Pharmacological Reports
2007, 59, 664–671
ISSN 1734-1140

Study into a possible mechanism responsible for the antidepressant-like activity of the selective 5-HT_6 receptor antagonist SB-399885 in rats

Anna Wesołowska

Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Anna Wesołowska, e-mail: wesolow@if-pan.krakow.pl

Abstract:
The mechanism of the antidepressant-like activity of the selective 5-hydroxytryptamine$_6$ (5-HT_6) receptor antagonist *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide (SB-399885) was studied in the forced swim test in rats. SB-399885 administered intraperitoneally at a single dose of 10 mg/kg potently shortened the immobility time in rats. That potential antidepressant-like effect of SB-399885 was not modified in animals with a lesion of the 5-HT system produced by *p*-chloroamphetamine (*p*-CA, 2 × 10 mg/kg). The anti-immobility effect of SB-399885 was blocked by the dopamine D_1- and D_2-like receptor antagonists SCH 23390 (0.063 mg/kg) and sulpiride (10 mg/kg), respectively, as well as by the α_2-adrenoceptor antagonist idazoxan (4 mg/kg), but it was not changed by the α_1-adrenoceptor antagonist prazosin (1 mg/kg). Neither sulpiride (10 mg/kg) or idazoxan (4 mg/kg) nor SCH-23390 (0.063 mg/kg) administered jointly with SB-399885 (10 mg/kg) noticeably changed the exploratory locomotor activity of rats evaluated by the open field test. The results described in the present paper indicate that the anti-immobility activity of SB-399885 is not connected with 5-HT innervation, and that D_1- and D_2-like receptors and α_2-adrenoceptors are involved in this action.

Key words:
5-HT_6 receptor antagonist, SCH 23390, sulpiride, idazoxan, prazosin, forced swim test, rats

Introduction

Pharmacological treatments that modify serotonergic transmission are widely used in the therapy of mental illnesses like depression. In the brain, serotonin (5-HT) is synthesized in restricted populations of neurons, located in the raphe nuclei which project to numerous brain regions. Multiple effects of 5-HT are mediated by its interaction with different receptor types which have recently been divided into seven families according to their cDNA-deduced primary sequences, signal transduction mechanisms and pharmacological profile [2].

5-HT_6 receptors, which are linked to G-protein stimulating adenylate cyclase [1, 20, 28, 36], occur exclusively in the mammalian central nervous system, and their highest densities have been found in the olfactory tubercle, striatum, nucleus accumbens, and moderate ones in the hypothalamus, thalamus, hippocampus and cerebral cortex [4, 14, 15, 43, 49]. The localization of 5-HT_6 receptors in corticolimbic regions and the relatively potent affinity and antagonistic activity of several antidepressants towards these receptors suggest that they may play a significant role in depression [3, 4, 25, 37].

The results presented by Yau et al. [48] showed that adrenalectomy and the blockade of glucocorticoid synthesis by metyrapone or aminoglutethimidine induced up-regulation of 5-HT_6 receptor mRNA in the rat hippocampus. As both above-mentioned blockers are used to treat drug-resistant depression in clinical practice [21, 26], the authors speculated that 5-HT_6 receptors may be involved in their effect. Furthermore, recent experiments with selective 5-HT_6 receptor antagonists (i.e. *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide (SB-399885) and SB-258585) have shown their significant antidepressant-like activity in the forced swim and tail suspension tests in mice and rats, which indicates that the blockade of 5-HT_6 receptors produces a potential antidepressant effect [27, 45, 46]. On the other hand, Svenningson et al. [40] reported that stimulation of 5-HT_6 receptors may evoke antidepressant-like activity, since 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine, a 5-HT_6 receptor agonist, decreased the immobility time of mice in the tail suspension test. Nevertheless, putative mechanisms involved in the antidepressant-like effects of 5-HT_6 receptor ligands have not been satisfactorily elucidated so far.

The present series of experiments were aimed at evaluating the mechanism of the antidepressant-like effect of SB-399885, a selective 5-HT_6 receptor antagonist [18], in the forced swim test in rats. SB-399885 was shown to be a potent ligand of human recombinant 5-HT_6 receptors (pK_i = 9.11) and of rat and human native receptors (pK_i = 8.81 and 9.02, respectively), with excellent selectivity (> 200-fold) over 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, 5-HT_7, α_{1B}, D_2, D_3, D_4 receptors, ion channels and enzymes [18]. SB-399885 displayed good central nervous system penetration and showed features of a 5-HT_6 receptor antagonist in the cAMP accumulation assay [17, 18]. Moreover, the extracellular levels of dopamine (DA), noradrenaline (NA) and acetylcholine (ACh) were elevated in the prefrontal cortex and hippocampus of freely moving rats after a single administration of SB-399885 [17, 18, 23]. In order to ascertain whether the integrity of 5-HT neurons was necessary to reveal the antidepressant-like activity of the selective 5-HT_6 receptor antagonist, the anti-immobility effect of SB-399885 was studied in the forced swim test in rats whose 5-HT neurons had been destroyed by prior administration of *p*-chloroamphetamine (*p*-CA). Moreover, the present paper examined the influence of D_1- and D_2-like receptor antagonists (SCH 23390 and sulpiride, respectively), as well as antagonists of α_1- and α_2-adrenoceptors (prazosin and idazoxan, respectively) on the antidepressant-like effect induced by SB-399885. The dosage and time schedules of SB-399885 were based on the results of our earlier studies [45], whereas the remaining antagonists were used at doses effective in blocking the effects induced by agonists of D_1- and D_2-like receptors as well as by agonists of α_1- and α_2-adrenoceptors [e.g. 16, 34, 35, 39, 41, 42].

Materials and Methods

Animals

The experiments were carried out on male Wistar rats (240–270 g) purchased from a licensed breeder (Górzkowska; Poland). The animals were kept under a natural dark-light cycle (January – June), in groups of eight in 60 × 38 × 20 cm cages at a temperature of 20 ± 1°C and with permanent free access to food (standard laboratory pellets) and water. All the experiments were conducted in the light phase between 09.00 and 14.00 h. Each experimental group consisted of 6–8 animals/dose, and the animals were used only once in each test. All the experimental procedures were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Substances used

p-Chloroamphetamine (*p*-CA; Sigma-Aldrich, Poland), *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide (SB-399885; GlaxoSmithKline, UK), idazoxan hydrochloride (Research Biochemicals Inc., USA), prazosin hydrochloride (Sigma-Aldrich, USA), SCH 23390 hydrochloride (Sigma, Poland), sulpiride (Sigma, USA) were used. Idazoxan, prazosin, SCH 23390 and *p*-CA were dissolved in distilled water, whereas SB-399885 and sulpiride were suspended in a 1% aqueous solution of Tween 80 immediately before administration. All the compounds were administered intraperitoneally (*ip*), except for idazoxan which was injected subcutaneously (*sc*) at a volume of 2 ml/kg. SB-399885 was given 30 min before the test, while the remaining antagonists tested were injected 60 min before. *p*-CA

was applied 9 and 8 days before the test. Control animals received a vehicle (a 1% Tween 80 or distilled water) according to the same schedule.

Forced swim test

The experiment was carried out according to the method of Porsolt et al. [30]. On the first day of experiment, the animals were gently individually placed in Plexiglas cylinders (40 cm high, 18 cm in diameter) containing 15 cm of water maintained at 25°C for 15 min. Upon removal from water, the rats were placed in a Plexiglas box for 30 min under a 60-W bulb to dry off. On the following day, the rats were placed again in the cylinder and the total duration of immobility was recorded throughout the 5-min test period. Fresh water was used for each animal.

Open field test

The experiment was performed in a darkened room according to the slightly modified method of Janssen et al. [19]. The centre of the open arena (1 m in diameter, divided into six symmetrical sectors without walls) was illuminated with a 75 W electric bulb hanging directly 75 cm above it. An individual vehicle- or drug-injected animal was gently placed in the centre of the arena and were allowed to explore freely. The time of walking, ambulation (the number of crossings of sector lines) and the number of rearing and peeping episodes (looking under the edge of the arena) were recorded for 5 min.

Data analysis

The results represent the mean ± SEM. The statistical significance of drugs' effects was evaluated using an analysis of variance (ANOVA), followed by Dunnett's test (when only one drug was given), or by the Newman-Keuls test (when two drugs were used).

Results

Forced swim test

The selective 5-HT_6 receptor antagonist SB-399885 (10 mg/kg) significantly [$F(3, 28) = 9.743$, $p < 0.001$] reduced the immobility time of rats in the forced swim test; its lower (3 mg/kg) and higher (20 mg/kg) doses had no pronounced effect in that test (Fig. 1). 5-HT depletion with *p*-CA (2 × 10 mg/kg) neither affected the immobility time by itself nor modified the anti-immobility action of SB-399885 (10 mg/kg) (Tab. 1). Sulpiride (10 mg/kg), SCH 23390 (0.063 mg/kg), prazosin (1 mg/kg) and idazoxan (4 mg/kg) administered alone were ineffective in the forced swim test (Fig. 2, 3). Sulpiride (10 mg/kg) significantly [$F(1, 28) = 5.921$, $p < 0.05$] inhibited the

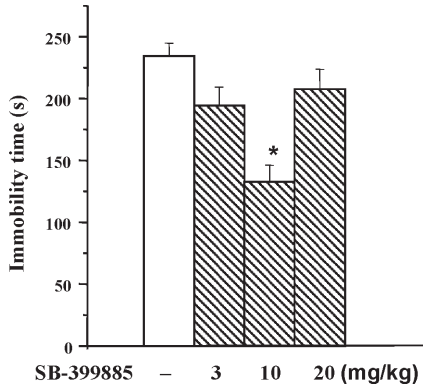

Fig. 1. The effect of SB-399885 on the immobility time in the forced swim test in rats. SB-399885 was administered 30 min before the test. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by Dunnett's test; * $p < 0.001$ *vs.* vehicle

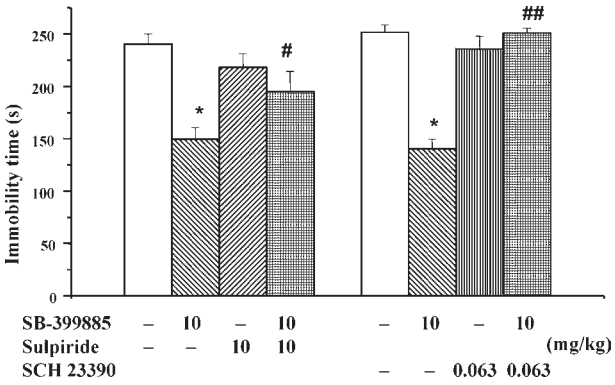

Fig. 2. The influence of sulpiride and SCH 23390 on the antidepressant-like effect induced by SB-399885 in the forced swim test in rats. SB-399885 was administered 30 min before the test, while sulpiride and SCH 23390 were given 60 min before. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by the Newman-Keuls test; * $p < 0.001$ *vs.* vehicle; # $p < 0.05$, ## $p < 0.001$ *vs.* SB-399885 group

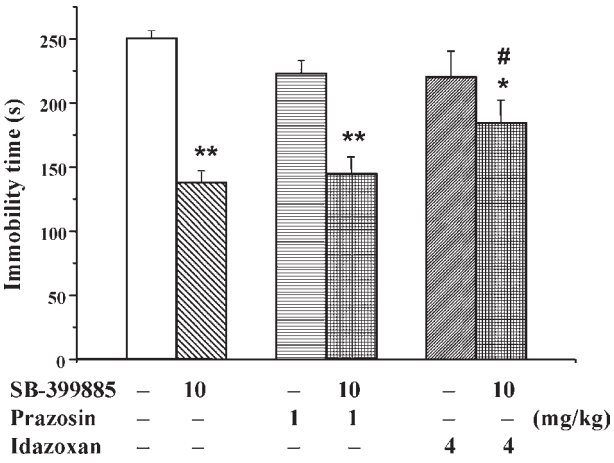

Fig. 3. The influence of prazosin and idazoxan on the antidepressant-like effect induced by SB-399885 in the forced swim test in rats. SB-399885 was administered 30 min before the test, while prazosin and idazoxan were given 60 min before. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by the Newman-Keuls test; * $p < 0.01$, ** $p < 0.001$ *vs.* vehicle; # $p < 0.05$ *vs.* SB-399885 group

Tab. 1. The effect of *p*-CA on the anti-immobility action of SB-399885 in the forced swim test in rats

Treatment and dose (mg/kg)	Immobility time (s)
Vehicle + vehicle	239.0 ± 8.8
p-CA + vehicle	227.6 ± 9.8
Vehicle + SB-399885 (10)	143.3 ± 11.5*
p-CA + SB-399885 (10)	161.4 ± 7.3*
	F(1, 28) = 2.422 ns

p-CA (10 mg/kg) was administered on two consecutive days (on the 9 and 8 days), while SB-399885 was given 30 min before the test. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by the Newman-Keuls test; * $p < 0.001$ *vs.* vehicle + vehicle group. ns – non-significant

antidepressant-like effect of SB-399885 (10 mg/kg) (Fig. 2). SCH 23390 (0.063 mg/kg) reversed the anti-immobility action evoked by SB-399885 (10 mg/kg) in a statistically significant manner [$F(1, 28) = 51.878$, $p < 0.001$] (Fig. 2). Prazosin (1 mg/kg) did not alter the anti-immobility effect of SB-399885 (10 mg/kg) [$F(1, 28) = 2.932$, ns] in the forced swim test, while idazoxan (4 mg/kg) significantly [$F(1, 28) = 6.875$, $p < 0.05$] reduced that effect in the same test (Fig. 3).

Open field test

SB-399885 (10 mg/kg) significantly decreased the time of walking, whereas the other parameters evaluated by the open field test, i.e. ambulation and peeping + rearing, remained unchanged (Tab. 2). Sulpiride (10 mg/kg) and idazoxan (4 mg/kg), administered alone or in a combination with SB-399885 (10 mg/kg), did not change the exploratory locomotor activity of rats in the open field test (Tab. 2). SCH 23390 (0.063 mg/kg) administered alone did not change the exploratory locomotor activity of rats, whereas the combined administration of SCH 23390 (0.063 mg/kg) and SB-399885 (10 mg/kg) significantly decreased peeping + rearing, the time of walking and ambulation having remained unchanged (Tab. 2).

Discussion

In line with our earlier study [45], the currently described results indicate that the selective 5-HT_6 receptor antagonist SB-399885 [18], used at a dose of 10 mg/kg, exerts antidepressant-like activity in rats by shortening the immobility time in the forced swim test. This effect seems to be specific, since SB-399885 at an antidepressant-like dose does not stimulate the activity of rats, as shown in the open field test. The antidepressant-like activity of SB-399885 is most probably connected with its 5-HT_6 receptor antagonistic properties, since this compound is a selective ligand and blocker of 5-HT_6 sites [18]. Hence, direct involvement of other receptors in its effect ought to be excluded.

The shortening of immobility time, induced by antidepressant drugs in the forced swim test, depends on the enhancement of the central 5-HT and catecholamine neurotransmission [5, 7, 31, 32]. Unfortunately, no information is available on the effect of SB-399885 on the levels of 5-HT. A microdialysis study has only shown that its analogue SB-271046, another selective 5-HT_6 receptor antagonist, has no influence on the basal release of 5-HT [11, 22]. The results obtained in the present experiment demonstrate that administration of *p*-CA, which under our laboratory conditions reduces cortical and hippocampal concentrations of 5-HT and 5-hydroxyindoleacetic acid in rats by ca. 85–89% and 81–86%, respectively [9, 47], does not

Tab. 2. The effect of SB-399885, sulpiride, SCH 23390 and idazoxan, given alone or in a combination, on exploratory activity evaluated by the open field test in rats

Treatment (mg/kg)	Exploratory activity		
	Walking time (s)	Ambulation	Peeping + rearing
Vehicle	60.0 ± 7.4	12.5 ± 1.0	13.0 ± 1.1
SB-399885 (10)	25.2 ± 3.9**	11.3 ± 1.0	10.3 ± 1.0
	F(1, 10) = 17.327 p < 0.01	F(1, 10) = 0.671 ns	F(1, 10) = 3.265 ns
Vehicle + vehicle	53.0 ± 3.1	17.5 ± 1.8	14.3 ± 2.1
Vehicle + sulpiride (10)	53.7 ± 3.6	18.3 ± 1.8	17.0 ± 1.8
Sulpiride (10) + SB-399885 (10)	44.3 ± 4.9	13.5 ± 1.2	10.5 ± 1.6
	F(2, 15) = 2.143 ns	F(2, 15) = 2.915 ns	F(2, 15) = 3.131 ns
Vehicle + vehicle	53.0 ± 6.6	14.8 ± 1.3	15.3 ± 1.9
SCH 23390 (0.063)	50.2 ± 7.3	15.8 ± 1.9	14.3 ± 1.3
SCH 23390 (0.063) + SB-399885 (10)	31.0 ± 6.3	11.2 ± 1.3	8.7 ± 1.1*
	F(2, 15) = 3.125 ns	F(2, 15) = 2.657 ns	F(2, 15) = 6.123 p < 0.05
Vehicle + vehicle	59.3 ± 3.5	17.0 ± 1.8	14.7 ± 2.3
Idazoxan (4)	54.0 ± 5.5	14.2 ± 1.2	13.5 ± 2.2
Idazoxan (4) + SB-399885 (10)	45.8 ± 7.8	11.0 ± 1.9	11.2 ± 2.2
	F(2, 15) = 1.348 ns	F(2, 15) = 3.367 ns	F(2, 15) = 0.636 ns

SB-399885 was administered 30 min before the test, while sulpiride, SCH 23390 and idazoxan were given 60 min before. The animals were observed for 5 min. The results represent the mean ± SEM of 6 rats. The data were statistically evaluated by ANOVA, followed by the Newman-Keuls test; * $p < 0.05$, ** $p < 0.01$ *vs.* vehicle. ns – non-significant

modify the effect of SB-399885 in the forced swim test. Hence, it is proposed that the anti-immobility effect of SB-399885 does not actually require any integrity of 5-HT neurons. Such concept is in good agreement with the neuroanatomical data showing that 5-HT_6 receptors are located outside 5-HT neurons [14]. Additionally, Ward et al. [43] found 5-HT_6 receptor mRNA in 5-HT projection fields, which may suggest their postsynaptic localization. It is noteworthy that also the anxiolytic-like effect of SB-399885 in rats does not seem to be conditioned by the integrity of 5-HT neurons, since it was not altered by the lesion of 5-HT neurons [44].

The present results also demonstrate that catecholamine systems play an important role in the anti-immobility action of SB-399885, since this effect 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; all three antagonists *per se* did not induce any antidepressant-like effect. It is noteworthy that neither SCH 23390 or sulpiride nor idazoxan at the doses used noticeably modified the exploratory locomotor activity of rats, hence, their antagonism towards SB-399885 in the forced swim test cannot be attributed to a competing behavior, like, for instance, locomotor activity. Interestingly, SB-399885 does not decrease the walking time of rats treated earlier with SCH 23390, sulpiride or idazoxan, as observed after administration of SB-399885 alone. On the other hand, prazosin, an α_1-adrenoceptor antagonist, did not change the anti-immobility action produced by SB-399885.

Some other data also seem to support the assumption that a dopaminergic mechanism may be involved in the functional effects of SB-399885. In fact, although SB-399885 does not bind to DA receptors [18], it increases basal extracellular DA concentration in rat hippocampus and prefrontal cortex and enhances the haloperidol- and risperidone-induced increases in DA efflux in both these regions [17, 23]. Moreover, combined administration of non-active doses of SB-399885 and the antidepressant bupropion, whose mechanism of action is connected with DA reuptake inhibition, produces significant anti-immobility action in the forced swim test in rats [27]. Furthermore, it has been shown that other 5-HT_6 receptor antagonists can potentiate the amphetamine-evoked behavioral actions and increases in the extracellular levels of DA in rat frontal cortex, nucleus accumbens and striatum [12, 13, 33]. All the above-described results seem to suggest that 5-HT_6 receptor blockade has modulatory influence on DA neurotransmission, and that D_1- and D_2-like receptors are important to the antidepressant-like activity of SB-399885.

The results of *in vitro* experiments have revealed that SB-357134, another selective 5-HT_6 receptor antagonist, induces glutamate release *via* AMPA receptors which, in turn, modulate DA efflux; in consequence, the released DA may exert facilitating effect on ACh release *via* D_1 receptors in the striatum and D_2 ones in the frontal cortex, since both D_1 and D_2 antagonists (SCH 23390 and haloperidol, respectively) block the SB-357134-induced ACh efflux [24].

It is unlikely that the antidepressant-like effect of SB-399885 develops as a consequence of enhanced ACh release [18], since Shytle et al. [38] have presented some data suggesting that depressed mood states are associated with hypercholinergic neurotransmission. Furthermore, anticholinergic drugs reduce the immobility time of mice in the forced swim and tail suspension tests [6, 10] and enhance the antidepressant-like effects of imipramine [29].

It has also been presented that SB-399885 shows no affinity for adrenergic receptors [18], but increases extracellular NA level in the prefrontal cortex of freely moving adult rats [17]. Recently, we have demonstrated that SB-399885 administered jointly with desipramine (both given at non-active doses) produces a pronounced anti-immobility effect in the forced swim test in rats [27], which suggests that NA-mediated neurotransmission is likely to be involved in the antidepressant-like activity observed after combined administration of the selective 5-HT_6 receptor antagonist (SB-399885) and desipramine. By showing that an α_2-adrenoceptor antagonist (idazoxan), but not an α_1-adrenoceptor antagonist (prazosin), inhibits the anti-immobility effect of SB-399885, the present results leave no doubt that NA neurotransmission plays some role in the potential antidepressant activity of the tested 5-HT_6 receptor antagonist, and that α_2-adrenoceptors are essential to this effect.

The importance of DA and NA systems to the antidepressant-induced anti-immobility effect has been thoroughly investigated. Indeed, it has been shown that D_1- and D_2-like antagonists, including SCH 23390 and sulpiride, abolish the anti-immobility activity of various antidepressants [7, 16, 35], and that the idazoxan-produced α_2-adrenoceptor blockade prevents the antidepressant-like effect of desipramine [8, 34]. On the other hand, idazoxan potentiates the anti-immobility effect evoked by combined administration of desipramine and fluoxetine or minalcipran [34].

In conclusion, the results obtained in the present study indicate that the antidepressant-like effect of SB-399885 in the forced swim test in rats is not connected with serotonergic innervation and activation of DA and NA systems – *via* D_1- and D_2-like receptors as well as α_2-adrenoceptors – seems to be crucial for the antidepressant-like activity of SB-399885 in the model used.

Acknowledgments:
The author is grateful to Prof. Ewa Chojnacka-Wójcik for valuable scientific discussion. She also wishes to thank GlaxoSmithKline for their generous gift of SB-399885, and gratefully acknowledges the excellent technical assistance of Ms. Lucyna Socha. The study was supported by the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

References:

1. Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K et al.: Stimulation of type 1 and type 8 Ca^{2+}/Calmodulin-sensitive adenylyl cyclases by the G_s-coupled 5-hydroxytryptamine subtype 5-HT_{7A} receptor. J Biol Chem, 1998, 273, 17469–17476.
2. Boess FG, Martin IL: Molecular biology of 5-HT receptors. Neuropharmacology, 1994, 33, 275–317.
3. Boess FG, Monsma FJ, Carolo C, Meyer V, Rudler A, Zwingelstein C, Sleight AJ: Functional and radioligand binding characterization of rat 5-HT_6 receptors stably expressed in HEK293 cells. Neuropharmacology, 1997, 36, 713–720.

4. Boess FG, Riemer C, Bös M, Bentley J, Bourson A, Sleight AJ: The 5-hydroxytryptamine$_6$ receptor-selective radioligand [^{3}H]Ro 63-0563 labels 5-hydroxytryptamine receptor binding sites in rat and porcine striatum. Mol Pharmacol, 1998, 54, 577–583.
5. Borsini F: Role of the serotonergic system in the forced swimming test. Neurosci Biobehav Rev, 1995, 19, 377–395.
6. Borsini F, Meli A: Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology, 1988, 94, 147–160.
7. Borsini F, Meli A: The forced swimming test: its contribution to the understanding of the mechanisms of action of antidepressants. Adv Biosci, 1990, 77, 63–76.
8. Cervo L, Grignaschi G, Samanin R: Alpha 2-adrenoceptor blockade prevents the effect of desipramine in the forced swimming test. Eur J Pharmacol, 1990, 175, 301–307.
9. Chojnacka-Wójcik E, Tatarczyńska E, Gołembiowska K, Przegaliński E: Involvement of 5-HT$_{1A}$ receptors in the antidepressant-like activity of gepirone in the forced swimming test in rats. Neuropharmacology, 1991, 30, 711–717.
10. Cryan JF, Mombereau C, Vassout A: The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev, 2005, 29, 571–625.
11. Dawson LA, Nguyen HQ, Li P: The 5-HT$_6$ receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology, 2001, 25, 662–668.
12. Dawson LA, Nguyen HQ, Li P: Potentiation of amphetamine-induced changes in dopamine and 5-HT by a 5-HT$_6$ receptor antagonist. Brain Res Bull, 2003, 59, 513–521.
13. Frantz KJ, Hansson KJ, Stouffer DG, Parsons LH: 5-HT$_6$ receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. Neuropharmacology, 2002, 42, 170–180.
14. Gerard C, Martres MP, Lefevre K, Miquel MC, Verge D, Lanfumey L, Doucet E et al.: Immuno-localization of serotonin 5-HT$_6$ receptor-like material in the rat central nervous system. Brain Res, 1997, 23, 207–219.
15. Gerard C, el Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, Hamon M, Martres MP: Quantitative RT-PCR distribution of serotonin 5-HT$_6$ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse, 1996, 23, 164–173.
16. Hirano S, Miyata S, Onodera K, Kamei J: Involvement of dopamine D$_1$ receptors and α_1-adrenoceptors in the antidepressant-like effect of chlorpheniramine in the mouse tail suspension test. Eur J Pharmacol, 2007, 562, 72–76.
17. Hirst WD, Moss SF, Bromidge SM, Riley G, Stean TO, Rogers DC, Sunter D et al.: Characterisation of SB-399885, a potent and selective 5-HT$_6$ receptor antagonist. Soc Neurosci Meet, 2003, 576.7. (abstr.).
18. Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM et al.: SB-399885 is a potent, selective 5-HT$_6$ receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol, 2006, 553, 109–119.
19. Janssen PAJ, Jagenau AHM, Schellekens KHC: Chemistry and pharmacology of compound related to 4-(4-hydroxy-4-phenylpiperidino)-butyrophenone. IV. Influence of haloperidol (R-1625) and chloropromazine on the behaviour of rats in an unfamiliar open field situation. Psychopharmacologia, 1960, 1, 389–392.
20. Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY et al.: Cloning, characterization, and chromosomal localization of a human 5-HT$_6$ serotonin receptor. J Neurochem, 1996, 66, 47–56.
21. Kramlinger KG, Peterson GC, Watson PK, Leonard LL: Metyrapone for depression and delirium secondary to Cushing's syndrome. Psychomatics, 1985, 26, 67–71.
22. Lacroix LP, Dawson LA, Hagan JJ, Heidbreder CA: 5-HT$_6$ receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. Synapse, 2004, 51, 158–164.
23. Li Z, Huang M, Prus AJ, Dai J, Meltzer HY: 5-HT$_6$ receptor antagonist SB-399885 potentiates haloperidol and risperidone-induced dopamine efflux in the medial prefrontal cortex or hippocampus. Brain Res, 2007, 1134, 70–78.
24. Marcos B, Gil-Bea FJ, Hirst WD, Garcia-Alloza M, Ramirez MJ: Lack of localization of 5-HT$_6$ receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT$_6$ receptor-mediated acetylcholine release. Eur J Neurosci, 2006, 24, 1299–1306.
25. Monsma FJ, Shen Y, Ward RP, Hamblin MW, Sibley DR: Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol, 1993, 43, 320–327.
26. Murphy BE, Dhar V, Ghadirian AM, Chouinard G, Keller R: Response to steroid suppression in major depression resistant to antidepressant therapy. J Clin Psychopharmacol, 1991, 11, 121–126.
27. Nikiforuk A, Wesołowska A, Popik P: Effect of combined administration of the selective serotonin$_6$ receptor antagonist SB-399885 and antidepressants in the forced swim test in rats. Soc Neurosci Meet, 2007, 709.7. (abstr.).
28. Plassat JL, Amlaiky N, Hen R: Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. Mol Pharmacol, 1993, 44, 229–236.
29. Popik P, Kozela E, Krawczyk M: Nicotine and nicotinic receptor antagonists potentiate the antidepressant-like effects of imipramine and citalopram. Br J Pharmacol, 2003, 139, 1196–1202.
30. Porsolt RD, Anton G, Blavet N, Jalfre M: Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol, 1978, 47, 379–391.
31. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M: Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol, 1979, 57, 201–210.
32. Porsolt RD, Bertin A, Jalfre M: Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther, 1977, 229, 327–336.

33. Pullagurla M, Bondareva T, Young R, Glennon RA: Modulation of the stimulus effects of (+)amphetamine by the 5-HT_6 antagonist MS-245. Pharmacol Biochem Behav, 2004, 78, 263–268.
34. Rénéric JP, Bouvard M, Stinus L: Idazoxan and 8-OH-DPAT modify the behavioral effects induced by either NA, or 5-HT, or dual NA/5-HT reuptake inhibition in the rat forced swimming test. Neuropsychopharmacology, 2001, 24, 379–390.
35. Rogóż Z, Skuza G: Mechanism of synergistic action following co-treatment with pramipexole and fluoxetine or sertraline in the forced swimming test in rats. Pharmacol Rep, 2006, 58, 493–500.
36. Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, Schwartz JC: A novel rat serotonin (5-HT_6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. Biochem Biophys Res Commun, 1993, 193, 268–276.
37. Sebben M, Ansanay H, Bockaert J, Dumuis A: 5-HT_6 receptors positively coupled to adenylyl cyclase in striatal neurons in culture. Neuroreport, 1994, 5, 2553–2557.
38. Shytle RD, Silver AA, Lukas RJ, Newman MB, Sheehan DV, Sanberg PR: Nicotinic acetylcholine receptors as targets for antidepressants. Mol Psychiatry, 2002, 7, 525–535.
39. Stuchlik A, Rehakova L, Telensky P, Vales K: Morris water maze learning in Long-Evans rats is differentially effected by blockade of D_1-like and D_2-like dopamine receptors. Neurosci Lett, 2007, 422, 169–174.
40. Svenningsson P, Tzavara ET, Qi H, Carruthers R, Witkins JM, Nomikos GG, Greengard P: Biochemical and behavioral evidence for antidepressant-like effects of 5-HT_6 receptor stimulation. J Neurosci, 2007, 27, 4201–4209.
41. Takamori K, Yoshida S, Okuyama S: Repeated treatment with imipramine, fluvoxamine and tranylcypromine decreases the number of escape failures by activating dopaminergic systems in a rat learned helplessness test. Life Sci, 2001, 69, 1919–1926.
42. Villegier A-S, Lotfipour S, McQuown SC, Belluzzi JD, Leslie FM: Tranylcypromine enhancement of nicotine self-administration. Neuropharmacology, 2007, 52, 1415–1425.
43. Ward RP, Hamblin MW, Lachowicz JE, Hoffman BJ, Sibley DR, Dorsa DM: Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. Neuroscience, 1995, 64, 1105–1111.
44. Wesołowska A: The anxiolytic-like effect of the selective 5-HT_6 receptor antagonist SB-399885: the impact of benzodiazepine receptors. Eur J Pharmacol, 2008, in press.
45. Wesołowska A, Nikiforuk A: Effects of the brain-penetrant and selective 5-HT_6 receptor antagonist SB-399885 in animal models of anxiety and depression. Neuropharmacology, 2007, 52, 1274–1283.
46. Wesołowska A, Nikiforuk A, Stachowicz K: Anxiolytic- and antidepressant-like effects produced by the selective 5-HT_6 receptor antagonist SB-258585 after its intrahippocampal administration to rats. Behav Pharmacol, 2007, 18, 439–446.
47. Wesołowska A, Paluchowska MH, Chojnacka-Wójcik E: Involvement of presynaptic 5-HT_{1A} and benzodiazepine receptors in the anticonflict activity of 5-HT_{1A} receptor antagonists. Eur J Pharmacol, 2003, 471, 27–34.
48. Yau JLW, Noble J, Widdowson J, Seckl JR: Impact of adrenalectomy on 5-HT_6 and 5-HT_7 receptor gene expression in the rat hippocampus. Mol Brain Res, 1997, 45, 182–186.
49. Yoshioka M, Matsumoto M, Togashi H, Mori K, Saito H: Central distribution and function of 5-HT_6 receptor subtype in the rat brain. Life Sci, 1998, 62, 1473–1477.

Received:
September 9, 2007; in revised form: November 20, 2007.