6$ receptor agonist attenuated sodium azide-stimulated glutamate release.

In summary, the above-cited results support the contention that selective 5-HT$_6$ receptor antagonists, when administered peripherally or into the hippocampus, may evoke an anxiolytic-like effect that can possibly be explained by a functional interaction between 5-HT$_6$ receptors and the benzodiazepine system. The selective blockade of 5-HT$_6$ receptors evoked by SB-399885 may also facilitate the anticonflict effect of diazepam. Alternately, stimulation of 5-HT$_6$ receptors can also produce potential anxiolytic activity. The ability of 5-HT$_6$ receptor agonists to enhance extracellular GABA levels and decrease stimulated glutamatergic neurotransmission seems to support the hypothesis that 5-HT$_6$ receptor agonists may be effective agents for the treatment of anxiety.

Conclusions and prospective future for 5-HT$_6$ receptor ligands

Over the last several years, a number of studies have attempted to evaluate the potential role of the 5-HT$_6$ receptor in affective disorders. From the data provided in this review, it is evident that the 5-HT$_6$ receptor has emerged as a very interesting molecular target that interacts with antidepressant/anxiolytic drugs. It is uncertain whether antagonists or agonists of this receptor will best serve the potential therapeutic indications, i.e., depression and/or anxiety. This uncertainty arises from the observation that equivalent antidepressant and anxiolytic potency and efficacy can be delivered in animal models by both 5-HT$_6$ receptor antagonists and agonists. However, it should be kept in mind that the most of presented results concern the effects of 5-HT$_6$ receptor ligands after their acute administration. Yet, such findings may underline further preclinical studies performed after repeated administration of selective 5-HT$_6$ receptor ligands. Of particular interest is the observation that inhibition of the 5-HT$_6$ receptor synergistically potentiates the effect of clinically used antidepressants and anxiolytics. Combining a lower dose of an antidepressant and/or anxiolytic drug with a 5-HT$_6$ receptor antagonist might accelerate the onset of action and minimize the side effect profiles. The combination might also be very useful for patients who either does not respond to classic antidepressant/anxiolytic treatment or for whom monotherapy provides insufficient efficacy. Alternately, 5-HT$_6$ receptor agonists administered alone may have some advantages compared with SSRI, including acute onset of action in the treatment of some types of anxiety-related disorders as well as depressive symptoms. This finding opens up numerous possibilities for new individual therapies targeting the 5-HT$_6$ receptor or combinations of new drugs and current antidepressants/anxiolytics. But only clinical testing will determine the extent to which this approach shows distinct advantages over existing therapies and finally demonstrate the true innovation associated with this novel potential mechanism.

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Received:
October 19, 2009; in revised form: December 4, 2009.