Blockade of Glycine Transporter-1 (GLYT-1) Potentiates NMDA Receptor-Mediated Synaptic Transmission in Hypoglossal Motoneurons

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Submitted 20 November 2003; accepted in final form 28 May 2004

INTRODUCTION

An essential requirement for activation of N-methyl-D-aspartate receptors (NMDAR) is the binding of glutamate and the co-agonist glycine (McBain and Mayer 1994). It remains controversial whether or not the glycine binding site of the NMDAR is saturated. The implication is that a nonsaturated glycine binding site may be a means by which glutamatergic NMDAR-mediated synaptic transmission is regulated. Previous research in hypoglossal motoneurons (HMs) has shown that NMDAR-mediated spontaneous miniature excitatory postsynaptic currents (mEPSCs) and NMDAR-induced responses are potentiated by the addition of glycine (Berger and Isaacson 1999; Berger et al. 1998). It has been suggested that extracellular glycine concentration is highly regulated by glycine transporters (GLYT), which may be localized in close proximity to NMDARs (Attwell et al. 1993). Two glycine transporters have been cloned, GLYT1 and -2 (Borowsky et al. 1993; Guastella et al. 1992; Liu et al. 1992, 1993; Smith et al. 1992). GLYT1 is expressed primarily in glial cells surrounding both glycineric and nonglycinergic neurons, whereas GLYT2 expression is predominantly neuronal and most likely at glycineric presynaptic terminals (Zafra et al. 1995b). The expression of GLYT2 is restricted to the spinal cord, brain stem, and cerebellum (Poyatos et al. 1997), where glycineric synaptic transmission is prevalent. In contrast, immunohistochemistry has shown GLYT1 is distributed throughout the brain and specifically in regions shown to express NMDARs including spinal cord, brain stem, cerebellum, hippocampus, and cortex (Zafra et al. 1995b).

GLYT1 is a protein with 12 transmembrane spanning segments which co-transport Na+ and Cl− with glycine (Aragon and Gimenez 1986; Olivares et al. 1997; Roux and Supplisson 2000). This stoichiometry allows both import and export of glycine. Immunohistochemistry of GLYT1 and -2 shows labeling throughout the hypoglossal nucleus. GLYT1 labeling is diffuse and becomes more intense and uniform during development consistent with its glial localization. In contrast, GLYT2 labeling is intense throughout the nucleus and increases in intensity with age. Our results demonstrate the glycine binding site of the NMDAR is not saturated in the brain stem slice during the first 2 wk of development. We suggest that modulation of glycine concentration by GLYT1 is an important mechanism to regulate NMDAR-mediated synaptic transmission.

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mission and inhibitory glycine transmission are both present in this motor nucleus. Previous research has shown that mEPSCs recorded in HMs are potentiated by bath applied glycine and by the nontransportable agonist of the NMDAR glycine binding site, d-serine (Berger et al. 1998). Glycine is an important neurotransmitter in the hypoglossal nucleus. This has been demonstrated by the abundant expression of glycine receptors in the hypoglossal nucleus and electrophysiological recordings of glycine input (O'Brien and Berger 1999; 2001; Singer et al. 1998). The presence of both glycineergic and NMDA synapses makes the hypoglossal nucleus an ideal location to investigate the role of GLYT1 in modulating glycine concentrations in close proximity to NMDA synapses. We have recorded whole cell currents from HMs after application of the GLYT1 antagonist, NFPS. The results from this study show for the first time that blockade of GLYT1 with NFPS causes an increase in the charge transfer of NMDAR-mediated mEPSCs, spontaneous EPSCs (sEPSCs), evoked EPSCs (eEPSCs), and NMDA-induced currents. Further, using immunohistochemistry we have shown extensive labeling of GLYT1 and -2 throughout the hypoglossal nucleus an ideal location to investigate the role of GLYT1 in modulating glycine concentrations in close proximity to NMDA synapses.

METHODS

Electrophysiology

Sprague Dawley rats were divided into two age groups, neonate (P2–4) and juvenile (P8–11 days old), and were anesthetized with halothane and decapitated. The brain stem was dissected, and slices (200–300 µM thick) containing the hypoglossal motor nucleus were cut using a DSK microlicer. The slices were transferred to an incubation chamber for 1 h at 37°C. After incubation, slices were kept at room temperature until recording. During dissection, slicing and incubation brain stem slices were perfused with Ringer solution containing (in mM) 120 NaCl, 2.5 KCl, 1.10 MgCl₂, 1.0 NaH₂PO₄, 26.2 NaHCO₃, 2.5 glucose, and 2.5 CaCl₂ equilibrated with 95% O₂-5% CO₂. Whole cell patch-clamp recordings from HMs were performed from a total of 66 cells. Cells were identified by their location and morphology (Umemiya and Berger 1994) under infra-red differential interference contrast optics. Glass electrodes (3–5 MΩ) were filled with (in mM) 100 Cs-gluconate, 10 HEPES, 5 QX-314, 10 BAPTA, 4 NaCl, 1 CaCl₂, 20 TEA-Cl, 4 ATP, and 0.2 GTP. Recordings from HMs held at -70 mV were performed 20 min after bathing the slice in 0 Mg²⁺ Ringer solution (the Ringer solution is the same as described in the preceding text with the exception of 0 MgCl₂ with noionic substitution). In this condition, the voltage-dependent Mg²⁺ block of the NMDAR was removed from the pore (Berger et al. 1998). In all recording conditions, except where we recorded glycine dependent postsynaptic currents (IPSCs; in this case we included just bicuculline), we included both 10 µM bicuculline (to block GABA_A receptors) and 2 µM strychnine (to block glycine receptors) in our bathing solution. When recording mEPSCs and NMDA-induced currents, tetrodotoxin (TTX; 1 µM) was included in the bathing solution to block Na⁺-dependent action potentials. e- and sEPSCs were collected from the same neuron; in these cases, only those sEPSCs that occurred 100 ms after the decay of the eEPSC were analyzed for the effect of NFPS. In addition to three HMs, only eEPSCs were recorded. When we compared control sEPSCs recorded with and without eEPSCs, we found that they were not different from each other: eEPSCs were evoked by placing a bipolar electrode lateral to the nucleus (0.1 mV 0.5–2 V, 0.03 Hz). Whole cell NMDA-induced currents were produced by brief (5–10 ms) pressure ejection (Picospritzer IID, General Valve) of 250 µM NMDA in 0 Mg⁺⁺ Ringer solution (described in the preceding text) onto the soma of HMs. In a series of experiments, 100 µM glycine was added to the NMDA puffing solution. Whole cell glutamate currents induced to activate AMPAR responses contained (in mM) 140 NaCl, 2.5 CaCl₂, 1 MgCl₂, 5 HEPES, and 1 glutamate. In a series of experiments, we recorded glycineergic mIPSCs. In these experiments, we included bicuculline (10 µM), TTX (1 µM), DNQX (20 µM), and d-2-amino-5-phosphonovaleric acid (d-APV; 50 µM). In all experiments, drugs were washed onto the slice for a minimum of 10 min before data were acquired. All experiments were performed (room temperature and 32°C. Damage to the cells and nuclei of the slice were determined at 10 kHz, and filtered at 5 kHz. Data were additionally filtered at 1 kHz using Clampfit (Axon Instruments, v. 8.2) and analyzed using Clampfit or Mini Analysis Program (Synaptosoft, v. 5.2.4). To measure the NMDAR component, we have integrated the area beneath the EPSC waveform and the baseline between two time points. We have referred to this measure as NMDA charge transfer. For m- and sEPSCs, the NMDA charge transfer was defined as the 90-ms time region beginning 10 ms after the start of the m- or sEPSC. A minimum of 200 events were analyzed for each HM. This time frame was used because in HMs the fast non-NMDA component of mEPSCs has been shown to have a rapid time course and decayed to 5% of the peak within 10 ms after the start of the response (Berger et al. 1998). For eEPSCs, the NMDA charge transfer was defined as the 160-ms time region beginning 13 ms after the start of the response. A minimum of 20 current traces were used for analysis of each HM. For NMDA-induced EPSCs, the entire trace from the beginning of the response and ending 1 s after the start of the response was used to define the NMDA charge transfer. A minimum of 10 current traces were averaged for analysis. To compare between experimental protocols, the NMDA charge transfer data are expressed as a percent of control. Data are expressed as means ± SE unless otherwise stated. Statistical analysis used was a one-tailed t-test for means performed in Excel (Microsoft, Office 97). Drugs added: bicuculline (10 µM; Tocris), strychnine (2 µM; Sigma), d-APV (50 µM; Tocris), DNQX (20 µM; Sigma), TTX (1 µM; Alomone Laboratories, Israel), NFPS (500 nM; gift from Allelix).

Immunohistochemistry

Neonate and juveniles rats were anesthetized using pentobarbital. Juvenile rats were initially transcardially perfused with 0.9% NaCl until the perfusate ran clear to wash blood from the tissue; this was followed by 4% paraformaldehyde for ~2 min. Brains were then removed and postfixed in 4% paraformaldehyde for 3 h. The brains of neonate rats were not perfused but removed and fixed for 24–48 h. Neonate and juvenile brains were washed in PBS after fixation and stored in 30% sucrose/PBS solution until the tissue was cut. Sections (30 µM) of brain were sliced using a slide microtome. Free-floating sections were then processed for immunofluorescence. Sections were incubated in PBS blocking solution containing 0.2% Triton X-100 (Sigma) and 10% donkey serum (Vector Laboratories, Burlingame, CA) for 1 h, followed by overnight incubation in PBS blocking serum plus primary antibody (1:2000 GLYT1, goat anti-GLYT1; 1:8000 GLYT2, guinea pig anti-GLYT2, both antibodies from Chemicon, Temecula, CA). Sections were then washed in PBS and incubated in secondary antibody (goat-anti-guinea pig Alexa Fluor 488 for GLYT1 and -2, respectively, 1:200) for 1 h, then mounted with Vectashield (Vector Laboratories, Burlingame, CA), and coverslipped. Controls were performed where blocking solution was substituted for the primary antibody and protocol was followed as described in the preceding text. Images were acquired using a Bio-Rad MRC-600 (Bio-Rad, Hercules, CA) confocal microscope. GLYT images were acquired using 488 excitation wavelength. Images were Kalman filtered using Comos software (v. 7, BioRad). Images were imported into Photoshop 5.5 (Adobe, San Jose, CA) for cropping and sizing. Final figures were created using Powerpoint 97 (Microsoft, Seattle, WA).
RESULTS

Electrophysiology

Data were recorded from a total of 66 cells. We have recorded responses to NFPS from both AMPARs and NMDARs postsynaptic currents (PSCs).

To investigate the role of GLYT1 in modulating NMDARs, we first recorded mEPSCs from HMs in the presence of TTX, bicuculline, and strychnine. Figure 1A1 shows an example of mEPSCs from a HM (postnatal day 3, P3) before (control) and after (NFPS) the addition of 500 nM NFPS. Similarly mEPSCs from a P10 HM show NFPS application results in an increase in NMDA charge transfer (Fig. 1A2). Data from six cells (P 2–4) show a significant 41 ± 17% increase in charge transfer in the presence of NFPS (500 nM; P < 0.05, Fig. 1A3). This increase in charge transfer is comparable to the increase seen after addition of 100 μM glycine described by Berger et al. (1998). In P8-10-day-old rats, there is a 26 ± 14% increase in charge transfer after NFPS, although this result is not statistically significant (n = 7 cells, P > 0.05).

We next recorded sEPSCs (Fig. 1B, I and 2). In both age groups, there was a significant increase in charge transfer to 156 ± 24 and 158 ± 14% of control in 2- to 4- and 8- to 10-day-old rats, respectively (Fig. 1B3, n = 6 for each age group, P < 0.05). Although sEPSCs showed a greater increase in charge transfer than observed for mEPSCs, this increase in charge transfer for sEPSCs versus mEPSCs was not significant. Nevertheless the larger increase in the NMDAR-mediated charge transfer in sEPSCs after NFPS application may be due to a greater accumulation of glycine when TTX is not present because of the greater spontaneous release of glycine from glycinergic presynaptic terminals. In support of this hypothesis, in five HMs, we recorded glycinergic m- and sIPSCs in the presence of bicuculline (10 μM), D-APV (50 μM), and DNQX (20 μM). We observed that the frequency of glycinergic sIPSCs is much lower when tetrodotoxin is present in the bath solution (Fig. 2A, bottom), compared with the frequency of sIPSCs observed when tetrodotoxin is absent (Fig. 2A, top). We observed that the frequency of glycinergic events signifi-
cantly decreased from 2.4 ± 0.3 to 0.8 ± 0.1 Hz (P < 0.01) after application of TTX (Fig. 2D). In contrast, the glycineric IPSC mean peak amplitude was not significantly changed due to TTX application 49 ± 12 versus 41 ± 11 pA (P > 0.05), respectively (Fig. 2, B and C). We believe that this higher frequency of glycine events in the absence of TTX results in a greater glycine accumulation when glycine uptake is blocked with NFPS and contributes to a greater potentiation by NFPS of NMDAR component in sEPSCs than mEPSCs.

Next we have investigated the effect of NFPS on eEPSCs. To electrically evoke synaptic inputs to HMs, we placed a bipolar stimulating electrode lateral to the hypoglossal nucleus. Stimulation at this site produces both excitatory and inhibitory inputs to the hypoglossal nucleus (Bellingham and Berger 1996; Rekling et al. 2000). We expect a large increase in NMDA charge transfer after application of NFPS because we are stimulating both excitatory and inhibitory inputs. This speculation arises due to the increased availability of glycine after stimulation of an inhibitory input. The time course of glycineric currents is determined by diffusion and not uptake in the hypoglossal nucleus (Singer and Berger 1999), which suggests glycine may escape the cleft and spillover to activate nearby NMDARs. Figure 3, A and B, shows the increase in the eEPSC charge transfer after treatment with NFPS in HMs from 3- and 8-day-old rats, respectively. Our results show a significant increase in charge transfer to 141 ± 13% (2–4 days) and 134 ± 8% (8–10 days) of control after treatment with NFPS (Fig. 3C, P < 0.01). It may be expected that GLYT2 would also have a significant role in regulating extracellular glycine concentration in this protocol due to activation of inhibitory glycineric inputs; however, we have not investigated this possibility.

To confirm NFPS did not have an effect on AMPARs, we recorded AMPAR-mediated PSCs in the presence of TTX, bicuculline, strychnine, and n-APV followed by the addition of NFPS. We first recorded AMPAR-mediated mEPSCs and found there was no significant difference in mEPSC amplitude before and after NFPS (27 ± 1.7 pA control vs. 33 ± 2.8 pA NFPS, P > 0.05, n = 6, data not shown). The absence of a presynaptic effect of NFPS is also indicated because there was no significant difference in AMPAR-mediated mEPSC frequency before and after NFPS (3.7 ± 1.0 Hz control vs. 4.1 ± 0.7 Hz NFPS, P > 0.05, n = 6, data not shown). We also recorded AMPAR-mediated PSCs after local application of glutamate. Bath application of NFPS did not alter the amplitude of the glutamate-induced AMPAR-mediated response (756 ± 148.2 pA control, 769 ± 148.8 pA NFPS, P > 0.05, n = 5, data not shown). Therefore any changes in NMDAR-mediated synaptic transmission that we observe are not due to an effect of NFPS on AMPAR-mediated synaptic transmission.

We also examined the effect of NFPS on whole cell currents generated by puffing NMDA (50 μM) onto the HM cell soma. We found that in the presence of NFPS, puffing NMDA onto HMs did not result in an enhancement of the NMDA charge transfer (Fig. 4A). This result was surprising because our mEPSC data from neonates showed sufficient extracellular glycine to potentiate mEPSCs. In an attempt to potentiate the NMDA-mediated response, we next included glycine (100 μM) in the puffer solution. Similarly, we did not observe an increase in charge transfer after application of NFPS (Fig. 4B). Finally, we recorded the response in the presence of bath-applied glycine (1 mM) followed by NFPS and found a significant increase in charge transfer (Fig. 4C). In the presence of exogenously applied glycine, NFPS application increased the NMDA charge transfer by 72 ± 37% (Fig. 4D, P < 0.05, n = 7).

Immunohistochemistry

Figure 5 illustrates the labeling of GLYT1 and -2 in neonate and juvenile rats. Transverse sections of brain stem, containing the hypoglossal nucleus show GLYT1 and -2 labeling in both age groups (A, C, E, and G). Labeling shows intense labeling of both transporters in the hypoglossal nucleus, while the adjacent dorsal motor nucleus of the vagus is devoid of such labeling. This result is consistent with those described by O’Brien and Berger (2001) who showed strong glycine receptor labeling in the hypoglossal nucleus and lack of labeling in the dorsal motor nucleus of the vagus. Figure 5, B and D, illustrates GLYT1 immunohistochemistry at higher magnification and show diffuse labeling surrounding HMs in both neonate and juvenile. The diffuse and uniform distribution of GLYT1 throughout the hypoglossal nucleus is consistent with glial localization. GLYT2 labeling shows intense labeling throughout the nucleus (Fig. 5, F and H). This labeling is consistent with its localization at glycineric synaptic boutons.
We have investigated four modes of NMDAR-mediated synaptic transmission. Our results indicate that even with reduced extracellular glycine levels by blocking glycine uptake results in a significant increase in NMDA charge transfer after NFPS application (94 ± 4% of control; n = 5, middle bar). In the presence of exogenously applied glycine (1 mM), NMDA-induced responses were significantly potentiated by 72 ± 39% of control after NFPS application (n = 7).

(Geiman et al. 2002; Zafra et al. 1995a). Both GLYT1 and -2 labeling become more intense with postnatal age.

**D I S C U S S I O N**

Our results clearly show that the glycine binding site of NMDARs in HMs is not saturated in the brain stem slice. We have used the GLYT1 antagonist NFPS to show that elevating extracellular glycine levels by blocking glycine uptake results in a potentiation of NMDAR-mediated synaptic transmission. We have investigated four modes of NMDAR-mediated synaptic transmission. Our results indicate that even with reduced levels of synaptic glycine release (in the presence of TTX), blocking glycine uptake resulted in a significant increase in NMDAR charge transfer. These results indicate that GLYT1 is an important modulator of NMDAR-mediated synaptic transmission and is necessary to maintain glycine at subsaturating concentrations.

A recent study by Chen et al. (2003), reports a maximum 15% increase in NMDAR response after NFPS application in the absence of extracellular glycine in prefrontal cortex. In contrast, we found that NFPS at a concentration of 500 nM resulted in an approximate 40% increase in the NMDAR response. This difference may be due to high intrinsic availability of glycine in the hypoglossal motor nucleus which has a large glycinergic innervation within the brain stem (Rampon et al. 1996). Consistent with this finding is the high rate of glycinergic spontaneous activity in the hypoglossal motor nucleus (sEPSCs; 2.4 Hz). Our results show a similar increase in the NMDAR response after NFPS application to those reported in the hippocampus, a region where GABA rather than glycine is used as an inhibitory neurotransmitter (Bergeron et al. 1998). There are five isoforms of GLYT1, 1a–1e (Adams et al. 1995; Borowsky and Hoffman 1998; Borowsky et al. 1993; Hanley et al. 2000; Kim et al. 1994), that are all blocked by NFPS (Aubrey and Vandenberg 2001). It may be possible that these isoforms have different regional expression and/or different affinities for glycine, which may account for differences in the degree of potentiation between different brain regions. Furthermore, the density of GLYT1 at each site may have an impact on the magnitude of potentiation. Differences in the degree of potentiation between different brain regions has implications for the use of transporter antagonists as therapies for numerous disease states.

Recent research has shown that GLYT1 is essential for survival (Gomeza et al. 2003). GLYT1 knockout mice die within 14 h of birth and display significant motor and respiratory problems. In GLYT1 knockout mice, it appears that elevated glycine levels result in the tonic activation of glycine receptors. This tonic activation of glycine receptors decreases input resistance and may contribute to inhibition of rhythmic network activity through shunting inhibition (Gomeza et al. 2003). This research suggests that during early development the regulation of glycine concentration by GLYT1 has a significant impact on glycinergic synaptic transmission. Evidence shows that the role of GLYT1 in modulating glycine levels is complex and influences both NMDAR- and glycine receptor-mediated synaptic transmission (Bergeron et al. 1998; Chen et al. 2003; Gomeza et al. 2003).

The m-, s-, and eEPSC data suggest synaptic NMDARs of HMs are closely localized to the sites of glycine release. Thus glycine may spill over from such sites to activate neighboring NMDARs. Classically, neurotransmitter spillover was thought to indicate a lack of specificity that would compromise a synapse’s ability to be modulated (Barbour and Hausser 1997). However, recent research currently suggests spillover is necessary to prolong synaptic currents (Carter and Regehr 2000), promote transmitter release (Turecek and Trussell 2001), and inhibit neurotransmitter release (Dittman and Regehr 1997). Recent work in the spinal cord has shown synaptically released glycine can facilitate NMDAR currents by spillover (Ahmadi et al. 2003). The presence of high uptake glycine transporters can limit this interaction (Bergeron et al. 1998; Supplisson and Bergman 1997).

Our experiments, which applied brief puffs of NMDA to the soma of HMs, did not result in an enhancement of the NMDA response unless glycine was present extracellularly in the bathing solution. Whole cell currents activated by puffing NMDA are most likely comprised of a combination of both synaptic and extrasynaptic NMDARs. Our results suggest that extrasynaptic NMDARs may not be localized to regions of glycine release and after NFPS application a sufficient amount of glycine does not accumulate to potentiate NMDARs. Similarly in experiments where glycine was added to the puff electrode, NFPS was not able to potentiate the NMDAR response. It was expected that adding glycine to the electrode...
would result in an enhanced response following NFPS application. However, our results indicate that the transient elevation in glycine may diffuse quickly from NMDAR sites. Alternately, the kinetics of GLYT1 may not be fast enough to affect transient glycine concentrations such as that produced by puffing NMDA and glycine. When a high glycine concentration (1 mM) is present in the bath solution, NFPS significantly potentiates the NMDA response. In this condition there is steady state level of glycine present, which GLYT1 may be capable of locally regulating. Elevating this steady state level of glycine by preventing uptake by GLYT1 using NFPS results in potentiation of both synaptic and extrasynaptic NMDARs.

Both GLYT1 and -2 labeling were more diffuse in neonates than in juveniles. GLYT1 and -2 mRNA and protein levels increase during the first two postnatal weeks (Zafra et al. 1995b). These findings are consistent with our results, which show increased intensity of labeling of both GLYT1 and -2 with development. Our immunolabeling revealed a similar staining pattern for both GLYT1 and -2. Both transporters show labeling throughout the hypoglossal nucleus but not in the adjacent dorsal motor nucleus of the vagus. The extensive labeling of both transporters throughout the hypoglossal motor nucleus suggest that this region exhibits strong glycine regulation and that these transporters regulate glycine concentration together (Herdon et al. 2001; Jursky and Nelson 1996). GLYT1 and -2 are proposed to regulate glycine uptake at NMDAR synapses and glycine receptors, respectively. The similarity in labeling of GLYT1 and -2 in the hypoglossal motor nucleus, a region that expresses both NMDAR and glycine receptors, suggests that excitatory and inhibitory inputs are closely situated. The proximity of these inputs is a further indication that glycine can “spill over” to potentiate neighboring NMDARs.

The glycine affinity of the NMDAR is determined by the NR2 subunit, whereas the glycine binding site is located on the NR1 subunit (Danysz and Parsons 1998). The presence of the NR2A subunit confers a receptor with a low affinity for glycine ($EC_{50} \approx 2.1 \mu M$) (Mishina et al. 1993). It has previously been suggested by Berger et al. (1998) that it is the presence of the NR2A subunit that allows increased glycine concentrations to potentiate the NMDAR response. Increased extracellular glycine concentrations in response to blocking glycine uptake by NFPS, most likely activates NR2A subunits in HMs. In many brain regions NR2A subunit expression is low in neonates and increases during early postnatal development (Flint et al. 1997;
Monyer et al. 1994). In the mouse hypoglossal motor nucleus, there is a high expression of NMDAR ε1 subunit (mouse equivalent of NR2A) at a very early age, appearing at embryonic day 13 (Oshima et al. 2002). Our results show NFPS blocks glycine uptake sufficiently to result in potentiation of the NMDA response in both age groups. This result is consistent with findings that show NR2A expression throughout this developmental period. Further, our whole cell responses to NMDA suggest that extrasynaptic NMDARs in HMs most likely comprise NR2A subunits.

Previous research in the hypoglossal nucleus has shown the presence of dual-component mEPSCs (O’Brien et al. 1997). We have recorded both AMPAR and NMDAR responses in the presence of NFPS. We showed that NFPS did not affect the amplitude of AMPAR currents. Any changes were due to effects of NFPS on NMDAR-mediated transmission. An increase in the NMDAR response may be due to increased glutamate release. There is evidence that presynaptic glycine receptors increase glutamate release (Turecek and Trussell 2001). However, our recordings were performed in the presence of 2 μM strychnine, which would block strychnine-sensitive presynaptic glycine receptors. Therefore we conclude that the enhancement of the NMDAR response, results from increased glycine binding after treatment with NFPS.

The modulation of NMDAR responses by glycine has important physiologic function. Research to date has shown that the enhancement of the NMDAR response, results from increased glycine binding at NMDAR-ε1 receptors by preventing uptake, may provide an important modulatory effect in modulating NMDAR responses are replicated in vivo and in vitro.

ACKNOWLEDGMENTS

We thank Dr. E. Eggers and J. Sebe for reviewing the manuscript and Dr. W. Satterthwaite and P. Huynh for technical assistance. We thank the NPS Allelix Corporation for supplying NFPS.

GRANTS

This work was supported by National Institutes of Health Grants NS-14857 and HL-49657.

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