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THE INFLUENCE OF REPEATED ADMINISTRATION OF CLOZAPINE AND HALOPERIDOL ON THE EFFECTS OF THE ACTIVATION OF 5-HT<sub>1A</sub>, 5-HT<sub>2</sub> AND 5-HT<sub>4</sub> RECEPTORS IN RAT FRONTAL CORTEX

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The effects of a repeated treatment with antipsychotic drugs, clozapine and haloperidol, on the modulation of network activity ex vivo by 5-HT receptors were examined in rat frontal cortical slices using extracellular recording. Rats were treated for 21 days with clozapine (30 mg/kg p.o.), or haloperidol (1 mg/kg p.o.). Spontaneous bursting activity was induced in slices prepared 3 days after the last drug administration by perfusion with a medium devoid of Mg<sup>2+</sup> ions and with added picrotoxin (30 mM). The application of 2-3 µM 8-OH-DPAT, acting through 5-HT<sub>1A</sub> receptors, resulted in a reversible decrease of bursting frequency. In the presence of 1 µM DOI, the 5-HT<sub>2</sub> agonist, or 5 µM zacopride, the 5-HT<sub>4</sub> agonist, bursting frequency increased. Chronic clozapine treatment resulted in an attenuation of the effect of the activation of 5-HT<sub>2</sub> receptors, while the effects related to 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptor activation were unchanged. Treatment with haloperidol did not influence the reactivity to the activation of any of the three 5-HT receptor subtypes. These data are consistent with earlier findings demonstrating a selective downregulation of 5-HT<sub>2A</sub> receptors by clozapine and indicate that chronic clozapine selectively attenuates the 5-HT-mediated excitation in neuronal circuitry of the frontal cortex while leaving the 5-HT-mediated inhibition intact.

**Key words:** antipsychotic drugs, 5-HT<sub>1A</sub> receptors, 5-HT<sub>2</sub> receptors, 5-HT<sub>4</sub> receptors, 8-OH-DPAT, DOI, zacopride, cortical slice

INTRODUCTION

In comparison to typical antipsychotic drugs, the atypical drug clozapine produces less extrapyramidal side-effects and has other advantages, including decreases of negative as well as of positive syndroms in schizophrenia (1, 2). The
clinical efficacy of typical antipsychotic drugs, such as haloperidol, has been attributed primarily to the antagonistic effect on the dopamine D₂ receptor (3). In contrast, clozapine is a weak antagonist at the D₂ receptor, but it also demonstrates considerable affinity for numerous other receptors, including five 5-HT receptor subtypes (reviewed in: 2). It has been hypothesized that the mechanism of action of atypical antipsychotic drugs is related to a relatively potent blockade of 5-HT₄ receptors coupled to weaker antagonism of D₂ receptors (4; reviewed in: 1, 5). It has also been suggested that these drugs may exert their effects, in part, through the activation of 5-HT₁A receptors (6).

Frontal cortex receives a dense and widespread serotonergic innervation originating from the brainstem nuclei. Frontal cortical neurons express 5-HT₁A/B/D, 5-HT₂A/C, 5-HT₃, 5-HT₄, 5-HT₆, and 5-HT₇ receptors (7), however, it has been established that the most abundant receptors belong to the 5-HT₂A and 5-HT₁A subtypes (8, 9, 10, 11). Repetitive administration of clozapine results in a decrease of the density of 5-HT₂ receptors in rats (12, 13, 14). This effect has not been observed after haloperidol administration (13, 15). Repeated clozapine administration resulted in a selective reduction in the abundance of cortical 5-HT₂A receptor mRNA, with no effect on 5-HT₂C and 5-HT₁A receptor mRNAs. Again, treatment with haloperidol did not result in changes in the level of mRNAs for any of these three 5-HT receptor subtypes (16). It has recently been reported that the administration of clozapine, but not of haloperidol, results in an increase in intracellular 5-HT₂A receptor-like immunoreactivity in pyramidal neurons, with concurrent decrease in labeling of dendrites of these cells (17).

Modulatory influence of 5-HT₂A receptors on excitability of cortical pyramidal neurons and on glutamatergic transmission may play an important role in the pathomechanism of psychosis (10, 18, 19), however, the functional significance of reported clozapine-induced downregulation of 5-HT₂A receptors for the serotonergic modulation of neuronal activity of the frontal cortex remains unexplored. We have recently shown that the synchronized network activity in brain slices represents a convenient model to assess the modulatory effects of 5-HT receptor agonists in the hippocampus (20) and frontal cortex (21, 22). Therefore, in the present study we aimed at determining the effects of a repeated treatment with two antipsychotic drugs, a typical and an atypical one, on the modulation of frontal cortical synchronized activity by 8-OH DPAT, a 5-HT₁A/5-HT, receptor agonist, DOI, a 5-HT₂A/C receptor agonist and zacopride, a 5-HT₄ receptor agonist.

MATERIALS AND METHODS

Animals

Experiments were conducted on male Wistar rats, weighing approx. 90 g at the beginning of experiment. Rats were housed under a 12 h light/dark cycle with free access to commercial food
and tap water. Animals received clozapine (30 mg/kg p.o., dissolved in 2 ml of water), or haloperidol (1 mg/kg p.o., dissolved in 2 ml of water) once daily for 21 days. Each treated group of animals had matched control group and both groups were investigated simultaneously. The experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences.

**Slice preparation and recording**

Animals were decapitated three days after the last drug administration. Their brains were removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl (130), KCl (5), CaCl$_2$ (2.5), MgSO$_4$ (1.3), KH$_2$PO$_4$ (1.25), NaHCO$_3$ (26) and glucose (10), bubbled with a mixture of 95% O$_2$/5% CO$_2$, pH 7.4. Coronal slices (450 µm) were cut from the frontal cortex (2-3 mm anterior to bregma) using a vibrating microtome. After a recovery for at least 1 hr at room temperature a single slice was transferred to the recording chamber of a submerged type and superfused at 1.5 ml/min with ACSF (2.5 ml/min), in which [NaCl] was raised to 132 mM and [KCl] was lowered to 2 mM, at 32 ± 0.5 °C. A glass micropipette filled with 2M NaCl (1-4 MΩ) was positioned approx. 2 mm lateral to the midline and approx. 0.3 mm below the pial surface (layer II/III). To induce spontaneous bursting, a modified ACSF devoid of Mg$^{2+}$ ions and containing 30 µM picrotoxin was introduced. Spontaneous bursting activity developed in a majority, but not in all tested slices, although the number of animals in treated and control groups were equal. There was no relationship between the occurrence of bursting and the treatment. The signal was band-pass filtered (1 - 1000 Hz), recorded using 1401 interface and SIGAVG software (CED, UK) and displayed using a chart recorder (Gould, USA).

**Chemicals**

After stabilization of bursting activity patterns, slices were superfused for 9-15 min with modified ACSF containing the tested 5-HT receptor agonist, which was subsequently washed out. The agonists used were: (+)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT; Sigma), (+)-2,5-dimethoxy-4-iodoamphetamine hydrochloride ((±)-DOI hydrochloride, Sigma) and 4-amino-5-chloro-2-methoxy-substituted benzamide ((R,S)zacopride, generously donated by Delalande, France). Haloperidol and clozapine were obtained from Sigma.

The results are expressed as means ± S.E.M. or as means ± S.E.M. percent change of baseline values.

**RESULTS**

Spontaneous bursting activity developed in frontal cortical slices within 15-25 min of superfusion with modified ACSF devoid of Mg$^{2+}$ ions and containing 30 µM picrotoxin. Repetitive bursts, whose mean frequency ranged between 0.012-0.021 Hz, consisted of a fast negative-going field potential followed by a slower, negative-going waveform and field potential oscillations of variable duration, and were similar to those obtained in earlier studies (21, 22). Sample recordings are shown in Fig. 1. It has previously been shown and confirmed in this study that bursting activity could be modulated by DOI and 8-OH-DPAT, applied by bath for 9 min, or zacopride, applied by bath for 15 min (21, 22).
These effects could be attributed to the activation of 5-HT<sub>2</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors, respectively (21, 22).

As illustrated in Fig. 2A, repeated administration of clozapine resulted in an attenuation of the excitatory effect of the activation of 5-HT<sub>2</sub> receptors by 1 µM DOI. While in control, untreated animals, the activation of 5-HT<sub>2</sub> receptors resulted in an increase of the discharge frequency by 71±9% (n=16), in clozapine-treated group the effect of DOI application was significantly weaker and reached 43±6% (n=15; P=0.013, t test). In contrast, neither the effect of the
activation of 5-HT₄ receptors by 5 µM zacopride (in control group: 49±6% increase), nor the inhibitory effect of the activation of 5-HT₁₆ receptors by 2 µM 8-OH-DPAT (in control group: 25±6% decrease) were significantly affected by clozapine treatment (Fig. 2A).

In the second set of experiments, the influence of a repetitive administration of haloperidol on modulatory effects of 5-HT₃, 5-HT₁₆ and 5-HT₄ receptors was investigated. As illustrated in Fig. 2B, no significant changes in the magnitude of either excitatory effect of 1 µM DOI (in control group: 56±5% increase) and 5 µM zacopride (in control group: 55±7% increase), or inhibitory effect of 3 µM 8-OH-DPAT (in control group: 25±3% decrease), were detected.

Fig. 2. The effects of repeated (21 days) treatment with clozapine (A) and haloperidol (B) on the DOI and zacopride induced increase as well as the 8-OH-DPAT induced decrease of epileptiform discharge rate. Numbers indicate the numbers of cases in each group. *P=0.013, t-test.
DISCUSSION

The incubation of neocortical slices in Mg\(^{2+}\)-free ACSF results in an enhancement of conductance through NMDA receptor channels and thus in an increase of mono- and polysynaptic excitatory postsynaptic potentials (23). Spontaneous epileptiform discharges depend on intrinsic bursting properties of pyramidal cells (24). Discharges are initiated in a small group of interconnected neurons located in layer V (25) and/or in other cortical layers (26, 27). Epileptiform activity is further enhanced due to the blockade of fast GABA\(_{A}\) receptor-mediated inhibitory postsynaptic potentials by picrotoxin (21, 22, 23, 27). It has previously been demonstrated that spontaneous epileptiform activity in frontal cortical slices could be modulated by 5-HT receptor agonists. While the activation of 5-HT\(_{1A}\) receptors by 2-3 µM 8-OH-DPAT results in a reversible decrease in discharge frequency, the activation of 5-HT\(_{2}\) and 5-HT\(_{4}\) receptors by DOI (1 µM) and zacopride (5 µM), respectively, results in an increase in the frequency of discharges (21, 22). Since fast GABAergic transmission remains blocked, these effects could be attributed to the known influence of the activation of 5-HT receptors on the excitability of single pyramidal neurons. 5-HT\(_{1A}\) receptors reduce the excitability of cortical pyramidal cells by inducing membrane hyperpolarization and a decrease in the input resistance through opening of inwardly rectifying potassium channels (GIRK) via G-proteins (28). 5-HT\(_{2}\) receptors increase the excitability of cortical neurons by inducing membrane depolarization, a decrease of spike frequency accommodation and an occurrence of the slow afterdepolarization, which follows the burst of spikes, by reducing outward potassium currents (29) and to the stimulation of phospholipase C-mediated phosphoinositide hydrolysis (30). Moreover, the activation of 5-HT\(_{2A}\) receptors enhances spontaneous EPSPs (18). Similarly, 5-HT\(_{4}\) receptors increase pyramidal cell excitability by depolarization as well as by a decrease of the slow afterhyperpolarization and spike frequency adaptation (31) due to a closure of potassium channels. These effects are mediated by an increase in cAMP level and activation of protein kinase A (32).

The results of the present study indicate that treatment of rats with the atypical antipsychotic drug, clozapine, induced a selective reduction of the electrophysiological effect of the activation of 5-HT\(_{2}\) receptors, which was evident three days after the end of the treatment. The presence of clozapine in the tissue at this time-point is unlikely (33). The difference between the magnitude of the effect of DOI in two control groups of animals are most likely due to seasonal variability. In contrast, no effects of treatment with haloperidol, a typical antipsychotic drug, on the reactivity of these receptors were detected. These results are consistent with a reported decrease of the density of 5-HT\(_{2}\) receptors in the cortex of rats after clozapine treatment (12, 13, 14) and a lack of such change after haloperidol administration (13, 15). Since haloperidol is a selective antagonist of the D\(_2\) receptor (3), a lack of the influence on 5-HT receptors is not
unexpected. On the other hand, the atypical antipsychotic drug clozapine demonstrates considerable affinity for five 5-HT receptor subtypes, among which the interaction with the 5-HT$_{2A}$ receptor is the strongest (reviewed in: 2). Consistent with the present results it has also been shown that repetitive administration of clozapine or haloperidol for two weeks does not induce changes in the density of cortical 5-HT$_{1A}$ receptors (16). While the data regarding the level of 5-HT$_4$ receptors after repetitive administration of neuroleptics in rats are unavailable, it has been reported that neither chronic clozapine nor haloperidol changed the reactivity of hippocampal neurons to the activation of 5-HT$_4$ receptors (34). The mechanisms of a selective decrease in the abundance of cortical 5-HT$_{2A}$ receptors in rats by clozapine may involve a diminished gene expression (16), most likely resulting from decreased gene transcription (35) and/or post-transcriptional mechanism dependent on protein kinase C (36). It has also been suggested that clozapine may induce 5-HT$_{2A}$ receptor internalization (17).

The results of the present study are in line with the view that repeated administration of clozapine exerts a stronger effect than haloperidol upon the cortical serotonergic system and that this effect is mediated via 5-HT$_2$ (possibly 5-HT$_{2A}$) receptors (16). A decrease in the number of 5-HT$_2$ receptors will reduce the excitatory effect of 5-HT released from serotonergic terminals on cortical neurons. If enhanced serotonergic transmission in frontal cortex contributes to positive syndroms of schizophrenia, as suggested by studies on hallucinogens (e.g. 19), then the therapeutic effects of clozapine include not only a modification of the excitability through a direct blockade of 5-HT$_{2A}$ and other receptors located on cortical neurons, but also through a sustained reduction in the responsiveness of these cells to 5-HT.

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