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Short communication

Effects of MK-801 on clozapine-induced potentiation of excitatory synaptic responses in the perforant path–dentate gyrus pathway in chronically prepared rabbits

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Abstract

We previously found that the atypical antipsychotic drug, clozapine, when intraperitoneally (i.p.) injected, long-lastingly potentiated excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations to the perforant path in chronically prepared rabbits, and called this phenomenon 'clozapine-induced potentiation'. In the present study, we likewise examined whether clozapine-induced potentiation is caused by NMDA receptor-mediated neurotransmission in the perforant path–dentate gyrus pathway of chronically prepared rabbits. The non-competitive NMDA receptor antagonist — 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imino hydrogen maleate (MK-801; 1.0 mg/kg, i.p.) — completely prevented the potentiation of synaptic responses induced by subsequent administration of 20 mg/kg clozapine, whereas the 0.5 mg/kg dose had virtually no effect on the potentiation. These results suggest that the effect of clozapine requires NMDA receptor activation.

Keywords: Clozapine-induced potentiation; NMDA receptor; MK-801; Haloperidol; Long-term potentiation; Perforant path–dentate gyrus pathway

1. Introduction

A 5-HT and dopamine receptor antagonist, clozapine, is an atypical antipsychotic because of its effectiveness in treating polar and refractory symptoms in schizophrenia — i.e., negative symptoms such as abulia, autism and affective flattening — and its low potential to induce extrapyramidal side effects. In this respect, clozapine is supposed to have a different pharmacological profile from typical antipsychotics such as haloperidol. We previously found that clozapine (intraperitoneally injected) long-lastingly potentiated excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations to the perforant path in chronically prepared rabbits, and called this phenomenon 'clozapine-induced potentiation' (Kubota et al., 1996). In a further study, we observed that the potentiation lasted for at least 48 h (unpublished). It has been reported that such a potentiation was not induced by haloperidol (Krug et al., 1983; Jibiki et al., 1993; Arvanov et al., 1997). In particular, we have recently found in a previous study (Jibiki et al., 1993) that even haloperidol — at low doses of 0.1 or 0.4 mg/kg (injected i.p.), as well as a high dose of 0.8 mg/kg — exerted virtually no effect on the excitatory synaptic responses elicited in the dentate gyrus by single electrical stimulations to the perforant path in chronically prepared rabbits (unpublished). Recently, a phenomenon similar to clozapine-induced potentiation has been reported by Arvanov et al. (1997), who found that clozapine enhanced the amplitude of excitatory post-synaptic potentiations (EPSPs) evoked by single electrical stimulation in the pyramidal cells of the medial prefrontal cortex in rat brain slices. It is well known that antipsychotic drugs, including clozapine, have a very low affinity for binding sites of glutamate receptors, but show high affinity for those of monoamine receptors such as the dopamine, 5-HT receptors and adrenoceptors. Therefore, it is unlikely that such a facilitating effect of clozapine on excitatory synaptic transmission results from a direct interaction with the glutamate receptors. Currently, the mechanisms underlying the extraordinary action of antipsychotics on excitatory or glutamatergic neurotransmission such as

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clozapine-induced potentiation are unknown, and it would be of interest to clarify these mechanisms. On the other hand, a role of a subtype of the glutamate receptors — the NMDA receptor in the pathogenesis of schizophrenia — has been recently suggested, as represented by the so-called 'PCP psychosis', which signifies schizophrenia-like psychotic symptoms caused by intake of the non-competitive NMDA receptor antagonist, phencyclidine (PCP) in humans (Olney and Farber, 1995). Therefore, the question of whether antipsychotics affect NMDA receptor activity is an important one. The present study was thus undertaken to investigate whether clozapine-induced potentiation is related to NMDA receptor activation, using a type of non-competitive NMDA receptor antagonist — 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imino hydrogen maleate (MK-801).

2. Materials and methods

2.1. Animal model

Chronic experiments were carried out on 15 adult male rabbits weighing 2.5–3.5 kg. Each surgical procedure was conducted under i.p. pentobarbital sodium anesthesia (20–30 mg/kg). A tungsten microelectrode for recordings (tip diameter: 1–2 μm, resistance: 1–5 kΩ) and a concentric stimulating electrode for laminar analysis (0.6 mm in diameter) were attached to a holder with the tips aligned 1 mm apart. The tungsten microelectrode was connected to a memory oscilloscope (Nihon Kohden; VC10, bandpath: 0.08–3000 Hz) through a preamplifier. After unilateral craniectomy, these electrodes were inserted from the pial surface at the P4 and L6 positions on Ridge's map to the dentate gyrus, using an oil hydraulic microdrive (Narishige), with laminar analysis every 50 or 100μm, as detailed in previous studies (Jibiki et al., 1993; Kubota et al., 1994, 1996). Next, another concentric stimulating electrode was inserted from the pial surface at the P4 and L1 positions to the perforant path ipsilateral to the dentate gyrus, while observing the maximal responses elicited in the dentate gyrus by single shocks at a constant intensity delivered from the stimulating electrode. After a 10-day post-surgical recovery period, chronic experiments were performed as described below. Animal care and use procedures were in accordance with the approved protocol of the Animal Research Committee of the Kanazawa Medical University.

2.2. Experiment 1

In 5 out of 15 rabbits, control experiments were performed to examine the magnitude of clozapine-induced potentiation without MK-801 administration. The threshold intensities of single shocks to the perforant path for inducing population spikes in the dentate gyrus were initially examined. The intensities just above the threshold were determined as those of single shocks to elicit control responses, which consisted of a small population spike with an amplitude of less than 0.5 mV preceded by the leading edge population EPSPs of a low positive wave, and the subsequent slow component (Fig. 1A, baseline recording). Then, the baseline recording was performed for 30 min with single stimuli at a fixed intensity (monopolar square pulse of 0.2–0.5 ms duration, 400–800 μA, 30-s stimulus intervals). Next, vehicle solution (Mg^{2+}-free Ringer's solution) without MK-801 was intraperitoneally administered as a single injection (0.5 ml). Soon thereafter, single stimuli at a fixed intensity (the same as used for the baseline recording) were given to the perforant path for 30 min to observe the response changes in the dentate gyrus. Next, 20 mg/kg (i.p.) of clozapine dissolved in dimethylsulfoxide (0.5 ml) was administered as a single injection. Soon thereafter, single stimuli at a fixed intensity (the same as used for control recording) were given to the perforant path for 60 min to observe the response changes in the dentate gyrus. Next, a tetanic stimulation for inducing long-term potentiation was delivered to the perforant path. It is well known that long-term potentiation is easily produced in the perforant path–dentate gyrus pathway, as shown in previous studies (Jibiki et al., 1993; Kubota et al., 1994, 1996), and further that long-term potentiation is an NMDA receptor-mediated phenomenon that is blocked by the preceding dosage of MK-801. Therefore, we included the long-term potentiation-inducing tetanic stimulation to confirm the effect of MK-801 in the present study.

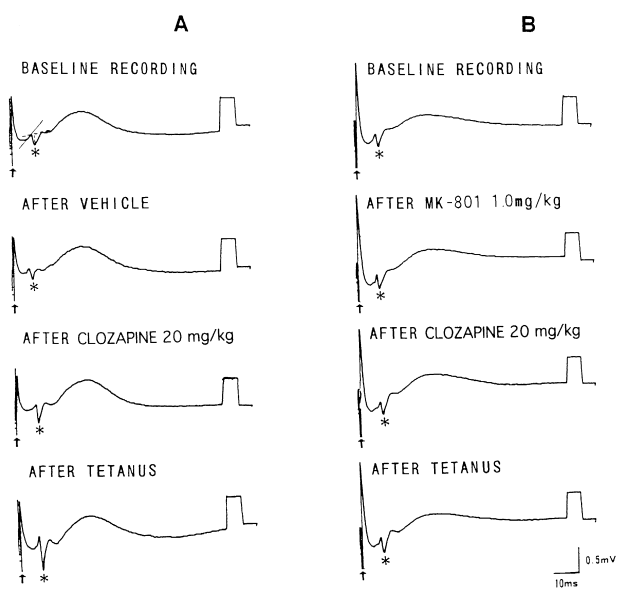

Fig. 1. (A) Typical averaged responses evoked in the dentate gyrus by single shocks at a fixed intensity to the perforant path during each session in a single rabbit in experiment 1. Arrow: single shock; asterisk: population spike. Dotted and solid lines in 'baseline recording' express how to measure the population spike amplitude from the tangent across the start and end of the spike to the peak of the spike and population EPSP slope, respectively. (B) Typical averaged responses in each session in a rabbit in experiment 2 (MK-801, 1.0 mg/kg injection). The same intensity of single shocks and symbols are as in (A).

The tetanic stimulation was repeated three times at 3-min intervals. The stimulus parameters were monopolar square pulses of 0.5-ms duration, 600 μA, 60 Hz and 1 s in total duration. Soon thereafter, single shocks at the fixed intensity were delivered again for about 30 min to observe the response in the dentate gyrus.

2.3. Experiment 2

In 10 rabbits, experiments were performed to examine whether clozapine-induced potentiation and long-term potentiation were induced after MK-801 administration. The baseline recording was initially performed as in experiment 1, after which MK-801, dissolved in Ringer's solution (0.5 ml), was administered as a single i.p. injection. The doses of MK-801 were 0.5 mg/kg (low-dose group) and 1.0 mg/kg (high-dose group), with five rabbits for each group. Soon after the MK-801 injection, single shocks at a fixed intensity were again given to the perforant path for 30 min to observe the response changes as in experiment 1. Subsequently, 20 mg/kg of clozapine dissolved in dimethylsulfoxide (0.5 ml) was administered as a single i.p. injection as in experiment 1. Then, single shocks at a fixed intensity were again given and the responses were observed for about 60 min. Next, long-term potentiation-inducing tetanic stimulation was delivered to the perforant path as in experiment 1. Soon thereafter, single shocks at the fixed intensity were delivered again for about 30 min.

2.4. Data analysis

In each experiment, four sets of responses were averaged using a DAT 1100 (Nihon Kohden) and recorded with an X–Y recorder. To analyze the response changes, the amplitude of the population spike and a slope of the population EPSP were measured as in previous studies (Jibiki et al., 1993; Kubota et al., 1994, 1996). Both the population spike amplitudes and the EPSP slopes in the 75 responses averaged during the whole experimental time of 150 min, were first analyzed by repeated measure analysis of variance (ANOVA) to examine whether there were significant differences between the three groups, i.e. the control group of experiment 1 and the low and high MK-801 dose groups of experiment 2. Then, the respective values in the 15 responses averaged over 30 min in each of the five sessions — baseline, after vehicle or MK-801 injection, the first and latter halves in the observation period of 60 min after clozapine injection, and after tetanus were analyzed by one-way ANOVA followed by Scheffe's multiple comparison — in order to examine whether they differed significantly between the five sessions in each group and, further whether they differed significantly between the three groups in each session.

3. Results

3.1. Experiment 1

In all five rabbits in experiment 1, the baseline responses were virtually unaltered during the baseline recordings (Fig. 1A, baseline recording). After subsequent vehicle injection before clozapine administration, the responses also showed no changes (Fig. 1A, after vehicle). Next, the responses showed no change during the first 30 min of the observation period (about 60 min) after the administration of 20 mg/kg of clozapine; however, they were markedly potentiated during the latter 30 min, with regard to both the PS and the EPSP slopes (Fig. 1A, after clozapine 20 mg/kg). Furthermore, the responses were still more potentiated soon after the tetanic stimulations, also with regard to population spikes and EPSP slope, showing the induction of long-term potentiation (Fig. 1A, after tetanus).

One-way ANOVA showed significant differences between the five sessions for both the population spike amplitudes and EPSP slopes [main time course effect, $F(4,20) = 11.026$, $P = 0.001$ in population spike and $F(4,20) = 5.110$, $P = 0.005$ in EPSP]. The subsequent Scheffe's multiple comparison showed no significant differences between baseline and after vehicle injection ($P = 0.998$ in population spike, $P = 0.999$ in EPSP) and the first half after clozapine injection ($P = 0.563$ in population spike, $P = 0.817$ in EPSP), whereas they showed significant differences between baseline and the latter half after clozapine injection ($P = 0.013$ in population spike, $P = 0.023$ in EPSP), and baseline and after tetanus ($P = 0.0003$ in population spike, $P = 0.017$ in EPSP) with regard to both the population spike amplitudes and the EPSP slopes (Fig. 2, control).

3.2. Experiment 2

In the low-dose group with MK-801 (0.5 mg/kg), the responses were virtually unaltered during the baseline recordings, after subsequent MK-801 injection and during the first half of the 60-min observation period after clozapine administration. In the latter half post-clozapine administration, the responses to single shocks were markedly potentiated as compared with previous responses for both population spike amplitudes and EPSP slopes. Then, after tetanic stimulation, the responses were still more potentiated with regard to both the PS amplitude and EPSP slope, showing the induction of long-term potentiation in all five rabbits.

One-way ANOVA showed significant differences between the five sessions for both the population spike amplitudes and EPSP slopes [main time course effects, $F(4,20) = 9.055$, $P = 0.0002$ in population spike and $F(4,20) = 5.330$, $P = 0.004$ in EPSP]. The subsequent Scheffe's multiple comparison showed no significant dif-

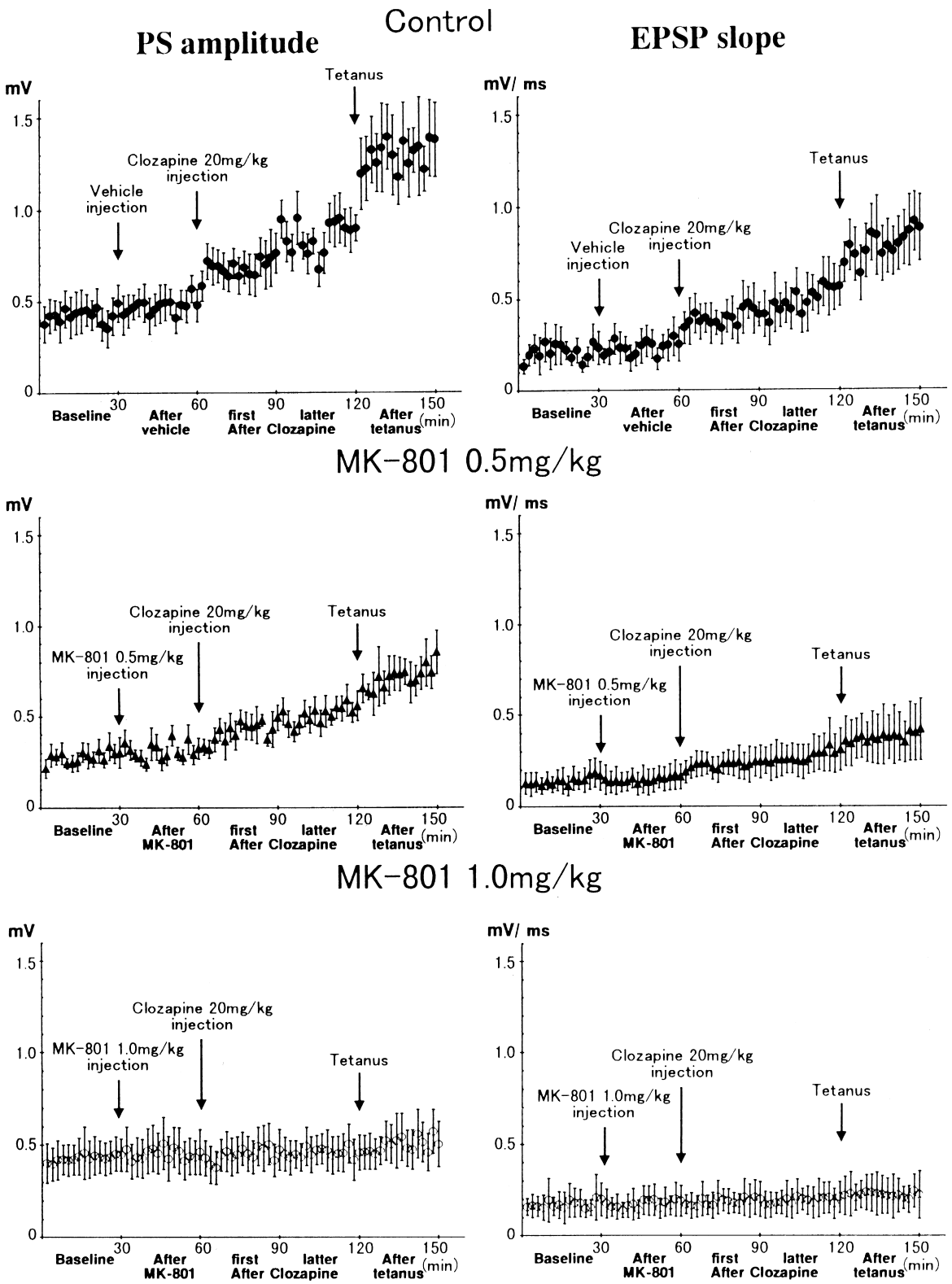

Fig. 2. Serial changes of population spike amplitudes and EPSP slopes in the 75 responses averaged, i.e. 300 real responses during the whole experimental period of 150 min divided into five sessions: baseline, after vehicle or MK-801 injection, the first half and latter half in the observation period of 60 min after clozapine injection, and after tetanus. Graphs show the mean and SE of the respective values from five rabbits in each group.

ferences between baseline and after MK-801 injection ($P = 0.997$ in population spike, $P = 0.999$ in EPSP) and the first half after clozapine injection ($P = 0.613$ in population spike, $P = 0.733$ in EPSP); whereas they showed significant differences between baseline and the latter half after clozapine injection ($P = 0.031$ in population spike,

$P = 0.020$ in EPSP), and between baseline and after tetanus ($P = 0.001$ in population spike, $P = 0.017$ in EPSP) with regard to both population spike amplitudes and EPSP slopes (Fig. 2, MK-801 0.5 mg/kg).

In the high-dose group of MK-801 (1.0 mg/kg), the responses were virtually unaltered throughout the experimental time, i.e. during the baseline recordings, after subsequent MK-801 injection, and after subsequent clozapine injection and tetanic stimulation (Fig. 1B).

One-way ANOVA showed no significant differences between the five sessions [main time course effect, $F(4,20) = 0.075$, $P = 0.988$ in population spike, $F(4,20) = 0.326$, $P = 0.856$ in EPSP] with regard to both the population spike amplitudes or EPSP slopes (Fig. 2, MK-801 1.0 mg/kg).

In addition, repeated measure ANOVA showed significant differences between the three groups with regard to both the population spike amplitude and EPSP slopes in the 75 responses averaged during the whole experimental time of 150 min [main group effect, $F(2,12) = 4.280$, $P = 0.0395$ in population spike and $F(2,12) = 4.104$, $P = 0.0438$ in EPSP, group × time course, $F(148,888) = 5.889$, $P = 0.001$ in population spike and $F(148,888) = 5.745$, $P = 0.001$ in EPSP]. Then, one-way ANOVA showed significant differences between the three groups with regard to both the population spike amplitude and EPSP slopes in the 15 responses averaged during each 30 min only in the last two of the five sessions, i.e. latter half after clozapine injection and after tetanus [main group effect, $F(2,12) = 7.313$, $P = 0.008$ in population spike and $F(2,12) = 4.259$, $P = 0.040$ in EPSP in the latter half after clozapine injection and $F(2,12) = 11.671$, $P = 0.001$ in population spike and $F(2,12) = 9.525$, $P = 0.003$ in EPSP after tetanus]. The subsequent Scheffe's multiple comparison showed significant differences between the control and MK-801 1.0 mg/kg dose groups in both the latter half after clozapine injection ($P = 0.014$ in population spike and $P = 0.047$ in EPSP, respectively) and after tetanus ($P = 0.019$ in population spike and $P = 0.042$ in EPSP, respectively). However, there were no significant differences between MK-801 0.5 and 1.0 mg/kg dose groups and between control and 0.5 mg/kg MK-801 dose groups with regard to both the population spike amplitudes or EPSP slopes (Fig. 2, comparison between control and 1.0 mg/kg MK-801, between 0.5 and 1.0 mg/kg MK-801, and between control and MK-801 0.5 mg/kg).

4. Discussion

In the present study, we found that 1.0 mg/kg MK-801 completely prevented clozapine-induced potentiation. This finding indicates that clozapine-induced potentiation is caused by NMDA receptor activation. The 1.0 mg/kg MK-801 doses may have been somewhat high. However, other experiments have shown that long-term potentiation induced in the rat hippocampus can be blocked with a single i.p. injection of 0.5–1.0 mg/kg MK-801 (Abraham and Mason, 1988). Perhaps, with systemic administration like intraperitoneal injection, high doses of MK-801 are required for blocking long-term potentiation. There is some evidence suggesting that antipsychotics might affect 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, it has been reported that the bath administration of either haloperidol or clozapine potentiated NMDA-evoked responses in a concentration-dependent manner in the pyramidal cells of the medial prefrontal cortex in rat brain slices, although haloperidol, but not clozapine, depressed the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-evoked responses (Arvanov et al., 1997). In view of these reports, the present finding showing that clozapine causes NMDA receptor activation may be reasonable.

In the mechanisms underlying clozapine-induced potentiation, a direct interaction of clozapine with the glutamate receptors — especially the NMDA receptors — is unlikely as mentioned above. The use of the clozapine dose of 20 mg/kg chosen for clozapine-induced potentiation in the present study was based on results of a previous study (Kubota et al., 1996), indicating that clozapine-induced potentiation is mostly induced by the dose of 20 mg/kg (i.p.). Further, our choice depended on experimental evidence that clozapine doses of 15–20 mg/kg are therapeutically equivalent (Rupniak et al., 1985). Studies with in vivo intracerebral microdialysis have shown that the acute and systemic administration of clozapine at more than 20 mg/kg dose-dependently produced an increase in extracellular concentrations of glutamate in the medial prefrontal cortex of freely moving rats, whereas haloperidol induced virtually no increase (Daly and Monghaddam, 1993; Yamamoto et al., 1994). In view of these findings, it is possible that clozapine may activate non-NMDA receptors by facilitating the release of glutamate from the presynapse in the axon terminals, which, in turn, cause membrane depolarization and remove the Mg^{2+} block of the NMDA receptor/ionophore complex and eventually activate NMDA receptors. It might be speculated that the increase in glutamate release results from clozapine's own action, i.e. the blockade of dopamine and 5-HT receptors, which causes the removal of the inhibitory action of dopamine and serotonin (Kornhuber and Kornhuber, 1986; Maura et al. 1988). Further, it is known that clozapine binds with high affinity to dopamine receptor subtypes, the dopamine D_1 and D_2 receptors, and 5-HT receptor subtypes, the 5-HT_2 and 5-HT_3 receptors, whereas haloperidol is a nearly selective dopamine D_2 receptor antagonist (Yamamoto et al., 1994). The difference in glutamate release between clozapine and haloperidol may be caused

by the difference in their binding potential to dopamine and 5-HT receptors or the subtypes.

In addition, clozapine is known to be associated with a higher prevalence of seizures than traditional neuroleptics (Haller and Binder, 1990). Clozapine-induced potentiation caused by NMDA receptor activation may contribute to the mechanisms underlying the clozapine-induced seizures, since it is probable that the potentiation leads to the excessive neuronal firing underlying seizures.

In conclusion, the present results indicate that clozapine-induced potentiation is a NMDA receptor-mediated event. This result may provide a new clue for the study of the pharmacological actions of antipsychotics or the pathogenesis of schizophrenia.

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