Clozapine and Haloperidol Modulate N-Methyl-D-aspartate-and Non-N-Methyl-D-aspartate Receptor-Mediated Neurotransmission in Rat Prefrontal Cortical Neurons In Vitro


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ABSTRACT

The effects of the antipsychotic drugs haloperidol and clozapine on N-methyl-D-aspartate (NMDA) and non-NMDA receptor-mediated neurotransmission were examined and compared in pyramidal cells of the medial prefrontal cortex in rat brain slices by using the techniques of intracellular recording and single-electrode voltage-clamp. The bath administration of either haloperidol or clozapine produced a marked facilitation (300–400%) of NMDA-evoked responses in a concentration-dependent manner. The EC_{50} values of haloperidol and clozapine were 38 and 14 nM, respectively. At concentrations of ≥100 nM, clozapine, but not haloperidol, produced bursts of excitatory postsynaptic potentials (EPSPs), which were blocked by glutamate receptor antagonists, suggesting that these EPSPs were the result of increasing release of excitatory amino acids. Haloperidol, but not clozapine, produced a concentration-dependent inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-induced current with an EC_{50} value of 37 nM. Haloperidol significantly decreased the amplitude of EPSPs evoked by the electrical stimulation of the corporces minor, whereas clozapine increased the amplitude of these EPSPs. The study of current-voltage relationship indicates that clozapine preferentially potentiates NMDA receptor-mediated transmission, whereas haloperidol depresses the non-NMDA receptor-mediated response, which probably obscures its potentiating effect on NMDA receptor-mediated EPSPs.

The relative ineffectiveness of dopamine antagonists to treat some symptoms of schizophrenia has prompted many investigators to postulate the involvement of other systems in the pathophysiology of this disease. The glutamate hypothesis of schizophrenia proposes a relationship between hypoactive glutamate neurotransmission, particularly NMDA receptor hypofunction, and the pathophysiology of schizophrenia (Carlsson and Carlsson, 1990; Deutsch et al., 1989; Kim et al., 1980; Moghaddam, 1994; Olney and Farber, 1995; Wachtel and Turski, 1990). This hypothesis stems from the observation that in normal human subjects, the NMDA receptor channel blockers ketamine and phencyclidine, can induce psychosis that includes many symptoms and cognitive disturbances commonly observed in patients with schizophrenia (Grotta, 1994; Herrling, 1994; Kristensen et al., 1992). Furthermore, in schizophrenics, NMDA receptor antagonists produce an exacerbation of psychotic symptoms (Lahti et al., 1995). However, evidence for a defect of NMDA receptor function in schizophrenia remains inconclusive. For example, it has been shown that [3H]kainate, d-[3H]aspartate, [3H]tenocyclidine and [3H]glycine binding is increased in the frontal cortex and [3H]dizocilpine (MK-801) binding is increased in the putamen of post-mortem schizophrenics, whereas the [3H]MK-801 binding sites in the temporal lobe areas including the hippocampus are not affected; in contrast, there is a decrease in non-NMDA receptor mRNA in cortex (Deakin et al., 1989; Harrison et al., 1991; Ishimaru et al., 1994; Kerwin et al., 1990; Kornhuber et al., 1989; Nishikawa et al., 1983; Simpson et al., 1991; Ulas and Cottman, 1993). The prefrontal cortex of schizophrenics has recently been shown to exhibit alterations in the expression of NR2 subunit mRNAs, which are potential indicators of deficits in NMDA receptor-mediated neurotransmission accompanying functional hypoactivity of the frontal lobe (Akbarian et al., 1996).

ABBREVIATIONS: aCSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; d-(-)-AP-5, d-(-)-2-amino-5-phosphonopentanoic acid; APD, antipsychotic drug; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N’-tetraacetic acid; AM, acetoxymethyl ester; CNQX, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline; CF, cerebrospinal fluid; EAA, excitatory amino acid; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; FS, fast-spiking; GABA, γ-aminobutyric acid; IB, intrinsic bursting; IM, intermediate; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartate; ROB, repetitive oscillatory bursting; RS, regular-spiking; TCP, tenocyclidine; TTX, tetrodotoxin; \( V_h \), holding potential.
There is evidence suggesting that some APDs might affect the NMDA receptor mediated transmission. For example, clozapine and olanzapine have been shown to reverse non-competitive NMDA antagonist-induced social withdrawal in rats (Corbett et al., 1995) and prevent MK-801 neurotoxicity (Farber et al., 1996). Moreover, the potency of a series of neuroleptics in blocking phencyclidine-induced hyperlocomotion correlated with the affinity for 5-hydroxytryptamine2A receptors (Gleason and Shannon, 1997; Maurel-Remy et al., 1995). In agreement with these findings, in vivo microdialysis studies indicate that the acute, systemic administration of clozapine, but not haloperidol, produces an increase in extracellular concentrations of glutamate (Daly and Moghaddam, 1993; Yamamoto et al., 1994) and aspartate (Daly and Moghaddam, 1993) in the mPFC of freely moving rats. Acute clozapine, however, does not alter glutamate levels in the neostriatum (Daly and Moghaddam, 1993). Electrophysiological studies have shown that both haloperidol and clozapine at low and high (>100 nM) concentrations augment and depress, respectively, the amplitude of field potentials in the rat neostriatum slices elicited by electrical stimulation of the overlying white matter (Banerjee et al., 1995). Clozapine has also been shown to potentiate population spikes in the entorhinal-dentate gyrus perforant pathway (Kubota et al., 1996). Currently, the exact explanation for the action of APD on glutamatergic neurotransmission is unknown.

Because a role for glutamate receptors has been suggested in schizophrenia, it is of interest to examine more closely whether APDs affect glutamate receptor subtype-mediated neurotransmission at the cellular level. The present study was designed to investigate and compare effects of haloperidol and clozapine on NMDA- and AMPA-induced responses in pyramidal cells of the mPFC, an area that has been suggested to play a key role in the pathogenesis of schizophrenia and in the working memory and cognitive functions (Berman and Weinberger, 1990), using the techniques of intracellular recording and single-electrode voltage-clamp. The effect of haloperidol and clozapine on NMDA- and non-NMDA receptor-mediated EPSPs/EPSCs evoked by electrical stimulation of the forceps minor was also examined. A portion of these results have appeared in a preliminary form (Wang and Arvanov, 1996).

**Preparation of mPFC slices.** The procedures for preparation of rat mPFC brain slices have been previously described (Arvanov and Wang, in press; Yang et al., 1996). Briefly, male Sprague-Dawley rats (body weight, 120–200 g; n = 87) were decapitated while under halothane anesthesia, and their brains were removed and cooled in ice-cold ACSF. The coronal (transverse) slices of mPFC (450 μm thick) were cut in ice-cold ACSF containing (in mM) NaCl 117, KC1 4.7, CaCl2 2.5, MgCl2 1.2, NaHCO3 25, NaH2PO4 1.2 and d-glucose 11, aerated with 95% O2/5% CO2 (pH 7.4) and kept submerged in aCSF at a constant rate of 1.5 ml/min.

**Identification of pyramidal and nonpyramidal neurons.** According to Connors and Gutnick (1990) and McCormick et al. (1985), there are three basic types of neocortical neurons: RS, IB and FS. For each type, classification is based on three general variables: characteristics of individual action potential/antlrorpotential complexes, response to a just-threshold intracellular current pulse and repetitive response to prolonged, intracellularly applied stimuli. RS and IB are identified as pyramidal neurons, and FS cells are nonpyramidal, presumably GABAergic interneurons (the spike width of FS cells is typically <0.5 msec at half-amplitude; when stimulated with a suprathreshold step of depolarizing current, they generate high frequencies that are sustained for the duration of the stimulus; Connors and Gutnick, 1990; Kawaguchi, 1993; McCormick et al., 1985). Recent studies, however, have revealed that nonpyramidal cells display a great diversity of intrinsic firing properties and can show frequency accommodation (Cauli et al., 1997; Kawaguchi and Kubota, 1996). In addition to RS and IB types pyramidal cells, Yang et al. (1996) added ROB and IM types. In general, the interneurons are characterized by a brief spike duration (<1 msec at half-maximum spike amplitude) and a lack of pronounced spike frequency adaptation in response to constant-current depolarizing pulses, whereas the pyramidal cells have a longer spike duration, particularly the second spike (1–3 msec at half-maximum spike amplitude), and show pronounced spike frequency adaptation (Cauli et al., 1997; Connors and Gutnick, 1990; Kawaguchi, 1993; Kawaguchi and Kubota, 1996; McCormick et al., 1985).

**Intracellular recording and single-electrode voltage-clamp.** Standard intracellular and single-electrode voltage-clamp recording techniques were used to record pyramidal cells in layers V and VI of the mPFC in slice preparations as previously described (Arvanov et al., 1996; Arvanov and Wang, in press; Wang and Arvanov, 1996). Sharp electrode recordings were preferable to minimize the washout of intracellular content occurring during the whole-cell recordings (Arvanov et al., 1992; Arvanov and Usherwood, 1991; McDonald et al., 1989). Intracellular recordings were performed using 4 M K-acetate- or 3 M KCl-filled microelectrodes (tip resistances, 60–90 MΩ) with an Axoclamp 2B (Axon Instruments, Burlingame, CA) amplifier. In current-clamp mode, the bridge balance was continually monitored and adjusted as necessary. Single-electrode voltage-clamp was achieved under discontinuous mode at a sampling rate of 5 to 6.2 kHz (30% duty cycle) and a gain of 2.5 to 5 nA/V. The efficacies of voltage-clamp, electrode “settling time” and input capacitance neutralization at the head stage were continuously monitored on an oscilloscope. Current and voltage records were acquired using digital/analog sampling and acquisition software (pClamp 6; Axon Instruments) and were filtered at 1 kHz and analyzed off-line. Voltage and current signals were also recorded on a Gould (Cleveland, OH) Easy Graph Thermal Recorder (TA 240) and two-channel videotape recorder (Instrutech VR-10B Digital Data Recorder, Westbury, NY). These traces were associated with this method in neurons with extended processes have been previously discussed (Finkel and Redman, 1985). As it has been pointed out, these problems faced during single-electrode voltage-clamp may be less acute when dealing with the relative changes after drug application (Madison et al., 1987; Schweitzer et al., 1993). The voltage-clamp conditions during our experiments were sound because we were able to clamp the fast EPSPs evoked by electrical stimulation (see below). By using a combination of current- and voltage-clamp recordings, the effect produced by APDs on NMDA- and AMPA-induced response could be examined with confidence. During voltage-clamp recordings, TTX (0.5 μM; to block action potentials), glycine (1 μM; to maximize NMDA-induced current), bicuculline (5–10 μM; to block GABA<sub>B</sub> receptors) and CGP 52432 (3-[3,4-dichlorophenyl)methyl]propyl(diethoxymethyl)phosphinic acid; 0.5 μM; to block GABA<sub>A</sub> receptors; Bittiger et al., 1993) were routinely included in the aCSF. All cells were held at −60 mV to minimize activating Is (I<sub>e</sub>) and I<sub>h</sub> (Halliwell and Adams, 1982). APDs were added to the superfusing aCSF. NMDA and AMPA were applied by placing a microdrop (10 μl) of concentrated solution (dilution factor, 1:100) on a marked spot in the inflow channel of the chamber (volume, 1 ml) as previously described (Holmes et al., 1996). Repeated microdrop application of NMDA to the same pyramidal cell with an interapplication interval of ~15 min produced a consistent inward current, although the base-line current caused by NMDA...
varied from cell to cell (30–70 pA). Typically, two or three stable consecutive control responses to NMDA were obtained, the average of which was counted as the baseline of NMDA, before drug tests.

**Electrical stimulation-evoked EPSPs/EPSCs.** Electrodes for voltage-clamp experiments were filled with 2 M CsCl plus 25 to 50 mM QX314 (lidocaine N-ethyl bromide quaternary salt; tip resistance, 30–50 MΩ) to improve the space clamp and block voltage-activated Na⁺ and K⁺ channels; under these conditions, the membrane resistance was usually increased by 30% to 50% (Wuarin et al., 1992). EPSPs/EPSCs were elicited by passing rectangular current pulses (0.3–0.5-msec duration; 50–250 µA) between the tips of a bipolar stainless steel electrode placed in the medial part of the forceps minor close to the recording electrode (Wuarin et al., 1992). Following the protocol of Tanaka and North (1993), experiments were carried out using a stimulus strength that was 70% of the threshold for evoking an action potential. A train of five electrical pulses was delivered at a rate of 0.05 Hz, three times before and after drug application (i.e., in all experiments), induced synaptic responses before and after drug application were averaged (n = 15). To isolate NMDA receptor-mediated EPSPs, the preparation was superfused with 20 µM CNQX, 5 to 10 µM bicuculline, 0.5 µM CGP 52432 and 1 µM glycine. Under these conditions, the amplitude of EPSPs was reduced to 17%, indicating that under normal conditions EPSPs were mediated primarily through non-NMDA receptors. The stimulus strength was increased 2-fold, and high-intensity electrical stimulation elicited a longer lasting EPSP.

**Data analysis.** The percent modulation produced by APDs on NMDA- and AMPA-evoked responses was calculated by subtracting the baseline peak amplitude of the responses from that evoked by bath application of APDs. This value was then divided by the baseline response and multiplied by 100. The results were presented as mean ± S.E., except when the data were transformed for analysis of variance (one-way and mixed level; see below). Paired t tests, Student’s t tests, analysis of variance and mixed-level analysis of variance models applying SAS Proc Mixed procedure were used; .05 was taken as the threshold for evoking a change from the baseline after 15-min wash. Traces 1 and 2 were expanded from time point of 1 and 2 in A to show clearly that clozapine markedly increased the frequency of NMDA-evoked EPSPs. B: Haloperidol at concentrations of 50 to 100 nM significantly potentiated NMDA-evoked responses. At concentrations of ≥100 nM, haloperidol tended to hyperpolarize the membrane potential.

**Presumed pyramidal neurons in the mPFC.** All experiments were routinely performed on presumed pyramidal neurons in layers five and six of the prefrontal cortex (anterior cingulate cortex areas 1 and 3; Zilles, 1985), which were located medial to the forceps minor and could be easily identified in the slice. A total of 93 presumed pyramidal cells has been recorded according to established criteria (Connors and Gutnick, 1990; Kawaguchi, 1993; McCormick et al., 1985; Yang et al., 1996). Stable recordings could be maintained for ≤5 to 5 hr, suggesting a relative lack of injury by the electrode penetration. It is rare to impale FS neurons with a relatively low-resistance unbeveled microelectrode (McCormick et al., 1985), which might account for the fact we have not encountered any FS nonpyramidal cells in our studies. As previously described (for review, see Connors and Gutnick, 1990), most (68%) pyramidal neurons encountered were the RS type. In normal aCSF, the great majority (>90%) of recorded cells were quiescent. They (n = 87) exhibited a mean resting membrane potential of –72.4 ± 1.2 mV, a spike amplitude of 82.9 ± 1.5 mV, a membrane resistance of 51.7 ± 4.1 MΩ (measured from the linear part of current-voltage curve) and a time constant of 10.4 ± 1.2 msec. These results are comparable to those reported by Tanaka and North (1993) and Yang et al. (1996).

**Effect of NMDA in presumed pyramidal cells of the mPFC.** In all 19 cells studied under the current-clamp mode, NMDA (10 µM) elicited EPSPs (frequency, 0.71 ± 0.03 Hz; amplitude, 7.4 ± 1.2 mV) followed by a membrane depolarization (amplitude, 8.5 ± 3.1 mV, range, 5–17 mV) and bursts of action potentials (fig. 1). In the voltage-clamp mode, at the holding potential (Vh) of –60 mV, NMDA 10 µM produced an inward current of 30–70 pA (46 ± 12 pA, n = 35; fig. 2).

In Ca²⁺-free (CaCl₂) was substituted by NaCl) aCSF and in normal aCSF containing either 0.5 µM TTX or 20 µM CNQX, the peak amplitude of NMDA-evoked depolarization was decreased by 61 ± 7% (n = 6), 28 ± 3% (n = 7) and 61 ± 5.5% (n = 6), respectively, whereas NMDA-evoked EPSPs were totally abolished. Moreover, NMDA-evoked EPSPs were blocked by bath application of the membrane permeable Ca²⁺-chelator BAPTA-AM (n = 5). Although BAPTA loaded in the recording electrode (200 mM, n = 4) blocked Ca²⁺-dependent inward current of 30–70 pA (46 ± 12 pA, n = 35; fig. 2).

**Drugs.** The compounds AMPA, NMDA, QX314, TTX and bicuculline methochloride were purchased from Research Biochemicals (Natick, MA). Clozapine, haloperidol and CGP 52432 were generous gifts from Sandoz (Hanover, NJ), McNeil Laboratories (Fort Washington, PA) and Ciba-Geigy (Basel, Switzerland), respectively.

**Results**

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activated slow afterhyperpolarization (obtained by passing 0.1-nA, 100-msec rectangular pulses as described by Lancaster and Nicoll, 1987), it failed to block NMDA-evoked EPSPs (not shown). In voltage-clamp experiments in the presence of TTX (which decreased ~30% of NMDA-induced current), the peak amplitude of NMDA-induced inward current was reduced by 64 ± 4% (n = 4) and 29 ± 2.8% (n = 9) in Ca²⁺-free and CNQX-added aCSF, respectively. Taken together, these results suggest strongly that in addition to interacting with NMDA receptors on pyramidal neurons, NMDA causes the release of EAAs, which in turn activates postsynaptic non-NMDA receptors to produce membrane depolarization and EPSPs.

Comparison of effects of clozapine and haloperidol on NMDA-induced response. Experiments were performed in both current-clamp and voltage-clamp mode to ensure that the effects of clozapine and haloperidol were not the result of altering membrane potentials (see below). To minimize actions of APDs on dopamine inhibition of GABAergic transmission (Kalivas et al., 1993), all voltage-clamp experiments were carried out in the presence of 5 to 10 μM bicuculline (a GABA_A receptor antagonist that has been shown in a separate study to completely block the inhibitory action of GABA).²

Both clozapine and haloperidol markedly potentiated NMDA-evoked responses, including the amplitude of membrane depolarization, frequency of EPSPs, number of bursts of action potentials (fig. 1) and peak amplitude of inward current (fig. 2) in pyramidal neurons of the mPFC. After 30 min of wash, the potentiated NMDA responses returned to basal levels (fig. 1); further administration of these drugs produced a reproducible facilitation of NMDA response (n = 7; data not shown).

The effect of clozapine and haloperidol on NMDA-evoked depolarization and inward current was concentration dependent. The concentration-response curves for both compounds were very steep (fig. 2). Clozapine, but not haloperidol, moderately increased and strikingly augmented at the concentration of 10 (n = 7) and 20 (n = 5; fig. 1A) nM, respectively. NMDA-induced membrane depolarization (current-clamp mode) and inward current (voltage-clamp mode; n = 5; fig. 2). Haloperidol did not produce a significant effect on NMDA response until the concentration was raised to 50 nM (figs. 1B and 2). At the concentration of 50 nM, haloperidol and clozapine, but not raclopride (a selective dopamine D2 antagonist and an APD) or fluoxetine (a selective serotonin uptake blocker and an antidepressant drug), markedly enhanced NMDA-activated inward current by 290 ± 36% (n = 5) and 390 ± 32% (n = 5), respectively. Clozapine and haloperidol reached the plateau at the concentration of 50 and 100 nM, with an EC50 value of 14 and 38 nM, respectively. Figure 3 summarizes the major findings of effects produced by haloperidol and clozapine.

In the current-clamp mode, in addition to facilitating NMDA-evoked depolarization, both clozapine and haloperidol induced a >2-fold increase of the frequency of NMDA-evoked EPSPs (figs. 1A and 3). Interestingly, haloperidol and clozapine produced a different effect on the amplitude of NMDA-evoked EPSPs [i.e. haloperidol (50 nM), but not clozapine, decreased the amplitude of NMDA-evoked EPSPs by 52 ± 11% (n = 5; fig. 3)]. This might have been related to the inhibitory action of haloperidol on the AMPA receptor-mediated response (see below).

Interestingly, 20 μM CNQX completely abolished the potentiating effect of both clozapine (n = 4) and haloperidol (n = 3) on NMDA-current (fig. 3). This result indicates that non-NMDA receptors are required for the expression of the facilitating effect of APDs on NMDA-evoked responses.

Effects of haloperidol and clozapine on membrane properties of mPFC pyramidal neurons. Facilitation of NMDA-evoked responses by 10 to 50 nM clozapine has not been associated with changes of membrane properties (e.g.,

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²X. Liang, V. L. Arvanov and R. Y. Wang, unpublished observations.
Clozapine-evoked EPSPs were not abolished in aCSF containing 0.5 mM to 1 mM clozapine. However, at concentrations of 100 nM to 1 μM, clozapine decreased AMPA-induced inward current to 66 ± 6% and 36 ± 4% (P < .05 for both cases, paired t tests; n = 5), respectively. Analyses of the current-voltage relationship of AMPA receptors caused by the bombardment of EAAs released by clozapine.

Effects of haloperidol and clozapine on AMPA-induced response. Haloperidol inhibited AMPA-induced inward current in a concentration-dependent manner with an EC50 value of 37 nM (fig. 5). In contrast, clozapine did not significantly alter AMPA current. Although at 100 nM, clozapine decreased AMPA-induced inward current to 66 ± 19% (n = 4) of control, the reduction did not reach statistical significance. The latter effect was associated with the appearance of EPSCs. It might be speculated that clozapine-induced reduction of AMPA current is the result of desensitization of AMPA receptors caused by the bombardment of EAAs released by clozapine.

Effects of haloperidol and clozapine on EPSPs/EPSCs evoked by electrical stimulation of the forceps minor. In normal aCSF, haloperidol depressed EPSPs evoked by electrical stimulation of the forceps minor in a concentration-dependent fashion; the depressed EPSPs returned to basal level after 30 min of washout of haloperidol (fig. 6A). At the concentrations of 50 and 100 nM, haloperidol markedly reduced the peak amplitude of EPSPs by 52 ± 7% and 68 ± 4% (P < .05 for both cases, paired t tests; n = 5), respectively. Analyses of the current-voltage relationship of electrical stimulation-evoked EPSCs revealed that the depressant action of haloperidol was voltage independent because 50 nM haloperidol depressed EPSCs to 45 ± 3% and 43 ± 3% of base line at the holding potential of −70 and −20 mV (n = 5; fig. 6, B and D), respectively. The inhibitory effect of haloperidol was blocked by CNQX (fig. 6D3) but undiminished in the presence of d-(-)-AP-5 (fig. 6D2). These results

either membrane potential nor input resistance (n = 8) has been altered significantly. However, at concentrations of 100 nM to 1 μM, clozapine elicited EPSPs (amplitude, 8.6 ± 1.1 mV; frequency, 1.1 ± 0.4 Hz; fig. 4A) and depolarized membrane potential (4.3 ± 1.1 mV) in all seven cells tested. The membrane potential recovered to control values and EPSPs were not observed 30 min after the washout of clozapine.

Comparison of EPSCs evoked by clozapine and by electrical stimulation of the forceps minor (see below) revealed that both EPSCs recorded from the same neuron reversed their polarity at the same holding potential (−8 to −3 mV; n = 3; fig. 4B). Both EPSCs were completely blocked by the non-NMDA-antagonist CNQX (20 μM) plus NMDA-antagonist d-(-)-AP-5 (40 μM, n = 3; data not shown). Interestingly, clozapine-evoked EPSPs were not abolished in aCSF containing 0.5 μM TTX (n = 5), although these EPSPs were slower and appeared as depolarizing waves with the duration of 0.4 to 1 sec. These results suggest that clozapine might have released EAAs in a largely TTX-independent manner and elicited EPSPs.

At lower concentrations, haloperidol did not have any effect on either membrane potential or input resistance. However, at the concentrations of 100 nM and 1 μM, haloperidol hyperpolarized the membrane potential by 3.5 ± 0.9 mV and 8.3 ± 2.1 mV (n = 5), which was associated with an increase of input resistance by 15 ± 6% and 36 ± 9%, respectively. Interestingly, haloperidol-induced alteration of membrane properties was prevented by 0.5 μM TTX (n = 7) and by loading QX314 in the recording micropipette (n = 3), suggesting that haloperidol may have inhibited TTX-sensitive voltage-activated Na+ channels to induce membrane hyperpolarization (Pencek et al., 1978; Westlind-Danielson et al., 1992).

Fig. 4. Voltage-current traces to illustrate clozapine-evoked EPSPs and membrane depolarizations in pyramidal cells of the mPFC. A, Representative voltage traces illustrating that clozapine at 100 nM induced EPSPs and produced a membrane depolarization. B, Comparison of EPSCs evoked by electrical stimulation of the forceps minor and induced by clozapine on the same pyramidal neurons at different levels of Vh. Both EPSCs reversed their polarity at the same Vh of −8 to −3 mV.
correspond to the finding that haloperidol markedly depresses AMPA-induced inward current.

In contrast to haloperidol-induced depression of EPSPs, clozapine (50–100 nM) increased the amplitude of EPSPs by 15.4 ± 6.4% (n = 6). Compared with normal controls, the increase was statistically significant (P < .05, paired t test). Current-voltage relationship analyses revealed that the potentiating effect of clozapine on EPSCs was voltage dependent. Thus, the potentiating effect of 50 nM clozapine increased from 9 ± 4% to 143 ± 12% (n = 6) when the V_h was changed from −70 to −20 mV (fig. 6B and C), supporting the finding that clozapine preferentially potentiates NMDA receptor-mediated transmission, which is voltage dependent. In addition, clozapine shifted the reversal potential of EPSCs to a more positive level (the reversal potential of EPSCs was −10 and 0 mV in the absence and presence of clozapine, respectively; fig. 6C1). The latter finding corroborates with the fact that the reversal potential of the NMDA component of EPSPs (see below) is more positive (Arvanov and Wang, in press; Burgard and Hablitz, 1993; Wuarin et al., 1992).

d-AP-5 (40 μM) abolished the potentiating effect of clozapine (fig. 6C2).

To isolate the NMDA receptor-mediated component of the synaptic responses, we included CNQX (20 μM), bicuculline...
(1 μM) and CGP 52432 (0.5 μM) in the aCSF (Tanaka and North, 1993; Wang and Arvanov, 1996). Clozapine was not capable of potentiating pharmacologically isolated, NMDA receptor-mediated EPSCs (n = 4). Moreover, the addition of 20 μM CNQX alone prevented the potentiating effect of clozapine (n = 5; fig. 6C3). These results reinforce the view that the potentiating effect produced by clozapine on NMDA receptor mediated neurotransmission is not a result of the direct interaction of clozapine with NMDA receptors on pyramidal neurons.

Discussion

The major finding of the present study is that both the classic APD haloperidol and the atypical APD clozapine potently facilitate NMDA-evoked depolarization and membrane current in the rat pyramidal cells of the mPFC in a concentration-dependent manner with an EC_{50} value of 38 and 14 nM, respectively. Although it is difficult to estimate the precise synaptic concentrations for haloperidol and clozapine to facilitate NMDA response because of the incomplete equilibrium conditions in which the APDs were typically applied for 10 min, the aCSF concentrations in the recording chamber needed for clozapine and haloperidol to exert their action are in a clinically relevant range. For example, serum levels for haloperidol and clozapine of schizophrenic patients are 10 to 70 and 447 to 3387 nM, respectively, after the administration of a range of clinical doses (Baldessarini et al., 1988; Farde et al., 1995; Nordstrom et al., 1995; Verghese et al., 1991). Moreover, it has been estimated that the majority of patients have an approximate CSF or plasma water molarity of 1 to 3 and 11.7 nM for haloperidol and clozapine, respectively (Seeman, 1992). Therefore, based on the estimation of free APDs concentrations in CSF or plasma water of schizophrenic patients, our results suggest that clozapine and, to a much lesser extent, haloperidol may exert some of their antipsychotic effects by altering glutamate neurotransmission in the mPFC if our findings can be generalized to humans. Obviously, further systematic studies of various APDs after both acute and chronic treatment must be performed to have a more thorough evaluation of the effect of APDs on glutamate neurotransmission. At any rate, the facilitating effect of clozapine and haloperidol on the NMDA response may contribute to the compensatory down-regulation of \[^{3}H\]MK-801 binding in the mPFC observed after subchronic administration of clozapine and haloperidol (Tarazi et al., 1996). On the other hand, it has also been reported that subchronic haloperidol produced an increase in NMDA receptors as quantified by \[^{3}H\]glutamate binding (Ulas et al., 1993) and that chronic treatment with either haloperidol or clozapine increases glutamate receptor subunit immunoreactivity in the rat mPFC (Fitzgerald et al., 1995). The alterations of glutamate receptor subtypes produced by repeated treatment with APDs must be verified by functional studies at the cellular level.

It is unlikely that the facilitating effect of clozapine and haloperidol resulted from a direct interaction with NMDA receptors because of the relatively low affinity of these drugs for the \[^{3}H\]MK-801 binding sites (Kohler et al., 1985; Lidsky et al., 1993; Lynch and Gallagher, 1996; Tarazi et al., 1996). This is supported by our finding that haloperidol and clozapine were not capable of producing the potentiating effect on NMDA-induced inward current in aCSF containing CNQX. Interestingly, we have shown in a separate study (Wang et al., 1997) that MDL 100907, a highly selective 5-hydroxytryptamine_{2A} receptor antagonist and a purported atypical APD (Kehne et al., 1996), like clozapine, dramatically augments NMDA responses in pyramidal cells of the mPFC; it may enhance NMDA responses by facilitating NMDA-induced release of EAAs, which in turn activate non-NMDA receptors, cause membrane depolarization and remove Mg\(^{2+}\) block of the NMDA receptor/ionophore complex, thereby facilitating strikingly NMDA responses. Thus, the ability of MDL 100907 to potentiate NMDA-induced inward current is abolished by CNQX and under the conditions [e.g., Ca\(^{2+}\)-free aCSF or low Ca\(^{2+}\) (0.1 mM) plus Cd\(^{2+}\) (0.2 mM) aCSF] that prevent Ca\(^{2+}\)-dependent release of neurotransmitters (Wang et al., 1997). It is possible that clozapine may act in the same fashion as MDL 100907 to enhance NMDA responses by facilitating the release of EAAs, although similar experiments as shown with MDL 100907 must be performed to determine the site of action of clozapine. It should be pointed out that TTX does not prevent the potentiating effect of clozapine or haloperidol, possibly because TTX depresses only action potential-dependent release of EAAs, which is ~30%, in rat brain slices (Martin et al., 1991, 1993); the result argues against the involvement of polysynaptic circuitry for APDs to enhance NMDA responses because TTX would have prevented the generation of action potentials.

It might be speculated that the biochemical mechanisms behind clozapine- and haloperidol-induced potentiation of NMDA responses are secondary to their binding to other receptor or effector systems. For example, the blockade of dopamine and serotonin receptors by clozapine and haloperidol could lead to an increase in glutamate release via removal of the inhibitory action of serotonin and dopamine (Kornhuber and Kornhuber, 1986; Maura et al., 1988a, 1988b, 1989; Peris et al., 1988). Our finding that raclopride did not modulate the NMDA response until its concentration was raised to micromolar range suggests that dopamine D_{2} receptors may not have a critical role in mediating the action of haloperidol and clozapine. However, it is important to note that raclopride will not occupy D_{2} receptors because the raclopride dissociation constant is very high for D_{2} receptors (Van Tol et al., 1991). Therefore, the possibility of the involvement of other dopamine receptor subtypes cannot be disregarded.

One of the major differences between haloperidol and clozapine is that clozapine, but not haloperidol, elicited EPSPs/EPSCs at concentrations of ~100 nM. The clozapine-evoked EPSPs/EPSCs were similar to those evoked by NMDA and by electrical stimulation of the forceps minor. Both clozapine- and electrical stimulation-evoked EPSCs reversed their polarity at the same V_{h}, and both were blocked by CNQX plus D(-)-AP-5. These findings indicate that EPSPs/EPSCs were the results of increased release of excitatory amino acids. In other words, at concentrations of ~100 nM, clozapine, but not haloperidol, produced a dramatic increase of release of EAAs, similar to that produced by electrical stimulation of the forceps minor. Our results are in excellent agreement with those obtained in \textit{in vivo} microdialysis studies showing that acute clozapine, but not haloperidol, increases extracellular concentrations of glutamate in the
mPFC of freely moving rats (Daly and Moghadam, 1993; Yamamoto et al., 1994), although it is difficult to directly compare the results obtained from in vitro slice vs. in vivo microdialysis. The clozapine-induced marked increase of the release of EAAs in the mPFC may account for, at least in part, the report that clozapine is associated with a higher incidence of seizures than traditional APDs in patients with no previous history of ictal events (Haller and Binder, 1990).

Another major difference between the two APDs is that clozapine markedly potentiated, whereas haloperidol decreased, EPSPs/EPSCs elicited by electrical stimulation of the forceps minor (white matter) in pyramidal neurons of the mPFC. Clozapine-induced facilitation of the evoked EPSPs was voltage dependent (i.e., the potentiating effect of clozapine increased dramatically when the Vθ was changed to a more depolarized potential), supporting the finding that clozapine preferentially potentiates NMDA receptor-mediated transmission because membrane depolarization markedly enhances the efficacy of activation of the NMDA receptor-ionophore complex (Nowak et al., 1984). In addition, there is a tendency for clozapine to shift the reversal potential of EPSCs to a more positive level, which presumably could be attributed to the more positive reversal potential of the NMDA component of EPSPs (Arvanov and Wang, in press; Burgard and Hablitz, 1993; Wuarin et al., 1992). Our results indicate that clozapine preferentially potentiated NMDA receptor-mediated transmission, whereas haloperidol produced an overall inhibitory action on glutamate receptor-mediated neurotransmission. The latter is likely due to the fact that the marked depressing action of haloperidol on the non-NMDA component of EPSPs (see below) prevented or obscured the potentiating action on the NMDA component.

Haloperidol, but not clozapine, depressed AMPA-induced response in a concentration-dependent manner. Furthermore, in the presence of d(−)-AP-5, haloperidol produced a voltage-independent inhibition of the non-NMDA component of EPSPs evoked by electrical stimulation of the forceps minor. The mechanisms of the inhibitory action of haloperidol on non-NMDA AMPA receptors are not clear at present, although the inhibition of Na+ channels by haloperidol (Pencek et al., 1978; Westlund-Danielsson et al., 1992) may in part account for the inhibitory effect. The inhibitory action of haloperidol on AMPA response may contribute to the result that haloperidol, but not clozapine, decreased the amplitude of EPSPs evoked by NMDA administration.

In summary, in the present study, we have shown that both the atypical APD clozapine and the typical APD haloperidol potentiate NMDA-evoked responses in pyramidal cells of the mPFC. In addition, clozapine enhances, whereas haloperidol depresses, electrical stimulation-evoked EPSPs/EPSCs, which might be due to the fact that haloperidol, but not clozapine, depresses AMPA-induced response in a concentration-dependent manner. Further systematic comparison of the similarities and differences in the effect of typical and atypical APDs on NMDA and non-NMDA receptors may help to more fully assess the role of glutamate in schizophrenia and the mechanisms of APD action with regard to therapeutic efficacy and side effects (for review, see Ashby and Wang, 1996). The results generated from these studies may provide a new paradigm to differentiate between typical and atypical APDs.

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References


