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Original article

Adaptive mechanisms following antidepressant drugs: Focus on serotonin 5-HT_{2A} receptors

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ABSTRACT

Background: There is a strong support for the role of serotonin (5-HT) neurotransmission in depression and in the mechanism of action of antidepressants. Among 5-HT receptors, 5-HT_{2A} receptor subtype seems to be an important target implicated in the above disorder.
Methods: The aim of the study was to investigate the effects of antidepressants, such as imipramine (15 mg/kg), escitalopram (10 mg/kg) and tianeptine (10 mg/kg) as well as drugs with antidepressant activity, including N-acetylcysteine (100 mg/kg) and URB597 (a fatty acid amide hydrolase inhibitor, 0.3 mg/kg) on the 5-HT_{2A} receptor labeling pattern in selected rat brain regions. Following acute or chronic (14 days) drug administration, rat brains were analyzed by using autoradiography with the 5-HT_{2A} receptor antagonist [^{3}H]ketanserin.
Results: Single dose or chronic administration of imipramine decreased the radioligand binding in the claustrum and cortical subregions. The [^{3}H]ketanserin binding either increased or decreased in cortical areas after acute N-acetylcysteine and URB597 administration, respectively. A similar shift towards reduction of the [^{3}H]ketanserin binding was detected in the nucleus accumbens shell following either acute treatment with imipramine, escitalopram, N-acetylcysteine and URB597 or repeated administration of imipramine, tianeptine and URB597.
Conclusions: In conclusion, the present result indicate different sensitivity of brain 5-HT_{2A} receptors to antidepressant drugs depending on schedule of drug administration and rat brain regions. The decrease of accumbal shell 5-HT_{2A} receptor labeling by antidepressant drugs exhibiting different primary mechanism of action seems to be a common targeting mechanism associated with the outcome of depression treatment.

Introduction

Depression disorder is one of the most common neuropsychiatric diseases worldwide leading to disability in the social and personal life. Although there is a wide pool of antidepressant drugs, responding to the available pharmacological treatment often is not sufficiently effective [1]. In addition, it often takes even more than 8 weeks to positive clinical conditions and until the treatment effects are visible, beyond present side effects. Moreover, approximately 40% of patients with drug resistant depression are not responsive to therapy and basic of pathophysiology still remains unclear [2]. Hence, researchers search for new drugs and targets for pharmacotherapy with elevated efficacy and fewer side effects.

The serotonin (5-HT) neurotransmission plays a significant role in physiological processes, such as the awake and sleep regulation, emotions and mood, sexual behavior, learning and memory, as well as pathological states, including eating disorders, anxiety or aggression. The irregularity in 5-HT system function had been implicated in pathogenesis of depression and target of its treatment since the discovery of the first antidepressant drugs in the 1950s. Research indicated that neuronal activation in the dorsal raphe nucleus, located in the midbrain is one of the primary sources of 5-HT neurons in the central nervous system, is implicated in characteristic antidepressant drug action [3,4]. The

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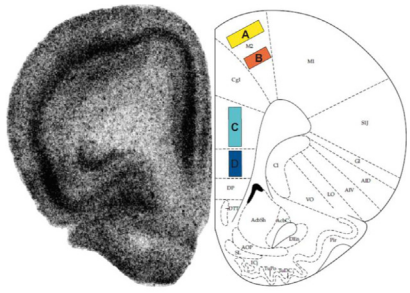
central 5-HT system originating from the dorsal raphe nucleus has widespread projections to emotion-related brain structures important for the development of symptoms of depression including the amygdala, hippocampus, basal ganglia and cortex [4]. The strongest evidence for the role of the 5-HT neurotransmission in the treatment of depression is related to the efficacy of antidepressants which modulate 5-HT levels in the brain. A majority of antidepressants block the 5-HT transporter (SERT) (such as tricyclic antidepressants and selective 5-HT reuptake inhibitors (SSRIs)) or inhibit degradation of 5-HT (monoamine oxidase inhibitors). SSRIs and the dual 5-HT and noradrenalin reuptake inhibitors (SNRIs) provide more than 90% of the global antidepressant drugs [5].

Among 5-HT receptors, 5-HT_{2A} receptors are widely distributed at varying densities throughout the brain, with the highest density in the neocortex, where they are localized on γ-aminobutyric acid (GABA)-ergic interneurons or glutamatergic projection neurons [6]. Numerous clinical studies highlight contribution of 5-HT_{2A} receptors to the pathogenesis of depression and antidepressant drug action. An association between specific HTR2A single nucleotide polymorphisms (SNPs) and susceptibility to major depression and/or severity of its episodes has been found. This association was also reported in severe forms of suicidal ideation, especially those with suicidal attempts or melancholic features [7]. Interestingly, variations in the gene encoding the 5-HT_{2A} receptor have been linked to the outcome of the depression treatment with SSRIs [8]. Additionally, reports of postmortem brain samples from adult and teenage suicide victims have shown higher levels of 5-HT_{2A} receptors [8]. Furthermore, a greater number of 5-HT_{2A} receptors in the platelets of suicidal victims were suggested as a biological marker of suicidal behavior [9]. Unfortunately, knowledge and evidence about the consequences of administration of antidepressant drugs on 5-HT_{2A} receptor expression in the brain are limited.

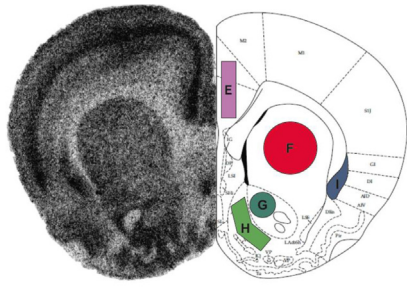
The aim of the present work was to investigate the potential changes in 5-HT_{2A} receptor labeling in different rat brain structures after acute and repeated administration of antidepressants. We focused on three antidepressants with different mechanisms of action, including imipramine (IMI; a SNRI), escitalopram (ESC; a

Table 1
Examples of 5-HT_{2A} receptor labeling in the control rat brain. Representative autoradiograms of [^{3}H]ketanserin binding in the brain of rats treated repeatedly with vehicle. The color outlines A–J show the brain areas in which optical densities were quantified.



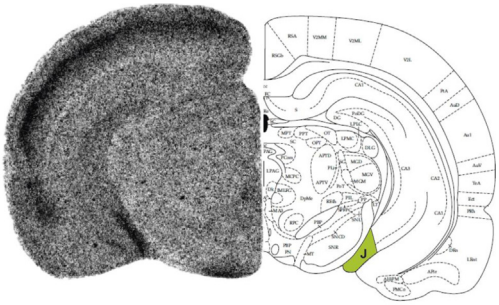
Interaural 11.70 mm
Bregma 2.70 mm

A - motor cortex (II–III layers)
B - motor cortex (V layer)
C - prelimbic cortex
D - infralimbic cortex



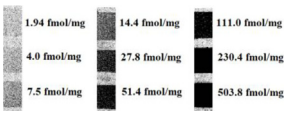
Interaural 10.70 mm
Bregma 1.70 mm

E - cingulate cortex
F - dorsal striatum (caudate putamen)
G - nucleus accumbens core
H - nucleus accumbens shell
I - claustrum



Interaural 3.70 mm
Bregma -5.30 mm

J - ventral dentate gyrus



SSRI) and tianeptine (TIA; a selective 5-HT reuptake enhancer), along with drugs in which antidepressant activity has been demonstrated in preclinical and clinical studies, including *N*-acetylcysteine (NAC) (mucolytic drug) and cyclohexylcarbamic acid 3-carbamoylbiphenyl-3-yl ester (URB597, a fatty acid amide hydrolase (FAAH) inhibitor) [10–12].

Material and methods

Animals

Adult male Wistar rats weighing 280–300 g were used for experiments. The animals were kept under standard laboratory conditions (12 h light/dark cycle at 22 ± 2 °C and 55 ± 10% humidity with free access to food and water). All procedures were carried out in accordance with directive 2010/63/EU and with approval of the Local Ethical Committee in Krakow, Poland (the approval number 59/2012). There were 7–8 rats/group.

Drugs

The following substances were used in experiments: *N*-acetylcysteine (NAC; Sigma-Aldrich, USA), URB597 (Sigma-Aldrich, USA), escitalopram oxalate (ESC; Lundbeck, Denmark), imipramine hydrochloride (IMI; Sigma-Aldrich, USA), and tianeptine sodium (TIA; Anpharm, Poland). ESC, IMI, NAC and TIA were dissolved in sterile 0.9% NaCl (pH of NAC and ESC solutions were neutralized with 10% NaOH). URB597 was dissolved in 2–3 drops of ethanol and diluted as required in a 1% Tween 80. Drugs were administered *ip* acutely on day 14 following 13 days of saline injections or chronically (14 days), while the control group received only saline for 14 days. The injection volume was 1 ml/kg. The doses for drugs were chosen based on effective doses used in our previous behavioral observations: URB597 (0.3 mg/kg; [10]) and NAC (100 mg/kg; [11]) as well as in other literature findings on IMI (15 mg/kg; [13]), ESC (10 mg/kg; [14]), and TIA (10 mg/kg; [15]) and following our previous biochemical studies on the same drugs [16,17].

Brain isolation

24 h after the last drug administration rats were decapitated, and their brains were rapidly removed, frozen by dry-ice bath using heptane and were stored at −80 °C until sectioned.

Quantitative autoradiography

Consecutive coronal sections (12 μm) of rat brains were cut on a cryostat microtome (Leica CM 1850, Germany) at −22 ± 2 °C and were thaw-mounted on polylysine-coated slides, and stored at −80 °C. Quantitative receptor autoradiography with the 5-HT_{2A} receptor antagonist [ethylene-^{3}H]ketanserin hydrochloride (PerkinElmer, USA; 1 nM, specific activity 67.0 Ci/mmol) as a radioligand was carried out according to the method of Syvälahti et al. [18] with some modifications [19].

The slides were first thawed at room temperature for 45 min and then pre-incubated in the Tris−HCl buffer (170 mM Tris−HCl; pH 7.5; Sigma-Aldrich, USA) for 15 min. Total binding was estimated by incubating the appropriate brain sections in Tris−HCl buffer with 1 nM [ethylene-^{3}H]ketanserin hydrochloride and 1 μM prazosin hydrochloride (Tocris Bioscience, UK) which blocked non-specific radioligand binding to α_1-adrenergic receptors. The non-specific binding was quantified by incubating separate sections in Tris−HCl buffer with the appropriate radioligand, prazosin hydrochloride and 10 μM M100,907 (5-HT_{2A} receptor antagonist; Abbott Healthcare Products B.V., Netherlands). Following the 2 h incubation period, tissue sections were washed twice in an ice-cold (4 °C) Tris−HCl buffer for 10 min and once in cold (4 °C) distilled water for 10 min, and then dried with cool air.

Radiolabeled, dried brain sections were exposed to tritium-sensitive screens ([^{3}H]-Fujifilm imaging plates; BAS-IP TR 2025E, Fuji Photo Film, Japan) in photosensitive cassettes (The Bass IP Cassettes, 20 cm × 25 cm, Fujifilm, Japan) for one week at 4 °C. The obtained autoradiograms were read out with a BAS-5000 IP Image Reader version 1.1. (Fujifilm, Japan), and quantitative analysis was performed using the MULTI GAUGE version 3.0. software (Fujifilm, Japan). Optical densities of gray values on the film were converted into bound radioactivity with a polynomial standard regression curve derived from autoradiographic [^{3}H]microscale (RPA 510, UK). The binding signal was measured in selected brain regions: motor cortex (II–III layers and V layer), prelimbic, infralimbic and cingulate cortices, dorsal striatum, nucleus accumbens core, nucleus accumbens shell, claustrum and ventral dentate gyrus. The brain regions were identified by comparing autoradiographic images with appropriate figures from The Rat Brain Atlas [20] (Table 1). Data (fmol/mg tissue) are expressed as the mean percentage of the control levels ± standard error of the mean (SEM).

Statistical analysis

All data were analyzed using an one-way analysis of variance (ANOVA), followed by *post hoc* Dunnett's test to analyze differences between group means (GraphPad Prism 7.04, GraphPad Software, USA). The criterion for statistically significant differences was set at $p < 0.05$.

Results

[^{3}H]Ketanserin binding to 5-HT_{2A} receptors showed different expression in rat brain areas of control rats (Table 1). The lowest binding density (20–60 fmol/mg tissue) was observed in the motor cortex (V layer), dorsal striatum, nucleus accumbens core and nucleus accumbens shell, the medium value (61–110 fmol/mg tissue) was seen in the claustrum and ventral dentate gyrus and the highest binding (>110 fmol/mg tissue) in the motor cortex (II–III layers), prelimbic, infralimbic and cingulate cortices.

Changes in [^{3}H]ketanserin binding following antidepressant drugs

IMI

IMI administration caused changes in the 5-HT_{2A} receptor labeling in the motor cortex (II–III layers) ($F_{2,20}$ = 21.03, $p < 0.001$), motor cortex (V layer) ($F_{2,20}$ = 42.17, $p < 0.001$), prelimbic cortex ($F_{2,20}$ = 30.62, $p < 0.001$), infralimbic cortex ($F_{2,20}$ = 25.55, $p < 0.001$), cingulate cortex ($F_{2,20}$ = 37.74, $p < 0.001$), dorsal striatum ($F_{2,20}$ = 5.03, $p < 0.05$), nucleus accumbens core ($F_{2,20}$ = 4.97, $p < 0.05$), nucleus accumbens shell ($F_{2,20}$ = 9.98, $p < 0.001$), claustrum ($F_{2,20}$ = 22.66, $p < 0.001$) and ventral dentate gyrus ($F_{2,20}$ = 5.25, $p < 0.05$). *Post hoc* analyses revealed the significant decrease (at least $p < 0.01$) in the 5-HT_{2A} receptor binding after acute IMI administration in cortical structures and dorsal striatum (ca. 50–60%), nucleus accumbens core and shell, claustrum and ventral dentate gyrus (ca. 40%). Moreover, repeated administration of IMI caused a decrease in 5-HT_{2A} receptor density (ca. 30–40%) in such brain regions as the motor cortex (II–III and V layers), prelimbic, infralimbic and cingulate cortices, nucleus accumbens shell and claustrum (at least $p < 0.01$) (Fig. 1).

ESC

After ESC administration radioligand binding to the 5-HT_{2A} receptor changed only in the nucleus accumbens shell ($F_{2,21}$ = 3.93,

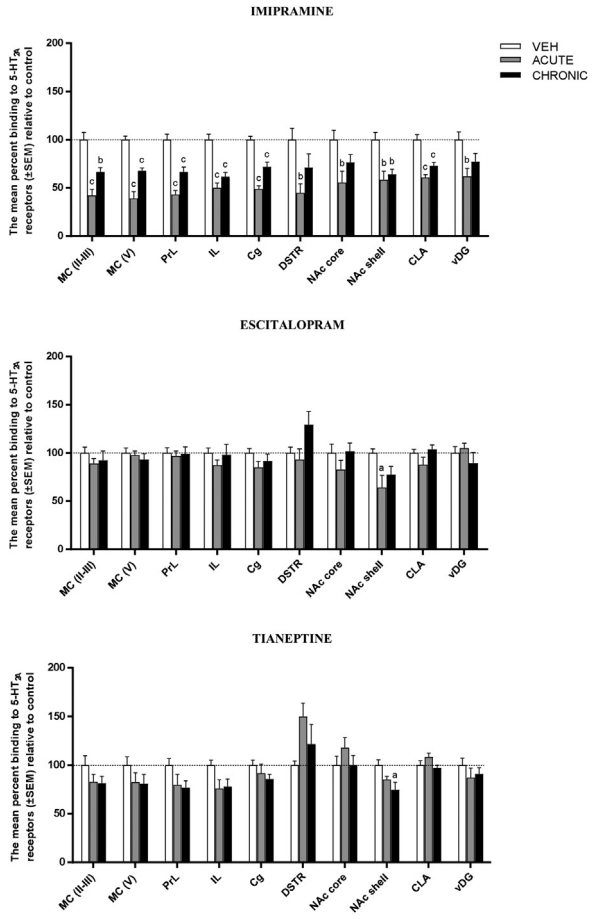


Fig. 1. The binding of [^{3}H]ketanserin to 5-HT$_{2A}$ receptors in various rat brain areas following acute and chronic administration of antidepressants. *MC (II–III)* – motor cortex II–III layers, *MC (V)* – motor cortex V layer, *PrL* – prelimbic cortex, *IL* – infralimbic cortex, *Cg* – cingulate cortex, *DSTR* – dorsal striatum (caudate putamen), *NAc core* – nucleus accumbens core, *NAc shell* – nucleus accumbens shell, *CLA* – claustrum, *vDG* – ventral dentate gyrus. All data are presented as the mean percent binding to 5-HT$_{2A}$ receptors (±SEM) relative to control (VEH). *VEH* – vehicle; imipramine (15 mg/kg); escitalopram (10 mg/kg); tianeptine (10 mg/kg). N = 7–8 rats/group. Data are analyzed using an one-way ANOVA and *post hoc* Dunnett's test: $^a p < 0.05$; $^b p < 0.01$; $^c p < 0.001$ *vs.* VEH group.

$p < 0.05$). Acute administration of ESC evoked a 35% decrease in 5-HT$_{2A}$ receptor labeling in the nucleus accumbens shell ($p < 0.05$), while ESC administered chronically did not change significantly the 5-HT$_{2A}$ receptor density in all examined rat brain areas (Fig. 1).

TIA

TIA treatment resulted in changes in the 5-HT$_{2A}$ receptor density in the nucleus accumbens shell ($F_{2,21} = 4.82$, $p < 0.05$). Acute administration of TIA did not change 5-HT$_{2A}$ receptor density, whereas TIA administered chronically showed a decrease (ca. 25%) in [^{3}H]ketanserin binding in the nucleus accumbens shell ($p < 0.05$) (Fig. 1).

Changes in [^{3}H]ketanserin binding following drugs with antidepressant activity

NAC

Administration of NAC induced changes in the 5-HT$_{2A}$ receptor labeling in the II–III layers of motor cortex ($F_{2,20} = 4.57$, $p < 0.05$), V layer of motor cortex ($F_{2,20} = 11.81$, $p < 0.001$), cingulate cortex ($F_{2,20} = 5.30$, $p < 0.05$) and nucleus accumbens shell ($F_{2,20} = 3.55$, $p < 0.05$). After acute administration of NAC, the 5-HT$_{2A}$ receptor density either increased (ca. 20–30%) in the motor (II–III, V layers) and cingulate cortices (at least $p < 0.05$) or a 30% decreased in the nucleus accumbens shell ($p < 0.05$). Also, chronic NAC administration revealed an increase in the 5-HT$_{2A}$ receptor density in the motor cortex (V layer) ($p < 0.01$) (Fig. 2).

URB597

URB597 evoked significant changes in [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptor in the II–III layers ($F_{2,21} = 3.96$, $p < 0.05$) and V layer of the motor cortex ($F_{2,21} = 5.39$, $p < 0.05$), prelimbic ($F_{2,21} = 7.47$, $p < 0.01$), infralimbic ($F_{2,21} = 8.25$, $p < 0.01$) and cingulate cortices ($F_{2,21} = 12.46$, $p < 0.001$), nucleus accumbens shell ($F_{2,21} = 5.42$, $p < 0.05$), claustrum ($F_{2,21} = 4.74$, $p < 0.05$) and ventral dentate gyrus ($F_{2,21} = 7.86$, $p < 0.01$). Acute administration of URB597 decreased the labeling of the 5-HT$_{2A}$ receptor in the motor cortex (II–III and V layers), prelimbic, infralimbic and cingulate cortices, nucleus accumbens shell, and ventral dentate gyrus (ca. 30%), the claustrum (ca. 20%) (at least $p < 0.05$), while chronic treatment with URB597 decreased (ca. 30%) 5-HT$_{2A}$ receptor binding only in the nucleus accumbens shell ($p < 0.05$) (Fig. 2).

Discussion

In this study, we evaluated the impact of the acute and repeated administration of clinically effective antidepressants (ESC, TIA), the classical antidepressant (IMI) and for the first time, compounds with antidepressant potential (NAC, URB597) on the density level of 5-HT$_{2A}$ receptors in selected rat brain structures. We observed, a reduction in the [^{3}H]ketanserin binding in the nucleus accumbens shell common to all tested drugs, while in the other examined brain areas, the effect depended on the particular drug. The most

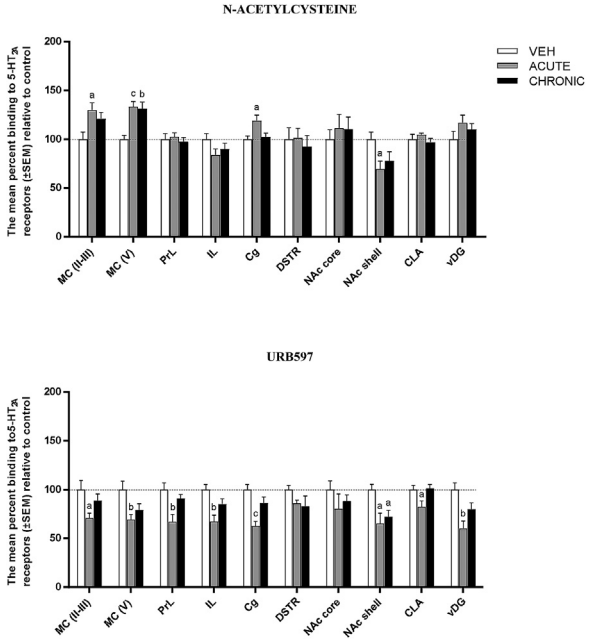


Fig. 2. The binding of [^{3}H]ketanserin to 5-HT$_{2A}$ receptors in various rat brain areas following acute and chronic administration of drug/compound with antidepressant activity in preclinical studies. *MC (II–III)* – motor cortex II–III layers, *MC (V)* – motor cortex V layer, *PrL* – prelimbic cortex, *IL* – infralimbic cortex, *Cg* – cingulate cortex, *DSTR* – dorsal striatum (caudate putamen), *NAc core* – nucleus accumbens core, *NAc shell* – nucleus accumbens shell, *CLA* – claustrum, *vDG* – ventral dentate gyrus. All data are presented as the mean percent binding (±SEM) relative to control (VEH). *VEH* – vehicle; N-acetylcysteine (100 mg/kg); URB597 - cyclohexylcarbamic acid 3-carbamoylbiphenyl-3-yl ester (FAAH inhibitor; 0.3 mg/kg). N = 7–8 rats/group. Data are analyzed using an one-way ANOVA and *post hoc* Dunnett's test: $^a p < 0.05$; $^b p < 0.01$; $^c p < 0.001$ *vs.* VEH group.

significant changes were caused by the administration of IMI, which reduced the labeling of 5-HT_{2A} receptors in all investigated rat brain structures.

Considering changes in the [^{3}H]ketanserin binding in the nucleus accumbens shell, it should be underlined that this brain structure is deeply associated with anhedonia. In fact, this core symptom of depression, defined as loss of interest in previously pleasurable activities, occurs in half of the patients with bipolar depression [21] and is linked with poor response to treatment outcome [2]. Anhedonia is regarded as a combination of different components including a lack of motivation to obtain a reward, a reduction in the experience of pleasure and a deficit in reward learning [22]. The reduced motivation is associated with dysregulation of the mesoaccumbal dopaminergic pathway from the ventral tegmental area to the nucleus accumbens, mainly to the shell subregion [23]. As previously shown, repeated treatment with antidepressant drugs having different mechanisms of action induced adaptive changes in the central dopamine D_1 and $D_{2/3}$ receptors at the behavioral level and in biochemical assays in the nucleus accumbens shell [*e.g.,* 24, 25]. More recent clinical trial with the use of positron emission tomography and the $D_{2/3}$ receptor-selective radiotracer [^{11}C]raclopride demonstrated that depressed patients show greater dopamine $D_{2/3}$ receptor availability in the bilateral ventral pallidum/nucleus accumbens, and that dopamine $D_{2/3}$ receptor availability in the nucleus accumbens correlated negatively with the severity of motivational anhedonia [26].

The nucleus accumbens receives the dense 5-HT-ergic afferents from the raphe nucleus that end mainly on the GABA-ergic medium spiny neurons which express dopamine receptors [27]. It is worth noting that in the nucleus accumbens shell 5-HT-labeled axon terminals are more numerous than in the nucleus accumbens core and they form mainly symmetrical synapses, which can postsynaptically inhibit GABA-ergic neurons, and also modulate them at the presynaptic level. In addition, 5-HT neurons affect dopamine in the nucleus accumbens by interacting with cholinergic interneurons (whose activation increases dopamine outflow). The significance of 5-HT neurotransmission within the nucleus accumbens in the context of depression is demonstrated by previous studies. Thus, single and chronic SSRI administrations increase extracellular levels of 5-HT in the nucleus accumbens [28,29] while the 5-HT infusion intra-nucleus accumbens results in an increased dopamine release [30], what correlates with antidepressant effects. Furthermore, studies on the genetic animal models of depression (Flinders Sensitive Line) demonstrated an increased inhibition of 5-HT and dopamine release compared to controls in the nucleus accumbens; the effect normalized by chronic antidepressant treatment [31].

5-HT_{2A} receptors seem to be a key player in the 5-HT – dopamine interaction in the nucleus accumbens since (i) these receptors are localized to GABA-ergic neurons with higher density in the shell [32]; (ii) local perfusion of the 5-HT_2 receptor agonist (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) into the nucleus accumbens results in dopamine increases that can be blocked by nonselective 5-HT_2 receptor antagonists [33]; (iii) 5-HT_{2A} receptor antagonists reduce the increase in dopamine in the nucleus accumbens resulting from stimulation of the dorsal raphe nucleus [34]. Thus, it was not surprising that in our study [^{3}H] ketanserin binding pattern was different in the accumbal subregions, and the changes after administration of the test drugs/compounds with antidepressant activity are visible mainly in nucleus accumbens shell. In the nucleus accumbens core decrease in the 5-HT_{2A} receptor density was observed only after acute IMI administration. At the same time, in nucleus accumbens shell, the density of 5-HT_{2A} receptor in rats decreased after a single dose of IMI, ESC, NAC and URB597 as well as the repeated administration of IMI, TIA and URB597. It may indicate that, in spite of various basic mechanisms of action of these antidepressants, the nucleus accumbens shell is a focal point for adaptive changes induced by these drugs. Of note, ayahuasca, a 5-HT_{2A} receptor agonist and harmine, a monoamine oxidase A inhibitor, induced fast-acting antidepressant effects in a small open-label trial conducted in depressive patients together with increased blood perfusion in the left nucleus accumbens assessed by single photon emission tomography [35].

Decreased binding of [^{3}H]ketanserin to 5-HT_{2A} receptors was also observed in the rat cortical structures after single administration of IMI or URB597 as well as in the ventral dentate gyrus and after chronic administration of IMI. The results are in line with the previous literature observations with chronic administration of IMI [*e.g.*, 36–38], mirtazapine, citalopram, desipramine [39,40], but not with reboxetine or fluoxetine [*e.g.,* 36,39,40]. Interestingly, a reduction in the 5-HT_{2A} receptor density was found early after 2 or 7 days of administration of IMI [41,42]. The effect observed after a single drug administration may be a result of the relatively high affinity of IMI for 5-HT_{2A} receptors and its antagonistic activity [42]. Differences between our and the latter authors' observations after a single IMI administration may be due to the fact that the brains for analysis were taken either 24 h or 48 h after the drug administration. Lower density of 5-HT_{2A} receptors may be explained by desensitization, intercalation and down-regulation caused by increasing the amount of 5-HT in the synaptic cleft resulting from inhibition of reuptake by antidepressants. Receptor down-regulation may be the result of protein degradation or disturbances at the gene expression stage [43]. As a consequence, a lower density of the 5-HT_{2A} receptors may increase the opposing neurotransmission *via* numerous co-occurring 5-HT_{1A} receptors, which also play a role in depression [42]. The 5-HT_{2A} receptor stimulation increases, and of the 5-HT_{1A} receptors reduces the release of 5-HT in the medial prefrontal cortex and limbic areas, and hence can normalize the amount of 5-HT, increased after administration of antidepressants [5]. The local intra-medial prefrontal cortex infusion of the selective 5-HT_{2A} receptor antagonist MDL 100,907 led (indirectly) to an increased dopamine release [44]. As shown in the neocortex, 5-HT_{2A} receptors located on GABA-ergic terminals tonically enhanced GABA release by endogenously released 5-HT as demonstrated by local administration of DOI and microdialysis study [45]. In the medial prefrontal cortex, by activating $GABA_B$ receptors, GABA released from these neurons may inhibit dopamine release [46]. Therefore, the reduced density of 5-HT_{2A} receptors observed in our study may result in the reduction of tonic inhibition of GABA-ergic interneurons and in the increase in dopamine release, thereby modulating disturbed dopaminergic system in depression.

URB597 reduced the immobility in the forced swim test in rats, and this effect was blocked by rimonabant (cannabinoid CB_1 receptor antagonist) and 5-HT synthesis inhibitor p-chlorophenylalanine (pCPA), which demonstrates the contribution of anandamide-CB_1 and 5-HT signaling to the antidepressant drug effect [47]. It is well known that CB_1 receptor agonists as well as acute or chronic URB597 administration increase 5-HT neural firing in the dorsal raphe nucleus [48,49] and 5-HT release in the medial prefrontal cortex and hippocampus leading to desensitization of postsynaptic 5-HT_{2A} receptors in the rat hippocampus [48], and in the prefrontal cortex of FAAH null-mutant mice [50]. A single dose of URB597 caused an increase in the level of anandamide in the hippocampus after 2 h from the administration [17], which might result in an increase in agitation of CB_1 receptor and compensative reduction of 5-HT_{2A} receptors observed in our study. Furthermore, subchronic administration (7 days) of URB597 caused an increase in brain-derived neurotrophic factor (BDNF) levels in the hippocampus which confirms negative modulation of the

plasticity associated with BDNF by 5-HT_{2A} [47]. So, our present findings may indicate that lowering 5-HT_{2A} receptor binding after URB597 may induce an antidepressant effect through increasing BDNF-dependent neuroplasticity.

In the present study, we observed for the first time that acute and repeated NAC administration resulted in an increased [^{3}H] ketanserin in the motor-cingular cortex and in motor cortex (V layer), respectively. To date there is no evidence that NAC directly interacts with 5-HT_{2A} receptors. However, NAC is a precursor of the antioxidant glutathione, a prodrug of cysteine and may modulate the glutamatergic system by increasing extrasynaptic glutamate through the activation of cystine-glutamate antiporter in glial cells and by stimulating proximal $mGlu_{2/3}$ receptors [51,52]. In addition, it has been shown that in the prefrontal cortex, 5-HT_{2A} receptors are found on glutamatergic neurons [6], and that in the cortical pyramidal neurons 5-HT_{2A} receptors and $mGlu_2$ receptors may form a heteromeric complex [53]. A single NAC administration (at a dose of 100 mg/kg) resulted in reduction of head twitch response induced by the 5-HT_{2A} receptor agonist DOI, and this effect was eliminated by a cystine-glutamate antiporter inhibitor (S)-4-carboxyphenylglycine (CPG) and LY341495 ($mGlu_2$ receptor antagonist). Therefore, it seems that in the 5-HT_{2A}-$mGlu_2$ receptor interaction, NAC can indirectly suppress the 5-HT_{2A} receptor activity through activating the $mGlu_2$ receptors in the brain cortex [54]. The complex mechanism by which NAC counteracts depression symptoms via indirect modulation of monoaminergic pathways was also postulated by other studies [55,56].

In line with previous literature findings, our present data show either increase or decrease in the cortical binding of 5-HT_{2A} receptors depending on the drug used and the time of exposure of animals. Therefore, it is difficult to find a common mechanism for all drugs and to definitely postulate that the observed changes can be regarded as adaptation mechanisms for antidepressive actions.

In conclusion, our studies confirmed the involvement of 5-HT neurotransmission via 5-HT_{2A} receptors in the action of various antidepressants and, for the first time, demonstrated the involvement of 5-HT_{2A} receptors in the mechanism of action of NAC and URB597. The present data indicate different sensitivity of 5-HT_{2A} receptor to antidepressant drugs depending on schedule of drug administration and rat brain structures. The 5-HT_{2A} receptors within the nucleus accumbens shell seem to be an interesting target down-regulated by drugs with different mechanisms of action.

Conflict of interest

The authors declare no conflict of interest.

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