

Original Article

Regulation of Ionotropic Glutamatergic Mechanisms Following Treatment with Chlorpromazine

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Abstract

Objective: Chlorpromazine hydrochloride (CPZ) refers to antipsychotic drugs. CPZ is drug of first choice for the treatment of schizophrenia. Currently interaction CPZ with dopamine receptors is the dominant view. However, its impact on the main excitatory neurotransmitter system, glutamatergic, is unclear. This issue is the aim of our study.

Methods: Experiments carried out on brain slices of the olfactory cortex of rats. We used electrophysiological techniques extracellular recordings in brain slice to investigate CPZ effects on the activity of the both glutamatergic ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors (AMPA) and N-methyl-D-aspartic acid type glutamate receptors (NMDARs).

Results: Application of CPZ on slices modified the incoming and outgoing sodium potassium currents involved in the generation of AMPA EPSP (excitatory postsynaptic potential). CPZ nonlinear way modified AMPARs activity with increasing concentrations. Opposite, NMDARs activity decreased in a linear manner with increasing concentrations CPZ. The neuroleptic actively interacted with glutamate and glycine sites of NMDARs. To specify of CPZ neurotrophic effects promazine, which is structurally similar to CPZ, was applied on slices. Promazine did not caused significant modifications of the activity both AMPARs and NMDARs. In order to improve functions of NMDARs slices pretreated by ammonium chloride (NH_4Cl , 20 mM), which is commonly recognized as fast and efficient lysosomal inhibitor. Such processing slices and subsequent action of CPZ protected the functioning of NMDARs.

Conclusions: Data presented in this study on the influence of CPZ on the ionotropic glutamatergic mechanisms allow to understand the multifaceted mechanisms action of neuroleptic in protection against deterioration of mental disorders such as schizophrenia.

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Introduction

Chlorpromazine (CPZ) is on the World Health Organization list of essential medicines remains drug of first choice for the treatment of schizophrenia

despite the emergence of other drugs (1, 2).

Chlorpromazine hydrochloride (some synonyms aminazine, largactil, thorazine, etc.), the most commonly used form of CPZ in the clinic, refers to a group of antipsychotic drugs. It is a dimethylamine derivative of phenothiazine, has a chemical formula of $C_{17}H_{19}ClN_2SHCl$ and molecular weight: 355.33 g/mol. In the blood of warm-blooded, it interacts with plasma proteins, its binding reaches 90–99 %. The time of its half-life was 30 ± 7 hours, which indicates its long-term stability.

The principal pharmacological actions are antipsychotropic. It also exerts sedative and antiemetic activity. CPZ has actions at all levels of the central nervous system, primarily at subcortical structures of the brain – reticular formation of the midbrain. CPZ has strong antiadrenergic and weak peripheral anticholinergic activity. It also possesses slight antihistaminic and antiserotonergic activity (3, 4, 5).

Currently interaction CPZ with dopamine receptors is the dominant view. At the same time, its impact on the main excitatory neurotransmitter system, glutamatergic, has poorly been studied, although some fragmentary data confirm such interactions (6, 7, 8). The importance of this question consists in that in recent years developed intensively theory pathogenesis of schizophrenia, where the main role in this disease play a disturbances glutamatergic mediator system. Confirmation “glutamatergic” hypothesis is based on the fact that the injection in animal NMDA-dependent antagonists of glutamate receptors (phencyclidine, ketamine, MK-801, etc.) is one of the most common and adequate model of schizophrenia (9, 10). NMDA-receptor antagonists induce behavioral disorders in humans, which are very similar to the clinical manifestations of schizophrenia (11, 12, 13). Phencyclidine injections can cause memory impairment without symptoms of psychosis. Chronic exposure NMDA-antagonists leads to cognitive deficits, very similar to the schizophrenic (11), which is believed to be associated with dysfunction of NMDARs (14).

Insufficient the specific data on mechanisms of

influence of CPZ on glutamatergic receptor mechanisms prompted us to investigate CPZ effects on the activity of key glutamatergic ionotropic receptors, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) and *N*-methyl-D-aspartic acid type glutamate receptors (NMDARs) (15) of these, AMPARs implement rapid transmission synaptic excitation throughout most of the vertebrate central nervous system. The NMDARs family have received special attention because of its distinct role in the regulation of synaptic plasticity – long-term potentiation/depression and experience-dependent synaptic refinement (16, 17) and because of its critical role in neurological and psychiatric disorders (18, 19). Hypo- or hyperactivation of NMDARs is critically involved in pain amplification, stroke, epilepsy, schizophrenia, post-traumatic stress disorder, dementia, depression and various neurodegenerative diseases (e.g. Alzheimer's and Parkinson's) (19).

For the experimental study, the influence of CPZ on AMPARs and NMDARs is important to choose an adequate and optimal experimental object, in which was not significant influence of the dopaminergic and serotonergic neurotransmitter systems, because CPZ blocks these receptors. Because our studies conducted on slices the olfactory cortex of Wistar rats. The advantages of these slices is that in them are present and actively operate AMPARs and NMDARs as in the base functions of excitation transfer, as well and in more complex processes of learning. Presence of dopaminergic and serotonergic receptors in these slices is a minimum (20). These, data allow us to ignore the involvement of these neurotransmitter systems under the action of CPZ and to submit involvement of AMPARs and NMDARs in slices in “pure form”.

In addition, in order to understand the initial processes of the CPZ we studied the changes of activity of the both AMPARs and NMDARs in neuronal network at stimulation the lateral olfactory tract, which is the main afferent input to the cells of the olfactory cortex. Thus the goals of our research is to elucidate the dynamic the impact CPZ on the activity of AMPARs and NMDARs, to determine the dose-dependent effects of this antipsychotic and also to

find method elimination of negative effects CPZ on ionotropic glutamate receptors.

Materials and methods

Animals

Wistar rats with body weight 100–150 g were obtained from vivarium (Pavlov Institute of Physiology, RAS Saint Petersburg, Russia) and kept in animal room with controlled temperature ($21 \pm 1^\circ\text{C}$) and humidity (55%), with food and water ad libitum in a 12 h dark/light cycle. All experiments were performed in compliance with ethical standards of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All efforts were made to minimise animal suffering and the number of animals used.

Preparation of slices

Studies were performed on male Wistar rats with body weight 100–150 g (vivarium of the Pavlov Institute of Physiology, RAS). In this work were used methods of the slices preparation and their incubation as described in our previous publications (21, 22, 23). Tangential slices of olfactory cortex 450–500 μm thick were cut from the brain of male rats of Wistar line with body weight 100–150 g.

The animals were decapitated by the guillotine (Pavlov Institute of Physiology RAS, Russia). Using special surgical instruments brain were rapidly removed and placed on a metal table, cooled to $+4^\circ\text{C}$ and covered with filter paper. With a scalpel along the midline the brain was dissected into two halves, gently rolled over so that the olfactory cortex was upstairs. With the help a glass slide with support guide and special knife – “cutter” (Pavlov Institute of Physiology, RAS) prepared olfactory cortex slices of rat brain. These tools were used to minimize injury to slices structures because our previous studies have shown that the use of vibroslicer leads to greater injury of the slices structures and worsens their vital activity (24). The prepared slices were transferred to a glass vial with a brush and every slice was preincubated for 1 h in 1 mL of artificial cerebrospinal fluid (aCSF)

at 37°C , pH 7.21–7.24. The composition of aCSF was as follows (mM): NaCl – 124.0, KCl – 5.0, CaCl_2 – 2.6, KH_2PO_4 – 1.24, MgSO_4 – 1.2, NaCHO_3 – 3.0, tris-HCL (pH 7.4) – 23.0, glucose – 10.0. aCSF was equilibrated with O_2 . The concentrations of Ca^{2+} and Mg^{2+} were optimized for maximal synaptic activity in olfactory cortex slices.

The duration of the entire procedure of slice preparation from the time decapitation and placing it in the incubation medium was 1 min. After placing, the slice in a glass vial gas atmosphere above a medium was replaced of oxygen for 1 min. The vials with slices placed in a Warburg apparatus (Germany) with a frequency of 120 swings per min and a temperature of 37°C , where the slices preincubated before being placed into the recording chamber. The incubation medium with slice was replaced by a fresh aCSF twice after 1 and 3 hours of preincubation in order to remove from medium the remnants of disrupted cells and their metabolites. The slices incubated at 37°C to restore full functioning of slice structures after their preparation. We early found that slicing using incubation medium warmed to near-physiological temperature (37°C), greatly enhance slice quality without affecting intrinsic electrophysiological properties of the neuronal network (24). The advantage of using such a method has been confirmed by recent studies Huang and Uusisaari (25).

Osmolarity of aCSF was 295–305 mOsmo (OMT-5-01, “Burevestnic”, Russia). After preincubation, slice-by-slice was transferred into the interface recording chamber. Drug solutions were prepared in extracellular solutions and applied to slices by perfusion system at a constant rate (2 ml/min), controlled by the electronic device (Pavlov Institute Physiology, RAS).

Electrical stimulation and recording techniques

Extracellular field potentials (FPs) were evoked using platinum custom-made bipolar stimulating electrodes positioned onto the proximal part of the lateral olfactory tract (LOT), which is the main afferent input to the neurons of the olfactory cortex. Stimulation was applied as the rectangular pulses (duration – 0.1 ms, intensity – 1.2–1.5 V, frequency – 0.003

Hz) using the stimulator ESU-1 (Russia).

The FPs were recorded using a glass microelectrode filled with 1M NaCl with tip resistance 1–5 mOm. Signals were registered with an NTO-2 amplifier (Russia), digitized by analog–to–digital converter MD-32 (Russia) and stored on the computer. The recording point was located in the piriform cortex of the olfactory cortex slice. A silver reference electrode was located in the chamber floor.

Typical FPs in the piriform cortex evoked by orthodromic stimulation of the LOT anterior part consist of two main components: namely, presynaptic (AP LOT) and postsynaptic: AMPA and NMDA EPSP. The components of FPs, their characteristics, pharmacological identification and methods of measuring their amplitudes were described in detail earlier (21, 23). In present study we recorded and analyzed the changes amplitudes the both of AMPA and NMDA EPSP. Amplitudes of these FP postsynaptic components we estimated from the isoline to the peak level as shown in Fig. 1A (the top row of records). The amplitudes of AMPA EPSP we assessed within an 2 ms window centered at the peak of the response. Peak NMDA EPSP was measured as the average potential observed in an 8 ms window (23).

Drugs

Chemical compounds for the preparation of aCSF were supplied by Chimreaktiv Company (Russia), Chlorpromazine hydrochloride was received from Moscow endocrine factory (Russia). CPZ was dissolved in modified aCSF, in which NaCHO_3 was eliminated because it forms insoluble precipitates upon contact with CPZ was substituted for an equal amount of NaCl.

CPZ was dissolved in aCSF immediately before testing. The prepared solution filtered and kept in the thermostat at 37 °C until use. CPZ applied on slices via bath perfusion at a constant rate (2 ml/min) and controlled by the electronic device (Pavlov Institute Physiology, RAS).

The design of the experiment

We studied the effects of the CPZ at concentrations 10^{-6} , 10^{-5} and 10^{-4} M. These concentrations of CPZ were selected based at concentrations similar to those attained in the brain of psychotic patients (26). At first, the slices perfused by control aCSF and FPs recorded during 15 min. Among the components of the FPs were analyzed the amplitudes of postsynaptic components, AMPA and NMDA EPSP which reflect the activities of AMPARs and NMDARs, respectively. These values considered as control for subsequent actions of CPZ. Then slices treated CPZ in one of the above concentrations for 30 min. During this time, the both AMPA and NMDA EPSPs were recorded and analyzed. The slices were washed by control aCSF during 30 min, and the activity of these receptors determined again. FPs recorded under control conditions and in the presence of CPZ (30 min). The LOT was stimulated under control conditions and during treatment with the test substances with frequency 0.003 Hz.

In order to determine the effect of CPZ on activation of sodium and potassium channels, shaping of AMPA EPSP, we determined by the formula $dV \text{ (mV)}/dt \text{ (ms)}$ the degree of change sodium and potassium influx (Fig. 2).

Statistical analyses

The statistical analyses of the changes in amplitudes of separate FP components were performed using the nonparametrical U test, Wilcoxon-Mann-Witney matched pairs signed–rank test ($P \leq 0.01$). The data are presented as mean \pm S.E.

Results

Effects of CPZ on FP amplitudes modification in brain slices

The synaptic responses in the slice recorded following addition of CPZ at different doses in the bathing medium. The FPs registered during 30 min of CPZ action.

As shown in Fig. 1A CPZ in concentrations of 10^{-6} M induced initial increase followed by a decrease of the amplitudes of the both AMPA and NMDA EPSP. In order to assess the degree of interaction CPZ with AMPARs and NMDARs and the duration its effects on these receptor mechanisms we has been used the test on reversibility of CPZ action during washing. This assay for reversible CPZ action at 10^{-6} M concentrations showed that the effects neuroleptic were resistant. At the end of the washing the both AMPA and NMDA EPSP amplitudes exceeded the control values (Fig. 1A, Wash, 30 min).

Increase concentration CPZ in the perfusion medium to 10^{-5} M did not caused activation of the AMPARs

during washing (Fig. 1B). In contrast, activation NMDARs increased (Fig. 1B, 10 min) and decreased by the end of action CPZ (Fig. 1B, 30 min). In the test for the reversibility of activities of the both AMPARs and NMDARs were equal to control values (Fig. 1B, Wash, 30 min).

CPZ in concentration 10^{-4} M induced the different responses of the AMPARs and NMDARs. So, amplitude AMPA EPSP increased in the initial period action CPZ (Fig. 1C, 7–20 min), and at 30 min was equal to the control value (Fig. 1C, 30 min). Surprisingly, activation NMDARs did not change during 20 min but at 30 min decreased below control values. In the test for the reversibility of activation of

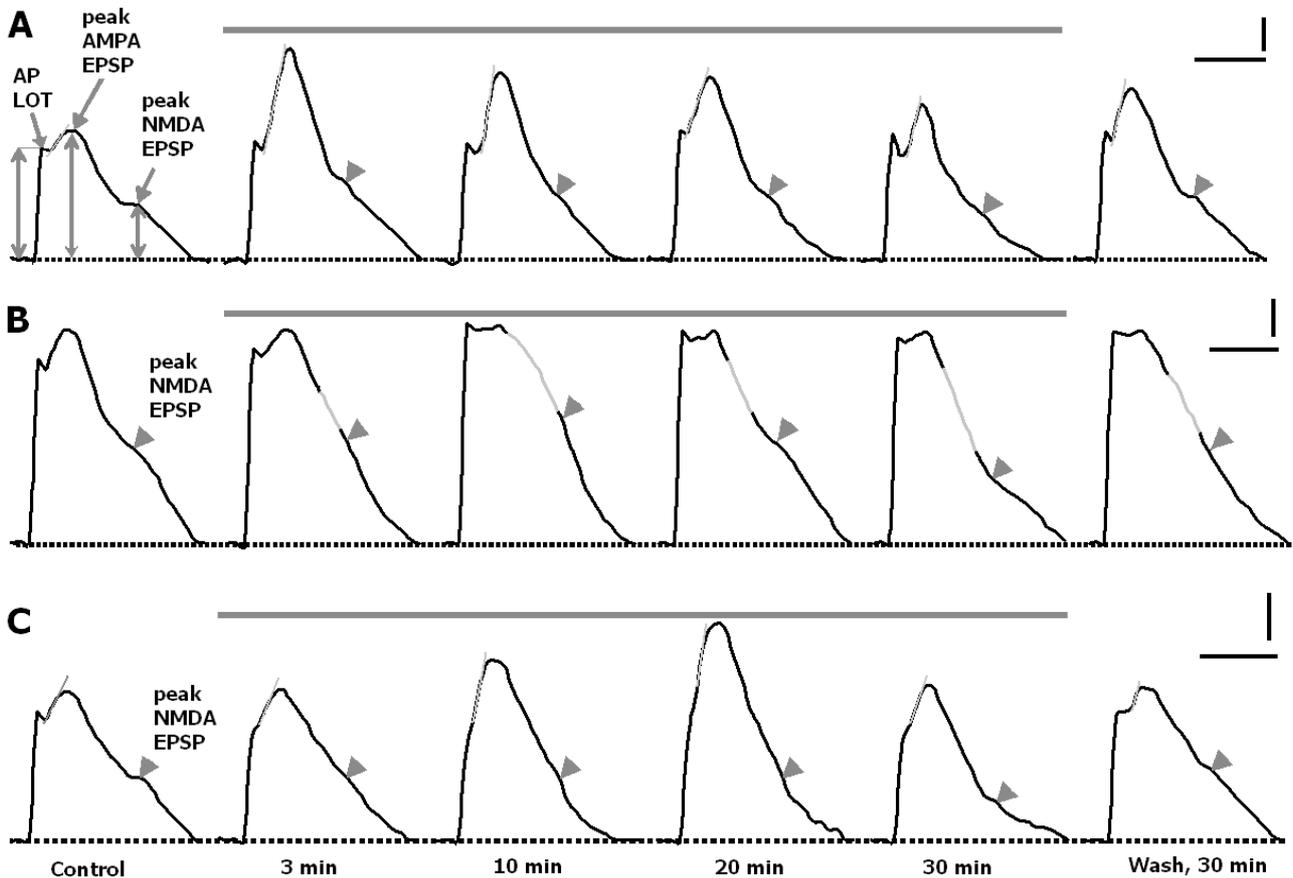


Fig. 1: Representative traces of synaptic responses recorded in piriform cortex of olfactory slices at perfusion CPZ with different concentrations.

FPs at perfusion with CPZ in concentrations 10^{-6} M (A), 10^{-5} M (B) and 10^{-4} M (C). The gray solid line in A, B and C – the duration of application CPZ. Horizontal dotted line – isoline. At the control FPs in A arrows indicate the separate components of FPs. Gray vertical arrows from the isoline to AMPA and NMDA of peaks of EPSP indicate methods of measurement the amplitudes of these components FPs. In A and C, the tangent gray lines represent the lines slope of the rising phase of AMPA EPSP. In B, a gray inclined lines indicates the slope of the descending phase AMPA EPSP. These labels are presented for estimation the degree of activation of sodium (A, C) and potassium channels (B). Numerals on the bottom of registration traces in C – time points of FPs registration. Thick gray arrows on the descending phase of the FPs indicate the peaks of NMDA EPSP. Calibration in A, B and C: 0.1 mV, 5 ms. At the FPs registration, the electronic device for artefact-rejection was used.

the both AMPARs and NMDARs were higher than control values (Fig. 1C, Wash, 30 min).

Effects of CPZ on input sodium and output potassium currents in AMPA EPSP in brain slices

Further, to understand how CPZ affects the basic mechanisms electrogenesis in piriform cortex we recorded the activity of sodium and potassium channels by the method described in section "Materials and methods", paragraph "The design of the experiment".

It has been found that the degree of activation sodium channels and respectively sodium influx, has a nonlinear wave-like nature when slices exposed CPZ in concentrations of 10^{-6} M and 10^{-5} M. CPZ in the initial period action increased the activation of sodium channels with a maximum effect on 7 min at

concentration 10^{-6} M. The minimum degree of activation sodium channels at this concentration CPZ was at 20 min and persisted up to 30 min. In the assay reversibility during washing the degree of activation sodium channels was decreased as compared with initial CPZ effects (control, 100 % vice versa CPZ Wash 30 min, 267%, Wilcoxon-Mann-Whitney, $U = 12$, $n = 5$, $P \leq 0.05$) (Fig. 3A).

CPZ in concentration 10^{-5} M evoked inhibition activity of sodium channels throughout the entire time of action antipsychotic and at washing (Fig. 3A). When analyzing of repolarization process AMPA EPSP been found that activation of potassium channels and, correspondingly, potassium outflux had a nonlinear character for CPZ in concentration 10^{-6} M. Maximal effect of CPZ (10^{-6} M) was on 10 min. With further action CPZ, activity of potassium channels decreased and remained until the end action of the

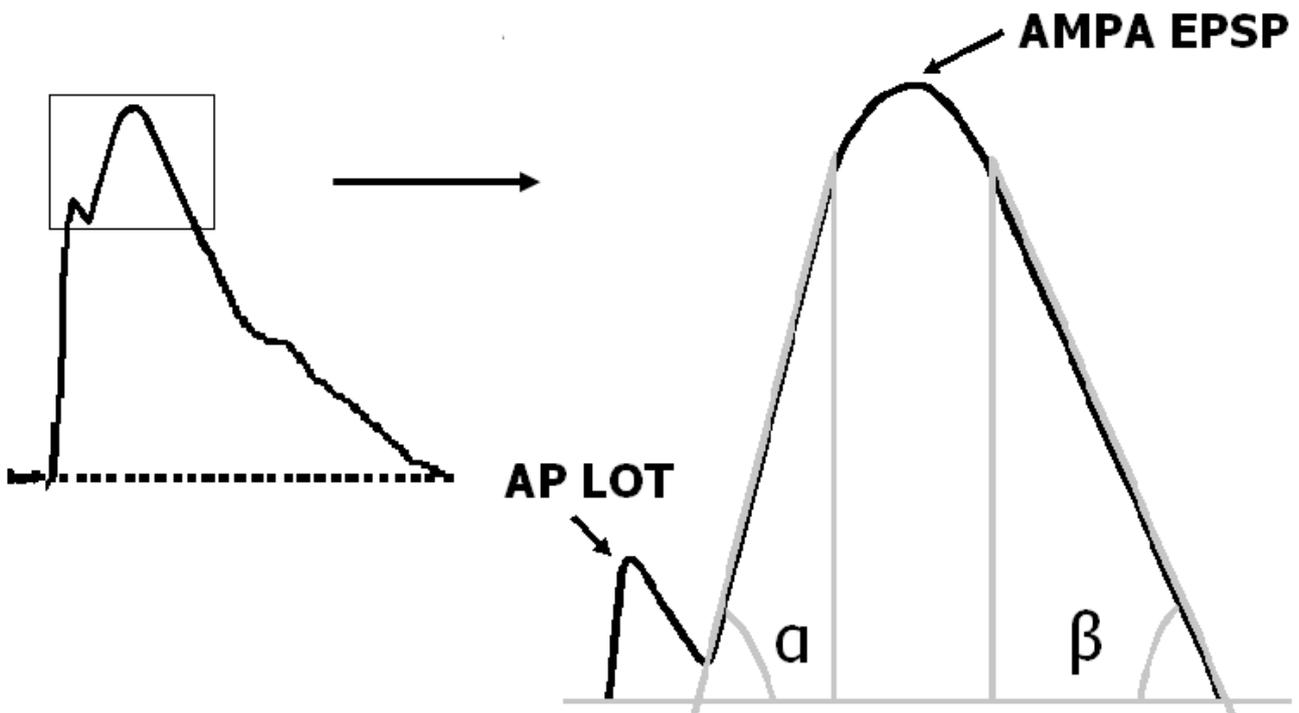


Fig. 2 : Method for measuring input sodium and output potassium currents in generation AMPA EPSP. In the left part figure is shown a typical FP, recorded in the olfactory cortex slice, in box AMPA EPSP and part of AP LOT were marked. In the right part figure, gray lines indicate methods measuring the slope of the rising phase (α) and the slope of the descending phase (β) AMPA EPSP. These tangents are localized on the rectilinear sites the rising and descending phases of AMPA EPSP. They reflect the degree of activation and time opening of sodium (rising phase) and potassium (descending phases) channels in formation of AMPA EPSP, respectively. The calculation of these currents, participating in the generation of AMPA EPSP was determined by formula dV (mV)/ dt (ms) using a special computer program. AMPA EPSP slopes were measured (bin size of 0.05 mV/ms). To determine of control response, the average slope from five AMPA EPSP for each slice was registered. This baseline values were considered as control and the slopes of individual responses were expressed to percentage to this level. Additionally, the slopes of the AMPA EPSP for every concentrations CPZ in fixed time points were measured.

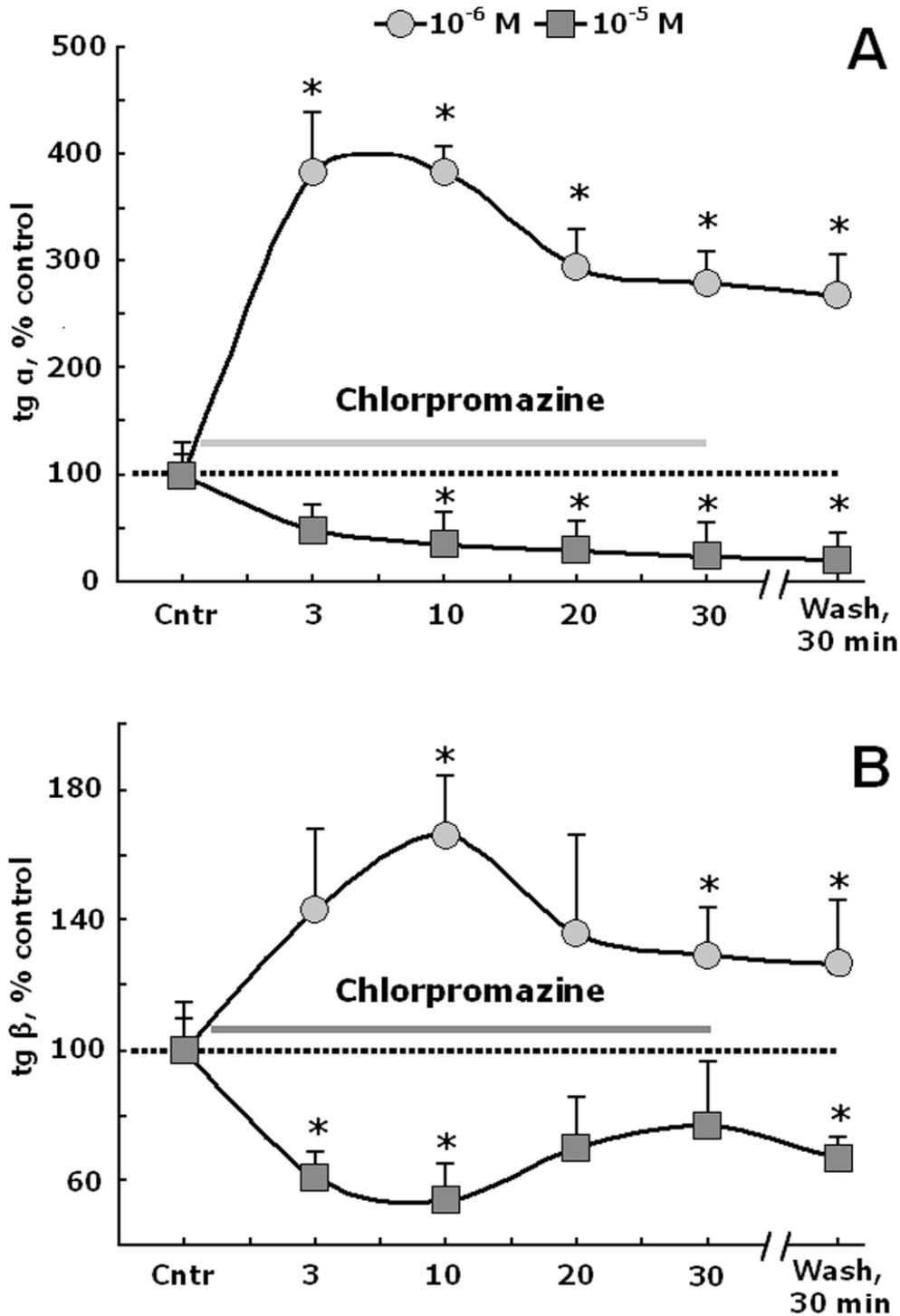


Fig. 3: Modifications rising and descending slopes of AMPA EPSP under the action of CPZ in different concentrations. A – changes of rising AMPA EPSP slope under the application of CPZ in different concentrations, as shown at the top of the figure. Ordinate – rising slope (A) and descending slope (B) of AMPA EPSP, % of control. Abscissa – Cntr, control, the initial AMPA EPSP slopes were measured and be referred to as control values. Numbers indicate time (min) registration of the averaged slopes value during action CPZ and these values were normalized in each experiment. Wash, 30 min value of the rising slope during washing. B – changes of descending slope of AMPA EPSP under the application of CPZ in different concentrations. Abscissa and ordinate – designations are the same as in A. * – P≤0.05, Wilcoxon-Mann-Whitney, U test. n = 5 for every point.

antipsychotic drug. In the test for reversibility during washing the activity of potassium channels remained higher than control values (control, 100% vice versa CPZ Wash 30 min, 127%, Wilcoxon-Mann-Whitney, $U = 10$, $n = 5$, $P \leq 0.05$) (Fig. 3B).

CPZ at a concentration of 10^{-5} M induced a wave-like inhibition of the potassium channels. It is interesting to note that when washing activity of these channels was lower than in control (control, 100% vice versa CPZ Wash 30 min, 67%, Wilcoxon-Mann-Whitney, $U = 8$, $n = 5$, $P \leq 0.05$) (Fig. 3B).

These findings indicate CPZ modifies the activity of sodium and potassium channels. These effects were not linear that indicate on possibility internalization of CPZ by nerve cells of brain slices.

Dose-relationship for changes activities of AMPARs and NMDARs

In order to understand the effects CPZ in different concentrations on the activity of AMPARs and NMDARs, we studied the dose-dependent pattern. AMPA and NMDA responses were studied depending on the time of action CPZ in different concentrations as well as in a test for reversibility at washing. It has been found that when the lowest concentration CPZ (10^{-6} M) activation AMPARs was increased. Moreover, these changes were phase: the initial transient increase in amplitude AMPA EPSP (1–7 min). At the end of the action CPZ, activation AMPARs reduced and was not statistically different from control values. In the test of reversibility at washing was discovered that activation AMPARs remained elevated and was significantly different from control values (Fig. 4A). The concentration of CPZ (10^{-5} M) did not lead to significant changes in AMPARs both in action and in washing (Fig. 4A).

Application on slices CPZ in higher concentration (10^{-4} M) caused a stepwise increase activation AMPARs. Small increase in amplitude AMPA EPSP was in the time range 7–15 min. Increase in activation AMPARs was significant from 16–20 min. However, further action CPZ in this concentration level of activation AMPARs declined up to control values at the end of his action. In the test for reversibility of

the activation of AMPARs increased and persisted for washing (Fig. 4A).

The pattern of changes of NMDA responses by the action CPZ at different concentrations differed from AMPA responses. When using the low concentrations CPZ (10^{-6} M and 10^{-5} M) was increase of activity NMDARs. Moreover, at a lower concentration CPZ (10^{-6} M), this was short-term excitation at 3–7 min, and then the curve was parallel to the abscissa. At both concentrations, CPZ (10^{-6} M, 10^{-5} M) activation NMDARs at the end of its actions dropped significantly compared to the control. In washing NMDARs, activation was increased, but the short-term at the 5th min. While continuing to washing AMPARs activity did not differ from control values (Fig. 4B).

The highest concentration CPZ (10^{-4} M) caused a slight inhibition of activation NMDARs with 7–15 min and maximal inhibition was at the end of action neuroleptic drug. In the test of reversibility at washing activation NMDARs increased, but these values were not statistically significant (Fig. 4 B).

For clarify the dose-dependent effect of CPZ we determined the AMPA and NMDA EPSP amplitudes during 15 min. This was made in order to isolate the direct effect of CPZ on AMPA and NMDA dependent mechanisms. Proportional dose effects were observed only for NMDARs, while for AMPARs such regularity was not detected (Fig. 5A, B). Probably, CPZ modifies activities separate subunits of the AMPA and NMDA receptor complexes (for further analysis, see below, paragraph "CPZ effects on glutamate and glycine sites NMDARs").

The effects of promazine on modifications of activity AMPARs and NMDARs

It is known that promazine is structurally similar to CPZ. This drug belongs to a phenothiazine families with actions similar to CPZ but it possess no antipsychotic activity. Promazine is primarily used in short-term treatment of disturbed behavior and as an antiemetic (pubchem.ncbi.nlm.nih.gov). Therefore, we chose promazine as the reference drug in order to control the specificity of CPZ effects on the both

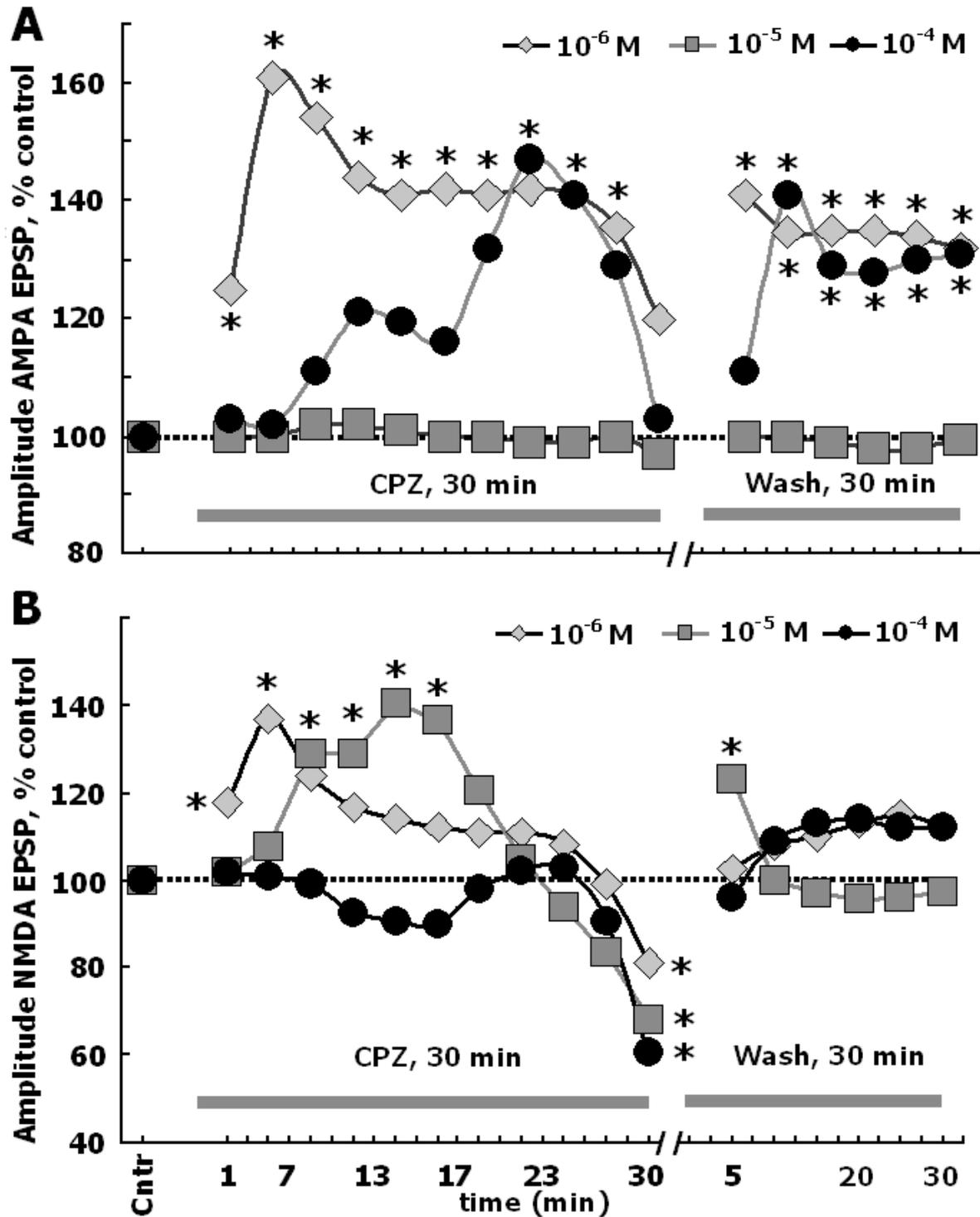


Fig. 4 : Effects treatment of slices with different concentrations of CPZ on the amplitudes of AMPA (A) and NMDA (B) EPSP as an index of activities AMPARs and NMDARs.

Horizontal dotted line - control level for AMPA (A) and NMDA EPSP (B). The gray solid line in A and B marked "CPZ, 30 min" indicate time applications on slices in different concentrations presented in the top of the charts. The gray solid line in A and B marked "Wash, 30 min", time washout of slices by control aCSF. Abscissa - Cntr, control, the control value of AMPA (A) and NMDA EPSP (B) amplitudes referred to as control values. Numbers indicate time (min) registration during action CPZ. Wash, 30 min bathing by control aCSF. Scale time is irregular. Ordinate - designation is indicated in Figure. * - P≤0.05 compared with control values, nonparametric Wilcoxon-Mann-Whitney U test. n = 9 for each timepoint. SD is omitted. Detailed explanations in the text.

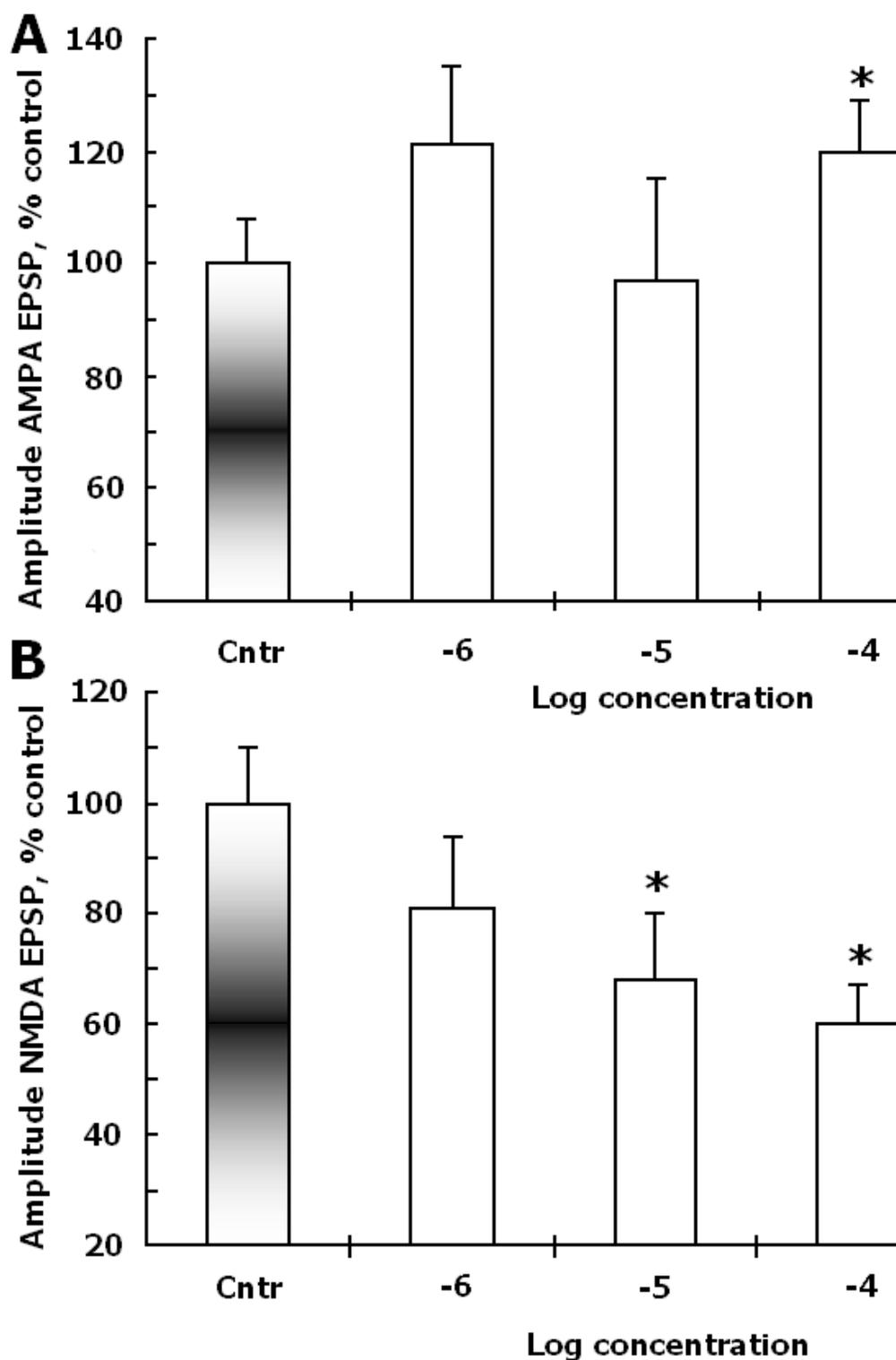


Fig. 5: Dose–response relationship for changes in the amplitudes of AMPA (A) and NMDA EPSP (B) under the action of different concentrations of CPZ.

Abscissa – Cntr, control values amplitude of AMPA (A) and NMDA EPSP (B); average values obtained within 15 min of perfusion slices control aCSF. The numbers, log concentrations of CPZ. Ordinate – designation is indicated in Figure. * – $P \leq 0.05$ compared with control values, nonparametric Wilcoxon-Mann-Whitney U test. $n = 6$ for each timepoint. Note, that proportional the dose-dependence was obtained only for the activity of NMDARs, whereas the rectilinear dose-dependence for AMPARs activity was not detected.

AMPA and NMDA activities. As a result of conducted experiments, it was found that promazine had no influence on the activity of both AMPARs and NMDARs. Changes in the activity of these receptors did not exceed 1% (Fig. 6A, B). At washing activities, the both AMPARs and NMDARs also were not changed significantly compared with control values (Fig. 6A, B). We assume that the above data indicate the specificity of the CPZ effects on the activities of both NMDARs and AMPARs.

CPZ effects on glutamate and glycine sites NMDARs

The results described above demonstrate that CPZ has the most pronounced neurotrophic effects on the NMDARs (paragraph "Dose-relationship for changes activities of AMPARs and NMDARs"). These receptors are tetrameric assemblies of the glutamate binding GluN2 subunits and glycine-binding GluN1 subunits. Glutamate and glycine molecules bind to different subunits of the NMDA receptor; two of each are thought to be required for maximum activation of the receptor. NMDA receptors also require glycine to act as a co-agonist with *L*-glutamate (27). Glutamate binds to GluN2 subunits and glycine binds to a homologous site on GluN1 and GluN3 subunits, to cause the opening of the receptor's Na⁺/K⁺/Ca²⁺-permeable ion channel. The influx of Ca²⁺ ions into neurons initiates many of the actions of NMDA receptors (28).

Given the considerable functional significance of these subunits in NMDARs, we presented the data in this paragraph on the effects of CPZ on glutamate and glycine sites. For these studies, slices were pretreated with *L*-glutamate or glycine for 15 min and then treated with CPZ 10⁻⁵ M for 30 min. *L*-glutamate enhanced the initial activation of NMDARs in slices. Late activation of these receptors from 13 to 20 min was retained. From 25 to 30 min, suppression of the NMDARs activity was the same as under the action of CPZ. Initial depression of NMDARs remained during 7–9 min in the test for reversibility during washing (Fig. 7A). These findings indicate that the initial activating effect of CPZ for NMDARs is caused by activation of the glutamate site of these receptors.

Next, we tested the participation of other significant

NMDARs site – glycine. It was revealed that activation of the glycine site by application of glycine (0.03 M) was as a result of partial activation of NMDARs (control, CPZ – 145%, vice versa glycine – 128%; nonparametric Wilcoxon-Mann-Whitney U = 11, n = 9, P ≤ 0.05). Importantly, glycine blocked late phase excitation of NMDARs induced by CPZ at its separate application (Fig. 7B, 23–30 min). Perhaps this is due to the action of glycine as a co-agonist at the glutamate site of NMDARs. The data obtained show that late phase excitation of NMDARs, induced by CPZ, is associated with activation of the glycine site of these receptors.

Discussion

In the present study, we investigated the effects of CPZ, a widely used antipsychotic, on the activity of ionotropic glutamatergic receptors, AMPARs and NMDARs. We found that CPZ interacts with both ionotropic glutamate receptors AMPARs and NMDARs. CPZ demonstrated neurotrophic effects on these receptor mechanisms: it modified the amplitude characteristics of these receptors and altered the kinetics of the complicated interactions with these receptor complexes. The effects of CPZ on activity of both AMPARs and NMDARs were studied in the brain slices of rat olfactory cortex with on-line recording of extracellular field potentials.

First, we were trying to understand the character of CPZ effects on input sodium and output potassium currents during generation of AMPA EPSP. It was found that the modification of activity of sodium and potassium currents under the action of CPZ were not linear. A smaller concentration of CPZ (10⁻⁶ M) caused an increase in inward sodium and outward potassium currents. Opposite, a higher concentration of CPZ (10⁻⁵ M) inhibited the activity of these currents when generating the AMPA EPSP.

In explaining these findings, one should take into account the different conductivity of NMDARs and AMPARs. It is revealed that the activation of NMDARs leads to opening of ion channels, which are selective for cations, resulting in an increase in the influx of Ca²⁺ ions in neurons and efflux of K⁺ ions

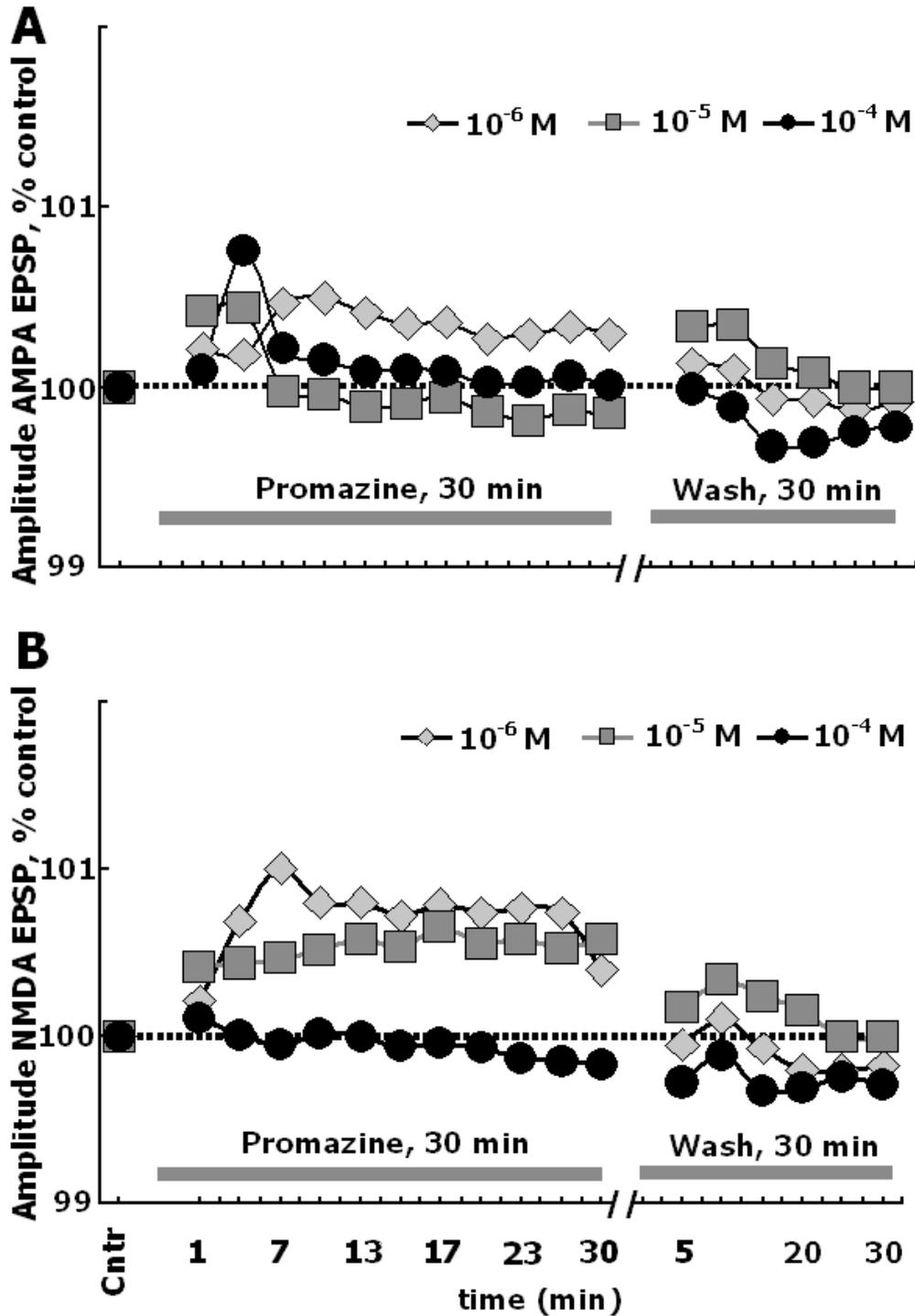


Fig. 6 : Effects of treatment brain slices with different concentrations of promazine on the amplitudes AMPA (A) and NMDA (B) EPSP. Horizontal dotted line – control level for AMPA (A) and NMDA EPSP (B). The gray solid line in A and B marked “Promazine, 30 min”, promazine time applications on slices in different concentrations indicated in the top of the charts. The gray solid line in A and B marked “Wash, 30 min”, time washout of slices by control aCSF. Abscissa – Cntr, control, the control value of AMPA (A) and NMDA EPSP (B) amplitudes referred to as control values. Numbers indicate time (min) registration of AMPA and NMDA EPSP during action CPZ. Wash, 30 min bathing by control aCSF. Scale time is irregular. Ordinate – designation is indicated in Figure. n = 6 for each timepoint. Statistically significant differences for each timepoint of curves were not revealed as compared with control values $P \geq 0.05$, nonparametric Wilcoxon-Mann-Whitney U test, the maximum amplitude changes of AMPA and NMDA EPSP did not exceed 1%. Promazine, was chosen as the reference preparation for CPZ effects, which is structurally similar to CPZ but lacking both D2-effects and antipsychotic potency. It had no influence on activities AMPARs and NMDARs. For clarity, SD on curves is omitted.

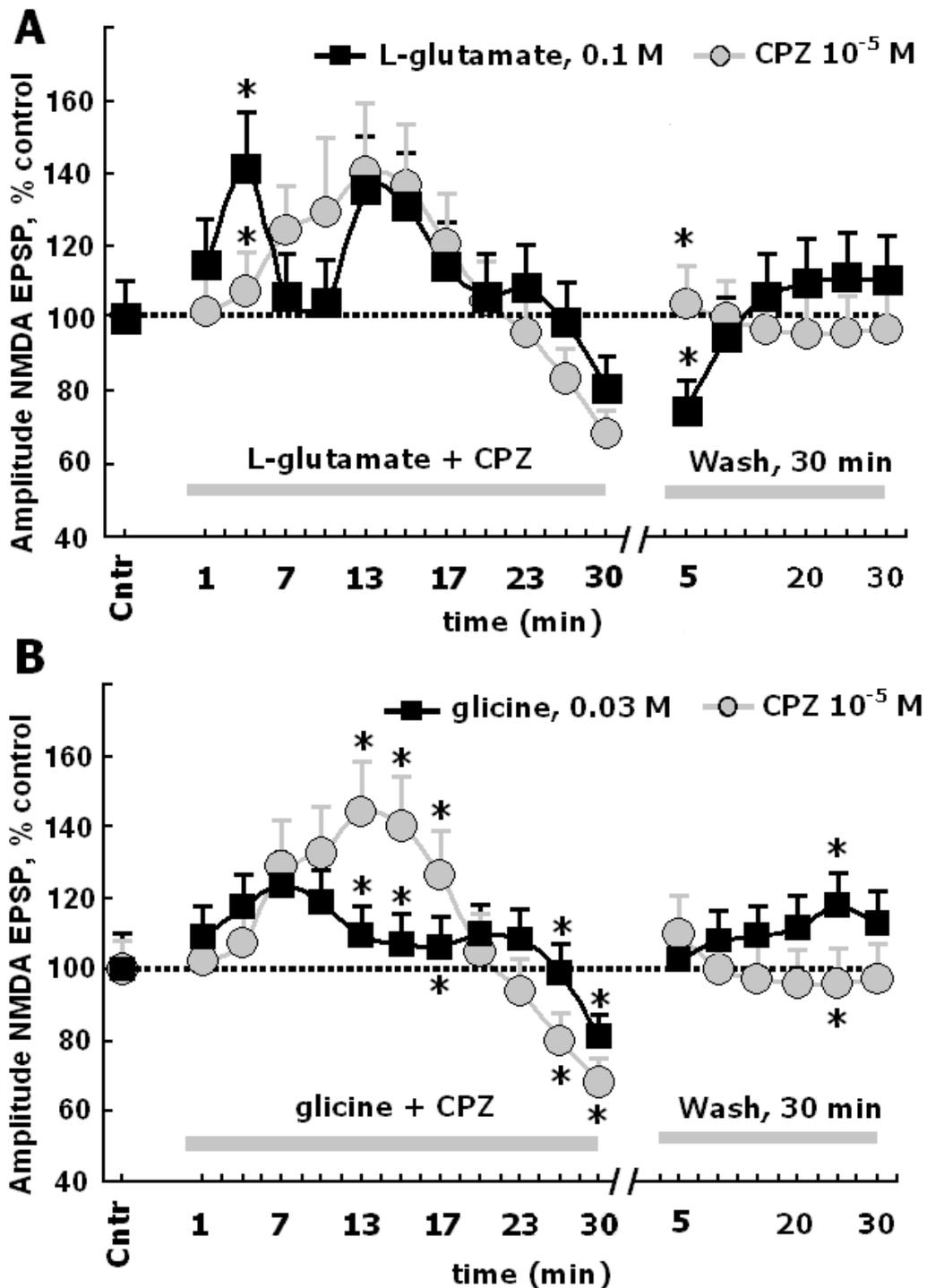


Fig. 7: The effects CPZ in concentration 10⁻⁵ M on L-glutamate (A) and glycine (B) sites of NMDARs in piriform cortex of brain slices. A, B – Horizontal dotted line – control level of AMPA and NMDA EPSP amplitudes. The gray solid line in A marked “L-glutamate + CPZ”, indicates pretreatment of slices by L-glutamate during 15 min and the subsequent impact CPZ within 30 min. The gray solid line in B marked “glycine + CPZ”, indicates pretreatment of slices by glycine during 15 min and the subsequent impact CPZ within 30 min. The gray solid line in A and B marked “Wash, 30 min”, time washing of slices by control aCSF. Abscissa – Cntr, control, the control value of AMPA (A) and NMDA EPSP (B) amplitudes. Numbers indicate time (min) registration of AMPA and NMDA EPSP during action CPZ, L-glutamate and glycine. Wash, 30 min bathing by control aCSF. Scale time is irregular. Ordinate – designation is indicated in Figure. * – P≤0.05 when comparing a data on curve (CPZ, 10⁻⁵ M) with the data on the curve “L-glutamate, 0,1 M,” nonparametric Wilcoxon-Mann-Whitney U test. n = 9 for each timepoint. For B * – P≤0.05 when comparing a data on curve (CPZ, 10⁻⁵ M) with the data on the curve “glycine, 0.03 M,” nonparametric Wilcoxon-Mann-Whitney U test. n = 9 for each timepoint. Further explanations in the text.

from their. The NMDARs has a high single channel conductance (130–150 pS) compared with AMPARs (14–15 pS). The open probability of agonist-bound receptors has been estimated to range between 0.04 and 0.3 pS, and open times can vary from 0.1 to 8 ms (29). Taking into account the above data concerning NMDARs properties, it can be assumed that CPZ with increases concentration linearly acted on NMDARs conductivity. Regarding the reactions of AMPARs, the CPZ nonlinear manner modified activity of these receptor mechanisms with increasing concentration. This indicates that the conductivity of AMPARs changed non-linearly under the influence of CPZ and perhaps a different types of sodium channels were activated.

We revealed that CPZ modified activities AMPARs and NMDARs phased manner: initial activation and subsequent depression. However, this pattern was observed for the changes in activity AMPARs at CPZ concentrations of 10^{-6} M and 10^{-4} M. As for the NMDARs, at low concentrations CPZ (10^{-6} M and 10^{-5} M) was the initial activation, and at the end of exposure, these receptors were depressed. At washing period (the test for the reversibility of effects antipsychotic) the initial activity of the both AMPARs and NMDARs restored.

It is known NMDARs require activation of glutamate and glycine as a co-agonist (27). We suggest that pattern of activation of these receptors is associated with the effect of CPZ on different sites of AMPARs and NMDARs. This hypothesis tested in our experiments with activation of glutamate and glycine sites of NMDARs in brain slices.

We have found that an increase the initial reactions of NMDARs (10–13 min) have been associated with activation of glutamate sites. Under the action of CPZ on the glycine site of the activation of the NMDARs was less expressed. With prolonged influence CPZ (30 min), activation of glutamate and glycine sites of NMDARs transformed into inhibition of these receptors. These findings can be interpreted as desensitization activity of NMDARs. Hence, in the late period of time (20–30 min), CPZ demonstrated properties of nonspecific and reversible inhibitor of

NMDARs. We believe that this property of CPZ on NMDARs explains the protective effects this antipsychotic, which detected as in vitro and well as in animal studies including hypoxia (30), ischemia (31) and glutamate-induced neurotoxicity (32).

To specify of CPZ neurotrophic effects we applied promazine on the brain slices. It is known that promazine is structurally similar to CPZ. This drug belongs to the phenothiazine family with effects similar to CPZ, but it no possesses antipsychotic activity. It is primarily used in short-term treatment of disturbed behavior and as an antiemetic (33). According to our data promazine did not caused significant modifications of the activity both AMPARs and NMDARs, the changes do not exceed 1% from control values. We assume these findings indicate the specificity of the neurotrophic effects of CPZ on the activity of both NMDARs and AMPARs.

Conclusion

Presented data extends the understanding a range of the CPZ effects in the CNS mammalian and possibly for human. Currently, the dominant idea is that the effects of CPZ are mainly manifested through dopamine and serotonergic receptors. However, our data indicate that CPZ actively interacts with ionotropic glutamatergic mechanisms. Note that the glutamatergic system is the main excitatory mediator system in the CNS mammals, including human. We believe data presented in this study on the influence of CPZ on the ionotropic glutamatergic mechanisms allow understanding the multifaceted mechanisms action of neuroleptics in preventing the deterioration of mental disorders such as schizophrenia.

Conflict of interest

None

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