ABSTRACT

An increasing body of evidence supports the hypothesis that diminished function of N-methyl-D-aspartate (NMDA) receptors and the associated increase in glutamate release and overstimulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors are critical elements of the pathophysiology of schizophrenia. Here, we describe a halogenated derivative of the aromatic amino acid L-phenylalanine that (1) activates NMDA receptors, (2) depresses presynaptic glutamate release, and (3) blocks AMPA/kainate receptors. The experiments were conducted in rat cerebrocortical cultured neurons by using the patch-clamp technique. 3,5-Dibromo-L-phenylalanine (3,5-DBr-L-Phe) augmented NMDA miniature excitatory postsynaptic currents (mEPSCs) and activated the steady-state current, effects that were eliminated by NMDA receptor antagonists DL-2-amino-5-phosphonopentanoic acid and MK-801 (dizocilpine maleate; 5H-dibenzo[a,d]cyclohepten-5,10-imine). 3,5-DBr-L-Phe was a partial agonist at the glutamate-binding site of NMDA receptors with an EC50 of 331.6 ± 78.6 μM and with an efficacy of 30.5 ± 4.7% compared with NMDA. 3,5-DBr-L-Phe depressed both amplitude and frequency of AMPA/kainate mEPSCs. The IC50 of 3,5-DBr-L-Phe to inhibit AMPA/kainate mEPSC frequency was 29.4 ± 4.3 μM. 3,5-DBr-L-Phe significantly decreased paired pulse depression of AMPA/kainate EPSCs and attenuated current activated by AMPA with higher efficacy at lower concentration of AMPA. 3,5-DBr-L-Phe neither affected GABA miniature inhibitory postsynaptic currents nor elicited action potentials. By enhancing NMDA receptor function, reducing glutamate release and blocking AMPA/kainate receptors 3,5-DBr-L-Phe represents a new type of polyvalent modulator of glutamatergic synaptic transmission with potential therapeutic applications.

In vitro, in vivo, and clinical evidence indicates that disturbed glutamatergic synaptic transmission may be a critical element in the pathophysiology of several brain disorders, including schizophrenia (Konradia and Heckersa, 2003; Moghaddam and Jackson, 2003). Abnormal glutamatergic neurotransmission has been reported in animal models of schizophrenia as well as in human post-mortem and glutamate receptor antagonist studies (Hirsch and Weinberger, 2003; Konradia and Heckersa, 2003; Moghaddam and Jackson, 2003). Noncompetitive antagonists of NMDA receptors (MK-801 and phencyclidine) increase the locomotor activity of animals and induce stereotyped behavior characteristic of human schizophrenia (Lipska and Weinberger, 2000). Likewise, NMDA receptor antagonists produce in otherwise healthy humans positive and negative symptoms of schizophrenia and exacerbate those in schizophrenic patients (Krystal et al., 1994; Jentsch and Roth, 1999). In addition to decreased NMDA receptor function, changes in glutamatergic transmission characteristic of schizophrenia include increased glutamate release and overstimulation of non-NMDA (AMPA/kainate) receptors (Moghaddam et al., 1997). Consistent with this hypothesis are findings showing an increase in the number of NMDA receptor subunits NR1 in cortical regions and a decrease in the expression of AMPA/kainate receptors in the hippocampus of schizophrenics (Meador-Woodruff and Healy, 2000). The observed decrease in AMPA/kainate receptors may be a down-regulation

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; MK-801, dizocilpine maleate; 5H-dibenzo[a,d]cyclohepten-5,10-imine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; 3,5-DBr-L-Phe, 3,5-dibromo-L-phenylalanine; EPSC, excitatory postsynaptic current; TTX, tetrodotoxin; mEPSC, miniature excitatory postsynaptic current; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline; mIPSC, miniature inhibitory postsynaptic current; NMDAR, N-methyl-D-aspartate receptor; AP-5, α-2-amino-5-phosphonopentanoic acid; LY 341495, (2S)-2-amino-2-[(1S,2S)-2-carboxycyclopent-1-yl]-3-(xanth-9-yl)propanoic acid.

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Cultures were maintained in an atmosphere of 5% CO$_2$, 95% air.

3,5-Dibromo-L-phenylalanine (3,5-DBr-L-Phe) acts as a partial agonist of NMDA receptors, inhibiting the glycine-binding site of NMDA receptors, 2) competing for the glutamate-binding site of AMPA/kainate receptors, and 3) attenuating glutamate release. The relative contribution of each individual mechanism to the antiglutamatergic effect of L-Phe can be modified by creating halogenated derivatives of the molecule (Kagiyama et al., 2004). Antagonists of AMPA/kainate receptors and agonists for group II metabotropic glutamate receptors reverse the psychotropic effects of phenycyclidine and inhibit conditioned avoidance responses and amphetamine-induced hyperactivity (Moghaddam and Adams, 1998; Lorrain et al., 2003; Takahata and Moghaddam, 2003). Despite the availability of many types of antipsychotic drugs the search for new efficacious agents without unwanted side effects remains a high priority in the treatment of schizophrenia (Hyman and Fenton, 2003; Moghaddam, 2003).

Here, we describe a compound that simultaneously produces all three desirable actions on glutamatergic synaptic transmission. During investigation of the cellular mechanisms of brain dysfunction seen in phenylketonuria, we found that L-Phe selectively depresses glutamatergic synaptic transmission by a combination of pre- and postsynaptic actions (Glushakov et al., 2002, 2003). Specifically, L-Phe depresses glutamatergic synaptic transmission by 1) competing for the glycine-binding site of NMDA receptors, 2) competing for the glutamate-binding site of AMPA/kainate receptors, and 3) attenuating glutamate release. The relative contribution of each individual mechanism to the antigu-}

Materials and Methods

All experiments were approved by the University of Florida Animal Care and Use Committee.

Neuronal Cultures. Cerebral cortices were dissected from newborn rats and treated with 0.25% trypsin to dissociate the cells. Dissociated cells were resuspended in Dulbecco’s modified Eagle’s medium containing 10% plasma-derived horse serum and were plated in poly-1-lysine-coated, 35-mm plastic tissue culture dishes medium containing 10% plasma-derived horse serum and were plated in poly-1-lysine-coated, 35-mm plastic tissue culture dishes.

Electrophysiological Recordings. Voltage- and current-clamp recordings of membrane ionic currents and potentials were conducted by using Axopatch 200B and Axoclamp 1B amplifiers (Axon Instruments Inc., Union City, CA). The perforated nystatin- and gramicidin-based patch-clamp recording techniques were used in some cases to reduce nonspecific rundown of intracellular processes. Neurons were used for electrophysiological recordings between 12 and 27 days in vitro. If a neuron showed either a marked change in holding current or a noticeable alteration in amplitude or shape of capacitance transients during the experiment, the data from that neuron were discarded. Patch microelec
trodes were pulled from 1.5-mm borosilicate glass tubing using a two-stage vertical pipette puller (Narishige, East Meadow, NY). When filled with recording solution, patch microelectrodes had a resistance of 3 to 5 MΩ. For rapid application of agonist-containing solutions to neurons, the SF-77B system (Warner Instrument, Hamden, CT) was used. Otherwise, a slower exchange of extracellular solution was done using a customized system. The slow onset of activation of 3–5-DBr-L-Phe-induced current seen in some figures is related to slow exchange of extracellular solution and not to a gradual onset of 3,5-DBr-L-Phe-induced activation of NMDA receptors.

The miniature EPSCs were recorded in TTX-containing (0.3–1 μM) extracellular solution at $V_h = -60$ mV. In NMDA receptor-mediated mEPSC recordings, Mg$^{2+}$ was omitted from the extracellular solution. To isolate the NMDA component of glutamate receptor-mediated currents, the non-NMDA (AMPA/kainate) receptor antagonist NBQX (10–20 μM) was added to extracellular solutions. Conversely, to isolate the non-NMDA receptor-mediated currents, the experiments were performed in the presence of NMDA receptor channel blocker MK-801 (5–10 μM) or in the presence of the NMDA receptor antagonist AP-5 (20 μM). Strychnine (1 μM) and picrotoxin (100 μM) were added to the extracellular solution to block glycine and GABA receptors, respectively. The basic extracellular solution contained 140 mM NaCl, 4 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES, and 11 mM glucose. The pH of the extracellular solution was adjusted to 7.4 using NaOH. The main solution for filling the patch electrodes contained 135 mM Cs gluronate, 5 mM NaCl, 10 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 11 mM EGTA, 10 mM HEPES, 2 mM Na$_2$ATP, and 0.2 mM Na$_2$GTP. The pH of the intracellular solution was adjusted to 7.4 using CsOH. To record GABA receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs), 100 μM picrotoxin in the extracellular solution and 135 mM Cs gluronate in the intrapipette solution were replaced with 5 μM NBQX and 135 mM KCl, respectively. Various concentrations of 3,5-DBr-L-Phe, NMDA, AMPA, and glycine were added to the extracellular solution according to the protocols described. 3,5-DBr-L-Phe was obtained from Showa Denko K.K. (Kawasaki, Japan). All other compounds were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris Cookson Inc. (Ellisville, MO).

The digitized data were analyzed off-line using the Mini-Analysis Program (Synaptosoft, Leonia, NJ) or pCLAMP9 (Axon Instruments Inc.). Miniature EPSCs were identified and confirmed by analyzing the rise time, decay time, and waveform of each individual spontaneous event.

General Data Analysis. Values are reported as mean ± S.E.M. Before parametric testing, the assumption of normality was validated using the Kolmogorov-Smirnov test with Lilliefors’ correction (SPSS version 10; SPSS Inc., Chicago, IL). Multiple comparisons among groups were analyzed using analysis of variance (two- or one-way repeated measures with two- or one-way replication where appropriate) followed by Student-Newman-Keuls testing. Single comparisons were analyzed using a two-tailed Student’s t test. A $P < 0.05$ was considered significant.

Results

Effect of 3,5-DBr-L-Phe on NMDA Receptor Function. The NMDA receptor-mediated mEPSCs in rat cultured neurons were recorded in Mg$^{2+}$-free extracellular solution in the presence of AMPA/kainate, glycine, and GABA receptor blockers and were analyzed as fluctuating background inward current (noise). 3,5-DBr-L-Phe, added to the extracellular solution, increased this current in a concentration-dependent manner (Fig. 1A). The augmenting effect of 3,5-DBr-L-Phe was detectable at concentrations as low as 10 μM.
and reached its maximum at concentrations of 1000 μM. The effect of 3,5-DBr-L-Phe at low concentrations (less than 30 μM) manifested as potentiation of the mEPSCs, whereas at higher concentrations, 3,5-DBr-L-Phe activated the steady-state inward current (Fig. 1A). The concentration of 3,5-DBr-L-Phe required to produce half-maximal effect (EC₅₀) was 331.6 ± 78.6 μM. The concentration-response relationships for 3,5-DBr-L-Phe to activate total steady-state and fluctuating background inward currents are shown in Fig. 1, B and C, respectively. Compared with NMDA-activated current, 3,5-DBr-L-Phe-activated current had a similar onset and reverted to baseline on washout of 3,5-DBr-L-Phe (Fig. 1A). The NMDA receptor antagonist AP-5 and the NMDA receptor channel blocker MK-801 (not shown) completely blocked current activated by 3,5-DBr-L-Phe (Fig. 1D).

To elucidate whether 3,5-DBr-L-Phe interacts with the glutamate- or glycine-binding sites on NMDA receptors, we measured its effects in the presence of different concentrations of glycine, glutamate, and NMDA added to the extracellular solution. A 100-fold increase in concentration of glycine from 0.1 to 10 μM did not prevent the augmenting effect of 3,5-DBr-L-Phe on NMDA receptor-mediated current (Fig. 2A). 3,5-DBr-L-Phe (100 μM) activated NMDA receptor-mediated current of 67.4 ± 16.4 and 144.4 ± 32.7 pA in the presence of 0.1 and 10 μM glycine, respectively. To obtain further evidence for whether 3,5-DBr-L-Phe interacts with the glycine-binding site of NMDA receptors, the dose-response curves for glycine to modulate NMDA current were generated in the absence and presence of 3,5-DBr-L-Phe. These dose-response curves overlapped (Fig. 2B), indicating that augmentation of NMDA receptor current by 3,5-DBr-L-Phe cannot be explained by its interaction with the glycine-binding site of NMDA receptors. The effect of 3,5-DBr-L-Phe on NMDA receptor function, however, did depend on the concentration of glutamate or NMDA. At low concentrations of glutamate and NMDA, total NMDA receptor-mediated current activated by glutamate or NMDA combined with 3,5-DBr-L-Phe was larger than the current recorded in response to application of glutamate or NMDA only. In contrast, combined current, activated by both 3,5-DBr-L-Phe and higher concentration of glutamate or NMDA, was either smaller or similar to the current recorded in the presence of glutamate or NMDA only (Fig. 2, C and D). Comparison of the concentration-response relationships for 3,5-DBr-L-Phe and NMDA to activate NMDA receptor-mediated currents showed that efficacy for 3,5-DBr-L-Phe was 30.5 ± 4.7% compared with NMDA (Fig. 2, E and F). Similar efficacy for 3,5-DBr-L-Phe was observed when instead of NMDA glutamate was used as agonist (data not shown). These results demonstrate that 3,5-DBr-L-Phe acts as a partial agonist of the glutamate-binding site of NMDA receptors.

**Effect of 3,5-DBr-L-Phe on Glutamate Release and AMPA/Kainate Receptor Function.** To isolate the non-NMDA receptor-mediated EPSCs, experiments were performed in the presence of NMDA receptor channel blocker MK-801 (5–10 μM) or in the presence of the NMDA receptor antagonist AP-5 (20 μM). Strychnine (1 μM) and 100 μM picrotoxin were added to the extracellular solution to block glycine and GABA receptors, respectively. Further addition of the non-NMDA receptor antagonist NBQX (10 μM)
abolished all postsynaptic currents, indicating that the recorded mEPSCs were mediated through activation of a non-NMDA receptor (AMPA/kainate) subtype of glutamate receptors. A typical record of membrane ionic current under such conditions is illustrated in Fig. 3A. In contrast to NMDA receptor-mediated currents, 3,5-DBr-l-Phe depressed both the frequency and amplitude of AMPA/kainate receptor-mediated mEPSCs. The frequency and amplitude of AMPA/kainate receptor-mediated mEPSCs were depressed from 2.4 ± 0.7 Hz and 20.0 ± 6.6 pA in control to 0.6 ± 0.2 Hz and 12.8 ± 3.9 pA in the presence of 100 μM 3,5-DBr-l-Phe, respectively (Fig. 3, B and C). The effect of 3,5-DBr-l-Phe had a fast onset and was reversible on washout. A clear reduction in the frequency and amplitude of detected mEPSCs was seen within seconds after application of 3,5-DBr-l-Phe. The depressant effect of 3,5-DBr-l-Phe on AMPA/kainate-activated currents recorded from the same neuron was concentration-dependent. Analysis of the concentration-response curve for the effect of 3,5-DBr-l-Phe to inhibit mEPSC frequency using a nonlinear logistic regression technique showed that the concentration of 3,5-DBr-l-Phe required to depress non-NMDA receptor-mediated mEPSCs by half (IC50) was 29.4 ± 4.3 μM. The finding that 3,5-DBr-l-Phe attenuates both the frequency and the amplitude of AMPA/kainate receptor-mediated mEPSCs indicates that 3,5-DBr-l-Phe may produce both pre- and postsynaptic effects.

To determine whether 3,5-DBr-l-Phe changes the probability of glutamate release, the extent of paired pulse depression for AMPA/kainate receptor-mediated component of EPSCs and its changes after application of 3,5-DBr-l-Phe was established. Low-density areas in dishes with cultured cells (two to five neurons in 400-μm-diameter view-field) were selected for the experiments. Synaptic responses were evoked by applying voltage pulses (0.2–1 ms, 20–90 V) to an extracellular electrode (a patch electrode filled with the extracellular solution) positioned in the vicinity of the presynaptic neuron soma or neurite (Fig. 4A). As a quantitative estimate of paired pulse depression, the paired pulse ratio, [i.e., the ratio of the amplitude of the second EPSC in the pair to that of the first (EPSC2/EPSC1)] was estimated. Application of 100 μM 3,5-DBr-l-Phe decreased the amplitude of single evoked EPSCs (first response in a paired pulse experiment) in cerebrocortical neurons by 47.5 ± 2.9% (Fig. 4B). Although the EPSC kinetics did not seem to change, 3,5-DBr-l-Phe decreased the paired pulse ratio by 41.4 ± 15.0%. These data suggest that a reduced probability of synaptic vesicle release may contribute to the inhibitory effect of 3,5-DBr-l-Phe on excitatory transmission at these synapses.
To assess whether 3,5-DBr-L-Phe may also depress activity of postsynaptic AMPA/kainate receptors, we investigated the effect of 3,5-DBr-L-Phe on current activated by AMPA. AMPA (3 μM) activated a rapidly rising inward current (i.e., IAMPA) that was depressed by 3,5-DBr-L-Phe (Fig. 4C). The effect of 3,5-DBr-L-Phe on IAMPA was concentration-dependent and reversible upon washout of the drug. The concentration-response relationships for 3,5-DBr-L-Phe to depress IAMPA obtained at two different concentrations of AMPA are shown in Fig. 4D. The increase in the concentration of AMPA from 3 to 10 μM markedly shifted the concentration-response relationship for 3,5-DBr-L-Phe to depress IAMPA rightward (Fig. 4D).

Fig. 3. 3,5-DBr-L-Phe depresses AMPA-kainate receptor-mediated mEPSCs in rat cerebrocortical cultured neurons in concentration-dependent manner. A, representative traces of AMPA-kainate mEPSCs recorded from a cortical neuron under the following conditions: control, in the presence of 100 μM 3,5-DBr-L-Phe, and after washout of 3,5-DBr-L-Phe. AMPA/kainate receptor-mediated currents were recorded in 0.3 mM TTX-containing extracellular solution at holding membrane potential of −60 mV. MK-801 (10 μM), 1 μM strychnine, and 100 μM picrotoxin were added to the extracellular solution to block NMDA, glycine, and GABA receptors, respectively. B and C, concentration-response relationships for 3,5-DBr-L-Phe to attenuate AMPA/kainate receptor-mediated mEPSC frequency and amplitude, respectively. Data were normalized to control values and plotted against the concentration of 3,5-DBr-L-Phe. Data are expressed as mean ± S.E.M. of six to seven cells. Intervention versus control, *, P < 0.01. Curve fitting and estimation of value of IC₅₀ for the frequency of AMPA/kainate mEPSCs was made according to the four-parameter logistic equation. The IC₅₀ for the effect of 3,5-DBr-L-Phe on the amplitude of AMPA/kainate mEPSCs was not determined because the small number of mEPSCs in the presence of 3,5-DBr-L-Phe concentrations higher than 100 μM made it impossible to adequately determine the average amplitude of non-NMDAR-mediated mEPSCs.

Fig. 4. 3,5-DBr-L-Phe causes depression of glutamate release and activity of postsynaptic AMPA-kainate receptors. A, effect of 3,5-DBr-L-Phe on the evoked EPSCs in rat cerebrocortical cultured neuron. Examples of average EPSCs (20 traces average) in control conditions (open circle) and in the presence of 3,5-DBr-L-Phe (closed circle). Synaptic responses were evoked by applying two subthreshold electric stimuli (0.4–1 ms, 50–90 V, 250 ms apart) to an extracellular electrode (a patch electrode filled with the extracellular solution) positioned in the vicinity of the presynaptic neuron. Sweeps were recorded at 10-s intervals. After 20 sweeps, 100 μM 3,5-DBr-L-Phe was added. Neuron was held in whole cell mode at Vₘ = −60 mV in 1 mM Mg²⁺-containing extracellular solution. Strychnine (1 μM) and 100 μM picrotoxin were added to the extracellular solution to block glycine and GABA receptors, respectively. B, values of the second/first amplitude ratio of the paired EPSC responses. The amplitude of the first and second EPSCs were measured against the baseline; each point represents an average of five subsequent sweeps. Data expressed as mean ± S.E.M. for seven cells. Intervention versus control, *, P < 0.01 compared with control. C and D, depressant effect of 3,5-DBr-L-Phe on AMPA-activated current (IAMPA). 3,5-DBr-L-Phe exposure was initiated 45 s before the start of AMPA application. Horizontal bar denotes 3 μM AMPA application. D, concentration-response relationships for 3,5-DBr-L-Phe to attenuate AMPA-activated currents. Data expressed as mean ± S.E.M. for three to five cells. Intervention versus control, *, P < 0.05 compared with control.
The IC50 values of 3,5-DBr-L-Phe in the presence of 3 and 10 μM AMPA were 294.4 ± 8.9 and 1140.1 ± 54.6 μM, respectively. These results indicate that 3,5-DBr-L-Phe most probably acts as a competitive inhibitor of the glutamate-binding site on the AMPA receptors.

**Effect of 3,5-DBr-L-Phe on GABA-ergic Transmission and Elicited Action Potentials.** GABA receptor-mediated mIPSCs were recorded in the presence of 5 μM MK-801, 10 μM NBQX, and 1 μM strychnine to block NMDA, AMPA/kainate, and glycine receptors, respectively. A typical record of membrane ionic current under such conditions is illustrated in Fig. 5A. 3,5-DBr-L-Phe (100 μM) significantly changed neither the frequency nor the amplitude of GABA receptor-mediated mIPSCs. The frequency and amplitude of GABA receptor-mediated mIPSCs were depressed from 1.0 ± 0.2 Hz and 25.1 ± 2.3 pA in control to 0.8 ± 0.2 Hz and 24.2 ± 1.6 pA in the presence of 100 μM 3,5-DBr-L-Phe, respectively (Fig. 5, A and B). Likewise, 100 μM 3,5-DBr-L-Phe did not cause any changes in elicited action potentials (Fig. 5C), indicating lack of the effect of 3,5-DBr-L-Phe on voltage-operated ionic channels.

**Discussion**

The results of the current study clearly demonstrate that 3,5-DBr-L-Phe produces a unique combination of actions on excitatory glutamatergic synaptic transmission in rat cerebrocortical cultured neurons. Specifically, 3,5-DBr-L-Phe activates NMDA receptor-mediated current, depresses activity of non-NMDA (AMPA/kainate) subtype of glutamate receptors, and diminishes presynaptic glutamate release. Such differential modulation of glutamatergic synaptic transmission may be beneficial in brain disorders characterized by alterations in the glutamate system.

**Mechanism of Action.** Current activated by 3,5-DBr-L-Phe was blocked by the specific NMDA receptor antagonists AP-5 and MK-801. These and other experimental evidence indicate that 3,5-DBr-L-Phe acts as agonist of NMDA receptors. NMDA receptors require two coagonists, glutamate and glycine, for their activation. The binding sites for glycine and glutamate are located on subunits 1 and 2, respectively (Laube et al., 1997). Previously, we have demonstrated that L-Phe depressed NMDA receptor function by competing for the glycine-binding site of NMDA receptors and depressed non-NMDA receptor activity by competing for the glutamate-binding site of these receptors (Glushakov et al., 2002, 2003). Similar to L-Phe, 3,5-DBr-L-Phe also is a competitive antagonist of AMPA/kainate receptors. Surprisingly, halogenation of the L-Phe molecule not only reversed its effect (activation versus depression) but also changed the site of its interaction with the NMDA receptors (glutamate-binding site versus glycine-binding site). Thus, the activating effect of 3,5-DBr-L-Phe on NMDA receptor function did not depend on concentration of glycine. Increases in the concentration of glycine augmented the 3,5-DBr-L-Phe-activated current as would be expected from an increased concentration of coagonist. On the other hand, the activating effect of 3,5-DBr-L-Phe on NMDA receptor function depended on the concentration of glutamate or NMDA added to the extracellular solution. The total current activated by 3,5-DBr-L-Phe and low concentrations of the agonists was bigger than the current activated by glutamate or NMDA only. The opposite changes in NMDA receptor-mediated current took place at higher concentrations of glutamate or NMDA. It is noteworthy that the maximal current activated by 3,5-DBr-L-Phe was approximately 30% of the maximal current activated by NMDA or glutamate. Together, these findings support the notion that 3,5-DBr-L-Phe acts as a partial NMDA receptor agonist at the glutamate-binding site.

Our results indicate that 3,5-DBr-L-Phe has a postsynaptic effect on non-NMDAR (AMPA/kainate)-mediated events as well. 3,5-DBr-L-Phe depressed the amplitude of non-NMDAR-mediated mEPSCs and the current activated by exogenous AMPA. Increases in the concentration of AMPA shifted the concentration-response curve for the effect of 3,5-DBr-L-Phe on I_{AMPA} rightward. This finding suggests that the glutamate-binding site on the AMPA receptor is a possible target for the inhibitory action of 3,5-DBr-L-Phe. The postsynaptic effects of 3,5-DBr-L-Phe on non-NMDA receptors may be further augmented by the presynaptic action of 3,5-DBr-L-Phe. Although the postsynaptic effects of 3,5-DBr-L-Phe can reduce the frequency of AMPA/kainate receptor-mediated mEPSCs by decreasing the mEPSC amplitude of some events below the threshold for detection, the predomi-
nant depression of mEPSC frequency over mEPSC amplitude strongly suggests a presynaptic action based on a decreased
decrease in the frequency of AMPA/kainate mEPSCs in combination
in the presence of AMPA/kainate mEPSCs. In fact, in our preliminary studies we found
preventing overactivation of NMDA receptors by excess glu-
normalization of the depressed NMDA receptor activity while
experiments.

Potential Application of 3,5-DBr-L-Phe. Given the fact that decreased function of NMDA receptors and accompanying
increased neurotransmitter release with overactivation of AMPA/kainate receptors are important contributors to the pathophysiology of schizophrenia (Moghaddam et al., 1997; Jentsch and Roth, 1999; Meador-Woodruff and Healy, 2000; Greene, 2001; Hirsch and Weinberger, 2000; Konradia and
Heckers, 2003), 3,5-DBr-L-Phe may represent the prototype of
mechanisms mediating the depressant effect of 3,5-DBr-L-Phe
on glutamate release. Elucidation of the mechanisms whereby
3,5-DBr-L-Phe depresses neurotransmitter release requires
detailed investigation and will be a subject for our future
experiments.

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