Abstract:
The mechanism of the antidepressant-like activity of the selective 5-hydroxytryptamine_6 (5-HT_6) receptor antagonist \( \text{N-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide} \) (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 \( \times \) 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 \( \alpha_2 \)-adrenoceptor antagonist idazoxan (4 mg/kg), but it was not changed by the \( \alpha_1 \)-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 \( \alpha_2 \)-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].
Mechanism of the antidepressant-like activity of SB-399885 in rats
Anna Wesołowska

The results presented by Yau et al. [48] showed that adrenalectomy and the blockade of glucocorticoid synthesis by metyrapone or aminoglutethimide 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; Glaxo-SmithKline, 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](image1)

![Fig. 2. The influence of sulpiride and SCH 23390 on the antidepression-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](image2)
In line with our earlier study [45], the currently described results indicate that the selective 5-HT6 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-HT6 receptor antagonistic properties, since this compound is a selective ligand and blocker of 5-HT6 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-HT6 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
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-HT6 receptors are located outside 5-HT neurons [14]. Additionally, Ward et al. [43] found 5-HT6 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 D1- and D2-like receptor antagonists SCH 23390 and sulpiride, respectively, and by the $\alpha_2$-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 $\alpha_1$-adrenoceptor antagonist, did not change the anti-immobility action produced by SB-399885.

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

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.
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-HT6 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-HT6 receptor blockade has modulatory influence on DA neurotransmission, and that D1- and D2-like receptors are important to the antidepressant-like activity of SB-399885.

The results of \textit{in vitro} experiments have revealed that SB-357134, another selective 5-HT6 receptor antagonist, induces glutamate release \textit{via} AMPA receptors which, in turn, modulate DA efflux; in consequence, the released DA may exert facilitating effect on ACh release \textit{via} D1 receptors in the striatum and D2 ones in the frontal cortex, since both D1 and D2 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-HT6 receptor antagonist (SB-399885) and desipramine. By showing that an \(\alpha_2\)-adrenoceptor antagonist (idazoxan), but not an \(\alpha_1\)-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-HT6 receptor antagonist, and that \(\alpha_2\)-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 D1- and D2-like antagonists, including SCH 23390 and sulpiride, abolish the anti-immobility activity of various antidepressants [7, 16, 35], and that the idazoxan-produced \(\alpha_2\)-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 – \textit{via} D1- and D2-like receptors as well as \(\alpha_2\)-adrenoceptors – seems to be crucial for the antidepressant-like activity of SB-399885 in the model used.

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