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Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex

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ABSTRACT

N-methyl-D-aspartic acid (NMDA) receptors are critical for both normal brain functions and the pathogenesis of schizophrenia. We investigated the functional changes of glutamatergic receptors in the pyramidal cells and fast-spiking (FS) interneurons in the adolescent rat prefrontal cortex in MK-801 model of schizophrenia. We found that although both pyramidal cells and FS interneurons were affected by in vivo subchronic blockade of NMDA receptors, MK-801 induced distinct changes in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors in the FS interneurons compared with pyramidal cells. Specifically, the amplitude, but not the frequency, of AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs) in FS interneurons was significantly decreased whereas both the frequency and amplitude in pyramidal neurons were increased. In addition, MK-801-induced new presynaptic NMDA receptors were detected in the glutamatergic terminals targeting pyramidal neurons but not FS interneurons. MK-801 also induced distinct alterations in FS interneurons but not in pyramidal neurons, including significantly decreased rectification index and increased calcium permeability. These data suggest a distinct cell-type specific and homeostatic synaptic scaling and redistribution of AMPA and NMDA receptors in response to the subchronic blockade of NMDA receptors and thus provide a direct mechanistic explanation for the NMDA hypofunction hypothesis that have long been proposed for the schizophrenia pathophysiology.

1. Introduction

Glutamatergic hypotheses of schizophrenia are based on the ability of *N*-methyl-D-aspartic acid (NMDA) receptor antagonists such as phencyclidine, ketamine, or MK-801 to induce psychotic symptoms closely resembling those of schizophrenia (Farber, 2003; Javitt, 2004; Javitt and Zukin, 1991; Jentsch and Roth, 1999; Krystal et al., 1994; Lahti et al., 1995; Marino and Conn, 2002; Moghaddam, 2003; Olney and Farber, 1995; Pinault, 2008). Although the exact mechanisms remain elusive, it is generally believed that NMDA antagonists reduce the stimulation of NMDA receptors in γ-aminobutyric acid-ergic (GABAergic) interneurons, leading to disinhibition of glutamatergic pyramidal neurons, which in turn induces hyper-excitability in pyramidal neurons and thus excessive glutamate release in the limbic systems (Benes, 2000; Jentsch and Roth, 1999; Lisman et al., 2008; Moghaddam et al., 1997; Olney et al., 1991). The critical players in this hypothesis are the GABAergic interneurons, which play an essential role in the regulation of activities of pyramidal cells in the cortical circuits (Lisman et al., 2008; Nakazawa et al., 2011). Indeed, clear deficits of GABAergic transmissions were reported in the brains of patients with schizophrenia (Lewis et al., 2005). Deficient GABA function is known to be closely associated abnormal oscillations and synchrony that are critical for the generation of cognitive deficits and other symptoms of the disorder (Uhlhaas and Singer, 2006, 2010). In animal models, repeated application of NMDA receptor antagonists evokes behavioral and neurochemical changes (Carpenter and Koenig, 2008; Gunduz-Bruce, 2009; Lindsley et al., 2006; Rujescu et al., 2006). For example, subchronic administration of NMDA receptor antagonists decreases the expression of parvalbumin (PV) in GABAergic interneurons (Abekawa et al., 2007; Braun et al., 2007; Cochran et al., 2003; Coleman et al., 2009), disrupts cortical inhibition (Grunze et al., 1996; Li et al., 2002; Zhang et al., 2008), induces working memory deficits (Coleman et al., 2009), attentional deficits and increased impulsivity (Amitai and Markou, 2009; Amitai et al., 2007).

Abbreviations: CI-AMPARs, calcium-impermeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; CP-AMPARs, calcium-permeable AMPA receptors; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EPSCs, excitatory postsynaptic currents; FS, fast-spiking; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartic acid; PFC, prefrontal cortex; RI, rectification index.

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Our recent studies showed that excitatory inputs onto immature fast-spiking (FS) interneurons have strong NMDA receptor-mediated currents that progressively weaken with age, becoming small or absent in adult FS interneurons in the rat prefrontal cortex (PFC) (Wang and Gao, 2009, 2010). These properties suggest that FS interneurons may be particularly vulnerable to environmental or drug stimulation (e.g., MK-801) during cortical development (Xi et al., 2009b). Indeed, in a strain of genetically modified mice in which the NMDAR subunit NR1 was conditionally knocked out in GABA neurons, including PV-positive cells, failed to produce significant effects when the knockout occurred after adolescence. However, when the deletion was conducted early in development, adult mice developed schizophrenia-like behavioral alterations (Belforte et al., 2010). Despite this large body of evidence, it remains unclear how NMDA receptor antagonist affects the synaptic functions of prefrontal neurons, particularly in FS interneurons. It is also unknown why GABAergic interneurons are more vulnerable to NMDA receptor antagonist among different cell-types (Wang et al., 2008a) and how the proposed hypofunction of glutamatergic systems occur in the pathogenesis of schizophrenia, particularly during the adolescent period. We therefore explored how sub-chronic treatment with MK-801 resulted in the disruption of glutamatergic transmission in adolescent rat prefrontal neurons by comparing the changes of NMDA and AMPA receptors in FS interneurons with those in pyramidal neurons. We found that MK-801 induced distinct cell-type specific alterations of glutamatergic receptors in the pyramidal neurons and FS interneurons.

2. Materials and methods

2.1. Animal treatment

We used 98 female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) at adolescent ages PD30 to PD39 (Spear, 2000; Tseng and O'Donnell, 2007; Wang and Gao, 2010). The rats were maintained on a 12-h light/dark cycle and were fed *ad libitum*. To be consistent, we continued to use female rats in which we have reported significant changes of NMDA receptors in the MK-801 model (Xi et al., 2009a, 2011, 2009b). In fact, although we did not observe a significant gender difference, some studies suggested that PCP-, MK-801-, and ketamine-induced effects are more reproducible in female animals (Dickerson and Sharp, 2006; Farber et al., 1995; Nakki et al., 1996). The animals were treated under National Institutes of Health animal use guidelines, and the experimental protocol was approved by the Institutional Animal Care and Use Committee at Drexel University College of Medicine. Because we could use only one rat each day for electrophysiological recording, the rats were treated with either MK-801 (0.1 mg/kg, intraperitoneally [i.p.], daily) or 0.9% saline as vehicle control for 5 consecutive days from PD30 to PD39. This dosage is based on our recent reports in which 0.1 mg/kg MK-801 were found to be effective in inducing changes of NMDA receptors in both transcription and protein levels (Xi et al., 2011, 2009b).

2.2. Cortical slice preparation

The detailed procedure is described in our previous publications (Gao et al., 2001; Wang and Gao, 2009, 2010). The rats (at ages of PD36-45) were deeply anesthetized with Euthasol (0.2 ml/kg, *i.p.*) 24 h after the last injection of MK-801 or saline. The rats were rapidly perfused with ice-cold (<4 °C) sucrose solution filled with 95% O_2 and 5% CO_2 and then were decapitated. The sucrose solution contained (in mM) NaCl 87; KCl 2.5; NaH_2PO_4 1.25; $NaHCO_3$ 25; $CaCl_2$ 0.5; $MgSO_4$ 7.0; sucrose 75; and glucose 25. The brains were quickly removed and immersed in ice-cold sucrose solution. The frontal cortex was cut into 300-μm slices with a Leica Vibratome (VT 1000S; Leica, Bannockburn, IL), and the brain slices were incubated in oxygenated sucrose solution at 35 °C for 1 h. The cortical slices were kept at room temperature until being transferred into a submerged chamber for recording. The recording chamber was perfused with Ringer's solution bubbled with 95% O_2 and 5% CO_2 at a perfusion rate of 2–3 ml/min. The Ringer's solution contained (in mM) NaCl 128; KCl 2.5; NaH_2PO_4 1.25; $CaCl_2$ 2; $MgSO_4$ 1.0; $NaHCO_3$ 26; and dextrose 10.

2.3. Electrophysiological recordings

Whole-cell patch-clamp recordings were conducted in the prelimbic region through an upright microscope (Olympus BX51WI, Olympus Optics, Japan) equipped with infrared-differential interference contrast optics. The recordings were conducted at ~35 °C, and the resistance of the recording pipette (1.2 mm borosilicate glass, Warner Instruments Inc., Hamden, CT) was 4.5–7 MΩ. As we recently reported (Wang and Gao, 2009, 2010), the pipette tips were first filled with a K^+-gluconate-based intracellular solution and then back-filled with Cs^+-containing solution. The K^+-gluconate solution contained (in mM) K^+-gluconate 120; KCl 6; ATP-Mg 4; Na_2GTP 0.3; EGTA 0.1; Hepes 10; and 0.3% biocytin (pH 7.3), whereas the Cs^+-solution contained (in mM) Cs-gluconate 120; lidocaine 5 (QX-314); $CsCl_2$ 6; ATP-Mg 1; Na_2GTP 0.2; Hepes 10; and 0.3% biocytin (pH 7.3, adjusted with CsOH). With this method, we were able to successfully record the action potentials (AP) immediately after forming a giga-seal. The membrane potentials were corrected for liquid junction potential (9.8 mV) of the K^+-gluconate solution.

To record the miniature excitatory postsynaptic currents (mEPSCs), we stabilized the neurons in regular Ringer's solution for 5 min to allow the Cs^+ diffusion into the neurons, and the spontaneous synaptic currents were continuously recorded for 5 min at a holding potential of −70 mV in the presence of picrotoxin (PTX; 50 μM, Sigma-Aldrich, St. Louis, MO) to block GABAergic transmissions. The mEPSCs were then recorded with the addition of tetrodotoxin (TTX, 1 μM, Ascent Scientific, Princeton, NJ) to block spontaneous action potential-induced synaptic currents. Twenty rats were used for this study. In one set of experiment ($n = 8$ rats), we recorded mEPSCs in pyramidal cells with 1 mM MK-801 loaded into recording pipette to block postsynaptic NMDA receptors; whereas in another group of rats ($n = 10$ rats), we examined mEPSC changes and spike properties following a single MK-801 (0.1 mg/kg, i.p.) treatment. In some neurons, selective NR2B antagonist ifenprodil (3 μM) were bath-applied to examine the effects on mEPSC amplitude and frequency, which would reflect the subunit components of presynaptic NMDA receptors.

The evoked EPSCs were recorded in layer II/III neurons of the PFC by stimulating layer II/III with a bipolar electrode placed ~300 μm away from the recorded cells. To examine presynaptic NMDA receptors, we first applied paired-pulse (0.1 ms, 10–100 μA, 0.1 Hz, inter-stimulus interval 50 ms) to evaluate paired-pulse ratio (PPR) changes before (pre-AP5) and after AP5 treatment ($n = 9$ rats). In addition, we also we induced EPSCs with minimal stimulation which produced about 50% failures in all stimulus trials to observe the changes of percent failures after AP5 treatment ($n = 6$ rats).

To examine the AMPA receptor subunit changes in MK-801 model, we calculated rectification index (RI) as recently reported (Wang and Gao, 2010). A single pulse (0.1 ms, 10–100 μA, 0.1 Hz) was applied for the extracellular stimulation. The current–voltage (I–V) curve of the AMPA receptor-mediated EPSCs was determined by recording at −60, 0, and +60 mV in Ringer's solution containing PTX and the NMDA receptor antagonist AP5 (50 μM, Tocris Bioscience, Ellisville, MO) ($n = 32$ rats).

In addition, in one set of experiment ($n = 6$ rats), the recorded neurons were clamped at −70 mV and bathed with PTX and AP5 to isolate AMPA receptor-mediated current. Then calcium-permeable AMPA receptor (CP-AMPAR) antagonist 1-naphthylacetyl spermine trihydrochloride (NASPM, 50 μM) was bath-applied to examine the changes in AMPA receptor-mediated currents in MK-801 model.

To investigate the Ca^{2+} permeability of AMPA receptor-mediated currents in MK-801 model, the normal Ringer's solution containing 2 mM Ca^{2+} was replaced with a solution containing 30 mM Ca^{2+}. The control (2 mM Ca^{2+}) and highly concentrated Ca^{2+} (30 mM) solutions were identical in composition except for the concentrations of $CaCl_2$ and NaCl, which (in mM) were 2 and 124 and 30 and 92.7, respectively. The evoked AMPA-EPSCs were first recorded in the control solution and then in the high-concentration Ca^{2+} solution for 10 min in the presence of PTX (50 μM) ($n = 8$ rats).

All electric signals were recorded with a MultiClamp 700B (Molecular Devices, Union City, CA) and acquired at sampling intervals of 20–50 μs through a DigiData 1322A and pCLAMP 9.2 software (Molecular Devices). The access resistances were constantly monitored through a pulse of negative 5 mV (200 ms duration) and were adjusted during recordings as needed.

2.4. Data analysis

The passive membrane properties in pyramidal cells and FS interneurons were measured as reported in our previous studies (Wang and Gao, 2009, 2010). These parameters included resting membrane potential, input resistance, membrane time constant, AP threshold, AP half-width, and afterhyperpolarization (AHP). The fast AHP and late AHP were analyzed on the basis of a previous report (Storm, 1987). The value of fast AHP was determined as the difference between the AP threshold and the lowest point of the first undershoot of the spike, whereas the late AHP was the second deep valley after the spike.

The mEPSCs recorded in the voltage-clamp mode were analyzed with Clampfit 9.2. A typical mEPSC was selected to create a sample template for event detection within a data period, and the AMPA mEPSCs were detected with a threshold set at three times the value of the root mean square of the baseline noise. The event numbers were sorted to make histogram at bin size of 2 pA and the data were fitted with Gaussian function in Clampfit 9.2. The rectification index (RI) of the evoked AMPA-EPSCs was calculated as the amplitude of AMPA-$EPSC_{+60\ mV}$/AMPA-$EPSC_{-60\ mV}$ in the extracellular stimulus recording.

2.5. Statistical analysis

For all experiments, treatment effects were statistically analyzed by one-way ANOVA, followed by the appropriate *post hoc* tests using the software SPSS 16.0.