Pharmacological Reports
2008, 60, 464–474
ISSN 1734-1140

Influence of serotonin 5-HT$_7$ receptor blockade on the behavioral and neurochemical effects of imipramine in rats

Anna Wesołowska[1], Magdalena Kowalska[2]

[1]Department of Behavioral Neuroscience and Drug Development, [2]Department of Pharmacology, 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 aim of the present study was to examine the effect of the selective 5-HT$_7$ receptor antagonist (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine (SB-269970), administered alone or in combination with imipramine, on the immobility time of rats in the forced swim test as well as on the extracellular levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT) and their metabolites in the prefrontal cortex of freely moving rats. Both compounds were administered intraperitoneally (*ip*). Like imipramine (30 mg/kg, but not 20 mg/kg), SB-269970 (1.25 and 2.5 mg/kg, but not 0.625 mg/kg) significantly shortened the immobility time of rats without affecting their exploratory locomotor activity measured in the open field test. SB-269970 (0.625 and 1.25 mg/kg) raised the extracellular levels of DA, NA, 5-HT and their metabolites in rat prefrontal cortex. In that structure, imipramine (20 mg/kg) produced an increase in all the neurotransmitters measured, but failed to affect the levels of their metabolites. A combination of the inactive doses of SB-269970 (0.625 mg/kg) and imipramine (20 mg/kg) found in the forced swim test produced antidepressant-like effect, which did not stem from the increased exploratory locomotor activity. At the same time, that combination evoked a vast increase in the output of NA – but not DA and 5-HT – compared to the effects of both those drugs given alone. These results open up a possibility that the stimulating effect of SB-269970 on DA, NA and 5-HT transmission in the prefrontal cortex plays some role in the antidepressant-like activity of this compound. Moreover, these findings suggest that the increase in cortical NA level seems to account for the anti-immobility action observed after joint administration of the selective 5-HT$_7$ receptor antagonist and imipramine in rats.

Key words:
5-HT$_7$ receptor antagonist, SB-269970, imipramine, forced swim test, microdialysis, rats

Introduction

There exists extensive scientific literature concerning involvement of the brain serotonin (5-HT) system in the pathogenesis of depression and its role in the action of antidepressant drugs. Since the delayed onset of their action and a fairly high percentage of non-responders to specific antidepressant drug treatments are the major drawbacks of commonly used antidepressants, efforts are made to improve their action. It has been conclusively documented that antidepressants with a different mechanism of action, combined with selective and non-selective antagonists of 5-HT$_{1A/1B}$, 5-HT$_{2A/2C}$, 5-HT$_3$, 5-HT$_6$ or 5-HT$_7$ receptors, exert a pronounced antidepressant-like effect in animal models of depression [1, 9, 21, 23, 25, 35, 36, 41, 42, 49, 52].

Recently marked attention has been focused on 5-HT_7 receptors because of their potential role in mood disorders including depression. In fact, 5-HT_7 receptors are localized in corticolimbic areas [13, 22, 30, 38, 39, 46, 48] which are involved in affective processes; also some studies indicate both down-regulation of 5-HT_7 receptors and reduction of the effectiveness of rat hippocampal 5-HT_7 receptor activation after chronic treatment with various antidepressants [29, 45]. In contrast, electrophysiological investigations carried out in rat hippocampal slices have shown an enhancement of the excitatory effect of the activation of 5-HT_7 receptors after electroconvulsive shocks delivered ten times and no influence of repeated administration of an atypical antidepressant tianeptine or zinc hydroaspartate, a compound exhibiting antidepressant-like activity, on the studied effect [32].

Despite the latter facts, further support of the role of 5-HT_7 receptors in depression comes from *in vivo* experiments conducted on 5-HT_7 receptor knockout mice, which have demonstrated antidepressant-like behavior of these animals compared to wild-type mice in the forced swim [11, 15] and the tail suspension [15] tests. Furthermore, a smaller amount of rapid eye movement (REM) sleep occurs in 5-HT_7 receptor knockout mice and they have less frequent REM episodes [15]. In agreement with these data, a significant antidepressant-like effect was observed after administration of the selective 5-HT_7 receptor antagonist (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine (SB-269970) in the forced swim and tail suspension tests in mice [3, 15, 50–52]. Moreover, selective 5-HT_7 receptor antagonists including SB-269970 have been shown to increase the latency of REM sleep and to decrease its duration [3, 14, 15, 43], and thus to induce changes in sleep parameters in a pattern opposite to that found in patients with clinical depression.

It has also been demonstrated that citalopram, a selective 5-HT reuptake inhibitor, decreases the immobility time of 5-HT_7 receptor knockout mice and wild-type controls in the tail suspension test, and that its effect on 5-HT_7 receptor knockout mice is additive to that of the genotype alone [15]. This observation is in line with the data provided by Wesołowska et al. [52], showing that the 5-HT_7 receptor blockade induced by SB-269970 facilitates the anti-immobility effect of citalopram in the mouse forced swim test without increasing spontaneous locomotor activity. The latter study was extended by Bonaventure et al. [3] who demonstrated that joint administration of SB-269970 and a non-effective dose of citalopram produced a significant decrease in immobility time in the tail suspension test in mice. Moreover, those authors ruled out a possible pharmacokinetic interaction between SB-269970 and citalopram. In addition, the combination of SB-269970 and citalopram significantly delayed REM sleep latency and enhanced the decrease in REM sleep duration compared to citalopram alone [3]. Wesołowska et al. [52] also found that the selective blockade of 5-HT_7 receptors may have a synergistic effect with the inhibition of noradrenaline (NA) reuptake, as well as with the inhibition of monoamine oxidase-A, since combined administration of non-active doses of SB-269970 and imipramine, desipramine or moclobemide significantly shortened the immobility time of mice in the forced swim test.

In the light of the above observations, we tried to ascertain whether the selective 5-HT_7 receptor antagonist SB-269970 was able to exert an antidepressant-like effect in the rat forced swim test; moreover, we simultaneously examined the modulation of the dialysate levels of dopamine (DA), NA and 5-HT in the prefrontal cortex of freely moving rats after SB-269970 administration. All the same, the primary aim of our present research was to study the effect of joint administration of non-active doses of SB-269970 and the 5-HT/NA reuptake inhibitor imipramine, observed in the forced swim test, on the immobility time of rats in that test and on DA, NA and 5-HT efflux in a microdialysis assay. To the best of our knowledge, the effect of combined administration of selective 5-HT_7 receptor antagonists and imipramine has not been studied in rats so far. Furthermore, the present study has also been the first to determine the influence of the 5-HT_7 receptor antagonist and its joint administration with imipramine on biogenic amine levels in the extracellular space in live, conscious and freely moving rats.

Materials and Methods

Animals

The experiments were conducted on male Wistar rats (250–300 g) bred at the Institute of Pharmacology, Polish Academy of Sciences in Kraków, Poland. The rats were housed in temperature- and humidity-

controlled rooms on a 12-h light/dark cycle, with *ad libitum* access to filtered tap water and standard pelleted laboratory chow throughout the study. All the experiments were performed in a light phase between 09.00 and 17.00 h, on separate groups of animals; each animal was used only once in a test. The testing was carried out by an observer unaware of the treatment. The experimental procedures and the housing conditions were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Drugs used

The following drugs were used: (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine (hydrochloride, SB-269970; Tocris, UK) and imipramine (hydrochloride; Polfa, Poland). Both compounds were dissolved in distilled water and administered intraperitoneally (*ip*) in a volume of 2 ml/kg. SB-269970 and imipramine were given at 30 and 60 min, respectively, before the forced swim test. Control animals received a vehicle injection according to the same schedule.

Forced swim test

The experiment was carried out according to the method of Porsolt et al. [33]. 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 a 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. [17]. 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. A single vehicle- or drug-injected animal was gently placed in the centre of the arena and 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.

Microdialysis study

The rats were anesthetized with ketamine (75 mg/kg *im*) and xylazine (10 mg/kg *im*) and placed in stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Their skulls were exposed, and small holes were drilled for insertion of the microdialysis probes into the prefrontal cortex using the following coordinates: 2.9 mm anterior from the bregma; 0.8 mm lateral from the sagittal suture; –4.5 mm ventral from the dura surface (Fig. 1) [31]. Microdialysis probes were constructed by inserting two fused silica tubes (30 and 35 mm long, 150 μm o.d.; Polymicro Technologies Inc., Phoenix, AZ, USA) into a microdialysis fiber (220 μm o.d.; AN69, Hospal, Bologna, Italy). The tube assembly was placed in a stainless steel cannula (22G, 10 mm) forming the shaft of the probe. Portions of the inlet and outlet tubes were individually placed inside polyethylene PE-10 tubing and were glued for protection. The free end of the dialysis fibre was sealed, and 3 mm of the exposed length was used for dialysis in the prefrontal cortex. One day after the surgery and probe implantation, the inlet of the dialysis probes was connected to a syringe pump (BAS, IN, USA) which delivered an artificial cerebrospinal fluid composed of [in mM] NaCl 145, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2; pH = 7.4 at a flow rate of 1.5 μl/min. Baseline samples were collected every 20 min after a washout period to obtain a stable extracellular neurotransmitter level. Respective drugs were then administered, and dialysate fractions were collected for 240 min. At the end of the experiment, the rats were sacrificed and their brains were histologically examined to validate the correct probe placement.

DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NA, as well as 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were analysed by HPLC with electrochemical detection. Chromatography was performed using an LC-10 AD pump (Shimadzu Europa GmbH, Warszawa, Poland), an LC-4B amperometric detector with a cross-flow detector cell (BAS, IN, USA) and BDS-Hypersil C18 analytical column (3 × 100 mm, a 3 μm, Thermo Electron Corp., UK). The mobile phase was composed of 0.1 M monochloroacetic acid adjusted to

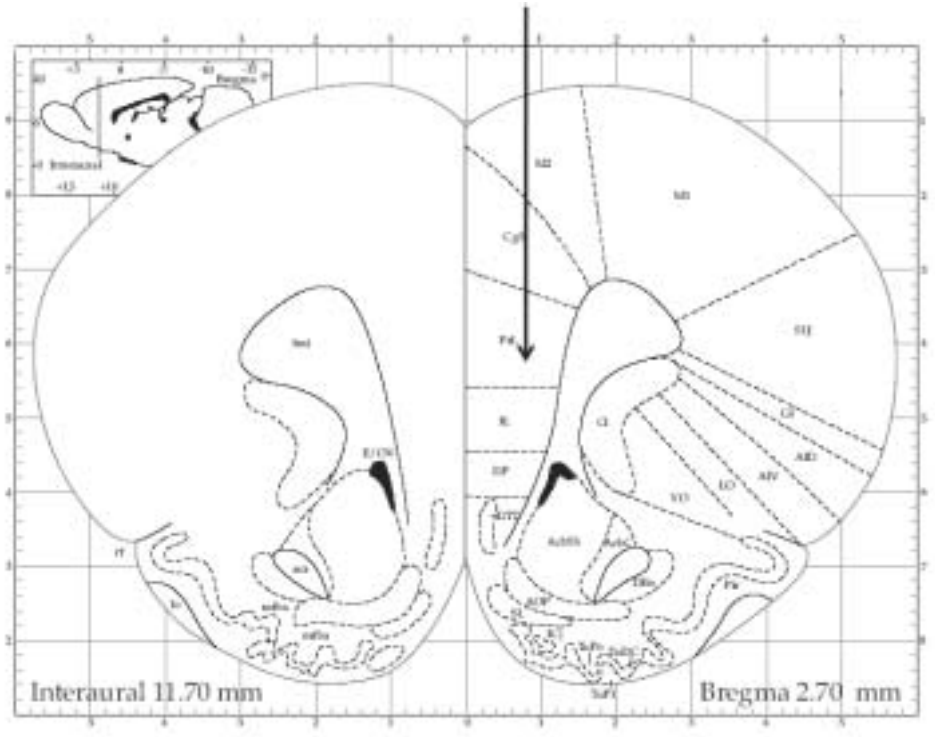

Fig. 1. Placement of microdialysis probe in the prefrontal cortex

pH = 3.7 with 3 M sodium hydroxide, 0.5 m M EDTA, 25 mg/l 1-octanesulfonic acid sodium salt, a 5.7% methanol and a 2.5% acetonitrile. The flow rate was 0.5 ml/min, and the applied potential of a 3-mm glassy carbon electrode was +600 mV with a sensitivity of 2 nA/V. NA was measured using the HPLC system equipped with a P580 pump (Dionex, CA, USA) connected to a BAS injection valve with a 10 μl injection loop and a BDS-Hypersil analytical column (2.0 × 100 mm, a 3 μm, Thermo Electron Corp., UK). The mobile phase was composed of 0.05 M KH_2PO_4 (adjusted to pH = 3.7 with orthophosphoric acid), 0.5 mM EDTA, 150 mg/l 1-octanesulfonic acid sodium salt, 10 mM NaCl and a 1.2% acetonitrile. The flow rate was 180 μl/min. NA was detected in dialysates with a BAS UniJet radial flow detector cell coupled to a LC-4B amperometric detector (BAS, IN, USA). The applied potential of a 3-mm glassy carbon electrode was +600 mV with a sensitivity of 2 nA/V. The chromatographic data were processed by Chromax 2001 (Pol-Lab, Warszawa, Poland) software run on a PC computer. The values were not corrected for an *in vitro* probe recovery, which was approximately 15%.

An average concentration of three stable samples prior to drug administration was regarded as a control value and was regarded as 100%.

Statistical analysis

The results represent the mean ± SEM. The statistical significance of drug effects was calculated by a repeated-measures ANOVA, followed by intergroup comparisons using Dunnett's test (when only one drug was administered), or Tukey's *post-hoc* test (when two drugs were used). The results were considered statistically significant when $p < 0.05$.

Results

Forced swim test

The selective 5-HT_7 receptor antagonist SB-269970 (1.25 and 2.5 mg/kg) significantly shortened the immobility time of rats in the forced swim test; its lower

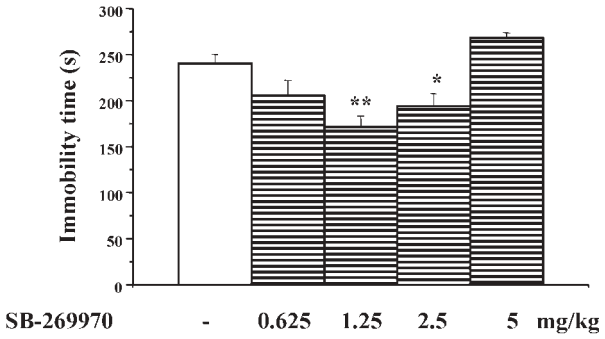

Fig. 2. The effect of SB-269970 on the immobility time of rats in the forced swim test. SB-269970 was administered 30 min before the test. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by Dunnett's test; * $p < 0.05$, ** $p < 0.01$ *vs.* saline

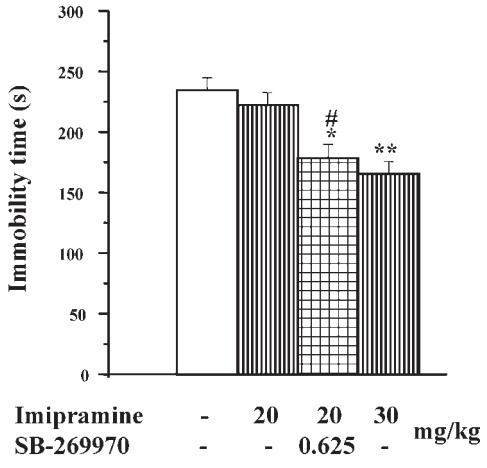

Fig. 3. The effect of imipramine alone or given jointly with SB-269970 on the immobility time of rats in the forced swim test. Imipramine and SB-269970 were administered 60 and 30 min, respectively, before the test. The animals were observed for 5 min. The results represent the mean ± SEM of 8 rats. The data were statistically evaluated by ANOVA, followed by Tukey's *post-hoc* test; * $p < 0.01$, ** $p < 0.001$ *vs.* saline; # $p < 0.01$ *vs.* imipramine (20 mg/kg)

(0.625 mg/kg) and higher (5 mg/kg) doses had no statistical effect in that test (Fig. 2). Imipramine (20 mg/kg) did not affect the immobility time of rats; when given at a higher dose of 30 mg/kg, the drug significantly reduced the immobility time of rats (Fig. 3). Therefore, a dose of 20 mg/kg of imipramine was chosen for an interaction study with SB-269970. Co-administration of SB-269970 (0.625 mg/kg) and imipramine (20 mg/kg) produced a statistically significant anti-immobility effect which was comparable to that of imipramine (30 mg/kg) alone (Fig. 3).

Open field test

SB-269970 (0.625–2.5 mg/kg) did not change the exploratory locomotor activity of rats, evaluated by the open field test. Imipramine (20 mg/kg), administered alone or jointly with SB-269970 (0.625 mg/kg), did not affect any parameters measured in that test (Tab. 1).

Tab. 1. The effect of SB-269970 and imipramine, given alone or jointly, on the exploratory activity evaluated by the open field test in rats

Treatment (mg/kg)	Exploratory activity		
	Walking time (s)	Ambulation	Peeping + rearing
Saline + saline	55.0 ± 3.1	17.5 ± 1.8	14.3 ± 2.1
SB-269970 (0.625) + saline	56.2 ± 1.9	21.3 ± 1.4	18.8 ± 2.1
SB-269970 (1.25) + saline	54.5 ± 8.3	17.8 ± 3.3	18.8 ± 2.3
SB-269970 (2.5) + saline	54.8 ± 7.7	19.2 ± 3.6	21.5 ± 3.3
	F(3, 20) = 0.015 ns	F(3, 20) = 0.423 ns	F(3, 20) = 1.436 ns
Saline + saline	53.3 ± 5.7	15.0 ± 2.0	13.7 ± 2.7
Saline + imipramine (20)	48.2 ± 7.9	13.5 ± 1.9	14.2 ± 2.0
SB-269970 (0.625) + imipramine (20)	43.8 ± 6.8	14.5 ± 3.7	12.7 ± 2.7
	F(2, 15) = 0.477 ns	F(2, 15) = 0.082 ns	F(2, 15) = 0.095 ns

SB-269970 and imipramine were administered 30 and 60 min, respectively, before the test. The animals were observed for 5 min. The results represent the mean ± SEM of 6 rats. ns – non-significant

Microdialysis study

The basal extracellular concentrations of biogenic amines in dialysates from the prefrontal cortex for all the rats tested were 1.45 ± 0.13 (DA), 3.43 ± 0.24 (NA) and 0.64 ± 0.07 (5-HT) pg/10 µl ± SEM. The selective 5-HT_7 receptor antagonist SB-269970 (0.625 mg/kg) markedly and significantly elevated the levels of DA, NA and 5-HT, the maximum effect being ca. 300%, 125% and 170%, respectively, of the basal release after 20 min of administration; it also in-

creased the dialysate levels of the metabolites DOPAC, HVA and 5-HIAA in the prefrontal cortex of freely moving rats. SB-269970 (1.25 mg/kg) produced an increase in the levels of biogenic amines and their metabolites, the maximum effect being ca. 240% of the basal release after 40 min of administration in the case of NA. The time-course of the above-described effects is shown in Figures 4 and 6, while the cumulative effect in the sampling period, i.e. during 240 min, expressed as an area under the curve (AUC), is presented in Figure 5.

Imipramine (20 mg/kg) increased the extracellular levels of DA, NA and 5-HT, the maximum effect being ca. 225%, 210% and 180%, respectively, at

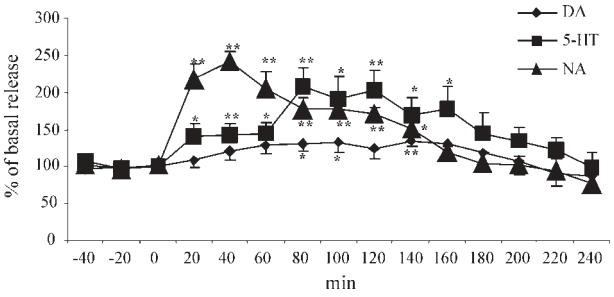

Fig. 4. The effect of SB-269970 (1.25 mg/kg) on the extracellular levels of DA, NA and 5-HT in rat prefrontal cortex, shown as a time-course. SB-269970 was given at the time-point "0". The collection of microdialysis samples was conducted every 20 min for 4 h. The results represent the mean ± SEM of 4 rats. The data were statistically evaluated by ANOVA, followed by Dunnett's test; * $p < 0.05$, ** $p < 0.01$ *vs.* basal level

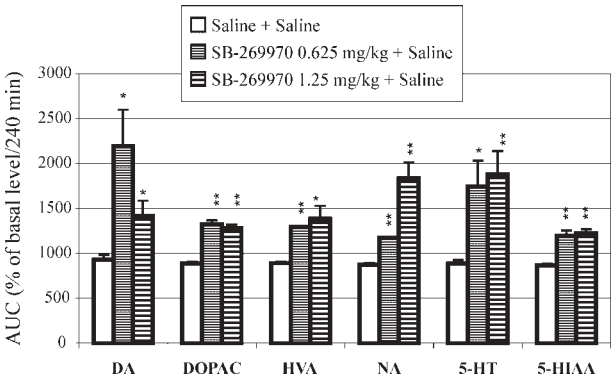

Fig. 5. The cumulative effect of SB-269970 on the extracellular levels of DA, NA, 5-HT and their metabolites in rat prefrontal cortex, shown as an area under the curve (AUC). The collection of microdialysis samples was conducted every 20 min for 4 h. The results represent the mean ± SEM of 4 rats. The data were statistically evaluated by ANOVA, followed by Dunnett's test; * $p < 0.05$, ** $p < 0.01$ *vs.* saline + saline

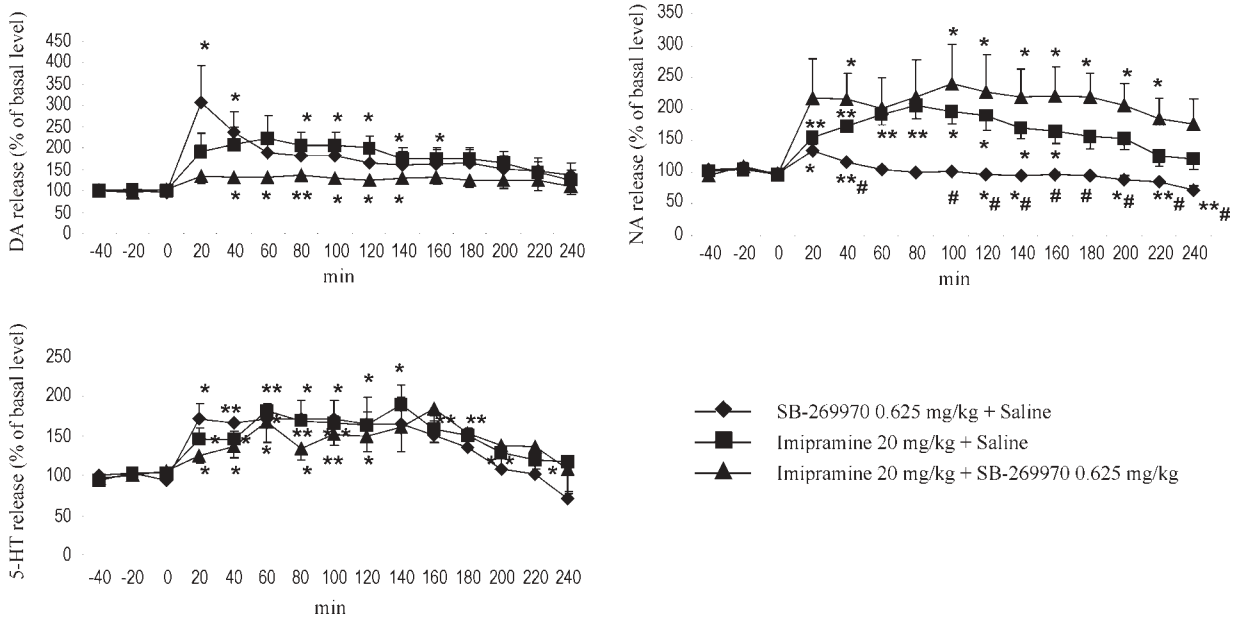

Fig. 6. The effect of SB-269970 (0.625 mg/kg) and imipramine (20 mg/kg) administered alone or jointly, on the extracellular levels of DA, NA and 5-HT in rat prefrontal cortex, shown as a time-course. SB-269970 and imipramine were given alone or jointly at the time-point "0". The results represent the mean ± SEM of 3–4 rats. The data were statistically evaluated by ANOVA, followed by Tukey's *post-hoc* test; * $p < 0.05$, ** $p < 0.01$ *vs.* basal level; # $p < 0.01$ *vs.* SB-269970 (0.625 mg/kg)

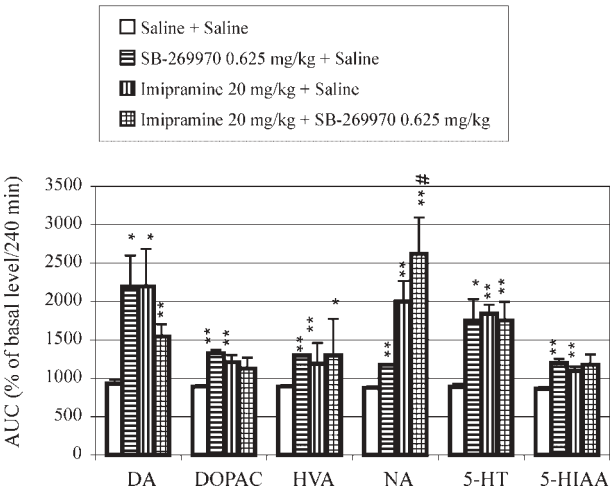

Fig. 7. The cumulative effect of SB-269970 and imipramine on the extracellular levels of DA, NA and 5-HT and their metabolites in rat prefrontal cortex, shown as an area under the curve (AUC). The collection of microdialysis samples was conducted every 20 min for 4 h. The results represent the mean ± SEM of 3–4 rats. The data were statistically evaluated by ANOVA, followed by Tukey's *post-hoc* test; * $p < 0.05$, ** $p < 0.01$ *vs.* saline + saline; # $p < 0.01$ *vs.* SB-269970 (0.625 mg/kg)

60–80 min after administration; the levels of the metabolites DOPAC, HVA and 5-HIAA were not increased upon imipramine injection (20 mg/kg). The time-course of the effect is shown in Figure 6, while the cumulative effect of imipramine expressed as AUC is presented in Figure 7.

Combined administration of SB-269970 (0.625 mg/kg) and imipramine (20 mg/kg) increased the dialysate levels of DA, NA and 5-HT, the maximum effect being ca. 140%, 240% and 180%, respectively, of the basal release after 60–100 min of administration. SB-269970 (0.625 mg/kg) given jointly with imipramine (20 mg/kg) also increased the level of HVA in the statistically significant manner. The time-course of the effect is shown in Figure 6, whereas the effect of both those compounds was averaged out over 240 min and expressed as AUC for a clear presentation of the results (Fig. 7).

Discussion

SB-269970, a pharmacological tool used in the present study, has been shown to be a potent ligand of cloned human [19] and guinea-pig [14] 5-HT_7 receptors (pK_i = 8.9 and 8.7, respectively). It displays excellent selectivity (> 250-fold) over 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_6, α_1-adrenergic, D_2 and D_3 receptors, except for 5-HT_{5A} ones (50-fold). Moreover, in a commercial screening package, SB-269970 has been found to be over 100-fold more selective over a total of 50 other receptors, transporters, enzymes and ion channels [14, 19]. SB-269970 shows *in vitro* and *in vivo* features of a 5-HT_7 receptor antagonist and displays good central nervous system penetration [12, 14, 19, 20, 44].

The present study demonstrates for the first time that SB-269970 administered systemically to rats in doses of 1.25 and 2.5 mg/kg produces anti-immobility action in the forced swim test. Its antidepressant-like activity cannot be attributed to changes in general activity, as this drug used at doses producing an anti-immobility effect does not change exploratory locomotor activity measured in the open field test. These results are in line with our earlier findings which indicated that SB-269970 given locally into the CA1 region of rat hippocampus may evoke an antidepressant-like effect [50]. The present observation is also consistent with the results of several other studies carried out on mice, which point to the antidepressant-like activity of SB-269970 in the mouse models of depression, i.e. the forced swim and the tail suspension tests [3, 15, 51, 52]. We have observed that SB-269970 produces antidepressant-like activity in rats, developing an apparent U-shaped dose-response relationship which is difficult to explain. Recent studies [12, 14] indicated that the hypothermic effect of the non-selective 5-HT receptor agonist 5-carboxytryptamine was reduced by SB-269970 in a dose-dependent manner in mice and guinea-pigs. Additionally, Bonaventure et al. [3] reported that SB-269970 dose-dependently produced anti-immobility action in the tail suspension test in mice; furthermore, in an autoshaping Pavlovian/instrumental learning task, SB-269970 dose-dependently reversed the amnesic effects of scopolamine or dizocilpine in rats [24]. On the other hand, the decrease in rat body temperature evoked by the 5-$HT_{1A/7}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin was inhibited by SB-269970, showing a tendency to develop a bell-shaped dose-response relationship. Moreover, Wesołowska et al. [51] found that SB-269970 exerted anxiolytic-like activity in mice and rats, as well as antidepressant-like activity in mice, producing a U-shaped dose-response effect.

Hence, it is only hypothesized that the lack of typical dose-dependence, observed in the present behavioral experiment, and the reduction of the effectiveness of a higher dose of SB-269970 indicate that all 5-HT_7 receptors seem to be saturated with lower doses of the 5-HT_7 receptor antagonist tested, and that further augmentation of its dose has no effect on the immobility time, or that some non-specific activity of SB-269970 prevents a further decrease in immobility time. However, there have been no data so far showing such non-specific action of SB-269970.

Our study was also aimed at determining the ability of SB-269970 to augment the effect of the 5-HT/NA reuptake inhibitor imipramine (which *per se* does not exhibit affinity for 5-HT_7 receptors [39]) on the antidepressant-like behavior of rats in the forced swim test. As previously observed in mice [52], a low dose of imipramine (which has no effect of its own) induces a statistically significant anti-immobility effect in rats when used in combination with an inactive dose of SB-269970. This effect seems to be specific, since neither SB-269970 nor imipramine, given alone or jointly, have influence on the exploratory locomotor activity of rats, as shown in the open field test. In some more recent articles, Bonaventure et al. [3] and Wesołowska et al. [52] reported that the blockade of 5-HT_7 receptors facilitated the anti-immobility effect of several antidepressants including imipramine in the mouse tail suspension and the forced swim tests, without increasing locomotor activity. Additionally, Bonaventure et al. [3] ruled out a possible pharmacokinetic interaction between citalopram and SB-269970 in favor of a pharmacodynamic one to explain the enhancement of citalopram activity by SB-269970 in mice. Our data on the 5-HT_7 receptor antagonist tested and imipramine reinforce the above-cited findings by using a different strain of animals. However, at this stage of experimentation we are not able to decide whether the synergistic effect observed for SB-269970 and imipramine in rats may be regarded as a result of a pharmacodynamic and/or a pharmacokinetic interaction, since the levels of both the compounds used (given alone or jointly) have not been analyzed so far. Hence, the present study focuses on a putative pharmacodynamic interaction.

It has been well established that the antidepressant-like activity detected in the forced swim test depends primarily on the enhancement of central catecholamine and 5-HT neurotransmission [4–7, 34, 37]. In the present neurobiochemical experiment imipramine *per se* caused a substantial increases in the extracellular concentrations of DA, NA and 5-HT. These findings are basically in agreement with other microdialysis data [2, 10, 18, 28, 40] on the acute effect of imipramine on biogenic amine levels in rat prefrontal cortex. On the assumption that the DA, NA and/or 5-HT systems may be involved in mediation of the anti-immobility effect of the 5-HT_7 receptor antagonist used, our study has described for the first time the action of SB-269970 alone, as well as the effect of joint administration of SB-269970 and imipramine on the release of these neurotransmitters in the prefrontal cortex of live, conscious and freely moving rats. In that model SB-269970 *per se* was examined in two doses: 1) 0.625 mg/kg, i.e. a dose inactive in the forced swim test, used for an interaction study, and 2) 1.25 mg/kg, i.e. a dose producing the strongest anti-immobility action. Microdialysis results showed that both a lower and a higher dose of SB-269970 alone significantly increased DA, NA and 5-HT efflux, as well as the levels of biogenic amine metabolites in rat prefrontal cortex. The dose of 0.625 mg/kg of SB-269970 produced a weaker effect than that of 1.25 mg/kg except for DA release. In contrast, Bonaventure et al. [3] reported that SB-269970 itself, administered in a dose of 10 mg/kg, did not increase cortical 5-HT, DA and NA release in rats. The substantial difference between our study and that of Bonaventure et al. [3] lies in the dose used of the 5-HT_7 receptor antagonist. The latter authors tested the dose of 10 mg/kg of SB-269970 in a microdialysis study with rats; the same dose produced an antidepressant-like effect in mice (a tail suspension test), but was ineffective in rats (sleep-wake states). In our behavioral study, SB-269970 given in a dose of 10 mg/kg was also ineffective in the forced swim test in rats (data not shown); hence, it is likely that pronounced effects of SB-269970 can be observed after administration of its lower doses to rats, but not to mice. Nevertheless, the dose of 10 mg/kg of SB-269970 significantly enhanced the effect of citalopram on the release of cortical 5-HT in rats [3].

The identification of 5-HT_7 receptor mRNA in the dorsal raphe nucleus, as well as the presence of 5-HT_7 receptor protein in 5-HT cell bodies and axon terminal fields suggest that these receptors may act as 5-HT release-mediating autoreceptors [13, 38]. Our results seem to confirm such a conclusion, since the blockade of 5-HT_7 receptors increases 5-HT and 5-HIAA levels in cortical terminals. An intriguing question arises

why the 5-HT_7 receptor antagonist also induces an increase in extracellular DA and NA concentrations. SB-269970 is a selective 5-HT_7 receptor antagonist, which has no affinity for DA and adrenergic receptors, nor does it show any DA and NA reuptake inhibition activity [14, 19]. Therefore, it may only be hypothesized that its effect is likely to be connected with the indirect modulating action of released 5-HT on DA and NA neurons *via* 5-HT_7 heteroreceptors localized on DA and NA neurons. However, there is no evidence in the literature pointing to such localization of 5-HT_7 receptors.

Several neuroanatomical and electrophysiological studies indicate a functional interplay between the 5-HT and DA systems. For instance, some ultrastructural reports reveal that 5-HT terminals enter into synaptic contact with DA neurons in the ventral tegmental area [16, 47] and substantia nigra pars compacta [8]. Moreover, Minabe et al. [26] reported that the depletion of brain 5-HT with *para*-chlorophenylalanine decreased the activity of midbrain DA cells. Such interactions between the two systems were further evidenced by Mnie-Filali et al. [27] who demonstrated that the *in vitro* inhibitory effect of amphetamine on DA neuronal firing activity was modulated by 5-HT_7 receptors in the ventral tegmental area. To the best of our knowledge, possible interactions between 5-HT_7 receptors and the dopaminergic and noradrenergic systems *in vivo* have not been studied so far.

We observed in the forced swim test the enhancement of the effect of imipramine (20 mg/kg) by SB-269970 administered in an inactive dose (0.625 mg/kg). In a microdialysis study, a significant interaction between SB-269970 and imipramine was demonstrated in the case of NA release only, whereas no enhancement of DA and 5-HT efflux after combined administration of SB-269970 and imipramine was detected; contrarywise, even a decrease in DA level was found after co-application of the 5-HT_7 receptor antagonist and imipramine.

Summing up, the present results suggest that SB-269970 shows antidepressant-like activity in the forced swim test in rats, possibly connected with increases in DA, NA and 5-HT levels in the prefrontal cortex. Moreover, SB-269970 can enhance the anti-immobility effect of imipramine in rats; the increase in cortical NA level seems to account for the behavioral enhancement observed in the forced swim test after combined administration of SB-269970 and imipramine to rats. Apparently, other biogenic amines (DA and 5-HT) do not seem to be important to the interaction tested. However, an adequate explanation of the influence of SB-269970 *per se* on DA and 5-HT levels in the context of its potential antidepressant activity requires further systematic studies.

Acknowledgments:
The authors are grateful to Prof. Ewa Chojnacka-Wójcik and Assoc. Prof. Krystyna Gołembiowska for their valuable scientific discussion of the results. The study was supported by the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

References:

1. Ago Y, Harasawa T, Itoh S, Nakamura S, Baba A, Matsuda T: Antidepressant-like effect of coadministration of sulpiride and fluvoxamine in mice. Eur J Pharmacol, 2005, 520, 86–90.
2. Bel N, Artigas F: In vivo effects of the simultaneous blockade of serotonin and norepinephrine transporters on serotonergic function. Microdialysis studies. J Pharmacol Exp Ther, 1996, 278, 1064–1072.
3. Bonaventure P, Kelly L, Aluisio L, Shelton J, Lord B, Galici R, Miller K et al.: Selective blockade of 5-hydroxytryptamine (5-HT)$_7$ receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. J Pharmacol Exp Ther, 2007, 321, 690–698.
4. Borsini F: Role of the serotonergic system in the forced swimming test. Neurosci Biobehav Rev, 1995, 19, 377–395.
5. Borsini F, Meli A: Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology, 1988, 94, 147–160.
6. Cervo L, Grignaschi G, Rossi C, Samanin R: Role of central serotonergic neurons in the effect of sertraline in rats in the forced swimming test. Eur J Pharmacol, 1991, 196, 217–222.
7. Cervo L, Grignaschi G, Samanin R: α_2-Adrenoceptor blockade prevents the effect of desipramine in the forced swimming test. Eur J Pharmacol, 1990, 175, 301–307.
8. Corvaja N, Doucet G, Bolam JP: Ultrastructure and synaptic targets of the raphe-nigral projection in the rat. Neuroscience, 1993, 55, 417–427.
9. Da-Rocha MA, Puech AJ, Thiebot M-H: Influence of anxiolytic drugs on the effects of specific serotonin reuptake inhibitors in the forced swimming test in mice. J Psychopharmacol, 1997, 11, 211–218.
10. Ferraro L, Fuxe K, Agnati L, Tanganelli S, Tomasini MC, Antonelli T: Modafinil enhances the increase of extracellular serotonin levels induced by the antidepressant drugs fluoxetine and imipramine: a dual probe microdialysis study in awake rat. Synapse, 2005, 55, 230–241.
11. Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, Bromidge F et al.: Genetic knock-

out and pharmacological blockade studies of the 5-HT$_7$ receptor suggest therapeutic potential in depression. Neuropharmacology, 2005, 48, 492–502.
12. Guscott MR, Egan E, Cook GP, Stanton JA, Beer MS, Rosahl TW, Hartmann S et al.: The hypothermic effect of 5-CT in mice is mediated through the 5-HT$_7$ receptor. Neuropharmacology, 2003, 44, 1031–1037.
13. Gustafson EL, Durkin MM, Bard JA, Zgombick J, Branchek TA: A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-HT$_7$ receptor in rat brain. Br J Pharmacol, 1996, 117, 657–666.
14. Hagan JJ, Price GW, Jeffrey P, Deeks N J, Stean T, Piper D, Smith MI: Characterization of SB-269970-A, a selective 5-HT$_7$ receptor antagonist. Br J Pharmacol, 2000, 130, 539–548.
15. Hedlund PB, Huitron-Resendiz S, Henriksen S J, Sutcliffe JG: 5-HT$_7$ receptor inhibition and inactivation induce antidepressant-like behavior and sleep pattern. Biol Psychiatry, 2005, 58, 831–837.
16. Herve D, Pickel VM, Joh TH, Beaudet A: Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res, 1987, 435, 71–83.
17. 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.
18. Jordan S, Kramer GL, Zukas PK, Moeller M, Petty F: In vivo biogenic amine efflux in medial prefrontal cortex with imipramine, fluoxetine, and fluvoxamine. Synapse, 1994, 18, 294–297.
19. Lovell PJ, Bromidge SM, Dabbs S, Duckworth DM, Forbes IT, Jennings AJ, King FD et al.: A novel, potent, and selective 5-HT$_7$ antagonist: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970). J Med Chem, 2000, 43, 342–345.
20. Mahé C, Loetscher E, Feuerbach D, Müller W, Seiler MP, Schoeffter P: Differential inverse agonist efficacies of SB-258719, SB-258741 and SB-269970 at human recombinant serotonin 5-HT$_7$ receptors. Eur J Pharmacol, 2004, 495, 97–102.
21. Marek GJ, Martin-Ruiz R, Abo A, Artigas F: The selective 5-HT$_{2A}$ receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. Neuropsychopharmacology, 2005, 30, 2205–2215.
22. Martin-Cora F, Pazos A: Autoradiographic distribution of 5-HT$_7$ receptors in the human brain using [^{3}H]mesulergine: comparison to other mammalian species. Br J Pharmacol, 2004, 141, 92–104.
23. Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R, Lucki I: Antidepressant-like behavioral effects in 5-hydroxytryptamine$_{1A}$ and 5-hydroxytryptamine$_{1B}$ receptor mutant mice. J Pharmacol Exp Ther, 2001, 298, 1101–1107.
24. Meneses A: Effects of the 5-HT$_7$ receptor antagonists SB-269970 and DR 4004 in autoshaping Pavlovian/instrumental learning task. Behav Brain Res, 2004, 155, 275–282.
25. Millan MJ, Brocco M, Veiga S, Cistarelli L, Melon C, Gobert A: WAY 100,635 enhances both the 'antidepressant' actions of duloxetine and its influence on dialysate levels of serotonin in frontal cortex. Eur J Pharmacol, 1998, 341, 165–167.
26. Minabe Y, Emori K, Ashby CR Jr: The depletion of brain serotonin levels by para-chlorophenylalanine administration significantly alters the activity of midbrain dopamine cells in rats: an extracellular single cell recording study. Synapse, 1996, 22, 46–53.
27. Mnie-Filali O, Dahan L, Zimmer L, Haddjeri N: Effects of the serotonin 5-HT$_7$ receptor antagonist SB-269970 on the inhibition of dopamine neuronal firing induced by amphetamine. Eur J Pharmacol, 2007, 570, 72–76.
28. Mochizuki D, Tsujita R, Yamada S, Kawasaki K, Otsuka Y, Hashimoto S, Hattori T et al.: Neurochemical and behavioural characterization of minalcipran, a serotonin and noradrenaline reuptake inhibitor in rats. Psychopharmacology, 2002, 162, 323–332.
29. Mullins UL, Gianutsos G, Eison AS: Effects of antidepressants on 5-HT$_7$ receptor regulation in the rat hypothalamus. Neuropsychopharmacology, 1999, 21, 352–367.
30. Neumaier JF, Sexton TJ, Yracheta J, Diaz AM, Brownfield M: Localization of 5-HT$_7$ receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. J Chem Neuroanatom, 2001, 21, 63–73.
31. Paxinos G, Watson C (Ed.): The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, 1998.
32. Pitra P, Tokarski K, Grzegorzewska M, Hess G: Effects of repetitive administration of tianeptine, zinc hydroaspartate and electroconvulsive shock on the reactivity of 5-HT$_7$ receptors in rat hippocampus. Pharmacol Rep, 2007, 59, 627–635.
33. 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.
34. 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.
35. Redrobe JP, Bourin M: Partial role of 5-HT$_2$ and 5-HT$_3$ receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol, 1997, 325, 129–135.
36. Redrobe JP, MacSweeney CP, Bourin M: The role of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in antidepressant drug actions in the mouse forced swimming test. Eur J Pharmacol, 1996, 318, 213–220.
37. Reneric 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 forced swimming test. Neuropsychopharmacology, 2001, 24, 379–390.
38. Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang J-M, Schwartz J-C: Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT$_7$) activating cAMP formation. Proc Natl Acad Sci USA, 1993, 90, 8547–8551.

39. Shen Y, Monsma FJ Jr., Metcalf MA, Jose PA, Hamblin MW, Sibley DR: Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. J Biol Chem, 1993, 268, 18200–18204.
40. Tanda G, Carboni E, Frau R, Di Chiara G: Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? Psychopharmacology, 1994, 115, 285–288.
41. Tatarczyńska E, Kłodzińska A, Chojnacka-Wójcik E: Effects of combined administration of 5-HT$_{1A}$ and/or 5-HT$_{1B}$ receptor antagonists and paroxetine or fluoxetine in the forced swimming test in rats. Pol J Pharmacol, 2002, 54, 615–623.
42. Tatarczyńska E, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik E: Effect of combined administration of 5-HT$_{1A}$ or 5-HT$_{1B/1D}$ receptor antagonists and antidepressants in the forced swimming test. Eur J Pharmacol, 2004, 487, 133–142.
43. Thomas DR, Melotto S, Massagrande M, Gribble AD, Jeffrey P, Stevens AJ, Deeks NJ et al.: SB-656104-A, a novel selective 5-HT$_7$ receptor antagonist, modulates REM sleep in rats. Br J Pharmacol, 2003, 139, 705–714.
44. Tokarski K, Zahorodna A, Bobula B, Grzegorzewska M, Pitra P, Hess G: Repeated administration of citalopram and imipramine alters the responsiveness of rat hippocampal circuitry to the activation of 5-HT$_7$ receptors. Eur J Pharmacol, 2005, 524, 60–66.
45. Tokarski K, Zahorodna A, Bobula B, Hess G: 5-HT$_7$ receptors increase the excitability of rat hippocampal CA1 pyramidal neurons. Brain Res, 2003, 993, 230–234.
46. Tsou AP, Kosaka A, Bach C, Zuppan P, Yee C, Tom L, Alvarez R et al.: Cloning and expression of a 5-hydroxytryptamine7 receptor positively coupled to adenylyl cyclase. J Neurochem, 1994, 63, 456–464.
47. Van Bockstaele EJ, Cestari DM, Pickel VM: Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. Brain Res, 1994, 647, 307–322.
48. Varnas K, Thomas DR, Tupala E, Tiihonen J, Hall H: Distribution of 5-HT$_7$ receptors in the human brain: a preliminary autoradiographic study using [^{3}H]SB-269970. Neurosci Lett, 2004, 367, 313–316.
49. Wesołowska A, Nikiforuk A: The selective 5-HT$_6$ receptor antagonist SB-399885 enhances anti-immobility action of antidepressants in rats. Eur J Pharmacol, 2008, 582, 88–93.
50. Wesołowska A, Nikiforuk A, Stachowicz K: Potential anxiolytic and antidepressant effects of the selective 5-HT$_7$ receptor antagonist SB 2269970 after intrahippocampal administration to rats. Eur J Pharmacol, 2006, 553, 185–190.
51. Wesołowska A, Nikiforuk A, Stachowicz K, Tatarczyńska E: Effect of the selective 5-HT$_7$ receptor antagonist SB 269970 in animal models of anxiety and depression. Neuropharmacology, 2006, 51, 578–586.
52. Wesołowska A, Tatarczyńska E, Nikiforuk A, Chojnacka-Wójcik E: Enhancement of the anti-immobility action of antidepressants by a selective 5-HT$_7$ receptor antagonist in the forced swimming test in mice. Eur J Pharmacol, 2007, 555, 43–47.

Received:
March 3, 2008; in revised form: April 30, 2008.