Glycine Binding Sites of Presynaptic NMDA Receptors May Tонically Regulate Glutamate Release in the Rat Visual Cortex

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Glycine binding sites of presynaptic NMDA receptors may tonically regulate glutamate release in the rat visual cortex. J Neurophysiol 97: 817–823, 2007. First published November 8, 2006; doi:10.1152/jn.00980.2006. In the CNS, activation of N-methyl-d-aspartate receptor (NMDA-R) glycine binding sites is a prerequisite for activation of postsynaptic NMDA-Rs by the excitatory neurotransmitter glutamate. Here we provide electrophysiological evidence that the glycine binding sites of presynaptic NMDA-Rs regulate glutamate release in layer II/III pyramidal neurons of the rat visual cortex. Specifically, our results reveal that the frequency of miniature excitatory postsynaptic currents is significantly reduced by 7-chloro-kynurenic acid (7-Cl KYNA), a NMDA-R glycine binding site antagonist, and glycine or d-serine reverses this effect. Similar results are obtained when the open-channel NMDA receptor blocker, MK-801, is included in the recording pipette. Our data indicate that the glycine binding site of postsynaptic NMDA-Rs is not saturated. Moreover, they suggest that presynaptic NMDA-Rs are located in layer II/III pyramidal neurons of the rat visual cortex and that the glycine binding site of presynaptic NMDA-Rs tonically regulates glutamate release.

INTRODUCTION

Johnson and Ascher (1987) first demonstrated that, in the vertebrate CNS, exogenously applied glycine potentiates the response of N-methyl-d-aspartate receptors (NMDA-Rs) to NMDA. Binding of glycine to the glycine binding site of NMDA-Rs is a prerequisite for NMDA-R activation by glutamate (Kleckner and Dingledine 1988). Thus glycine and glutamate are co-agonists for NMDA-Rs. It is generally assumed that the glycine binding site is constantly saturated due to high concentrations of glycine in cerebrospinal fluid (CSF) (Ferraro and Hare 1985). However, glycine in the synaptic cleft is subject to a powerful uptake system (i.e., glycine transporter 1 and 2, GlyT1 and GlyT2) (Bergeron et al. 1998; Betz et al. 2006; Chen et al. 2003; Supplisson and Bergman 1997). In addition, NMDA-R isoforms exhibit different affinities to glycine (Kew et al. 1998; Kutsuwada et al. 1992). A surge of in vivo and in vitro studies has suggested that saturation of the glycine binding varies among different brain areas. Some studies have shown that the application of exogenous glycine increases NMDA-R responses (Ahmadi et al. 2003; Baptista and Varanda 2005; Berger et al. 1998; Czepita et al. 1996), whereas others have found no modulatory effect (Fletcher et al. 1989; Obrenovitch et al. 1997).

D-serine, which is a potent agonist of the glycine binding site (Kleckner and Dingledine 1988; Martineau et al. 2006; Schell et al. 1995) but is not taken up by glycine transporters (Supplisson and Bergman 1997), has been shown to potentiate NMDA synaptic currents (Baptista and Varanda 2005; Mothet et al. 2000). The ability of glycine or d-serine to potentiate NMDA-R responses clearly indicates that the glycine binding site of NMDA-Rs is not saturated.

To date, a number of studies have shown that the NMDA-Rs are present on the presynaptic membrane in addition to the postsynaptic area. Presynaptic NMDA-Rs have been found to enhance neurotransmitter release in different regions of the CNS including the spinal cord, cerebellum, entorhinal cortex, and neocortex (Bardoni et al. 2004; Berretta and Jones 1996; DeBiasi et al. 1996; Glitsch and Marty 1999; MacDermott et al. 1999; Yang et al. 2006). They may also mediate synaptic plasticity (i.e., LTP and LTD) (Casado et al. 2002; Duguid and Sjostrom 2006; Sjostrom et al. 2003). Immunocytochemical studies have demonstrated that presynaptic NR1 and NR2B-containing NMDA-Rs are present in rat visual cortex (Aoki et al. 1994). Moreover, presynaptic NMDA-Rs enhance neurotransmitter release in the visual cortical layer V neurons as proven by application of APV, a competitive antagonist of these receptors (Sjostrom et al. 2003). However, whether the glycine binding site of presynaptic NMDA-Rs may modulate glutamatergic neurotransmission in CNS is still uncertain.

In this study, we examined whether the glycine binding site of presynaptic NMDA-Rs regulates glutamate release in the rat visual cortex. Our results show that the glycine binding site on the postsynaptic NMDA-Rs is not saturated and that glycine binding sites at presynaptic NMDA-Rs regulate glutamate release in the layer II/III pyramidal neurons of the rat visual cortex.

METHODS

Slice preparation

Visual cortical slices were prepared from Sprague-Dawley rats aged 13–15 days. All animals were housed in a standard environment on a 12/12-h light/dark cycle with light on at 07:00, and they were allowed ad libitum access to water and food. The use and care of animals in this study follow the guidelines of the Xi’an Jiaotong University Animal Research Advisory Committee. Rats were initially anesthetized with ether and then immersed in ice-cold water, with the nose exposed, for 3 min to reduce brain temperature. Immediately after decapitation, the brain was placed in an ice-cold artificial cerebrospinal fluid (ACSF) bubbled with 95% O2–5% CO2 (pH 7.4). The ACSF consisted of (in mM) 124 NaCl, 5 KCl, 1.2 KH2PO4, 1.3 MgSO4, 2.4 CaCl2, 26 NaHCO3, and 10 glucose, and it had an osmolality of 305–310 mosM/kg H2O. A block of tissue containing the primary visual cortex was cut into 350-μm-thick slices with a vibratome (Campden Instruments, London, UK). Slices were trans-
ferred to an incubating chamber containing ACSF equilibrated with carbogen (95% O2-5% CO2) and incubated for ≥1.5 h at room temperature (20°C) prior to electrophysiological recording.

Recordings

For recording, slices were individually transferred to a recording chamber where they were perfused (2.5–3 ml/min) with oxygenated Mg-free ACSF at 31 ± 0.5°C. The temperature of the recording chamber was continuously monitored and controlled by a custom-made temperature controller. The slices were placed on an upright infra-red video microscope with differential interference contrast (DIC) optics (OLYMPUS BX51WI), which was mounted on a Gibbaltar X–Y table. Slices were observed through a ×40 water-immersion objective using an infrared-sensitive camera (DAGE-MTI, IR-1000). Layer II/III pyramidal neurons of the visual cortex were visually selected (Mason and Larkman 1990). Patch-clamp recordings were performed in the whole cell configuration. Unpolished and uncoated patch pipettes (1.5 mm/1.1 mm; Sutter Instruments, Novato, CA) with a resistance of 4–6 MΩ were pulled using a horizontal puller (model P-97, Sutter Instruments, Novato, CA). The pipette solution contained (in mM) 130 cesium methanesulfonate, 5 NaCl, 5 QX-314, 0.1 CaCl2, 1 EGTA, 10 HEPES, 2 Na2ATP, and 0.25 Na2GTP (pH 7.3–7.4, adjusted with CsOH, 280–290 mosM/kg H2O). To block postsynaptic NMDA-Rs, the following pipette solution was used (in mM): 124 cesium methanesulfonate, 5 NaCl, 5 QX-314, 2 MgCl2, 10 BAPTA, 1 (pH 7.3–7.4, adjusted with CsOH, 280–290 mosM/kg H2O). To block postsynaptic NMDA-Rs, the following pipette solution was used (in mM): 124 cesium methanesulfonate, 5 NaCl, 5 QX-314, 2 MgCl2, 10 BAPTA, 1 (pH 7.3–7.4, adjusted with CsOH, 280–290 mosM/kg H2O). To block postsynaptic NMDA-Rs, the following pipette solution was used (in mM): 124 cesium methanesulfonate, 5 NaCl, 5 QX-314, 2 MgCl2, 10 BAPTA, 1 (pH 7.3–7.4, adjusted with CsOH, 280–290 mosM/kg H2O). To block postsynaptic NMDA-Rs, the following pipette solution was used (in mM): 124 cesium methanesulfonate, 5 NaCl, 5 QX-314, 2 MgCl2, 10 BAPTA, 1 (pH 7.3–7.4, adjusted with CsOH, 280–290 mosM/kg H2O).

Mg-free ACSF was obtained by omitting MgSO4 (1.3 mM) from the ACSF without compensation for the loss of osmolarity or for the amount of divalent ions. Mg-free ACSF at 31°C began 10 ms after the start of the mEPSCs (Beradaia and Trouslard 2002). Detection criteria also included rise time <5 ms (Sjostrom et al. 2003), which may eliminate the purely NMDA-R-mediated mEPSCs because the average 10–90% rise time of purely NMDA-R-mediated mEPSC events is 8 ± 2 ms (O’Brien et al. 1997). This means that our data do not include the silent synapses. Multiple peak events were discarded. Averaged mEPSCs were obtained by aligning events on the rising phase. Events occurring during 120 s were averaged and analyzed in every group. To quantify the NMDA component of mEPSCs, we integrated the average mEPSCs waveforms occurring over a 90-ms time period, which began 10 ms after the start of the mEPSCs (Berger et al. 1998). This integral was used as an index of NMDA-R-mediated charge transfer. Comparison of mEPSC distributions was performed using the Kolmogorov-Smirnov test, and values of P < 0.01 were accepted as significant (Beretta and Jones 1996). Group means were compared using a paired Student’s t-test, and P < 0.05 was considered significant. All data are expressed as means ± SE.

Results

Glycine or D-serine potentiates NMDA-R–mediated mEPSCs

Whole cell mEPSC recordings were performed on 58 pyramidal neurons in layer II/III of the visual cortical slices. Spontaneous mEPSCs were treated, 0.98 ± 0.5 Hz vs. control, 1.50 ± 0.5 Hz, P < 0.001, n = 8) but not the peak amplitude of the mEPSCs (control, 46.9 ± 4.4 pA vs. treated, 46.0 ± 4.6 pA, P > 0.18, n = 8). This result suggests that glycine binding site on synaptically active NMDA-Rs is not saturated. However, application of glycine (100 μM) did not alter the frequency or the peak amplitude of the mEPSCs (Fig. 1, D and E). Application of D-APV (50 μM) decreased the frequency (control, 2.82 ± 0.5 Hz vs. treated, 1.50 ± 0.5 Hz, P < 0.001, n = 8) but not the peak amplitude of the mEPSCs (control, 46.9 ± 4.4 pA vs. treated, 46.0 ± 4.6 pA, P > 0.18, n = 8). This result suggests that glycine binding site on presynaptic NMDA-Rs is saturated and the NMDA-Rs may tonically regulate glutamate release.

D-serine, another agonist at the glycine-site of NMDA-Rs, is not transported by glycine transporters (Supplisson and Bergman 1997). Therefore we examined the effect of D-serine on NMDA-mediated mEPSCs to verify the results obtained with glycine. Application of exogenous D-serine (100 μM) also increased the NMDA-R-mediated component of mEPSCs, which was abolished by D-APV (50 μM; Fig. 2, A and B). This enhancement of NMDA-R-mediated mEPSCs or increased NMDA charge transfer (tC), is shown in Fig. 1C. Charge transfers under control conditions and in response to glycine treatment were 622 ± 111 and 795 ± 104 fC, respectively (28% increase, P < 0.007, n = 8). These results indicate that the glycine binding site on synthapically active NMDA-Rs is not saturated. However, application of glycine (100 μM) did not alter the frequency or the peak amplitude of the mEPSCs (Fig. 1, D and E). Application of D-APV (50 μM) decreased the frequency (control, 2.82 ± 0.5 Hz vs. treated, 1.50 ± 0.5 Hz, P < 0.001, n = 8) but not the peak amplitude of the mEPSCs (control, 46.9 ± 4.4 pA vs. treated, 46.0 ± 4.6 pA, P > 0.18, n = 8). This result suggests that glycine binding site on presynaptic NMDA-Rs is saturated and the NMDA-Rs may tonically regulate glutamate release.

Glycine or D-serine reverses the inhibitory effects of 7-CI Kyna on the frequency of NMDA-R–mediated mEPSCs

To determine whether the glycine binding site of the NMDA-Rs may be involved in the modulation of glutamate
release, we employed 7-Cl KYNA, an antagonist of the glycine binding site of the NMDA-Rs. As shown in Fig. 3D, inclusion of 7-Cl KYNA (20 μM) in the extracellular solution blocked the postsynaptic NMDA component and reduced the frequency of the mEPSCs (control, 1.9 ± 0.7 Hz vs. treated, 1.1 ± 0.4 Hz; *P < 0.001, n = 10). Combined treatment with glycine (100 μM) and 7-Cl KYNA reversed this effect (control, 1.9 ± 0.7 Hz vs. treated, 1.8 ± 0.6 Hz; *P > 0.08, n = 10) and recovered the postsynaptic NMDA component (Fig. 3, B and D). As shown in Fig. 3C, the peak amplitude of the mEPSCs was not influenced by 7-Cl KYNA or glycine (control, 41.0 ± 3.3 pA; 7-Cl KYNA, 39.6 ± 4.0 pA; 7-Cl KYNA + glycine, 40.4 ± 3.9 pA, n = 10).

As shown in Fig. 4, D-serine (100 μM) also reversed the inhibitory effects of 7-Cl KYNA (20 μM) on the frequency of NMDA-mediated mEPSCs (control, 1.69 ± 0.4 Hz; 7-Cl KYNA, 1.1 ± 0.2 Hz; 7-Cl KYNA + D-serine, 1.65 ± 0.3 Hz, *P < 0.001, n = 12). The peak amplitude of the mEPSCs was unaffected by treatment either with 7-Cl KYNA only or along with D-serine (control, 40 ± 3.8 pA; 7-Cl KYNA, 38.6 ± 5.1 pA; 7-Cl KYNA + D-serine, 39.0 ± 4.4 pA; n = 12; Fig. 4C). These results suggest that the glycine binding site is involved in the modulation of the glutamate release.

Glycine or D-serine reverses the inhibitory effects of 7-Cl KYNA in the presence of MK-801

It is possible that 7-Cl KYNA reduced the frequency of the mEPSCs by acting on the glycine binding site of presynaptic NMDA-Rs or postsynaptic NMDA-Rs. In the latter case, presynaptic neurotransmitter release could be affected through retrograde messengers. To test these possibilities, we included the open-channel NMDA-R blocker, dizocilpine maleate (MK-801; 1 mM), in the recording pipette as described by Berretta and Jones (1996). Blockade of the postsynaptic NMDA-Rs with MK-801 completely abolished the NMDA component of the mEPSCs (Fig. 5). Inclusion of 7-Cl KYNA (20 μM) under these conditions reduced the frequency of the mEPSCs. Moreover, this effect could be reversed by the addition of glycine (100 μM; control, 2.6 ± 0.5 Hz; 7-Cl KYNA, 1.7 ± 0.3 Hz; 7-Cl KYNA + glycine, 2.6 ± 0.6 Hz; *P < 0.001, n = 10; Fig. 5C). As before, the peak amplitude of the mEPSCs was not affected by treatment with 7-Cl KYNA and glycine. (control, 34.3 ± 1.5 pA; 7-Cl KYNA, 34.4 ± 1.2 pA; 7-Cl KYNA + glycine, 34.5 ± 1.7 pA; n = 10).

As shown in Fig. 6C, D-serine (100 μM) also reversed the effects of the 7-Cl KYNA on mEPSC frequency in the presence of MK-801 (control, 2.8 ± 0.7 Hz; 7-Cl KYNA, 1.6 ± 0.5 Hz; 7-Cl KYNA + D-serine, 2.8 ± 0.6 Hz; *P < 0.001; n = 9). The peak amplitude of the mEPSCs was not affected by these treatments (control, 32.8 ± 3.9 pA; 7-Cl KYNA, 32.4 ± 4.1 pA; 7-Cl KYNA + D-serine, 33.1 ± 4.2 pA; n = 9; Fig. 6B). These results suggest that presynaptic, but not postsynaptic, NMDA-R glycine binding sites tonically regulate glutamate release in the visual cortex.

Additional experiments showed that there were no effects of glycine, D-serine or 7-Cl KYNA on the frequency and amplitude of mEPSCs in the presence of D-APV, which blocked both pre- and postsynaptic NMDARs (data showing in Supplemental materials).
DISCUSSION

In the present study, we have used a whole cell recording technique to examine the effects of glycine and D-serine on the spontaneous synaptic activities in layer II/III pyramidal neurons of the rat visual cortex. All data were obtained in the presence of TTX (0.5 μM), strychnine (1 μM), and picrotoxin (100 μM) in the Mg-free ACSF and at a holding potential of −60 mV. Under these conditions, the spontaneous spikes as well as glycinergic and GABAergic synaptic activities could be excluded, and the NMDA-R-mediated mEPSCs could be

FIG. 2. D-serine enhances the NMDA-R-mediated mEPSCs. A: traces of mEPSCs recorded from the same neuron (holding potential = −60 mV) under control conditions (top), after the addition of D-serine (middle), and after addition of D-APV (bottom). The NMDA component of mEPSCs was increased by D-serine and decreased by D-APV. B: average mEPSCs from the same neuron shown in A. C: NMDA charge transfer occurring between 10 and 100 ms after mEPSC onset was increased in the presence of D-serine (P < 0.005, n = 9). D: pooled data from nine neurons shows the peak amplitudes of the mEPSCs were not altered by treatment with D-serine and D-APV. E: D-serine (100 μM) did not alter the frequency of the mEPSCs. However, application of D-APV (50 μM) decreased the frequency (P < 0.001, n = 9).

FIG. 3. Glycine reverses the inhibitory effect of 7-chloro-kynurenic acid (7-Cl KYNA) on NMDA-R-mediated mEPSCs. A: traces of mEPSCs recorded from the same neuron (holding potential = −60 mV) under control conditions (top) and in the presence of only 20 μM 7-Cl KYNA (middle) and along with 100 μM glycine (bottom). B: average mEPSCs from the same neuron shown in A. C: pooled data from 10 neurons shows the peak amplitudes of the mEPSCs were not altered by treatment with 7-Cl KYNA and glycine. D: frequency of mEPSCs was reduced by 7-Cl KYNA (P < 0.001, n = 10), and this effect was reversed by addition of glycine (P > 0.08, n = 10).
facilitated. The finding that mEPSCs were abolished by the presence of d-APV (50 μM) and CNQX (10 μM) in extracellular fluid indicates that the mEPSCs were composed of both NMDA and non-NMDA glutamatergic components. On the other hand, mEPSCs were made up of only non-NMDA glutamatergic components when MK-801 was included in the recording pipette.

As described in the preceding text, application of exogenous glycine potentiated NMDA-R-mediated mEPSCs. Glycine may achieve this effect through at least three mechanisms. First, glycine might act through presynaptic, strychnine-sensitive glycine receptors to facilitate the release of glutamate, an action that has been demonstrated in the brain stem (Turecek and Trussell 2001). In this study, the contribution of such a mechanism is probably limited because mEPSCs were recorded in the presence of strychnine (1 μM) and picrotoxin (100 μM). Second, glycine might act on the NMDA-Rs containing NR3A or NR3B subunits (Chatterton et al. 2002), which are activated by glycine in the absence of glutamate. The finding that d-serine, which blocks NMDA-Rs containing NR3A or NR3B subunits, potentiates NMDA-R–mediated mEPSCs argues against this possibility. Third, glycine might act postsynaptically by activating the glycine binding site of NMDA-Rs. To test this hypothesis, we employed D-serine, another NMDA-R glycine binding site agonist. The ability of D-serine to increase the NMDA component provides evidence that glycine potentiates NMDA-R–mediated mEPSCs via the NMDA-R glycine binding site. In addition, the non-NMDA

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**FIG. 4.** D-serine reverses the inhibitory effect of 7-Cl KYNA on NMDA-R-mediated mEPSCs. A: traces of mEPSCs recorded from the same neuron (holding potential = −60 mV) under control conditions (top) and in the presence of 20 μM 7-Cl KYNA (middle) and along with 100 μM d-serine (bottom). B: average mEPSCs from the same neuron shown in A. C: pooled data from 12 neurons shows the peak amplitudes of the mEPSCs were not altered by treatment with 7-Cl KYNA and d-serine. D: frequency of mEPSCs was reduced by 7-Cl KYNA, and this effect was reversed by addition of D-serine ($P < 0.001, n = 12$).

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**FIG. 5.** Glycine reverses the inhibitory effects of 7-Cl KYNA on mEPSC frequency in the presence of MK-801. A: NMDA-R open channel blocker, MK-801 (1 mM), was applied via the recording pipette, and mEPSCs were recorded under control conditions (top) and in the presence of only 20 μM 7-Cl KYNA (middle) and along with 100 μM glycine (bottom). The mEPSC tracings from the same neuron (holding potential = −60 mV) are shown. Blockade of postsynaptic NMDA-Rs via MK-801 completely abolished NMDA-mediated mEPSCs. B: average mEPSCs shown in A. C: pooled data from 10 neurons shows the peak amplitudes of the mEPSCs were not significantly changed under any of these conditions. D: in the presence of MK-801, the frequency of mEPSCs was reduced by 7-Cl KYNA, and this effect was reversed by addition of glycine ($P < 0.001, n = 10$).
component was not influenced by glycine or \( \text{d}-\text{serine} \), and the application of \( \text{APV} \) or \( \text{7-Cl KYNA} \) abolished the NMDA component of the mEPSCs. Taken together, these results suggest that the glycine binding site on the postsynaptic NMDA-Rs is not saturated as has been shown in cat visual cortex (Czepita et al. 1996).

The levels of synaptic glycine are known to be tightly controlled by glycine transporters. There are two types of glycine transporters, \( \text{GlyT1} \) and \( \text{GlyT2} \). \( \text{GlyT1} \), which is localized to glia and neurons, is closely associated with the NMDA-Rs (Cubelos et al. 2005); whereas, \( \text{GlyT2} \) is colocalized with strychnine-sensitive glycine receptors (Gomeza et al. 2003). As has been described in the hippocampus (Watanabe et al. 2001), \( \text{d}-\text{serine} \) was capable of potentiating NMDA synaptic currents. Here, application of exogenous \( \text{d}-\text{serine} \) increased the NMDA component of the mEPSCs by \( \sim 52\% \), and glycine increased the NMDA component of the mEPSCs by \( \sim 28\% \). The greater effect of \( \text{d}-\text{serine} \), which is not taken up by glycine transporters (Supplisson and Bergman 1997), suggests that levels of synaptic glycine are relatively low and are tightly controlled by glycine transporters.

Our results also show that \( \text{APV} \) or \( \text{7-Cl KYNA} \) reduce the frequency but not the peak amplitude of mEPSCs, regardless of the presence of MK-801 in the recording pipette. These findings support the existence of presynaptic NMDA-Rs and are consistent with studies showing that presynaptic NMDA-Rs tonically facilitate glutamate release as a result of increased calcium influx into the terminals (Berretta and Jones 1996; Sjostrom et al. 2003; Yang et al. 2006). It was reported that mEPSCs events rely on presynaptic \( \text{Ca}^{2+} \) transient either in hippocamal area CA1 or in layer II neurons of the rat barrel cortex (Rusakov 2006; Simkus and Stricker 2002). Presynaptic \( \text{Ca}^{2+} \) transient could produce \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release (CICR), and then the postsynaptic EPSCs. The amplitude of CICR from internal \( \text{Ca}^{2+} \) stores depends on the amount of \( \text{Ca}^{2+} \) influx and probably on the cytosolic \( \text{Ca}^{2+} \) concentration (Rusakov 2006). Activation of presynaptic NMDA-Rs could induce the \( \text{Ca}^{2+} \) influx and then improve the CICR and finally facilitate the neurotransmitter release.

The difference in saturation of the glycine site between pre- and postsynaptic NMDA-Rs seems to have different affinities for glycine (Kew et al. 1998; Kutsuwada et al. 1992). There is strong evidence that at P14 the postsynaptic NMDA receptors are mostly \( \text{NR1-NR2B} \), whereas the presynaptic ones are mostly \( \text{NR1-NR2B} \) (Sjostrom et al. 2003; Woodhall et al. 2001; Yang et al. 2006). The EC50s of glycine and of \( \text{d}-\text{serine} \) for \( \text{NR1-NR2A} \) receptors are known to be higher than those for \( \text{NR1-NR2B} \). Thus a concentration of glycine will be saturating for the (presynaptic) \( \text{NR2B} \)-containing receptors and not saturating for the (postsynaptic) \( \text{NR2A} \)-containing receptors. Also consistent with our findings, presynaptic \( \text{NR1} \)- and \( \text{NR2B} \)-containing NMDA-Rs are known to exist (Aoki et al. 1994) and enhance neurotransmitter release in the visual cortex (Sjostrom et al. 2003).

The ability of glycine or \( \text{d}-\text{serine} \) to reverse the effects of \( \text{7-Cl KYNA} \) when MK-801 was present in the recording pipette argues against the possibility that glycine acts on postsynaptic receptors to facilitate glutamate release via retrograde messengers. Additionally, the finding that \( \text{d}-\text{serine} \) is able to reverse the effects of \( \text{7-Cl KYNA} \), combined with the fact that strychnine was present in all experiments, makes it unlikely that presynaptic, strychnine-sensitive glycine receptors facilitated glutamate release (Turecek and Trussell 2001).

In summary, our data show that glycine can act on glycine binding sites at presynaptic and postsynaptic NMDA-Rs to enhance the NMDA-R function, which could be indicative of a general role for the glycine binding site of presynaptic...
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NMADa-Rs in regulating glutamate release in the CNS. Moreover, these findings may be clinically relevant to schizophrenia, where enhancing NMADa-R function is considered to be a promising strategy for treatment of the disease (Coyle et al. 2002; Duncan et al. 1999).

GRANTS

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