Published in final edited form as:

Biol Psychiatry. 2007 October 1; 62(7): 739–746. doi:10.1016/j.biopsych.2006.12.003.

Positive Allosteric Modulation of Metabotropic Glutamate 5 (mGlu5) Receptors Reverses N-Methyl-D-Aspartate Antagonist-Induced Alteration of Neuronal Firing in Prefrontal Cortex

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Abstract

Background—Several lines of evidence suggest that N-methyl-D-aspartate (NMDA) receptor hypofunction may be associated with schizophrenia. Activation of metabotropic glutamate 5 (mGlu5) receptors enhances NMDA receptor mediated currents in vitro, implying that allosteric modulation of mGlu5 receptors may have therapeutic efficacy for schizophrenia. The aim of this study was to determine if positive allosteric modulators of mGlu5 receptors are effective in reversing two cellular effects of NMDA receptor antagonists that are relevant to schizophrenia: increases in corticolimbic dopamine neurotransmission and disruption of neuronal activity in the prefrontal cortex (PFC).

Methods—In freely moving rats, we measured the effects of the positive modulator of mGlu5 receptor 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) alone or in combination with the NMDA antagonist MK801 on 1) spontaneous firing and bursting of medial PFC (mPFC) neurons, and 2) dopamine release as measured by microdialysis in the mPFC and nucleus accumbens (NAc).

Results—The predominant effect of CDPPB on mPFC neurons was excitatory, leading to an overall excitatory population response. Pretreatment with CDPPB prevented MK801-induced excessive firing and reduced spontaneous bursting. In contrast, CDPPB had no significant effect on basal dopamine release as compared with control rats and did not alter MK801-induced activation of dopamine release in the mPFC and NAc.

Conclusions—These results show that positive modulation of mGlu5 receptors reverses the effects of noncompetitive NMDA antagonists on cortical neuronal firing without affecting dopamine neurotransmission. Thus, these compounds may be effective in ameliorating PFC mediated behavioral abnormalities that results from NMDA receptor hypofunction.

Keywords

Antipsychotic drugs; cognition; dopamine; ensemble recording; schizophrenia

Dopamine D2 receptor antagonists have been used effectively to treat psychosis in patients with schizophrenia (Davis *et al.* 1991; Delay and Deniker 1955; Miyamoto *et al.* 2005; Snyder *et al.* 1974). Chronic exposure to these drugs, however, is associated with profound side effects such as dysphoria, secondary negative symptoms (Carpenter *et al.* 1985), motor deficits (Llorca *et al.* 2002), weight gain (Schwartz *et al.* 2004), hyperprolactinemia

(Hummer and Huber 2004), and diabetes (Sathyaprakash and Henry 2004). Moreover, D2 antagonists are generally ineffective in treating negative symptoms and cognitive deficits associated with schizophrenia (Miyamoto *et al.* 2005). Hence, there is an acute need to develop alternative treatments for schizophrenia that have fewer side effects and are more effective in treating cognitive and negative symptoms of the disorder.

Design of novel therapeutic approaches for schizophrenia is contingent on a better understanding of the pathophysiology of the disease. Although the antipsychotic efficacy of D2 receptor antagonists supports the idea of a hyperactive dopamine system in schizophrenia (Carlsson 1978; Seeman 1987), limited efficacy of these drugs for treating cognitive deficits has prompted efforts to investigate the role of nondopaminergic systems in influencing these deficits. One example is the *N*-methyl-D-aspartate (NMDA) receptor channel (Coyle *et al.* 2002; Javitt 2002; Moghaddam 2003). *N*-methyl-D-aspartate receptors have been implicated in the etiology and pathophysiology of schizophrenia, primarily because NMDA receptor antagonists produce schizophrenia-like symptoms in healthy individuals (Honey *et al.* 2005; Javitt and Zukin 1991; Krystal *et al.* 1994; Luby *et al.* 1959; Malhotra *et al.* 1996; Newcomer *et al.* 1999; Olney and Farber 1995) and exacerbate preexisting symptoms in individuals with schizophrenia (Lahti *et al.* 1995; Malhotra *et al.* 1997). Postmortem and genetic linkage studies (Harrison and Weinberger 2005; Kristiansen *et al.* 2006; Moghaddam 2003), as well as a preliminary imaging study (Pilowsky *et al.* 2006), also support a role for NMDA receptor dysfunction in schizophrenia. *N*-methyl-D-aspartate receptor antagonists such as dizocilpine (MK801), phencyclidine (PCP), or low doses of ketamine are routinely used in preclinical studies to model schizophrenia. Systemic administration of these drugs to rodents and nonhuman primates produces behavioral deficits that parallel some positive and negative symptoms, as well as the cognitive deficits of schizophrenia (Bakshi and Geyer 1998; Hauber and Andersen 1993; Jentsch *et al.* 1997; Stefani and Moghaddam 2005; Verma and Moghaddam 1996). At a cellular level, NMDA antagonist administration increases dopamine release in the prefrontal cortex (PFC) of rodents (Verma and Moghaddam 1996) and humans (Aalto *et al.* 2005), suggesting that they work, in part, by disrupting dopamine neurotransmission.

Pharmacological approaches that may reduce the impact of NMDA receptor deficiency on behavior have primarily focused on stimulating the (co-agonist) glycine site on the NMDA receptors (Javitt 2002; Tsai *et al.* 1998). Another approach is to target various subtypes of metabotropic glutamate receptor to indirectly augment NMDA receptor function (Gasparini *et al.* 2002; Marino and Conn 2002; Moghaddam 2004). For example, in most of the cortical and subcortical areas of the central nervous system associated with schizophrenia pathology, NMDA receptors have synergistic relationship with the metabotropic glutamate 5 (mGlu5) receptors at the postsynaptic level (Alagarsamy *et al.* 2002). Agonists of mGlu5 receptor enhance NMDA receptor-mediated currents in hippocampal (Doherty *et al.* 1997) and subthalamic nucleus (Awad *et al.* 2000) slices in the rat. Conversely, the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) potentiates the effects of NMDA receptor antagonists on spontaneous burst and spike activity of cortical neurons (Homayoun and Moghaddam 2006). Behavioral studies also show that MPEP enhances the effects of NMDA antagonist blockade on prepulse inhibition, locomotion, working memory, and instrumental learning impairments (Campbell *et al.* 2004; Homayoun *et al.* 2004; Kinney *et al.* 2005a; Spooren *et al.* 2000). The worsening of the detrimental effects of NMDA receptor antagonists by mGlu5 receptor antagonist suggests that activation of mGlu5 receptors may represent a plausible approach for ameliorating symptoms of schizophrenia (Marino and Conn 2002; Moghaddam 2004). Recent behavioral studies using 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB), a positive allosteric modulator of the mGlu5 receptor (Kinney *et al.* 2005b; O'Brien *et al.* 2004), show that pretreatment with this compound reverses amphetamine-induced hyperlocomotion and prepulse inhibition deficit,

two tests which serve to model some aspects of schizophrenia in rodents (Kinney *et al.* 2005a).

This study sought to characterize the cellular interaction of the mGlu5 receptor positive modulator CDPPB with an NMDA antagonist in freely moving rats. Two measures that may subserve the disruptive behavioral effects of NMDA antagonists are increased dopamine release in corticolimbic regions (Verma and Moghaddam 1996) and increased random firing of prefrontal cortex (PFC) neurons (Homayoun *et al.* 2005; Homayoun and Moghaddam 2006; Jackson *et al.* 2004). Thus, we determined the effects of CDPPB administration alone or in combination with the NMDA antagonist MK801 on spontaneous firing and bursting of medial prefrontal cortex (mPFC) neurons and dopamine release in the mPFC and nucleus accumbens (NAc).

Methods and Materials

Animals

A total of 76 adult male Sprague-Dawley rats (Harlan, Somerville, New Jersey), weighing 250 to 420 g were used in this study. Animals were housed on a 12-hour light/12-hour dark cycle (lights on at 07:00 AM) for at least 1 week after their arrival in the animal facilities before surgery. Microdialysis and electrophysiological recordings were performed during the light phase on awake and freely moving animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (<http://grants.nih.gov/grants/olaw/olaw.htm>).

Drugs

All drugs and vehicles were administered intraperitoneally (IP). The compound CDPPB was synthesized in-house (Lindsley *et al.* 2004) and dissolved in a vehicle composed of 10% dimethyl sulfoxide (DMSO) + 90% ethylene glycol. The MK801 (Sigma-RBI, St. Louis, Missouri) was dissolved in saline (.9% sodium chloride [NaCl]). The route of administration (IP) and doses of CDPPB (3 and 10 mg/kg) were chosen according to Kinney *et al.* (2005a).

Electrophysiology Procedure

Extracellular activity of PFC single units was studied in awake unrestricted rats ($n = 9$). Microelectrode arrays (NB Labs, Denison, Texas) consisting of eight 50- μ m diameter Teflon-insulated, stainless steel wires were chronically implanted under halothane anesthesia in the mPFC (target coordinates in millimeters for the center of the array at anteroposterior (AP), +3.0 from bregma; mediolateral (ML), .7; and dorsoventral (DV), 3.5) (according to the atlas of Paxinos and Watson 1998). Arrays were arranged in a 2×4 pattern measuring approximately .25 $\times$.7 mm. Animals were allowed 5 to 7 days of recovery and 2 days of habituation to recording chamber before first recording session. Habituation also included sham injections. Each animal received two to four acute intraperitoneal injections based on a pseudo-random order with 7–10 washout period. Each recording session included 30 min of baseline activity and a minimum of 2 hours of postinjection recording. All recordings were performed in a standard transparent rodent housing cage with a modified open top. Animals were connected to a field-effect transistor headstage (NB Labs) using lightweight cabling that passed through a commutator and allowed the animal unrestricted movement during recording. Unit activity was recorded using multiple channel amplifiers with 500 $\times$ gain and 220 to 5900 Hz band pass filters (Plexon Inc., Dallas, Texas). The amplified signal from each electrode was digitized (30 kHz sampling rate) on computer hard disk for offline spike sorting. Spike sorting was performed with Off-Line Sorter software (Plexon Inc.) using a combination of automatic and manual sorting techniques previously

described (Homayoun and Moghaddam 2006). Only units with well-isolated waveform clusters that had stable waveforms during the length of the recording session were accepted as single unit. Data reported here are based on regular firing neurons with a baseline firing rate less than 10 Hz (putative pyramidal neurons). The paucity of recorded fast firing single units (>10 Hz, putative interneurons) did not allow analysis of their responses.

Microdialysis Procedure

Microdialysis probes were implanted under halothane anesthesia (Adams and Moghaddam 1998). Probes (outer diameter, 330 μ m; exposed tip, 3.0 mm for mPFC, 2.0 mm for NAc) were gently lowered to the mPFC (AP, +3–4 from bregma; ML, .8; DV, 5.8) and NAc (AP, +1.7 from bregma; ML, .8; DV, 8.5) and secured to the skull and skull screws using dental cement. Immediately after the surgery, rats were placed in a standard rodent housing cage (44 $\times$ 22 $\times$ 42 cm) with a modified open top and probes connected to a liquid swivel-balance arm assembly. Animals were allowed to recover for 24 hours prior to the start of experiment. Animals had ad libitum access to food and water during the recovery period but not during the experiment. Probes were perfused with Ringer's solution (in mmol/L: 145 NaCl, 2.7 potassium chloride [KCl], 1.0 magnesium chloride [MgCl₂], and 1.2 calcium chloride [CaCl₂]) at a flow rate of 1.4 μ L/min during the recovery period and 2.0 μ L/min during sample collection. Dialysis samples were collected every 20 min and injected immediately onto a high-performance liquid chromatography (HPLC) system with electrochemical detection for the analysis of dopamine as previously described (Adams and Moghaddam 1998).

Pharmacological Treatments

Electrophysiology Experiment—In the first experiment, the effects of CDPPB (3 and 10 mg/kg) were compared to vehicle. Animals received a single injection 30 min after the start of recording. In the second experiment, rats were treated with CDPPB (10 mg/kg) or vehicle, followed by MK801 (.1 mg/kg) 30 min later. Animals received four to six treatments in random order with a 7–10 day washout period.

Microdialysis Experiment—After 60 to 80 min of baseline collections, rats received a first injection of either CDPPB (10 mg/kg) or vehicle. Twenty minutes later, each rat received a second injection of either MK801 (.1 mg/kg) or saline.

Data Analysis

Electrophysiology Experiment—Electrophysiological data were analyzed using NeuroExplorer (Plexon Inc.) and Matlab (MathWorks, Natick, Massachusetts). Firing rate statistics were calculated using firing rate histograms with 5 min bins normalized to the mean baseline firing (30 min) of individual units. K-means cluster analysis (SPSS, Inc., Chicago, Illinois) was used to detect the predominant patterns of responses to treatment. Clustering used the normalized rate histogram bins of each unit as variables and was performed separately for neurons in single and double injection sessions. The number of clusters used for K-means analysis was initially set at 2 and was incremented to 10 at steps of 1. The clusters isolated at each step were visualized by plotting the average firing rates of all neurons allocated to each cluster and comparing their response patterns. Whenever neurons with similar response patterns had been clustered separately based on a small difference in response magnitude, the clusters were merged. The minimum number of clusters that could explain at least 95% of variability of response patterns was determined as the number of main clusters of responses. The final grouping was verified by obtaining a significant analysis of variance (ANOVA) with time as repeated measure and cluster membership as factor. The distribution of different types of responses between groups was

compared using the Pearson chi-square (χ^2) test. The temporal profile of normalized firing rates of all neurons in each group was plotted against time and was compared between groups using two-way ANOVA with time as repeated measure. The spontaneous bursting activity, defined as periods of non-Poisson high-frequency firing, was detected in each spike train using Poisson surprise method of Legendy and Salzman (1985) as implemented in NeuroExplorer. For each neuron, the relative change in the percentage of spikes in bursts during the postdrug period (minutes 30 to 150 in single injection groups and minutes 60 to 150 in double injection groups) versus baseline was calculated. One-way ANOVA with Bonferroni post hoc test was used to compare average bursting between groups ($p < .05$ as the significance criteria). A total of nine rats was used for the study. The number of recorded neurons in each treatment group was as follows: vehicle, $n = 123$; CDPPB (3 mg/kg), $n = 109$; CDPPB (10 mg/kg), $n = 85$; vehicle + MK801, $n = 123$; CDPPB (10 mg/kg) + MK801, $n = 160$. The number of rats in each treatment group was as follows: vehicle, $n = 4$; CDPPB (3 mg/kg), $n = 4$; CDPPB (10 mg/kg), $n = 5$; vehicle + MK801, $n = 4$; CDPPB (10 mg/kg) + MK801, $n = 6$.

Microdialysis Experiment—Statistical difference between groups was analyzed by two-way analysis of variance. When there was a significant group effect, post hoc pairwise comparison with the Bonferroni test was performed.

Histology

After the termination of the electrophysiology and microdialysis experiments, animals were anesthetized with chloral hydrate (Sigma-RBI) and intracardially perfused with 9% saline followed by 10% buffered formalin. Brains were removed and stored in 10% buffered formalin. The 250- μ m serial sections of the fixed brains were stained with cresyl violet acetate (Sigma-RBI) and electrode and microdialysis probe placements were verified under a microscope. Only rats showing correct placements were included in the statistical analysis (Figure 1).

Results

Electrophysiology Experiments

Effects of CDPPB Treatment Alone—Single neurons showed different firing rate responses to CDPPB treatment (Figure 2A). K-means clustering identified five patterns of firing rate responses in single injection groups (CDPPB or vehicle) that included: 1) an early increase, 2) a sustained increase, 3) an early decrease, 4) a sustained decrease, and 5) no change in firing rate (Figure 2A, black lines). The distribution of these response types was significantly different between vehicle and CDPPB groups (Figure 2B, $\chi^2 = 104.55$, $p < .001$). The effect of CDPPB on distribution of response types was dose-dependent, with the higher dose causing more sustained responses (both excitatory and inhibitory) than the lower dose (between two doses, $\chi^2 = 24.35$, $p < .001$). The predominant effect of CDPPB at both doses was to increase excitatory responses in PFC neurons. The CDPPB also dose-dependently increased the average firing rate of PFC neurons (Figure 2C, average normalized rate of all units). Two-way ANOVA revealed significant effects for treatment [$F(2,314) = 14.45$, $p < .000$], time [$F(29,9106) = 11.57$, $p < .000$], and treatment $\times$ time interaction [$F(58,9106) = 8.14$, $p < .000$]. In addition, CDPPB changed the pattern of firing of PFC units (Figure 2D) by significantly increasing the percentage of spikes in bursts compared with vehicle [one-way ANOVA, $F(2,314) = 3.22$, $p < .05$, post hoc test, $p < .05$].

Effects of the Combined Administration of CDPPB and MK801—K-means clustering identified four patterns of firing rate responses in double injection groups that included: 1) an early increase, 2) a sustained increase, 3) a sustained decrease, and 4) no

change in firing rate (Figure 3A). The majority of neurons in the vehicle pretreatment group responded to MK801 with a sustained increase in firing rate (Figure 3B). In contrast, CDPPB pretreatment decreased the proportion of this response type and instead increased the proportion of neurons with either early transient increase or sustained decrease responses ($\chi^2 = 121.85, p < .001$). As a result, the robust excitatory effect of MK801 on the average firing activity of PFC neurons was blocked by CDPPB pretreatment (Figure 3C). Two-way ANOVA showed significant effects for both treatment [$F(1,281) = 74.66, p < .000$] and time [$F(29,8149) = 55.03, p < .000$], as well as for treatment $\times$ time interaction [$F(29,8149) = 86.41, p < .000$]. In vehicle pretreated group, MK801 decreased the percentage of spikes in bursts in the majority of neurons (compared with single vehicle group, Figure 3D). This effect was blocked by CDPPB pretreatment [one-way ANOVA including the single vehicle group, $F(2,403) = 4.48, p < .05$, post hoc test, $p < .05$, t test between double injection groups, $p < .05$].

Microdialysis Experiments

Effects of the Administration of CDPPB Alone—In the mPFC, a small increase in dopamine release was observed after injection of CDPPB ($n = 8$) and the corresponding vehicle ($n = 7$). The two-way ANOVA with group as factor and sample as repeated measure showed a significant effect of sample [$F(12,156) = 12.85, p < .0001$]. There was no between-group differences in the amplitude of the response, as shown by a lack of effect of group [$F(1,13) = 1.38, p > .05$] and of sample $\times$ group interaction [$F(12,156) = .63, p > .5$] (Figure 4A).

Similar to the results in the mPFC, injection of CDPPB ($n = 10$) or vehicle ($n = 8$) produced a small increase in dopamine release in the NAc. Two-way ANOVA with group as factor and sample as repeated measure indicated an effect of sample [$F(12,192) = 4.74, p < .0001$], no significant effect of group [$F(1,16) = .006, p > .05$], and no significant sample $\times$ group interaction [$F(12,192) = .30, p > .05$] (Figure 4B).

Effects of the Combined Administration of CDPPB and MK801—In the mPFC, injection of MK801 produced the expected large and prolonged increase in dopamine release (vehicle + MK801, $n = 6$). Comparison of vehicle + saline, vehicle + MK801, and CDPPB + MK801 groups, by means of a two-way ANOVA with sample as repeated measure, showed a significant effect of group [$F(2,18) = 3.77, p < .05$] compared with vehicle + saline group. Pretreatment with CDPPB (CDPPB + MK801, $n = 8$) produced a similar magnitude of increase on dopamine release. Comparison between both vehicle + MK801 and CDPPB + MK801 groups with a two-way ANOVA with group as factor and sample as repeated measure showed no difference [no effect of group: $F(1,12) = .63, p > .05$; a significant effect of sample: $F(12,144) = 21.83, p < .001$; no significant sample $\times$ group interaction: $F(12,144) = .43, p > .05$], demonstrating that CDPPB lacks in reversing MK801 effects on dopamine release (Figure 4A).

Similar results were obtained in the NAc in that vehicle + MK801 ($n = 8$) and CDPPB + MK801 ($n = 12$) produced similar increases in dopamine release that were significantly larger than the increase observed after vehicle + saline. Comparison of vehicle + saline, vehicle + MK801, and CDPPB + MK801 groups, by means of a two-way ANOVA with sample as repeated measure, showed a significant effect of group [$F(2,25) = 3.86, p < .05$], a significant effect of sample [$F(12,300) = 16.04, p < .0001$], and a significant sample $\times$ group interaction [$F(24,300) = 2.92, p < .0001$] (Figure 4B).

Discussion

Positive allosteric modulation of mGlu5 receptors by CDPPB produced a predominately excitatory influence on population response and burst activity of mPFC neurons in awake rats. In contrast, CDPPB inhibited the profound excitatory effects of the NMDA antagonist MK801 on spontaneous activity of mPFC neurons. The CDPPB also normalized MK801-induced disruptions in burst activity. These effects of CDPPB were not related to changes in dopamine neurotransmission, as CDPPB alone did not change dopamine release in the mPFC or NAc as compared with control rats and did not modify the magnitude of MK801-induced dopamine release in either region.

The excitatory effects of CDPPB, when given alone, are consistent with previous findings demonstrating that the mGlu5 antagonist MPEP decreases firing rate and number of bursts in the mPFC of awake rats (Homayoun and Moghaddam 2006) and inhibits bicuculine-induced persistent spontaneous bursting in rat hippocampal slices (Stoop *et al.* 2003). These effects of CDPPB may be direct, as the activation of mGlu5 receptors produces excitatory postsynaptic potentials (EPSP) in vitro (Fitzjohn *et al.* 1999; Sharifullina *et al.* 2004; Wisniewski and Car 2002), or indirect through increasing the efficacy of endogenous glutamate neurotransmission at NMDA receptors. For example, positive allosteric modulation of mGlu5 receptors has been shown to enhance phosphorylation of the NR1 subunit of the NMDA receptors and the expression of signaling molecules such as cyclic adenosine monophosphate (cAMP) response element-binding (CREB) and extracellular signal-regulated kinase (ERK), all of which are critical for glutamate-mediated signal transduction mechanisms (Liu *et al.* 2006).

The significant effect of CDPPB was long lasting in that the firing rate remained elevated above baseline for over an hour after injection, suggesting that this drug does not lead to rapid desensitization of mGlu5 receptors. This is interesting given that agonist stimulation of mGlu1/5 receptors leads to rapid desensitization (Contractor *et al.* 1998; Gereau and Heinemann 1998). Another reason for the sustained effects of CDPPB may be that functional mGlu5 receptors have been localized on the nuclear membrane of cortical and midbrain neurons, where their activation increases intranuclear calcium release (Jong *et al.* 2005; O'Malley *et al.* 2003). Such an increase may stimulate signaling pathways and favor changes in gene expression, leading to sustained firing rate activation.

Pretreatment with CDPPB blocked the MK801-induced disruption of spontaneous firing and bursting activity. Several lines of evidence suggest a synergistic interaction between mGlu5 and NMDA receptors, whereby stimulation of one receptor may increase ligand affinity for, or signal transduction mechanism mediated by, the other receptor (Marino and Conn 2006). Systemic administration of NMDA antagonists is thought to initiate a disinhibition process by inhibiting NMDA-mediated firing rate of gamma-aminobutyric acid (GABA) interneurons, resulting in increased firing rate of pyramidal neurons and glutamate release in the PFC (Jackson *et al.* 2004; Moghaddam *et al.* 1997; Olney 1990). The origin of glutamatergic projections on GABA neurons that are disinhibited by NMDA antagonist is not clear but may involve afferents from the hippocampus (Greene 2001). Pretreatment with CDPPB may reduce the extent of this disinhibition by "recruiting" more NMDA receptors that are activated by endogenous glutamate. This mechanism is consistent with our previous findings showing that pretreatment with the mGlu5 receptor antagonist MPEP exaggerates the effects of MK801 on firing rate of mPFC neurons (Homayoun and Moghaddam 2006). One caveat with the present finding is that MK801 (and psychotomimetic NMDA antagonists such as ketamine or phencyclidine) are noncompetitive use-dependent antagonists; therefore, increased activity of NMDA receptors before administration of this class of antagonists should potentiate their effect. Thus, pretreatment with mGlu5 receptors

positive modulators, which are thought to increase the efficacy of endogenous glutamate neurotransmission at NMDA receptors (Gasparini *et al.* 2002; Liu *et al.* 2006; Marino and Conn 2002; Moghaddam 2004), would be expected to exacerbate the effects of MK801. This was not the case with our findings, suggesting that other mechanisms such as mGlu5 receptor-mediated activation of independent pathways that balance or counteract the effects of MK801 on cortical firing may play a role in systemic effects of CDPPB.

Antagonists of NMDA receptor such as ketamine or MK801 produce a behavioral syndrome in humans and laboratory animals that resemble some aspects of schizophrenia (Coyle 1996; Javitt and Zukin 1991; Krystal *et al.* 2003; Moghaddam 2003; Olney and Farber 1995). In particular, these compounds disrupt PFC-dependent cognitive functions, such as working memory and set shifting, in a manner similar to schizophrenia (Adler *et al.* 1999; Aultman and Moghaddam 2001; Krystal *et al.* 2000; Stefani *et al.* 2003). Our finding that CDPPB reduces the disruptive effects of MK801 on PFC neurons suggests that this compound may ameliorate some of the adverse cognitive effects of NMDA receptor hypofunction (Darrah *et al.*, unpublished data, 2007).

Systemic administration of CDPPB did not alter baseline and MK801-induced increases in dopamine release in either mPFC or NAc. Given that the mGlu5 receptor antagonist MPEP enhances dopamine release in mPFC (Homayoun *et al.* 2004), we had expected that administration of the positive modulator of mGlu5 receptor would decrease dopamine release in this region. One concern with the microdialysis study is that the vehicle used to dissolve CDPPB, a noxious mixture of DMSO and ethylene glycol, also increased the release of dopamine in mPFC and NAc. This finding is consistent with the literature showing that mild stressors increase dopamine neurotransmission in both structures (Abercrombie *et al.* 1989; Thierry *et al.* 1976). Therefore, the stress-induced increases in dopamine release may have masked a small decrease in dopamine release by CDPPB. Nonetheless, the lack of a significant difference in dopamine release between CDPPB-injected and vehicle-injected animals suggests that reversal of MK801-induced alterations on spontaneous firing and bursting of mPFC neurons occurs independently of dopamine-mediated mechanisms. This finding also suggests that CDPPB may be devoid of effects on mood and motivation that usually confounds the use of therapeutic ligands that affect dopamine neurotransmission.

Conclusions

Positive allosteric modulators of mGlu5 receptors have promising therapeutic potential for cognitive disorders including schizophrenia (Gasparini *et al.* 2002; Moghaddam 2003; Marino and Conn 2006). The present study revealed that systemic treatment with CDPPB, a positive allosteric modulator of mGlu5 receptor, increased spontaneous firing and burst activity of PFC neurons in awake rats, suggesting that this class of compounds influences both the rate and pattern of firing of cortical neurons. More importantly, pretreatment with CDPPB inhibited the increased random firing and reduced spontaneous bursting that results from NMDA receptor hypofunction. The CDPPB did not have a significant effect on dopamine neurotransmissions, suggesting that a clinical application of this compound may not be associated with motor side effects, dysphoria, or abuse liability. Although further characterization is necessary to predict the clinical usefulness of this class of compounds, the present results indicate that allosteric modulation of mGlu5 receptors has beneficial physiological effects on the function of PFC neurons in awake animals.

Acknowledgments

This work was supported by the National Institute of Mental Health and Pittsburgh Life Sciences Greenhouse.

We thank Alicia Defrancesco for technical assistance.

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Figure 1.

Placements of electrode arrays and microdialysis probes. Placement of electrode arrays are depicted by black circles [mPFC: (A) 2.7 mm from bregma; (B) 3.2 mm from bregma]. Location of the active surface of microdialysis probes is depicted by straight lines [(C) NAc, 1.7 mm from bregma; (D) mPFC, 3.2 mm from bregma]. (Plates are modified from Paxinos and Watson 1998). mPFC, medial prefrontal cortex; NAc, nucleus accumbens.

Figure 2.

Effect of CDPPB on spontaneous firing rate of PFC single units. **(A)** Different types of PFC single unit responses to CDPPB. Neuronal responses to CDPPB (3 or 10 mg/kg) and vehicle were classified as one of five patterns using K-means clustering (see Methods and Materials). Each panel shows the normalized firing rate histogram (bin = 5 min) of a representative single unit treated with 10 mg/kg of CDPPB. Each superimposed black line shows the average firing rate of the cluster of neurons with the corresponding type of response. Arrow indicates time of injection. **(B)** Distribution of neuronal response types. The proportion of neurons (% of total) that exhibited any of the response types in each treatment group is compared. Most of the neurons did not change their firing in response to vehicle. However, CDPPB predominantly increased the number of neurons with an excitatory response. **(C)** Temporal profile of average population activity of PFC neurons. Average ($\pm$ SEM) normalized firing rates of all neurons in each treatment group is plotted against time using 5-min bins; 1.0 indicates no change from baseline activity. **(D)** Effect of CDPPB on spontaneous bursting of PFC single units. The average change in percentage of spikes in bursts during postinjection period relative to baseline is compared. Both doses of CDPPB enhanced the bursting activity; 1.0 indicates no change from baseline. * $p < .05$ compared with vehicle group. CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; PFC, prefrontal cortex.

Figure 3.

Blockade of MK801-induced effects on PFC firing rate by CDPPB. **(A)** Different types of PFC single unit responses to CDPPB (10 mg/kg, or vehicle) plus MK801 (.1 mg/kg) treatment. Neuronal responses were classified as one of four patterns using K-means clustering. Each panel shows a single neuron treated with CDPPB + MK801. Each superimposed color line shows the average firing rate of the cluster of neurons with the corresponding type of response. Arrows indicate times of two injections. **(B)** Distribution of neuronal response types. Most of the neurons displayed a sustained increase response to MK801 in vehicle pretreated group. The CDPPB pretreatment blocked this effect and instead enhanced the proportion of neurons with either an early transient increase or a sustained decrease. **(C)** Temporal profile of average population activity of PFC neurons. Average ($\pm$ SEM) normalized firing rates of all neurons in each treatment group is plotted against time using 5-min bins; 1.0 indicates no change from baseline activity. **(D)** Blockade of MK801-induced effect on spontaneous bursting by CDPPB. The average change in percentage of spikes in bursts during post-MK801 period relative to baseline is compared. The single vehicle group (from Figure 2, dashed bar) has been shown for comparison; 1.0 indicates no change from baseline. * $p < .05$ compared with single vehicle group. # $p < .05$ compared with vehicle + MK801 group. MK801, dizocilpine; PFC, prefrontal cortex; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide.

Figure 4.

Effects of the administration of CDPPB, alone or in combination with MK801, on dopamine release in mPFC (A) and NAc (B). Results (dopamine release, mean $\pm$ SEM) are shown as percentage of baseline value. Each sample represents a 20-minute period. The first injection was performed after sample 3 was collected and the second after sample 4 was collected. * p < .05 Veh-MK801 versus Veh-Sal and # p < .05 CDPPB-MK801 versus Veh-Sal (Bonferroni post hoc test). CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; MK801, dizocilpine; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; Veh, vehicle; Sal, saline.