Pharmacological Characterization of Glycine-Gated Chloride Currents Recorded in Rat Hippocampal Slices

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Chattipakorn, Siriporn C. and Lori L. McMahon. Pharmacological characterization of glycine-gated chloride currents recorded in rat hippocampal slices. J Neurophysiol 87: 1515–1525, 2002; 10.1152/jn.00365.2001. An inhibitory role for strychnine-sensitive glycine-gated chloride channels (GlyRs) in mature hippocampus has been overlooked, largely due to the misconception that GlyR expression ceases early during development and to few functional studies demonstrating their presence. As a result, little is known regarding the physiological and pharmacological properties of native GlyRs expressed by hippocampal neurons. In this study, we used pharmacological tools and whole cell patch-clamp recordings of CA1 pyramidal cells and interneurons in acutely prepared hippocampal slices from 3- to 4-wk old rats to characterize these understudied receptors. We show that glycine application to recorded pyramidal cells and interneurons elicited strychnine-sensitive chloride-mediated currents (Igly) that did not completely desensitize in the continued presence of agonist but reached a steady state at 45–60% of the peak amplitude. Additionally, the inhibitory amino acid, taurine, which has been shown to activate GlyRs in other systems, activated GlyRs expressed by both pyramidal cells and interneurons, although with much less potency than glycine, having an EC50 10-fold higher. To examine the potential subunit composition of hippocampal GlyRs, we tested the effect of the GABA_A receptor antagonist, picrotoxin, on Igly recorded from both cell types. At low micromolar concentrations of picrotoxin (≤100 μM), which selectively block α-homeric GlyRs, Igly was partially attenuated in both cell types, indicating that α-homeric receptors are expressed by pyramidal cells and interneurons. At picrotoxin concentrations ≤1 mM, ~10–20% of the whole cell current remained, suggesting that αβ heteromeric GlyRs are also expressed because this subtype of GlyR is relatively resistant to picrotoxin antagonism. Finally, we examined whether hippocampal GlyRs are modulated by zinc. Consistent with previous reports in other preparations, zinc elicited a bidirectional modulation of GlyRs, with physiological zinc concentrations (1–100 μM) increasing whole cell currents and concentrations >100 μM depressing them. Furthermore, the same concentration of zinc that potentiates Igly suppressed currents mediated by the N-methyl-D-aspartate subtype of the glutamate receptor. Thus we provide a pharmacological characterization of native GlyRs expressed by both major neuron types in hippocampus and show that these receptors can be activated by taurine, an amino acid that is highly concentrated in hippocampus. Furthermore, our data suggest that at least two GlyR subtypes are present in hippocampus and that GlyR-mediated currents can be potentiated by zinc at concentrations that suppress glutamate-mediated excitability.

INTRODUCTION

Strychnine-sensitive glycine-gated chloride channels (GlyRs) are the major provider of neuronal inhibition in spinal cord and brain stem (Aprison 1990). However, in forebrain regions of mature CNS, such as hippocampus, a role for GlyRs in modulating neuronal excitability has been largely ignored. This is due in part to numerous reports from many brain regions demonstrating that fast synaptic inhibition is abolished by GABA_A receptor (GABA_A R) antagonists (Mody et al. 1994), implicating GABA_A Rs as the only ligand-gated ion channel responsible for mediating fast neuronal inhibition in brain, while in brain stem and spinal cord fast inhibition is accomplished by both GlyRs and GABA_A Rs working in conjunction (Chery and de Koninck 1999; Jonas et al. 1998; O’Brien and Berger 1999). Furthermore, a current perception exists that functional GlyR expression ceases following early postnatal stages of development that likely stems from too few studies demonstrating their presence. In fact it has been reported that functional GlyRs in hippocampus are not expressed beyond the second postnatal week (Ito and Cherubini 1991; but see Ye et al. 1999). However, in several animal models of epilepsy, exogenous application of glycine can depress seizure activity in hippocampus of adult rats (Cherubini et al. 1981; Seiler and Sarhan 1984), suggesting the interesting possibility that GlyRs may mediate this inhibitory influence of glycine and implying that GlyRs are expressed in mature hippocampus, challenging current perceptions.

The relative lack of attention given to GlyRs in hippocampus by investigators interested in neuronal inhibition is surprising in light of evidence that the machinery necessary for GlyR-mediated inhibition is documented to exist in hippocampus. For instance, synaptoneurosomes obtained from adult rat hippocampus contain glycine in concentrations similar to GABA (36 nmol GABA/mg protein vs. 42 nmol glycine/mg protein) and release of both inhibitory amino acids using Ca^{2+}-dependent and -independent mechanisms, suggesting the possibility of both vesicular and transporter-mediated release (Burger et al. 1991; Engblom et al. 1996). Additionally, taurine, a known GlyR agonist in other systems (Flint et al. 1998; Horikoshi et al. 1988; Hussy et al. 1997) that is present in high concentrations in hippocampus (del Rio et al. 1987; Saransaari and Oja 1994, 1997), can protect against excitotoxic cell death and depress pyramidal cell excitability (French et al. 1986; Taber et al. 1986). Presumably these effects of taurine in hippocampus are mediated via activation of GlyRs; however, this mechanism has never been tested. In situ hybridization and immunohisto-
potentiate found that hippocampal GlyRs are bidirectionally modulated not only GABA A Rs but also GlyRs are important providers of functions facilitate neuronal inhibition. Our

Electrophysiology

Hippocampal slices were prepared from 3- to 4-wk-old Sprague-Dawley rats as previously described (McMahon and Kauer 1997b) and maintained in a submersion holding chamber at room temperature. Artificial cerebrospinal fluid (ACSF) was used for slice preparation and recording and contained (in mM) 119 NaCl, 2.5 KCl, 2.5 CaCl2, 1.3 MgSO4, 1 NaH2PO4, 26 NaHCO3, 10 glucose, and 1 kynurenic acid, and was saturated with 95% O2-5% CO2 (pH: 7.4). For experiments, slices were placed in a submersion recording chamber and continually perfused with ACSF at 2–3 ml/min at 28–30°C. Whole cell recordings of visually identified CA1 pyramidal cells and GABAergic interneurons in s. radiatum were obtained using an Olympus BX50WI fixed-stage microscope with IR-DIC optics. Patch electrodes had resistances between 4 and 6 MΩ and were filled with (in mM) 100 CsCl or 100 Cs-glucolate, 0.6 EGTA, 5 MgCl2, 2 ATP-Na2, 0.3 GTP-Na, and 40 HEPES, pH: 7.2, 260–270 mOsm. In some recordings, 5 mM QX 314 was added to the internal solution to block voltage-dependent Na+ channels and to enhance space-clamp. Cells were held at −70 mV, except where noted.

An Axoclamp 2A amplifier was used to amplify current signals, and the output was continuously monitored on an oscilloscope and Gould chart recorder. Data were filtered at 3 kHz, stored on tape using a Vetter PCM data recorder, and analyzed off-line. Cell input resistance and series resistance (≥18 MΩ) were continually monitored throughout the recording using software written in Labview and kindly provided by Dr. Richard Mooney (Duke University). Experiments were terminated when these values increased by ≥20%. Recorded neurons were identified as pyramidal cells or interneurons by their electrical properties (e.g., input resistance, firing pattern). In addition, neurons were filled with 0.4% biocytin and processed to allow for post hoc neuronal identification (McManon and Kauer 1997a,b; McMahon et al. 1998). Biocytin filled cells were viewed at the light level to confirm cell identity as pyramidal cells versus interneurons and images were captured with digital microscopy. Representative images of a recorded pyramidal cell and interneuron are shown in Fig. 1B.

Drug delivery

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO). Agonists and antagonists were prepared as stock solutions and diluted to appropriate concentrations in the re-
Glycine (or taurine) was most often applied to recorded cells via a picrospritzer with the drug-containing pipette placed within 100 μM of the recorded cell. Agonists were also applied by bath perfusion of a fixed concentration (2–3 ml/min) or by a drug pipette (100 μM from the cell) connected to a valve system which permits switching between drug solutions (4–5 ml/min) for obtaining dose-response measurements. This latter method permits faster time to peak and recovery of responses than the standard bath perfusion method (bath exchange within 30 s rather than 1–2 min so peak response occurs within 20–30 s), although the faster bath exchange did not alter the amount of desensitization as measured by the peak versus steady-state current amplitude. The glycine concentration (300 μM) used in this study was chosen because it elicits robust responses facilitating characterization of the currents and is near the glycine EC50 concentration for these receptors as shown in Fig. 2A. Note that in recordings in brain slices, the exact concentration of the agonist that the receptors actually “see” is not known for sure because there are powerful re-uptake mechanisms in both neurons and glial cells that cannot be accounted for. Thus the EC50 concentration we observe in slices is higher than that reported for GlyRs recorded in dissociated hippocampal cultures (40–72 vs. 270 μM) (Shirasaki et al. 1991; Ye et al. 1999; Yoon et al. 1998). This discrepancy in agonist EC50 concentration between culture and slice preparations have been previously observed (Kaneda et al. 1995). Additionally, the modest speed in which agonists can be applied to recorded neurons in slices and the varying distances these cells are from the slice surface means that receptor desensitization will likely take place during the rising phase of the response that will affect the amplitude of the current; however, the extent of the desensitization is unknown (Kaneda et al. 1995).

Data analysis

Data were normalized to the maximum current for each cell. Reported mean and standard error were determined from normalized data. Glycine and taurine dose-response relationships were fit to the Hill equation \( I = I_{\text{max}}/1 + (\text{EC50/agonist})^n \), where \( I \) is the peak current for a given agonist concentration, \( I_{\text{max}} \) is the current at the maximal agonist concentration, \( \text{EC50} \) is the concentration of agonist...
GlyRs are functionally expressed in the CA1 region of rat hippocampus

To demonstrate that GlyRs are expressed by rat hippocampal neurons beyond the early stages of postnatal development, we obtained whole cell recordings of CA1 pyramidal cells and s. radiatum interneurons in hippocampal slices prepared from 3- to 4-wk-old animals and used GlyR immunohistochemistry to anatomically localize these receptors. In immunohistochemical staining experiments using the monoclonal anti-GlyR antibody (mAb 4a), which recognizes α1, α2, and β subunits of GlyRs (Pfeiffer et al. 1984), we consistently observed specific labeling of cell bodies and dendrites of both pyramidal cells and GABAergic interneurons located throughout all CA1 cell layers (Fig. IA). An absence of staining was observed when the primary antibody was omitted from the reaction (Fig. IA). These findings indicate that GlyRs are strongly and widely expressed by both pyramidal cells and interneurons in hippocampus. In Fig. IB, we show that short pulses (3 s) of glycine (300 μM) pressure-applied to a recorded pyramidal cell and interneuron elicited inward currents (Igly; ECl = 0 mV) that developed over a few seconds and decayed to baseline within 10 s following cessation of glycine application. Responses were observed in 100% of recorded pyramidal cells (n = 30) and interneurons (n = 40) and ranged in amplitude from 120 to 420 pA (240 ± 46 pA) in pyramidal cells. In strychnine or picrotoxin inhibition plotted to 10 mM glycine required for a half-maximal response, and IC50 is the concentration of strychnine or picrotoxin required to block glycine by 50% and n is the Hill coefficient (slope factor). Strychnine or picrotoxin inhibition were performed using Origin 5.0 or IGOR Pro 6.0 software. Data were expressed as means ± SE (pyramidal cells, n = 6; and interneurons, n = 6). Currents were normalized to the peak amplitude of the response at 1 mM glycine. Each point is the mean ± SE. Dose-response data were fit with the Hill equation (see METHODS). A, left: representative traces of Igly recorded from an interneuron at increasing glycine concentrations. Glycine was applied via a drug pipette (see METHODS) for a duration of 1 min and at increasing concentrations as noted. Glycine was reapplied at 1-min intervals. A, right: plot compares glycine dose-response curve recorded from pyramidal cells (n = 6) and interneurons (n = 6). B: control.
midal cells and 160 to 1,600 pA (782 ± 142 pA) in interneurons. Bath application of the GABA_A antagonist bicuculline (10 μM; n = 8) had no effect on I_gly. However, strychnine (1 μM) reversibly depressed or abolished I_gly (n = 10), reaching maximal block within 10 min. A partial recovery was obtained following a 30- to 45-min washout of the antagonist. These data indicate that functional strychnine-sensitive GlyRs are indeed expressed by pyramidal cells and interneurons beyond the second postnatal week of development, challenging a previous report (Ito and Cherubini 1991).

To further characterize the sensitivity of GlyRs to glycine and strychnine, we recorded dose-response curves from both pyramidal cells and interneurons (Fig. 2). Normalized dose-response curves demonstrate that GlyRs expressed by pyramidal cells and interneurons respond similarly to glycine (EC_{50} = 270 μM; Hill coefficient n = 1.4–1.9; Fig. 2A, right), although the mean current amplitude recorded at any given glycine concentration is 30–40% larger (38 ± 4% at 300 μM, approximate EC_{50}) in interneurons compared with pyramidal cells (data not shown). This finding together with a larger GlyR-induced decrease in cell input conductance in interneurons (on average a decrease of 14% in pyramidal cells vs. 30% in interneurons) suggests a higher density of somatic GlyRs expressed by these cells. Furthermore, we observed that I_gly recorded from both pyramidal cells and interneurons only partially desensitize during the 1-min exposure to glycine (concentrations ≤1 mM, Fig. 2A, left) and reach a steady-state level of conductance, 59 ± 10% of peak for pyramidal cells (n = 4) and 47 ± 19% of peak for interneurons (n = 4; values not significantly different; calculated at 300 μM glycine, approximate EC_{50}). Even with continuous glycine exposure ≤10 min, I_gly did not completely desensitize back to baseline, but continued at the steady-state conductance level reached at 1 min of exposure (data not shown).

Current-voltage relationship of glycine-evoked currents

Next, we sought to confirm that I_gly is carried by chloride ions (Fig. 3). The reversal potential for I_gly recorded from pyramidal cells (n = 4) and interneurons (n = 11) was −2.1 ± 0.7 and −0.7 ± 0.4 mV, respectively, when cells were recorded with 110 mM internal chloride (calculated Nernst potential for 110 mM [Cl\textsuperscript{−}] is −3.5 mV). In cells recorded with 10 mM [Cl\textsuperscript{−}], the I_gly reversal potential was −45.1 ± 1.7 mV for pyramidal cells (n = 4) and −41.1 ± 3.5 mV for interneurons (n = 7; calculated Nernst potential for 10 mM internal chloride is −63.9 mV). The ~40–45 mV shift observed in the I_gly reversal potential with a 100 mM increase in the internal chloride concentration is consistent with I_gly being carried by chloride ions and is near the predicted change in the reversal potential. The inexact shift between the observed and predicted values is likely do either to incomplete dialysis of the pipette solution with the cytosol of the recorded neuron or a result of varying activity of efficient chloride co-transporters having

![Current-voltage relationship of glycine-evoked currents](image-url)
differing activities depending on internal chloride concentration (DeFazio et al. 2000).

**Taurine is an agonist at GlyRs in hippocampus**

The inhibitory amino acid, taurine, is an agonist at glycine receptors in other systems (Flint et al. 1998; Hussy et al. 1997; Schmieden et al. 1992). Taurine has been shown to depress pyramidal cell excitability (Taber et al. 1986) and have an antiepileptic activity (Cherubini et al. 1981; Seiler and Sarhan 1984), although the mechanism underlying these effects is unknown. Thus we are interested to determine whether taurine also activates native GlyRs expressed in hippocampal neurons because this could be the mechanism by which taurine mediates an inhibitory effect. We obtained whole cell patch-clamp recordings from interneurons (*n* = 5) and observed large inward currents (*I*taurine*)* 400–600 pA) following application of taurine (1 mM, 3 s). One millimolar taurine was used in these experiments because this concentration was close to the EC50 (3.5 mM) as seen in Fig. 4, and experiments because this concentration was close to the EC50 (3.5 mM) as seen in Fig. 4, C and D. To ensure that *I*taurine* is due to activation of GlyRs and not to activation of GABA<sub>A</sub>Rs, taurine was applied in the presence of 10 μM bicuculline. Bicuculline had no effect on *I*taurine* while strychnine (1 μM) reversibly blocked this current (Fig. 4A).

To compare the activation of GlyRs by glycine and taurine, equimolar concentrations of glycine and taurine (300 μM) were bath applied to the same recorded cell (*n* = 6; Fig. 4B). We found that glycine induced larger inward currents (800–2,000 pA) than taurine (60–110 pA) at the same concentration. Additionally, in dose-response experiments, the taurine dose-response curve was shifted to the right of that for glycine with an EC50 of taurine (3.54 mM) 10-fold higher than the EC50 for glycine (0.27 mM) (Fig. 4D). These data demonstrate that taurine is an agonist at GlyRs but has a lower affinity for these receptors than glycine.

**Hippocampal GlyRs are blocked by picrotoxin**

In situ hybridization studies indicate that mRNA encoding α2, α3, and β subunits is present in hippocampus (Malosio et al. 1991), suggesting the possibility that hippocampal neurons may express more than one GlyR subtype. Furthermore, the possibility exists that hippocampal neurons may express both α homomeric receptors, the immature extrasynaptic GlyR subtype and αβ heteromeric GlyRs, the mature form of the receptor that is synaptically located (Becker et al. 1988; Kungel and Friauf 1997; Malosio et al. 1991; Takahashi et al. 1992). To pursue whether pyramidal cells and interneurons in acute slices at this stage of maturity express α homomeric and/or αβ heteromeric GlyRs, we tested whether the GABA<sub>A</sub>R antago-

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**Fig. 4.** Taurine is an agonist at GlyRs. A: taurine-mediated currents (*I*taurine*)* induced by a 3-s pressure application of 1 mM taurine (↓) is completely unaffected by 10 μM bicuculline but reversibly blocked with 1 μM strychnine (n = 4). B: comparison of *I*gly* (300 μM glycine bath applied, 1 min) and *I*taurine* (300 μM taurine bath applied, 1 min) recorded from the same s. radiatum interneuron (n = 4). C: representative traces of *I*taurine* recorded from an interneuron with increasing taurine concentrations. Taurine was bath applied for 1 min at the concentrations as noted and at an interval of 1 min. D: plot compares the dose-response relationship of glycine (*n* = 6) and taurine-mediated currents (*n* = 6). The taurine dose-response curve is shifted to the right of the glycine curve indicating that taurine is a less potent agonist at GlyRs in hippocampus. Currents are normalized to the peak amplitude of the response at the highest concentration of glycine (1 mM) and taurine (30 mM). Each point is the mean ± SE. In some cases, the error bars are with the symbol. Dose-response data were fit with the Hill equation (see METHODS). In all experiments, cells were held at *V*<sub>h</sub> = −70 mV. In A, C, and D, cells were recorded with a CsCl pipette solution; in B, cells were recorded with a Cs-gluconate pipette solution.
nistent, picrotoxin (1–1,000 μM) could suppress $I_{\text{gly}}$, taking advantage of the knowledge that the presence of the $\beta$ subunit in functional GlyRs renders these receptors insensitive to blockade by low micromolar concentrations of the antagonist (Pribilla et al. 1992). This antagonist was shown in a previous study of heterologously expressed GlyRs (Pribilla et al. 1992) to inhibit $\alpha$ homomeric receptors but not $\alpha\beta$ heteromeric GlyRs at $\leq$10 μM picrotoxin (equivalent to 20 μM picrotoxin). Additionally, this strategy has been used to demonstrate receptor heterogeneity in Mauthner cells (Legendaire 1997) and cultured neonatal hippocampal neurons (Yoon et al. 1998) where the authors found that low concentrations of picrotoxin ($\leq$50 μM or its equivalent) selectively blocked homomeric GlyRs but not heteromeric receptors. The presumed heteromeric receptors were not completely blocked at a concentration of 1 mM. As shown in Fig. 5, we observed that at picrotoxin concentrations $\leq$100 μM, $I_{\text{gly}}$ was partially depressed (≤50% of peak) in both cell types (Fig. 5A), consistent with the presence of $\alpha$ homomeric GlyRs. However, even at 1 mM picrotoxin, complete suppression of $I_{\text{gly}}$ was not achieved in pyramidal cells ($n = 10$) or interneurons ($n = 11$), with some cells, >20% of the peak amplitude still remaining. This finding is consistent with the expression of $\alpha\beta$ heteromeric receptors by these cells as well. Figure 5B shows a plot of the relationship between the peak of $I_{\text{gly}}$ (normalized to the maximum) and the picrotoxin concentration. Inhibition curves were fit to these data and yielded a picrotoxin IC$_{50}$ of 37.1 μM for pyramidal cells and 83.6 μM for interneurons (concentrations not significantly different, $P \geq 0.05$). In these experiments, we observed a large variability in the amount of block at any given picrotoxin concentration between neurons in both cell types, as indicated by the error bars in the dose-inhibition plot (Fig. 5B). Additionally, the blockade of $I_{\text{gly}}$ elicited by picrotoxin was not use dependent and a steady-state block could be observed following only a 10-min exposure of the slice to picrotoxin. Thus the partial blockade of $I_{\text{gly}}$ at low concentrations of picrotoxin and the lack of a complete block at 1 mM picrotoxin is implies that both $\alpha$ homomeric and $\alpha\beta$ heteromeric GlyRs are expressed by single neurons in hippocampus.

Bidirectional modulation of hippocampal GlyRs by zinc

The divalent cation zinc modulates many ligand-gated ion channels including GlyRs. Several studies show that GlyRs in forebrain regions are modulated by zinc. For example, zinc depresses $I_{\text{gly}}$ in cerebellar neurons (Virgino and Cherubini 1997) and septal cholinergic neurons (Kumamoto and Murata 1996), but bidirectionally modulates (facilitates as well as depresses) $I_{\text{gly}}$, depending on the zinc concentration, in spinal cord neurons (Bloomenthal et al. 1994; Laube et al. 1995) and retinal ganglion cells (Han and Wu 1999). We sought to determine whether native GlyRs in hippocampus are modulated by zinc and to investigate whether the modulation of $I_{\text{gly}}$ by zinc is similar or different to the well-characterized zinc modulation of $I_{\text{NMDA}}$. Short pulses (3 s) of 300 μM glycine ($n = 20$) or 300 μM NMDA ($n = 6$) were applied to recorded interneurons in the absence (control) and presence of increasing concentrations of zinc (1 μM to 1 mM) included in the extracellular solution. As shown in Fig. 6, the peak amplitude of $I_{\text{gly}}$ was increased 14 ± 2% ($n = 15$) and 27 ± 2% ($n = 10$) in the presence of 1 and 10 μM zinc, respectively. However, at 100 μM and 1 mM zinc, $I_{\text{gly}}$ was depressed 17 ± 4% ($n = 11$) and 52 ± 8% ($n = 4$), respectively, compared with control. In contrast, $I_{\text{NMDA}}$ was depressed by all zinc concentrations applied (1–100 μM). Moreover, the effects of zinc on $I_{\text{gly}}$ and $I_{\text{NMDA}}$ at all zinc concentrations were significantly different ($P < 0.01$) as shown in Fig. 6B. Our findings show that zinc modulation of $I_{\text{gly}}$ is bidirectional, with low concentrations of zinc potentiating and high concentrations depressing $I_{\text{gly}}$. Additionally, we show that zinc concentrations that potentiate $I_{\text{gly}}$ deppress $I_{\text{NMDA}}$.

DISCUSSION

Our data convincingly show that both major neuronal cell types in hippocampus, the excitatory pyramidal cells and inhibitory GABAergic interneurons, express GlyRs. Moreover, we find that GlyRs do not completely desensitize during pro-
longed glycine application. This finding indicates that when glycine levels increase in CSF for extended periods of time, as occurs following seizures and ischemia, activated GlyRs will continue to conduct current (Andine et al. 1991; Castillo et al. 1996; Sherwin 1999). The data presented in this study suggest the interesting hypothesis that activation of GlyRs, in conjunction with GABA_ARs, may provide an underappreciated, fundamental inhibitory mechanism in hippocampus that will modulate neuronal excitability. In fact, activation of these receptors may be the mechanism underlying the ability of glycine and taurine to depress seizure activity in some animal models of epilepsy (Cherubini et al. 1981; Seiler and Sarhan 1984).

Is taurine a ligand acting at hippocampal GlyRs?

Taurine is an abundant amino acid in hippocampus (del Rio et al. 1987; Saransaari and Oja 1994, 1997) and interestingly, taurine is released in high levels during conditions that elicit excitotoxic cell death (Saransaari and Oja 1994, 1997; Schurr et al. 1987). Taurine has been reported to counteract excitotoxicity (Saransaari and Oja 1998a,b) and has been shown to hyperpolarize hippocampal neurons (Taber et al. 1986). However, the mechanisms underlying these actions of taurine are presently unknown. Several studies have shown that taurine activates heterologously expressed GlyRs and native GlyRs expressed in hypothalamus and cerebral cortex (Flint et al. 1998; Hussy et al. 1997; Schmieden et al. 1992). In this study, we have shown that taurine can activate hippocampal GlyRs. The action of taurine at inhibitory GlyRs could be the mechanism responsible for taurine’s ability to protect against excitotoxic cell death because GlyR activation depresses neuronal excitability (Chattipakorn and McMahon 2000; Taber et al. 1986). This mechanism would counteract the pathological excitation elicited by glutamate at NMDA receptors.

Studies of GlyRs in heterologous expression systems has demonstrated that α1-containing GlyRs are more efficiently gated by taurine than α2-containing GlyRs (Schmieden et al. 1992). Our data show that taurine is less potent than glycine at activating GlyRs in hippocampal neurons, suggesting that GlyRs in mature hippocampus possibly contain α2 rather than α1 subunits. This interpretation is supported by an in situ hybridization study suggesting that the α2-to-α1 subunit switch during development does not occur in hippocampus as it does in spinal cord and brain stem (Malosio et al. 1991).

Picrotoxin antagonism suggests multiple GlyR subtypes

GlyRs in mammalian CNS are formed by a combination of five membrane spanning protein subunits, and as with other ligand-gated ion channels, the physiological and pharmacological properties of GlyRs is dependent on the subunit combination. Native GlyRs in spinal cord and brain stem are either α homomeric GlyRs (immature, extrasynaptic subtype) or αβ heteromeric GlyRs (mature, synaptic subtype) (Becker et al. 1988; Kungel and Friauf 1997; Malosio et al. 1991; Takahashi et al. 1992). Fortunately, the GABA_A antagonist picrotoxin is a useful tool in differentiating between homomeric and heteromeric GlyRs because the presence of the β subunit in heteromeric receptors confers picrotoxin resistance (Pribilla et al. 1992). In situ hybridization studies indicate that hippocampal neurons primarily contain mRNA encoding α2, some α3 (and not α1) and β subunits (Malosio et al. 1991). Therefore we used picrotoxin to test the possibility that some fraction of the receptors expressed by hippocampal neurons is α2 homomers and that both pyramidal cells and interneurons express these receptors. Low concentrations of picrotoxin (<100 μM) partially blocked the I_gly recorded from pyramidal cells and interneurons, suggesting that a subpopulation of the GlyRs expressed by both cell types are likely to be α2 homomeric receptors. The unblocked current at the lower picrotoxin concentrations may be the result of activation of another GlyR subtype, likely αβ heteromeric GlyRs, which have a much lower sensitivity to picrotoxin (Pribilla et al. 1992). This interpretation is consistent with a recently published report in dissociated hippocampal cultures (Yoon et al. 1998). The finding of possible αβ heteromeric GlyRs in hippocampus raises the question that some GlyRs may be synaptically located because of evidence showing that the β subunit of GlyRs is required for receptor clustering (Kirsch et al. 1993; Meyer et al. 1995).
**Zinc modulation of hippocampal GlyRs**

Zinc is abundant in forebrain regions, including hippocampus, where it is especially concentrated in mossy fibers (Assaf and Chung 1984; Howell et al. 1984). Zinc is contained in vesicles with glutamate, is co-released on membrane depolarization (Assaf and Chung 1984; Howell et al. 1984), and is estimated to reach 300 μM at the synaptic cleft during strong stimulation (Frederickson et al. 1983). At physiological concentrations, zinc has been shown to modulate ligand-gated ion channels. For example, zinc significantly inhibits NMDA receptor function, slightly potentiates the function of non-NMDA receptors (Westbrook and Mayer 1987), and blocks certain subtypes of GABA<sub>A</sub> receptor channels (GABA<sub>A</sub>Rs) (Westbrook and Mayer 1987). Although GlyRs and GABA<sub>A</sub>Rs are highly homologous, zinc concentrations <100 μM elicit opposing effects on hippocampal GlyRs and GABA<sub>A</sub>Rs, potentiating GlyRs (present report) but depressing GABA<sub>A</sub>Rs (Westbrook and Mayer 1987).

Zinc modulation of GlyRs is variable and the specific zinc effects on GlyR function depend on the brain region in which the receptors are expressed. For example, zinc depresses I<sub>gly</sub> in rat cerebellar granular cells (Virginio and Cherubini 1997) and rat septal cholinergic neurons (Kumamoto and Murata 1996). However, zinc modulation of I<sub>gly</sub> in rat embryonic spinal cord, human GlyR α1 and GlyR α2 expressed in HEK 293 cells and *Xenopus* oocytes (Bloomenthal et al. 1994; Laube et al. 1995) and isolated retinal cells (Han and Wu 1999) is bidirectional, eliciting facilitation and depression depending on the zinc concentration.

The data presented in this paper demonstrate that zinc modulates native GlyRs expressed by hippocampal neurons and that this modulation is bidirectional. At low concentrations, zinc potentiates I<sub>gly</sub> and at high concentrations suppresses these currents. The zinc concentrations eliciting potentiation and suppression of GlyRs in our study are similar to previous studies in other brain regions. Interestingly, we show that zinc concentrations (<100 μM) that potentiate I<sub>gly</sub> suppress I<sub>NMDA</sub> recorded from hippocampal neurons. These results suggest that a low concentration of zinc should increase inhibition of hippocampal circuits via its differential effects on I<sub>gly</sub> and I<sub>NMDA</sub>, which may contribute to the control of excitability in hippocampus.

**Are GlyRs involved in fast inhibitory transmission in hippocampus?**

The answer to this question is presently unknown. In spinal cord and brain stem, glycine and GABA are co-released from inhibitory interneurons and simultaneously activate GlyRs and GABA<sub>A</sub>Rs located in the postsynaptic density (Jonas et al. 1998; O’Brien and Berger 1999). Elegant electrophysiological recordings from motoneurons in spinal cord and brain stem slices show that miniature IPSCs are mediated by the activation of both receptors, demonstrating cotransmission of these two inhibitory neurotransmitters (Jonas et al. 1998; O’Brien and Berger 1999). A recent study in cerebellar slices has also demonstrated GABA and glycine co-release at synapses onto Golgi cells (Dumoulin et al. 2001). However, in recordings from identified lamina I neurons in spinal cord slices, it appears that although glycine and GABA are coreleased, GABA<sub>A</sub>Rs seem to be located extrasynaptically because the GABA<sub>A</sub>R-mediated component of the synaptic current is observed only following large but not minimally evoked monosynaptic IPSCs (Chery and de Koninck 1999).

These studies raise important questions as to whether glycine and GABA could be coreleased from GABAergic interneurons in hippocampus and as to the synaptic versus extrasynaptic location of the GlyRs expressed by pyramidal cells and interneurons. In hippocampus, GABA<sub>A</sub>R antagonists are reported to block IPSCs, indicating that GABA<sub>A</sub>Rs, but not GlyRs, are located synaptically (Mody et al. 1994). However, GlyRs could be located just outside the synapse, similarly to the distribution of GABA<sub>A</sub>Rs in spinal cord as discussed in the preceding text. Although we have not yet investigated the presence of GlyR-mediated synaptic currents, we speculate that GlyRs in hippocampus are located at both synaptic and extrasynaptic sites. Our picrotoxin data support this idea because it suggests that αβ heteromeric receptors make up some portion of the GlyRs expressed by hippocampal neurons and the presence of the β subunit in functional GlyRs is required for synaptic clustering by the intracellular protein gephyrin (Kirsch et al. 1993; Meyer et al. 1995).

Finally, glycine transporters, GlyT1 and GlyT2, known to be responsible for terminating glycineergic transmission in spinal cord and brain stem, are present in hippocampus and are colocalized with GlyRs, indicating that they will modulate the activity at GlyRs by regulating the local glycine concentration (Jursky and Nelson 1995; Klancnik et al. 1992). The source of glycine in hippocampus is unknown however the fact that glycine release is partially calcium and action potential dependent strongly indicates that some glycine is of neural origin (Engblom et al. 1996; Klancnik et al. 1992).

In summary, we propose that GlyRs participate in an inhibitory mechanism in hippocampus, modulating neuronal activity. We speculate that specifically enhancing GlyR activity, similarly to increasing GABA<sub>A</sub>R activity, could be beneficial in depressing hyperexcitability that ensues in epilepsy, encouraging future investigations into the precise location and function of these understudied inhibitory receptors.

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