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M100907, A Selective 5-HT_{2A} Receptor Antagonist and a Potential Antipsychotic Drug, Facilitates *N*-Methyl-D-Aspartate–Receptor Mediated Neurotransmission in the Rat Medial Prefrontal Cortical Neurons In Vitro

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The technique of intracellular recording was used to examine the effect of M100907 (formerly MDL 100907), a highly selective 5-HT_{2A} receptor antagonist and a potential antipsychotic drug (APD), on N-methyl-D-aspartate (NMDA) and (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-mediated responses in pyramidal cells of the rat medial prefrontal cortex in in vitro brain slice preparations. Bath administration of M100907, but not its inactive stereoisomer M100009, produced a 350% to 550% increase of NMDA-induced responses in a concentration-dependent manner with an EC_{50} value of 14 nmol/L, reminiscent of the action of clozapine. M100907 did not alter AMPA responses. Moreover, M100907 significantly increased the amplitude and duration of excitatory postsynaptic potentials and currents evoked by electrical stimulation of the forceps minor. We have generated several lines of evidence indicating that M100907 enhances glutamate receptor-mediated neurotransmission in pyramidal cells of the medial prefrontal cortex by facilitating NMDA-induced release of excitatory amino acids. The robust potentiation of NMDA receptor-mediated neurotransmission may explain, at least partly, the potential antipsychotic action of this compound. Furthermore, if M100907 proves to be an effective APD and if our findings can be extended to other atypical APDs, which are known to possess a relatively high affinity to 5-HT_{2A} receptors, they may account for the purported efficacy of atypical APDs in alleviating some negative symptoms such as cognitive and executive functions.
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KEY WORDS: *NMDA receptor; 5-HT_{2A} receptor; Antipsychotic drugs; Schizophrenia; Medial prefrontal cortex; Pyramidal cells*

There is ample evidence indicating that dopamine (DA) hyperactivity is inadequate to explain the pathogenesis and pathophysiology of schizophrenia (Davis et al. 1991; Meltzer 1991). For example, a substantial proportion of schizophrenic patients is resistant to treatment with neuroleptics, suggesting that other neurotransmitter systems may have a pathogenetic role in these patients. Moreover, neuroleptics are only partially effective in alleviating the negative or deficit symptoms of schizophrenic patients, particularly after resolution of the acute phase of the illness. In addition, there is no conclusive biochemical evidence indicating DA hyper-

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activity in schizophrenics. Thus, it has become clear that a hypothesis of excessive DA activity in all brain regions of schizophrenic patients is untenable. Most recently, preliminary evidence suggests that a highly selective serotonin$_{2A}$ (5-HT$_{2A}$) receptor antagonist M100907 [R-(+)-α-(2,3-dimethoxyphenil)-1-[4-fluorophenylethyl)]-4-piperidinemethanol; Johnson et al. 1996; Kehne et al. 1996; Marek and Aghajanian 1994; Sorensen et al. 1993], which is currently under phase IIB clinical studies, is an effective antipsychotic drug (APD; Dr. S. Sorensen, Hoechst Marion Roussel, personal communications). Where and how M100907 exerts its action to alleviate schizophrenic symptoms becomes a subject of fundamental importance with both theoretical and clinical relevance.

The glutamate hypothesis of schizophrenia proposes a relationship between hypoactive glutamatergic neurotransmission, particularly *N*-methyl-D-aspartate (NMDA) receptor hypofunction, and the behavioral deficits associated with schizophrenia (Carlsson and Carlsson 1990; Deutsch et al. 1989; Kim et al. 1980; Moghaddam 1994; Olney and Farber 1995; Wachtel and Turski 1990). The hypothesis stems from the observation that phencyclidine (PCP, a noncompetitive NMDA receptor antagonist) abused by people can induce psychosis that includes many symptoms and cognitive disturbances commonly observed in patients with schizophrenia (Grotta 1994; Herrling 1994; Kristensen et al. 1992). In addition, when ketamine, another noncompetitive NMDA antagonist, is given to schizophrenic individuals, it produces a short-lived, discrete activation of their psychotic symptoms, which have striking similarities to symptoms of their usual psychotic episodes (Lahti et al. 1995).

Evidence has been accumulating to suggest a disturbed glutamatergic neurotransmission in schizophrenics. For instance, an increase of ^{3}H-kainate, ^{3}H-aspartate, ^{3}H-tenocyclidine (TCP, an analog of PCP), and ^{3}H-glycine binding in the cerebral cortex, a decrease of non-NMDA receptor mRNA in cortex, and an increase of 3H-MK-801 binding in the putamen have been shown in post mortem schizophrenic brains (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 increase of NMDA binding sites and mRNA might be a compensatory response to the functional hypoactivity of the NMDA receptors in the cerebral cortex of patients with schizophrenia. Moreover, the prefrontal cortex of schizophrenics has recently been shown to exhibit alterations in the expression of NR2 subunit, which are potential indicators of deficits in NMDA receptor-mediated neurotransmission accompanying functional hypoactivity of the frontal lobe (Akbarian et al. 1996). Thus, it appears that a hypofunction of NMDA receptors in the prefrontal cortex and perhaps a disturbed balance between glutamatergic and other neurotransmitter systems may play a key role in the pathophysiology of schizophrenia.

We have begun to study the action of various typical and atypical APDs on glutamatergic neurotransmission in the medial prefrontal cortex (mPFC), an area that has been suggested to play a key role in the pathogenesis of schizophrenia and that contains a high density of both 5-HT$_{2A}$ and NMDA receptors (Blue et al. 1988; Dure and Young 1995; Mengod et al. 1990; Weinberger and Lipska 1995), using the techniques of intracellular recording and single-electrode voltage-clamp to record pyramidal cells of the rat mPFC in in vitro brain slice preparations (Arvanov et al. 1997a; Wang and Arvanov 1996). Our results have shown that the atypical APD clozapine produces a marked, three- to fourfold potentiation of the NMDA-activated inward current in pyramidal cells of the rat mPFC brain slices with an EC_{50} value of 14 nmol/L. Haloperidol, but not raclopride (up to 100 nmol/L), also potentiates NMDA-induced responses (EC_{50} = 38 nmol/L). However, it appears that haloperidol is less potent and less efficacious than clozapine in the potentiating action. Furthermore, clozapine, but not haloperidol, markedly potentiates glutamate neurotransmission elicited by electrical stimulation of the forceps minor (white matter) in the mPFC. The latter finding might be related to the fact that haloperidol, but not clozapine, depresses (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-induced responses in a concentration-dependent manner (IC_{50} = 37 nmol/L).

The purposes of the present study were twofold: (1) to examine the possible actions of M100907 on NMDA- and AMPA-receptor mediated neurotransmission, and (2) to compare these results with those obtained previously with clozapine and haloperidol.

MATERIALS AND METHODS

Preparation of mPFC Slices

The procedures for preparation of rat mPFC brain slices have been described previously (Arvanov et al. 1997a; Yang et al. 1996). Briefly, male Sprague-Dawley rats (body weight, 160 to 200 g) were decapitated under halothane anesthesia and their brains removed and cooled in ice-cold artificial cerebrospinal fluid (ACSF). The coronal (transverse) slices of mPFC (450 μm thick) were cut in ice-cold ACSF containing (in mmol/L): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, and D-glucose 11, aerated with 95% O_2/5% CO_2 (pH 7.4), and kept submerged in room temperature for at least 1 h to allow for recovery. A single slice was then transferred to a recording chamber (32°C) where it was held submerged between two nylon nets. The chamber was continuously superfused with ACSF at a constant rate of 2 ml/min.

Intracellular Recording and Single-Electrode Voltage Clamp

Standard intracellular recording techniques were used to record pyramidal neurons of the mPFC. Intracellular electrodes were pulled from Borosilicate glass capillaries (Kwik-Fil, WPI, Sarasota, FL) on a Brown and Flaming type horizontal puller (Model P-87, Sutter Instruments, San Rafael, CA). Recording electrodes were filled with 4 mol/L K-acetate or 3 mol/L KCl (tip resistances 55 to 80 MΩ). The procedures for intracellular recording and single-electrode voltage clamp using an Axoclamp 2B amplifier have been described elsewhere (Arvanov et al. 1995, 1997a). Neurons were impaled by briefly increasing the capacity compensation to cause the head-stage to oscillate and were subsequently hyperpolarized periodically by passing current through the electrode, a procedure that appeared to contribute to the recovery and "sealing" of the cells. Current and voltage records were acquired using a D/A sampling and acquisition software (pClamp 6, Axon Instruments, Foster City, CA) and were filtered at 1 KHz and analyzed off-line. Voltage and current signals were also recorded on a Gould Easy Graph Thermal Recorder (TA 240) and two-channel video tape recorder (Instrutech VR-10B Digital Data Recorder, Elmont, NY). The problems (e.g., space clamping) associated with this method in neurons with extended processes have been discussed elsewhere (Halliwell and Adams 1982; Johnston and Brown 1983; 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 relative changes after drug application (Madison et al. 1987; Schweitzer et al. 1993). This method was deemed suitable for studying the interaction of membrane receptors and the role of intracellular second messengers, because the cell could be recorded for a long time period without significant washout of intracellular components, which may be a major limitation for the whole-cell patch clamp (McDonald et al. 1989; Arvanov and Usherwood 1991) even though it would have provided a better space clamp.

During voltage-clamp recordings, tetrodotoxin (TTX, 0.5 μmol/L, to block action potentials), glycine (1 μmol/L, to maximize NMDA-induced current) and bicuculline (5 μmol/L, to block $GABA_A$ receptors) were routinely included in the ACSF. All cells were held at −60 mV to minimize activating I_M and I_h (Halliwell and Adams 1982).

Except where noted, drugs were administered via the superfusion medium. In the Ca^{2+}-free and low Ca^{2+} ACSF, Ca^{2+} was substituted by equi-osmolar Na^+. To load the recording cells with BAPTA (tetrapotassium salt), the recording electrode was filled with 2 mol/L K-acetate plus 0.2 μmol/L BAPTA (pH = 7). Although the intracellular concentration of BAPTA was unknown, it has been estimated to be about 7 mmol/L based on the shift of the inhibitory postsynaptic potentials (IPSPs) reversal potential caused by the KCl filled electrode (Lancaster and Nicoll 1987).

NMDA was applied by placing a microdrop (10 μl) of concentrated solution (1 mmol/L; the estimated final bath concentration was 10 μmol/L with a dilution factor 1:100) on a marked spot in the inflow channel of the chamber as previously described (Arvanov et al. 1997a; 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 baseline current caused by NMDA varied from cell to cell (30 to 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, prior to any drug tests.

For comparison, NMDA was also applied by superfusion in a few experiments. Bath application of 10 μmol/L NMDA for 3 min evoked a gradual increase of inward current. It often required 3 to 4 min for the inward current to reach a plateau. Thus, it was difficult to obtain a true maximum due to excitotoxicity, desensitization, and/or incomplete equilibrium. Moreover, a 25- to 30-min interapplication interval was needed to avoid an obvious desensitization and maintain a stable baseline. Therefore, using this method to study the drug effect on NMDA current was rather impractical. We have also tried micropressure injection of NMDA. The difficulty for the techniques of micropressure injection was twofold: (1) estimating the actual concentration of drug and (2) maintaining a stable baseline. Whereas applying a negative pressure to the pipette caused an obvious dilution of the drug solution in the pipette, without using the retaining pressure, NMDA slowly diffused out of the pipette and caused receptor desensitization.

Electrical Stimulation-Evoked EPSPs/EPSCs

Excitatory postsynaptic potentials (EPSPs, recorded in current-clamp mode)/ excitatory postsynaptic currents (EPSCs, recorded in voltage-clamp mode) were elicited by passing rectangular current pulses (pulse-width, 0.3 to 0.5 ms; stimulation strength, 50 to 250 μA) between the tips of a bipolar stainless steel electrode placed in the medial part of the forceps minor, about 1 mm from the recording site. 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 three times at a rate of 0.05 Hz before and after drug application. We have used the recording electrodes filled with 2 mol/L CsCl plus 25 to 50 mmol/L QX 314 (lidocaine *N*-ethyl bromide quaternary

salt, tip resistance 30 to 50 MΩ) to improve the space clamp (Wuarin et al. 1992) and block the late IPSPs (Nathan et al. 1990). Under these conditions, the Na^+ spikes were blocked and the membrane resistance increased by 30% to 50%.

Paradigm of Paired-Pulse Facilitation (PPF)

To determine the pre- or postsynaptic actions of M100907, we have examined the PPF, which is considered to be a presynaptic phenomenon, resulting from a transient increase of presynaptic Ca^{2+}, caused by a conditioning synaptic response (Hess et al. 1987; Zucker 1989). In addition to PPF, we have also adopted variance analysis to determine the statistical parameter m_{CV} = mean^2/variance (M^2/σ^2), the alterations of which have been reported to be closely related to presynaptic sites (Malinow and Tsien 1990; Lupica et al. 1992; Manabe et al. 1993). It was found that paired-stimulation of the forceps minor may induce PPF, paired-pulse depression (PPD), or no changes at neurons in layers V-VI. After extensive explorations and experimentations, we have decided that the following experimental conditions might be optimal for inducing PPF in presumed pyramidal cells of the mPFC (>80% of cells exhibited PPF): (1) adjusting stimulus intensity to elicit near threshold (minimal) $EPSP_1$ responses (Hess et al. 1987) that could be distinguished from baseline noise on 90% of the trials (0.05 ms pulse-width, 15 to 25 μA pulse-strength; same parameters were used for both $EPSC_1$/$EPSP_1$ and $EPSC_2$/$EPSP_2$), (2) setting the inter-pulse interval at 40 ms (Berretta and Jones 1996), (3) perfusion of slices with 1 μmol/L bicuculline (Nowicky and Bindman 1993), and (4) use of electrodes filled with 2 mol/L CsCl plus 25 mmol/L QX-314. Unitary EPSCs were evoked at a rate of 0.1 Hz, holding potential was −70 mV, unless stated otherwise. Sixty response traces per epoch were collected and used in the subsequent statistical analyses. The peak amplitude of EPSCs were measured using pClamp-6 "Spike" Data Analyses Systems. Noise-contaminated signal variance made by the background noise (Lupica et al. 1992) was <25%.

The results were presented as mean ± SEM. Paired *t*-tests, Student's *t*-tests, analysis of variance, and least significance difference test were used; .01 and .05 were selected for testing the level of significance.

Drugs

The compounds riluzole, BAPTA-AM, thapsigargin, glycine, (-)-bicuculline methchloride, CNQX-HBC complex, and d-AP5 were all purchased from Research Biochemicals International (RBI, Natick, MA). TTX (citrate buffer) was purchased from Sigma (St. Louis, MO). M100907 was a gift from Hoechst Marion Roussel, Inc. (Bridgewater, NJ).

The concentration of stock solution of drugs was prepared 1,000-fold higher than that of the final target concentration. Drugs were first dissolved in 50 to 100 μl of either 5% lactic acid (e.g., M100907) or 99% DMSO (e.g., riluzole, BAPTA-AM and thapsigargin), and then brought to stock concentration by the addition of purified water. Some compounds were dissolved directly in purified water [e.g., TTX (citrate buffer), glycine, (-)-bicuculline methchloride, CNQX-HBC complex and d-AP5].

RESULTS

A total of 137 presumed pyramidal cells has been recorded; the electrophysiological criteria for distinguishing presumed pyramidal versus nonpyramidal cells have been published previously (Arvanov et al. 1997a; Cauli et al. 1997; Connors and Gutnick 1990; Kawaguchi 1993; Kawaguchi and Kubota 1996; McCormick et al. 1985; Yang et al. 1996). Stable recordings could be maintained for up to 4 to 5 h, suggesting a relative lack of injury by the electrode penetration. All experiments were routinely performed on presumed pyramidal neurons in layers five and six of the prelimbic cortex (anterior cingulate cortex areas 1 and 3; Zilles 1985), which was located medial to the forceps minor and could be easily identified in the slice.

Effect of M100907 on NMDA-Induced Responses

MDL100907 produced a robust facilitation of NMDA-evoked depolarization (in current-clamp mode) and inward current (in voltage-clamp mode). The facilitating effect of M100907 on NMDA-activated inward current was not associated with changes of membrane properties (Table 1).

The concentration-response curve for M100907 to potentiate NMDA-induced responses was very steep; the effect was almost all or none. At 10 nmol/L or below (n = 19), M100907 did not alter NMDA-evoked response. At 20 nmol/L, M100907 produced a dramatic facilitation (mean ± SEM; 373 ± 17%, n = 7) and at 50 nmol/L, the potentiating effect of M100907 reached the plateau (435 ± 52%, n = 7). Further increase in the concentrations of M100907 did not significantly alter the maximal facilitation of NMDA response. The inactive isomer M100009 did not alter NMDA-induced inward current at 100 nmol/L (n = 4). However, it did potentiate NMDA current at the concentration of ≥2 μmol/L (Figure 1C).

The facilitating effect of M100907 was time dependent. Superfusion of M100907 did not significantly increase NMDA-induced inward current for the first few min, and the full effect of 100 nmol/L M100907 gradually developed toward the end of 10 to 15 min continuous superfusion.

Table 1. Comparison of Resting Membrane Potential (RMP), Membrane Resistance (Ri), Amplitude of Spikes, Time Constant, sAHP, and Spike Frequency Adaptation of mPFC Cells in Control, after Superfusion of M100907 (100 nmol/L) and in Preparation from the Reserpine-Treated Animals

Conditions	RMP (mV)	Ri (MΩ)[a]	Amplitude of Spikes (mV)	sAHP (mV)[b]	Spike Frequency Adaptation[c]
Control (n = 109)	−72.1 ± 1.3	53.8 ± 4.2	83.5 ± 1.5	4.3 ± 0.2	3–4
M100907 (n = 18)	−72.3 ± 1.4	54.9 ± 5.1	83.3 ± 1.7	4.1 ± 0.6	3–4
Reserpine (0.1 mg/kg, SC) animals (n = 4)	−71.1 ± 2.3	50.3 ± 5.9	81.1 ± 2.6	1.1 ± 0.2[d]	6–7
Reserpine (1 mg/kg, SC) animals (n = 5)	−67.5 ± 3.2	42.7 ± 6.1	78.9 ± 2.7	0.7 ± 0.4[d]	6–7

[a]Ri was measured at the linear part of I/V curve.
[b]Cells were held at −65 mV; sAHP was obtained by passing 0.7 nA through microelectrode.
[c]Number of action potentials induced by a depolarizing pulse 0.5 nA, 600 ms from RMP.
[d]Significantly smaller than that of the control, $p < .05$ (ANOVA and least significance difference test).
Values are expressed in mean ± SEM; n represents number of neurons.

There was no sign of tachyphylaxis of the potentiating effect of M100907. The effect was fully reversible and the NMDA responses returned to the basal level after a 30-min wash. Repeated administration of M100907 after the washing period produced a facilitation of NMDA response with a similar magnitude (n = 5, Figure 1A). Moreover, when the recorded pyramidal cell was continuously superfused with M100907-containing ACSF for an hour, the enhanced NMDA response was not diminished (n = 5).

Evidence for Presynaptic Site of Action of M100907 on NMDA-Evoked Responses

We have recently reported that NMDA-evoked response in the mPFC consists of both NMDA and non-NMDA components (Arvanov and Wang, 1997). The latter results from NMDA's activating presynaptic NMDA receptors, causing the release of excitatory amino acids (EAAs), which in turn activate postsynaptic non-NMDA receptors, thereby enhancing the NMDA receptor mediated response. To determine whether M100907 may facilitate NMDA-induced responses by enhancing the ability of NMDA to release EAAs, we studied the effect of compounds and manipulated the recording conditions, which are known to diminish the release of neurotransmitters, on M100907's facilitating action. Representative current traces were shown in Figure 2 to illustrate that M100907 was no longer effective in potentiating NMDA-induced inward current in either Ca^{2+}-free ACSF, low Ca^{2+} (0.1 mmol/L) plus Cd^{2+} 0.2 mmol/L ACSF, ACSF containing 20 μmol/L BAPTA-AM (a membrane permeable Ca^{2+}-chelator), ACSF containing 1 μmol/L thapsigargin (a membrane permeable Ca^{2+}-ATPase blocker that depletes intracellular Ca^{2+} stores), or ACSF containing 50 μmol/L riluzole (a non-specific inhibitor for the release of EAAs; Martin et al.

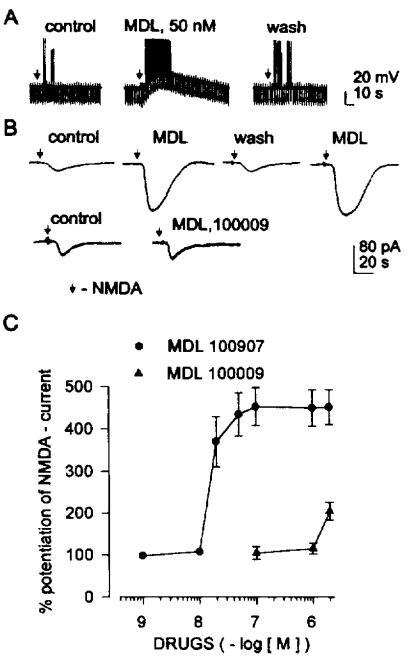

Figure 1. M100907 markedly potentiates NMDA-evoked membrane responses. Representative voltage **(A)** and current **(B)** traces illustrating that M100907 markedly enhanced NMDA-induced membrane depolarization, bursts of action potentials **(A)** and inward current **(B)**; the enhanced NMDA response returned to baseline after a 15-min wash. There were no signs of tachyphylaxis because repeated administration of M100907 produced a reliable facilitation of NMDA responses. In contrast to the action of M100907, bath application of M100009 (the inactive stereoisomer of M100907, 50 nmol/L) failed to potentiate NMDA responses. **(C)**: Comparison of concentration-response curves for M100907 and M100009 to potentiate NMDA-induced inward current. Each point represents the mean ± SEM of three to 12 experiments (n = 3, 12, 7, 7, 11, 4, 4 for the experiments of M100907 at concentration of 1, 10, 20, 50, 100, 1000, and 2000 nmol/L, respectively).

1993). Under the above-mentioned conditions, M100907 nonsignificantly increased NMDA current for 7.5 ± 4.3% (n = 5), 0.7 ± 0.5% (n = 5), 4.2 ± 2.8% (n = 4), 19.5 ± 9.8% (n = 5), and 7.6 ± 5.1% (n = 4), respectively. In contrast, diffusion of BAPTA into the recorded pyramidal neuron by loading 200 mmol/L BAPTA in the recording electrode (the potentiating effect of M100907 was examined 30 to 60 min after recording) failed to prevent the potentiating effect of M100907 on NMDA current (n = 7). BAPTA must have diffused into the neuron because in all seven cells studied with BAPTA, the Ca^{2+}-dependent slow after-hyperpolarizations (sAHPs) were completely blocked within 20 to 25 min of penetration (Figure 2 inset). Furthermore, we have demonstrated in separate experiments (Arvanov et al. 1996, 1997b) that the inhibitory effect of 20 μM DOB

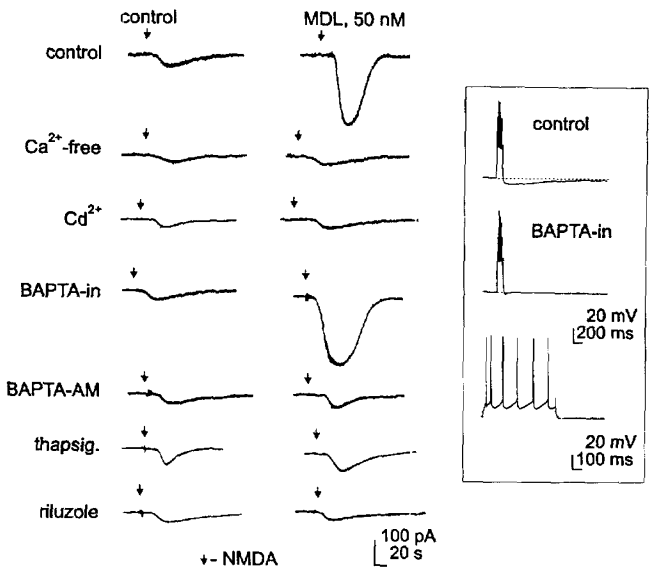

Figure 2. Comparison of the effect of M100907 on NMDA-induced inward current under various conditions, indicating a presynaptic site of action for M100907 to facilitate NMDA current. M100907 was no longer effective in potentiating NMDA current under the following conditions: (1) in Ca^{2+}-free ACSF, (2) in ACSF containing low Ca^{2+} (0.1 mmol/L) plus 0.2 mmol/L Cd^{2+}, (3) in ACSF containing the membrane permeable Ca^{2+}-chelator BAPTA-AM (20 μmol/L), (4) in ACSF containing thapsigargin (1 μmol/L, after 45 min of preincubation in thapsigargin, a membrane permeable Ca^{2+}-ATPase blocker that depletes intracellular Ca^{2+} stores), and (5) in ACSF containing riluzole (50 μmol/L, a nonspecific inhibitor of release of EAAs). In contrast, chelating of Ca^{2+} in the recorded neuron by loading 200 mmol/L BAPTA in the recording electrodes did not alter the ability of M100907 to enhance NMDA current. *Inset*: A slow AHP was apparent 5 min after the penetration of this presumed pyramidal neuron using a BAPTA-loaded micropipette (*upper trace*) but no longer existed 20 min later (*middle trace*), although a fast AHP remained intact (*lower trace*). This result indicates that BAPTA must have diffused into the cell.

[(-)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane, a 5-$HT_{2A,2C}$ receptor agonist] on NMDA responses was abolished in three pyramidal cells recorded with the BAPTA-loading electrodes, whereas the facilitating effect of M100907 in these cells remained intact. These combined results strongly indicate that M100907 may facilitate NMDA-induced release of EAAs, the process of which is Ca^{2+}-dependent, to produce its effect.

Effect of CNQX and Alteration of Extracellular Concentration of Mg^{2+} on M100907's Potentiating Action

It was surmised that NMDA-induced release of EAAs activates primarily the non-NMDA receptors on the somadendritic sites because NMDA-evoked membrane depolarization and inward current could be markedly attenuated by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dion (CNQX; Arvanov and Wang 1997). Interestingly, as shown in Figure 3, bath application of CNQX (20 μmol/L) also effectively blocked the facilitating effect of M100907, which produced a nonsignificant increase of NMDA responses by 12 ± 3% (n = 6; Figure 3). Therefore, non-NMDA receptors are required for the expression of M100907's potentiating effect. It should be pointed out that in CNQX-containing ACSF, where the non-NMDA component of NMDA's action was abolished, it was necessary to increase the concentration of NMDA from 10 to 15 μmol/L to obtain NMDA responses of comparable amplitude to that in the absence of CNQX.

It is possible that activation of non-NMDA receptors by released EAAs causes membrane depolarization and removes the Mg^{2+} block of the NMDA receptor-ionophore complex, thereby potentiating dramatically NMDA-induced inward current. To test this possibility, experiments were designed to examine the effects of altering the extracellular concentration of Mg^{2+} on M100907's facilitating effect. Figure 4 shows that M100907-induced facilitation of NMDA current is dependent on extracellular Mg^{2+} concentration. In Mg^{2+}-free ACSF (Figure 4A; n = 7), NMDA-induced current

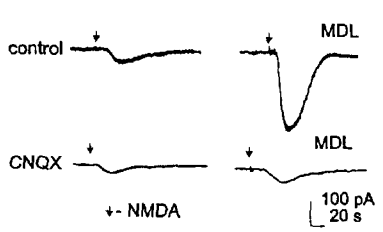

Figure 3. Blockade of M100907's potentiating action on NMDA-induced inward current by the non-NMDA receptor antagonist CNQX (20 μmol/L). In the presence of CNQX, M100907 failed to potentiate NMDA current.

was dramatically increased; addition of M100907 did not increase the amplitude of NMDA current even when the concentration of NMDA was reduced to 2 μmol/L, which elicited a comparable response to that elicited by 10 μmol/L NMDA in normal (1.2 mmol/L Mg^{2+}) ACSF. Therefore, the potentiating effect of M100907 was occluded in Mg^{2+}-free ACSF. The facilitating effect of M100907 was restored by increasing extracellular Mg^{2+} concentration, which significantly inhibited NMDA-induced current. Figure 4B illustrates the results of comparison of the effect of alteration of extracellular Mg^{2+} concentration on NMDA-induced inward current in the absence and in the presence of 100 nmol/L M100907. The IC_{50} values for Mg^{2+} block at the holding potential of −60 mV were 51 μmol/L and 4.5 mmol/L for NMDA alone ($n = 7$) and in the presence of M100907 ($n = 5$), respectively. The difference between the two IC_{50} values is highly significant ($p < .005$, t-test).

Effect of Reserpine on M100907's Potentiating Action

It has been demonstrated that M100907 causes release of DA in the rat prefrontal cortex (Schmidt and Fadayel 1995). To rule out the possibility that M100907's facilitating effect on NMDA responses is the result of releasing monoamines, experiments were performed in rats pretreated with reserpine [0.1 ($n = 4$) or 1 mg/kg ($n = 5$), SC, 24 h prior to experiments]. In reserpine-treated rats (in which the concentrations of DA and 5-HT in the forebrain were reduced to ≤5% of controls, Wang et al. unpublished observations), the ability of M100907 to enhance NMDA responses was not altered, although the reserpine treatment produced a significant decrease of sAHPs and spike frequency adaptation (Table 1). It should be pointed out that NMDA-induced inward current was marked increased in reserpine-treated rats. This may result from the release from the inhibitory action of monoamines on the release of EAAs (see Discussion).

Effect of Bicuculline and Glycine on M100907's Potentiating Action

The potentiating effect of M100907 on NMDA-induced inward current was not affected by alteration of concentrations of glycine or bicuculline. Thus, M100907 (100 nmol/L) increased NMDA current by 460 ± 33% ($n = 4$), 453 ± 30% ($n = 11$), and 470 ± 41% ($n = 4$) in ACSF containing 0, 5, and 20 μmol/L bicuculline, respectively; it also potentiated NMDA current by 465 ± 40% ($n = 4$) and 483 ± 42% ($n = 3$) in ACSF containing 0 and 10 μmol/L glycine, respectively. The percentage of increase in controls experiments, which were performed with 1 μmol/L glycine in ACSF, was 453 ± 35% ($n = 5$).

Effect of M100907 on EPSPs/EPSCs Evoked by Electrical Stimulation of the Forceps Minor

In controls, EPSCs evoked by electrical stimulation of the forceps minor at the holding potential of −60 mV had a duration of 83 ± 3 ms ($n = 15$). The latter was significantly reduced to 62 ± 2 ms ($n = 4$; $p < .05$) by bath application of d-2-aminophosphono-pentanoic acid (d-AP5, 40 μmol/L). In contrast, it was potentiated by 20 μmol/L CNQX to 125 ± 9 ms ($n = 5$; $p < .05$). EPSPs/EPSCs were completely blocked by d-AP5 plus CNQX. M100907 (100 nmol/L) significantly increased the peak amplitude and duration of EPSCs by 25 ± 4% and 58 ± 5%, respectively ($n = 7$, $p < .05$). Similarly, M100907 augmented the peak amplitude and duration of EPSPs by 28 ± 5 % and 53 ± 7%, respectively ($n = 5$, $p < .05$).

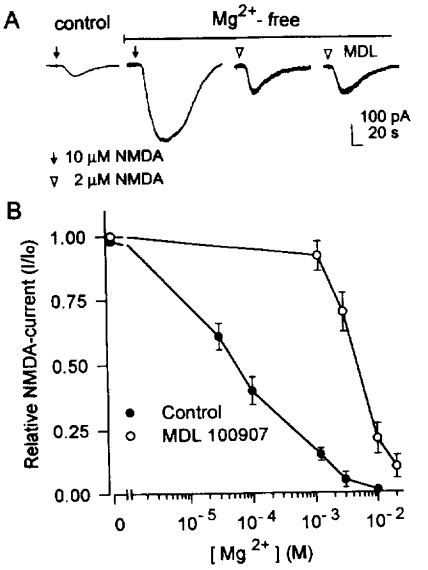

Figure 4. M100907-induced facilitation of NMDA current is dependent on extracellular Mg^{2+} concentration. **(A)**: Representative current traces to illustrate that NMDA current was strikingly increased in Mg^{2+}-free ACSF; the concentration of NMDA was reduced to 2 μmol/L to get a comparable response to that obtained in normal (1.2 mmol/L Mg^{2-}). Addition of M100907 failed to potentiate NMDA current. **(B)**: A plot illustrating M100907's effect on NMDA current is Mg^{2+}-dependent. The peak amplitude of NMDA currents (expressed as percentage of the maximal current I_0 obtained in Mg^{2+}-free ACSF) was plotted as a function of extracellular [Mg^{2+}] at the holding potential of −60 mV. In normal ACSF (1.2 mmol/L Mg^{2+}), M100907 markedly potentiates NMDA-induced inward current. In Mg^{2+}-free ACSF, the amplitude of NMDA-current was dramatically increased; addition of M100907 failed to further increase NMDA current; increasing extracellular Mg^{2+} concentration reinstated the Mg^{2+} block of NMDA receptor-ionophore complex and restored the facilitating action of M100907. Further increase of the extracellular concentration of Mg^{2+} depressed the ability of M100907 to enhance NMDA current.

The potentiating effect of M100907 was blocked by 40 μmol/L d-AP5 (n = 4) and also by 20 μmol/L CNQX (n = 5; Figure 5).

Effect of M100907 on PPF and EPSC Variance

An alternative method to determine possible presynaptic action of M100907 is to examine the effect of M100907 on PPF and EPSC variance. Of 19 pyramidal cells tested, the ratio of $EPSP_2/EPSP_1$ was 1.9 ± 0.04. The addition of 100 nmol/L of M100907 to the superfusing solution produced a significant increase of the amplitude of $EPSC_1$ by 138 ± 8%, which was clearly associated with a decrease of PPF to 1.28 ± 0.03 (71% of controls) and an increase of m_{CV} to 213 ± 12% of controls (n = 8; $p < .05$, paired t-tests for $EPSC_1$, PPF, and m_{CV}; Figure 6). Bath administration of d-AP5 (40 μmol/L) effectively prevented (n = 4) and reversed (n = 4) the effect produced by M100907 (Figure 6).

To ensure that alteration of m_{CV} is the result of actions of agents at presynaptic sites, we have performed the following experiments. Figure 6C shows that increasing the ratio of Ca^{2+}/Mg^{2+} in the ACSF, which is known to increase the release of neurotransmitters, produced a significant increase of EPSCs amplitude and an accompanying increase in m_{CV} (n = 4), reminiscent of the effect of M100907. In contrast, the non-NMDA receptor antagonist CNQX (2 μmol/L), which blocked non-NMDA receptors on somadendritic sites, decreased the amplitude of EPSCs to 36 ± 6% of controls without causing significant changes of m_{CV} (Figure 6C, average of 300 traces before and after CNQX from four cells). Moreover, in voltage-clamp mode, changing the holding potential (Vh) to a less negative level and to 0 mV, which produced a decrease of the amplitude of EPSCs and changed the EPSCs to the opposite direction, respectively, failed to alter m_{CV} significantly (Figure 6C, average of 120 traces from four cells). Figure 6D, where the relative change in average EPSC size is plotted against the relative change in m_{CV}, summarizes the results from all of the above manipulations. As shown, M100907, paired-pulse facilitation and increasing the Ca^{2+}/Mg^{2+} ratio in the ACSF all caused a parallel shift in the mean of the amplitude of EPSCs and m_{CV}. The result suggests that alteration of m_{CV} reflects changes of the amount of neurotransmitter released. A linear regression fit to these points (dotted line) yielded a coefficient of r^2 = 0.91 and a slope of 1.7 (95% confidence interval, $p < .01$), indicating that the alterations in m_{CV} were sufficient to account for the changes in EPSC amplitude.

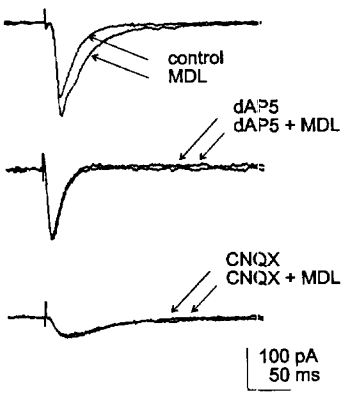

Figure 5. The effect of M100907 on the synaptic responses evoked by electrical stimulation of the forceps minor in pyramidal cells of the mPFC. In pyramidal cells, which were clamped at −60 mV, M100907 (100 nmol/L) potentiates the peak amplitude and duration of EPSCs evoked by electrical stimulation of the forceps minor. Superfusion of the NMDA-receptor antagonist d-AP5 (40 μmol/L) completely blocked the M100907-induced facilitation of evoked EPSCs (note that d-AP5 decreased the duration of EPSCs as compared with controls in the top trace). The non-NMDA-receptor antagonist CNQX (20 μmol/L) markedly decreased the amplitude and increased the duration of EPSCs; M100907 did not alter EPSCs in CNQX-containing solution, a finding that is consistent with the view that non-NMDA receptors are required for the expression of M100907's potentiating effect on NMDA receptor mediated neurotransmission.

DISCUSSION

Our results have shown that similar to the action of clozapine and haloperidol, M100907 dramatically increased NMDA-induced inward current by 3.5- to 5.5-fold. The ED_{50} value of M100907's effect is 14 nmol/L, which is comparable to that of clozapine (ED_{50} = 14 nmol/L) but is smaller than that of haloperidol (ED_{50} = 38 nmol/L; Arvanov et al. 1997a). The effect of M100907 on NMDA responses was not associated with the changes of membrane properties, e.g. resting membrane potential and input resistance. M100009, the much less active isomer of M100907, was inactive up to 100 nmol/L, but it did increase NMDA-induced inward current at concentration ⩾2 μmol/L. Based on our results, it appears that at clinically relevant concentrations, clozapine, M100907, and to a lesser extent haloperidol share the property of potentiating NMDA-induced responses, although at present we do not know whether these compounds share the same mechanisms of action to exert their potentiating effect. In contrast to the action of M100907, clozapine, and haloperidol, the antidepressant Prozac (fluoxetine, a selective 5-HT reuptake inhibitor, 100 nmol/L) did not affect NMDA-induced inward current (Arvanov et al. 1997a).

Although we do not know the mechanisms of action of M100907, our results obtained from several lines of experiments suggest the following scenario: M100907 probably facilitates NMDA-induced release of EAAs

from presynaptic terminals; the released EAAs then activate postsynaptic glutamate receptors, depolarize the membrane potential of pyramidal neurons, and remove the Mg^{2+}-block of the NMDA receptor-ionophore complex, thereby dramatically potentiating NMDA-induced inward current. The evidence that supports this view may be summarized as follows: (1) the potentiating effect of M100907 on NMDA-induced responses was markedly diminished or completely abolished when experiments were performed in Ca^{2+}-free ACSF or in ACSF containing either Cd^{2+}, BAPTA-AM, thapsigargin, riluzole, or CNQX; (2) the potentiating effect of M100907 was dependent upon extracellular concentration of Mg^{2+}; and (3) M100907 produced a decrease of PPF of the electrically evoked EPSCs, accompanied by an increase of m_{CV}. Interestingly, the potentiating effect of M100907 on NMDA-induced inward current required unobstructed non-NMDA receptors. This find-

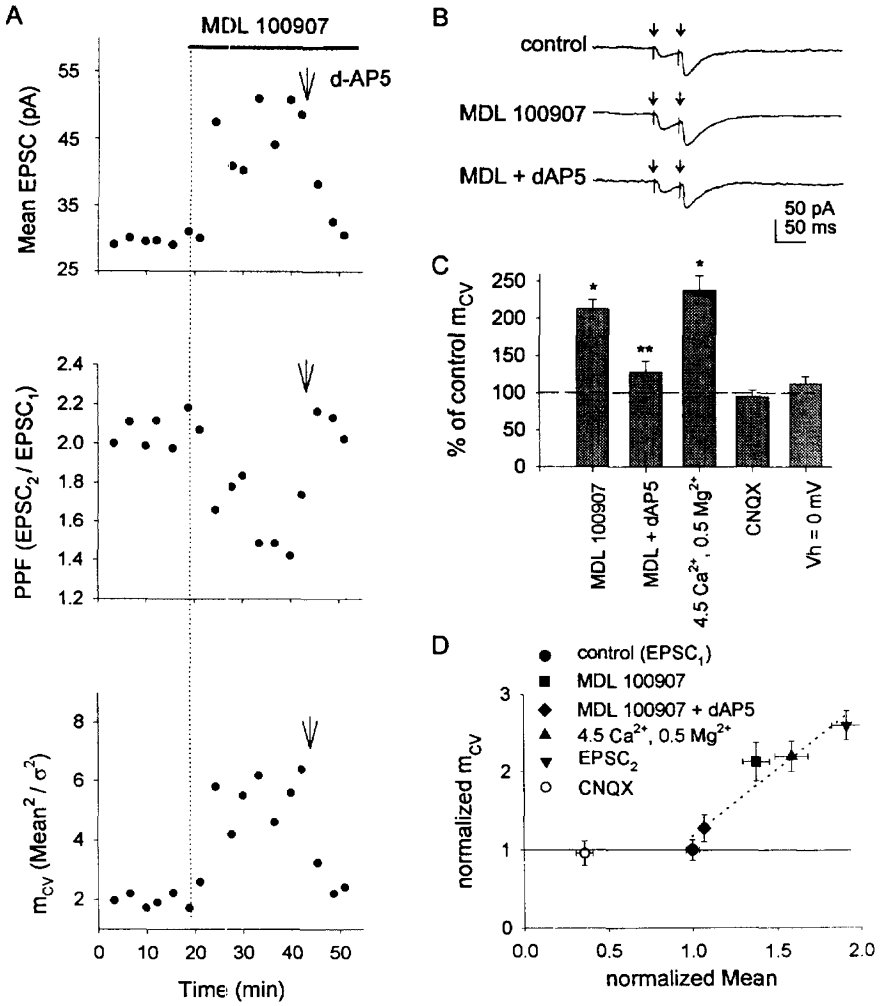

Figure 6. Comparison of the effects of M100907 and other treatments on unitary EPSCs, PPF, and m_{CV}. **(A)**: The time course of changes in $EPSC_1$, PPF, and m_{CV} ($Mean^2/\sigma^2$) induced by 100 nmol/L M100907. M100907 increased the amplitude of $EPSC_1$ and m_{CV} and decreased PPF; this effect was reversed by the selective NMDA receptor antagonist d-AP5 (40 μmol/L). Each point represents the average of 20 EPSCs evoked by paired-stimuli. **(B)**: Comparison of representative traces of averaged EPSCs evoked by 20 consecutive paired-stimuli in the control condition, in ACSF which contained 100 nmol/L M100907 (15 min after the start of M100907 perfusion) and in ACSF with further addition of 40 μmol/L d-AP5 (for 15 min), respectively. M100907 increased significantly the amplitude of $EPSC_1$ and this effect was blocked by d-AP5. **(C)**: A summary of the effects of various treatments on m_{CV}, which was the mean ± SEM of 60 responses obtained from four to eight cells. *Significantly greater than that of controls, $p < .05$; **significantly smaller than that of the M100907, $p < .05$. **(D)**: Comparison of the effects of all manipulations upon the relationship between the mean EPSC amplitude and the m_{CV}, normalized to the control $EPSC_1$. The broken line represents a regression fit. As can be seen from this figure, all of the manipulations affected mean EPSC amplitude and m_{CV} approximately equally except for CNQX, which significantly reduced mean EPSC amplitude without altering m_{CV}.

ing rules out the possibility that M100907 may interact directly with the Mg^{2+}-site of NMDA receptor-ionophore complex or metabotropic glutamate receptors to produce its potentiating effect. Rather, it suggests that activation of non-NMDA receptors is an indispensable link for the expression of M100907's potentiating action on NMDA responses.

M100907 up to 1 μmol/L did not alter AMPA-induced current. This is in marked contrast with the action of haloperidol, which inhibits AMPA responses in a concentration-dependent manner (IC_{50} = 37 nmol/L; Arvanov et al. 1997a). Clozapine, on the other hand, did not have any effect on AMPA responses until the concentration reached 100 nmol/L or higher. At these relatively high concentrations, clozapine effectively evoked EPSPs. It might be speculated that the clozapine-induced release of EAAs activated repeatedly the AMPA receptors and caused the receptor desensitization. At any rate, the effects produced by haloperidol and clozapine on AMPA receptors may account for the findings that haloperidol is ineffective in enhancing glutamatergic neurotransmission evoked by electrical stimulation of the forceps minor, whereas clozapine is slightly less effective than M100907 in potentiating glutamatergic neurotransmission.

It is unlikely that the facilitating effect of clozapine, haloperidol, and M100907 on NMDA-induced inward current results from a direct interaction with NMDA receptors because of the relatively low affinity of these drugs for the ^{3}H-MK-801 binding sites (Lidsky et al. 1993; Tarazi et al. 1996) and because of the blockade of the potentiating effect by CNQX and by compounds that diminish the release of neurotransmitters (see below). It might be speculated that the biochemical mechanisms behind clozapine and haloperidol-induced potentiation of NMDA response are secondary to their binding to other receptor or effector systems. For example, clozapine and haloperidol may block DA and/or 5-HT receptors and release the inhibitory effects of monoamines on glutamate release (Kornhuber and Kornhuber 1986; Maura et al. 1988a,b, 1989; Peris et al. 1988). However, the facilitating effect of haloperidol and clozapine on NMDA-induced response is not the result of their ability to block DA D_2 receptors because raclopride failed to potentiate NMDA responses (Arvanov et al. 1997a) and because clozapine has a lower affinity than haloperidol to D_2 receptors but is more potent than haloperidol in facilitating NMDA-induced responses. In addition, although M100907 possesses a negligible affinity to DA receptor subtypes (Kehne et al. 1996), it strikingly increased NMDA-induced responses and enhanced electrical stimulation-evoked EPSPs/EPSCs, reminiscent of the result produced by clozapine. Because haloperidol, clozapine, and M100907 possess a K_i value of 66, 22, and 0.7 nmol/L, respectively, to inhibit [^{3}H]ketanserin binding to 5-HT_{2A} receptors (Johnson et al. 1996; Kehne et al. 1996; Meltzer et al. 1989), it is possible that the potentiating effect of these compounds on NMDA-induced inward current might be the result of their interaction with 5-HT_{2A} receptors. Indeed, our preliminary results show that other 5-HT_2 receptor antagonists including ketanserin, ritanserin, metergoline, and LY 53857 all produced a marked potentiation of NMDA responses (Liang and Wang, unpublished observations). Further systematic examination and comparison of the actions of 5-HT_{2A} receptor agonists and antagonists on NMDA and AMPA receptor-mediated neurotransmission are needed to verify this view.

It has been demonstrated that M100907 may cause release of DA in the rat prefrontal cortex (Schmidt and Fadayel 1995). However, the potentiating effect of M100907 was not altered in rats pretreated with reserpine, indicating that M100907 probably did not produce its facilitating action by releasing monoamines. We have also demonstrated that alteration of the glycine concentration in the ACSF and bath application of either bicuculline or WAY 100635 (a selective 5-HT_{1A} receptor antagonist; not shown) did not affect the action of M100907.

Ample evidence indicates that NMDA receptor antagonists impair attention, learning and memory, and cognition (Malhotra et al. 1996; Witkin 1995). Accordingly, potentiating glutamatergic, particularly NMDA receptor-mediated, neurotransmission may account for, at least partly, the purported beneficial effect of atypical APDs in improving negative symptoms of schizophrenia including the executive function and cognition (haloperidol has a potent depressant action on AMPA response, which obscures haloperidol's action on NMDA responses; Arvanov et al. 1997a). To test this hypothesis, it will be necessary to examine and compare systematically actions of various typical and atypical APDs.

In summary, in the present study, we have demonstrated that M100907 may enhance NMDA receptor-mediated neurotransmission in pyramidal cells of the mPFC by facilitating NMDA-induced release of EAAs. If M100907 proves to be an effective APD, our results suggest that the modulatory action of M100907 and other putative APDs on glutamatergic neurotransmission may contribute to their antischizophrenic action, particularly their ability to alleviate some negative symptoms.

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