Electrophysiological Evidence for Expression of Glycine Receptors in Freshly Isolated Neurons from Nucleus Accumbens

GILLES MARTIN and GEORGE ROBERT SIGGINS
Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California
Received January 23, 2002; accepted May 13, 2002

ABSTRACT
In the course of studying N-methyl-D-aspartate (NMDA) receptors of the nucleus accumbens (NAcc), we found that 20% of freshly isolated medium spiny neurons, as well as all interneurons, responded in an unexpected way to long (5-s) coapplication of NMDA and glycine, the coagonist of NMDA receptors. Whereas the reversal potential of the peak NMDA current of this subset of neurons was still around 0 mV, the desensitizing current became outward at hyperpolarized potentials around −30 mV. A Cl⁻-free solution shifted the equilibrium potentials of the desensitized currents to around 0 mV. This outward current was not blocked by a Ca²⁺-free, Ba²⁺-containing solution, suggesting that the anionic conductance was not activated by Ca²⁺ influx through NMDA receptor channels. Interestingly, glycine alone also evoked a current with a similar hyperpolarized reversal potential in this subset of neurons. The glycine current reversed around −50 mV, rectified outwardly, and inactivated strongly. Its desensitization was best fitted with a double exponential. Only the slow desensitization showed clear voltage dependence. The glycine current was not blocked by 200 µM picrotoxin and 10 µM zinc, was weakly antagonized by 1 µM strychnine, and was not enhanced by 1 µM zinc. In addition, 1 mM taurine, but not GABA, inactivated glycine currents, and 1 mM glycine occluded 10 mM taurine-mediated currents. These data indicate that a subset of nucleus accumbens neurons expresses glycine receptors and that either glycine or taurine could be an endogenous agonist for these receptors.

The nucleus accumbens (NAcc), an interface region between limbic structures (hippocampus, amygdala, and prefrontal cortex) and the extrapyramidal motor system, modulates cognitive and motivational aspects of behavior that are translated into motor activity (Dunah et al., 1996). Projecting GABAergic neurons, also containing mostly either substance P or enkephalin, account for 90% of the neuronal population of NAcc (Meredith, 1999). These neurons receive massive dopaminergic input from the ventral tegmental area, glutamatergic input from cortical and subcortical regions, and GABA innervation from interneurons. The physiology of the receptors for these transmitters is relatively well known, because they have been heavily studied with regard to addiction to psychostimulants, opiates, and alcohol (Koob et al., 1996). NAcc-projecting neurons also receive inputs from acetylcholine- and somatostatin-containing interneurons (Groe newegen et al., 1991; Meredith, 1999), but relatively little is known about the physiology of receptors for these ligands in NAcc. Furthermore, some data suggest that other receptors, including glycine receptors, remain to be identified and characterized.

Glycine receptors, along with GABA receptors, represent the primary fast inhibitory mechanisms in the central nervous system. Activation of glycine receptors opens anionic channels that hyperpolarize neurons. Until recently, it was generally believed that glycine receptors were almost exclusively found in the spinal cord and brainstem of adult rats (Rajendra et al., 1997). However, recent findings suggest that these receptors may also be expressed in upper brainstem regions (Rampon et al., 1996). Recent studies also reported the presence of glycine receptors in forebrain structures (Yoon et al., 1998; Mori et al., 2002).

Glycine receptors are heteromultimeric receptors. Molecular approaches have revealed a certain diversity of glycine receptor subunits. To date, four different α and one β subunit have been identified (Legendre, 2001). Examination of the organization of glycine receptors revealed that glycine receptors are pentameric with an invariant stoichiometry of three α and two β subunits (Langosch et al., 1988). Although the expression of the β subunit matches that of α subunits in spinal cord, pons, and midbrain, there is evidence that β subunits in spinal cord, pons, and midbrain, there is evidence that β...
mRNA expression does not fully match that of α subunits in the brain (Fujita et al., 1991; Malosio et al., 1991). Thus, it was established that forebrain regions such as nucleus accumbens and striatum express β subunits, although in moderate levels (Fujita et al., 1991; Kirsch and Betz, 1993; Kirsch et al., 1993), but lack the α subunit (Sato et al., 1991; Malosio et al., 1991) that carries the binding site for glycine (Pfeiffer et al., 1982).

This absence of a systematic overlap between α and β subunit expression has prompted some investigators to suggest that additional α subunits, which could confer different properties for glycine receptors, remain to be discovered (Betz et al., 1999). It was also suggested that β subunits may form part of another receptor complex. Herein, we demonstrate for the first time in a subpopulation of NAcc neurons the existence of a functional receptor with electrophysiological properties reminiscent of glycine receptors but with a distinctive pharmacological profile.

Materials and Methods

Animals, Slice Preparation, and Experimental Solutions.

We used male Sprague-Dawley rats (100–200 g) to prepare NAcc slices. The rats were anesthetized with 4% halothane, decapitated, and the brains rapidly transferred into a cold (4°C) oxygenated, low-calcium HEPES-buffered salt solution: 334 mM sucrose, 2.5 mM KCl, 2 mM NaH2PO4, 11 mM glucose, 4 mM MgSO4, 2 mM CaCl2, and 1.5 mM HEPES. We glued a tissue block containing NAcc to a Teflon chuck and cut it transversally with a Vibroslicer (Campden Instruments, Loughborough, UK). Then we incubated the slices (400 μm in thickness) for up to 6 h at room temperature (20–22°C) in a gassed (95% O2 and 5% CO2) NaHCO3-buffered saline solution: 116.4 mM NaCl, 1.8 mM CaCl2, 0.4 mM MgSO4, 5.36 mM KCl, 0.89 mM NaH2PO4, 5.5 mM glucose, 24 mM NaHCO3, 100 mM glutathione, 1 mM nitro-arginine, and 1 mM kynurenic acid. pH was adjusted to 7.35 with NaOH (osmolarity 300–305 mOsm/l). After 1 h of incubation, we dissected out the region of the nucleus accumbens with the aid of a dissecting microscope. We incubated the tissue for 25 min in an oxygenated (100% O2 and constant stirring) HEPES-buffered solution in the inner chamber of a Cell-Stirr flask (Wheaton Instruments, Loughborough, UK). Then we incubated the slices (400 μm in thickness) for up to 6 h at room temperature (20–22°C) in a gassed (95% O2 and 5% CO2) NaHCO3-buffered saline solution: 116.4 mM NaCl, 1.8 mM CaCl2, 0.4 mM MgSO4, 5.36 mM KCl, 0.89 mM NaH2PO4, 5.5 mM glucose, 24 mM NaHCO3, 100 mM glutathione, 1 mM nitro-arginine, and 1 mM kynurenic acid. pH was adjusted to 7.35 with NaOH (osmolarity 300–305 mOsm/l). After 1 h of incubation, we dissected out the region of the nucleus accumbens with the aid of a dissecting microscope. We incubated the tissue for 25 min in an oxygenated (100% O2 and constant stirring) HEPES-buffered solution in the inner chamber of a Cell-Stirr flask (Wheaton, Millville, NJ) that contained papain (1 mg/ml) and 136 mM NaCl, 0.44 mM KH2PO4, 2.2 mM KCl, 0.35 mM NaH2PO4, 5.5 mM glucose, 10 mM HEPES, 100 mM glutathione, 1 mM nitro-arginine, 1 mM kynurenic acid, and 1 mM pyruvic acid (pH 7.35 with NaOH, osmolarity 300–305 mOsm/l). The temperature of this solution was kept constant (36°C) by a circulating water bath in the outer chamber of the flask (Stefani et al., 1994; Martin et al., 2002).

After enzymatic digestion, we transferred the tissue into a centri-fuge tube and rinsed it three to four times with the Na2-isethionate solution. We then filled the tube with 5 ml of Na2-isethionate solution and after 10 min, triturated the tissue using fire-polished Pasteur pipettes with successively smaller tip diameters. We plated the supernatant onto a 35-mm Petri dish placed on the stage of the inverted microscope. The cells were allowed to attach to the dish for 10 min before replacing the Na2-isethionate solution with normal external solution at a rate of 1.5 ml/min. This solution was composed of 142 mM NaCl, 2 mM KCl, 1 mM CaCl2, 23 mM glucose, 15 mM HEPES, and 10 mM glucose (pH 7.35 with NaOH, osmolarity 300 mOsm/l). The chloride-free solution was prepared by replacing NaCl with Na2-isethionate. Na2-free solution was prepared by substituting NaCl with N-methyl-d-glucamide; chloride was provided by HCl when the pH was readjusted. For study of the effects of Ba2+, we replaced CaCl2 with BaCl2.

Whole-Cell Recordings. We used standard whole-cell recording methods (Hamill et al., 1981). Briefly, we pulled patch electrodes from borosilicate capillary glass (Sutter Instruments, Novato, CA) on a Flaming-Brown puller (Sutter Instruments) to a final resistance of 1.8 to 2.2 MΩ. We filled the electrodes with a solution that consisted of 120 mM CsF, 10 mM CsCl, 11 mM EGTA, 10 mM HEPES, and 0.5 mM CaCl2 (pH 7.35 with CsOH; osmolarity 270–275 mOsm/l). The capillaries were first filled through the tip and then backfilled with the recording solution. We recorded in voltage-clamp mode with an Axopatch 1D amplifier and a DAC Digidata 1200 interface from Axon Instruments (Union City, CA). The signal was filtered at 5 kHz and digitized at 1 kHz. The series resistance was compensated (70–80%) only when the ramp protocol was used. We subtracted the NMDA current obtained with the ramp from the membrane response recorded in absence of NMDA and glycine. Potentials were not corrected for the liquid junction potential but are estimated to be +4 mV.

Superfusion and Drug Application. Control and drug-containing solutions were applied by gravity at a rate of 1.5 ml/s, using a rapid three-barrel capillary superfusion device (Warner Instrument, Hamden, CT) with the pipette tips placed about 200 μm from the recorded cell. The flow of solutions was controlled by solenoid valves. Each capillary had a tip diameter of 500 μm, and the distance from center to center was 700 μm. The barrel was attached to a motor, allowing fast lateral motions controlled by pClamp 6 (Axon Instruments), our data acquisition program. A drug onset time of 20 ms for the application system was determined by measuring the changes in the tip potential of the recording pipette filled with intracellular solution as the perfusion was switched from a normal to a 1:2 dilution of the extracellular recording solution.

Our standard drug-testing protocol was as follows. After recording a stable control current in ACSF alone, we superfused the cells with ACSF containing the drug for 2 to 3 min before recording. This was followed by a washout with the control ACSF. We examined the reversal potential of early NMDA currents by measuring the peak NMDA current amplitudes at various holding membrane potentials that were set manually between −70 and +20 mV with voltage steps of 10 mV. We also used a ramp protocol to determine the reversal potential of NMDA/glycine and glycine-desensitized currents. The membrane potential was held at −100 mV for 8 s, whereas the cells were superfused with the agonists and depolarized at a rate of 10 mV/s up to +40 mV.

Effects of zinc, bicuculline, and picROTOxin were tested by exposing the neurons with these drugs for at least 1 min before applying either GABA or glycine. Struckhine usually was coapplied with glycine although in some cells strychnine was applied before glycine. We purchased glycine, picrotoxin, taurine, ZnCl2, BaCl2, bicuculline, strychnine, and GABA from Sigma-Aldrich (St. Louis, MO). CGP 55845 is a gift from Novartis Pharma (Basel, Switzerland).

We fitted the glycine dose-response curve with a Hill equation as follows: 

\[
I = I_{\text{max}} / (1 + (EC_{50}/C)^n)
\]

where \(I, I_{\text{max}}, C, EC_{50}, \) and \(n\) are glycine-activated current, maximal glycine-activated current, glycine concentration, the concentration for 50% of maximum response, and the Hill coefficient, respectively. We measured the voltage-dependent glycine current desensitization using a linear regression formula in DeltaGraph 4.5 (SPSS, Chicago, IL).

Results

Freshly Isolated NAcc Neurons. Mechanical trituration after enzymatic treatment of NAcc slices yielded cells of various sizes. Most neurons had a small soma (Fig. 1, A–C) and only a small population had a larger cell body (Fig. 1, D and E). Neuronal shape also varied markedly; some neurons showed multipolar processes (Fig. 1B), whereas others were bipolar and ovoid (Fig. 1, A and C). Nevertheless, most of the small neurons recorded were likely to be medium spiny cells, because they represent up to 90% of the total number of cells in NAcc (Meredith and Totterdell, 1999). The larger neurons (Fig. 1D) are likely to be interneurons due to their size; few of
them are seen after an incubation of 30 min with papain (Bargas et al., 1994; Stefani et al., 1994). Only neurons presenting a smooth and shiny membrane were recorded, because these criteria usually correlate with healthy cells.

Currents Evoked by Coapplication of NMDA and Glycine. For 80% of medium spiny neurons tested (class I; \( n = 54 \)), coapplication of 200 \( \mu M \) NMDA and 100 \( \mu M \) glycine (the NMDA receptor coagonist) evoked characteristic NMDA currents that were inward at negative holding potentials and outward at positive ones (Fig. 2A), with the reversal potentials of both peak and desensitizing currents near 0 mV (Fig. 1B). Interestingly, in the remaining medium spiny neurons (class II; Fig. 2C), as well as in all large interneurons (Fig. 2E, arrow), an equivalent coapplication of NMDA and glycine evoked a transient inward current that reversed around 0 mV, followed by a desensitizing current that became outward around −30 mV. To determine the nature of this desensitized outward current, we examined its sensitivity to various ions. Because NMDA receptors can be coupled to calcium-dependent chloride channels (Scott et al., 1995), we studied the NMDA effects in a Cl\(^{-}\)-free solution that would weaken the NMDA inward currents opposing the outward current and thus shift the apparent reversal potential of the latter to the left. Although glycine currents were generally very stable, as shown by the experiments on current inactivation (see below), it is possible that exposure to Ba\(^{2+}\), which sequentially preceded the Na\(^{+}\)-containing solution, may prevent glycine currents from fully recovering.

Effects of Glycine Alone on Class II NAcc Neurons. We asked whether glycine alone could activate a similar outward current. At −70 mV, application of 100 \( \mu M \) glycine evoked an inward current that decreased at more depolarized holding potentials and reversed around −50 mV (Fig. 4A). Using a ramp protocol in 10 cells, the mean reversal potential of the glycine current was −50 ± 2 mV (−54 mV corrected for the liquid junction potential), a value close to the theoretical value for Cl\(^{−}\) given by the Nernst equation (−55.4 mV) with [Cl\(^{−}\)]\(_{o}\) of 145 mM and estimated [Cl\(^{−}\)]\(_{i}\) of 10 mM. This current rectified outwardly at both depolarized and hyperpolarized holding potentials (Fig. 4B). It is possible that part of the rectification may be due to poor space clamping or to the

Fig. 1. Phase-contrast micrographs of neuron types acutely isolated from the nucleus accumbens of 100- to 140-g rats. A to C, typical neurons representing the majority of isolated cells in NAcc. These had a relatively small soma and were either bipolar (A) or multipolar (B and C). D and E, neurons with a larger cell body believed to be a cholinergic interneuron. These neurons represent only a small fraction of isolated NAcc cells. Scale bars (D and E), 10 \( \mu m \).
participation of unblocked voltage-gated channels. It is also possible that fluoride, used in the recording pipette, passing through glycine receptors may contribute to some extent to the rectification. However, because the permeability of glycine receptors for this anion is the lowest of the six tested by Fathima-Shad and Barry (1989), its contribution is probably minimal.

We assessed the affinity of glycine for the receptor by examining currents evoked by 5-s epochs of glycine at increasing concentrations. The relationship between the peak current amplitude and glycine concentration (3 μM–1 mM) measured at 0 mV holding potential is shown in Fig. 5A (responses normalized to the peak current amplitude produced by 1 mM glycine). The experimental data are well fitted by a theoretical curve constructed from the Hill equation, with a Hill coefficient of 1.8 and an EC50 of 11 μM (Fig. 5B), suggesting that at least two glycine molecules are needed to activate each receptor. Although not systematically studied, we observed that the desensitization of the current was profoundly altered as the concentration of glycine increased (Fig. 5A). To characterize these receptors further, we measured the desensitization (decay of the current measured in continued presence of glycine) of glycine currents at several holding potentials from −70 to +10 mV. At all potentials (Fig. 6A; Vh = 0 mV), glycine-mediated currents desensitized very rapidly then returned close to base-
line levels within the 5-s application. The desensitization was best fitted with a double exponential (Fig. 6A), as also shown for hypothalamic neurons and Mauthner cells (Faber and Korn, 1987; Akaike and Kaneda, 1989). Between −80 and +10 mV, the mean fast and slow decay taws were 0.381 ± 0.23 and 1.705 ± 0.26 s, respectively (n = 8; Fig. 6B). Interestingly, the fast tau did not seem to be voltage-dependent, unlike the slow desensitization that showed a clear voltage dependence between −80 and +10 mV when fitted by linear regression. A similar voltage dependence was clearly seen for hypothalamic neurons and Mauthner cells (Faber and Korn, 1987; Akaike and Kaneda, 1989). Between

To further characterize glycine-mediated currents, we studied their inactivation, a property associated with glycine receptors (Akaike and Kaneda, 1989), by measuring the responses of test glycine currents after conditioning glycine applications at various intervals. Such conditioning applications strongly inactivated test glycine currents at 2-s intervals. As the interval increased, the inactivation clearly diminished (Fig. 6C): the mean average amplitude (n = 5) of the test glycine current at 2-s intervals was 48 ± 9% of the conditioning current (Fig. 6D), increasing to 95 ± 4% at 14-s intervals.

Pharmacology of NAcc Glycine-Like Receptors. We first examined the effects of strychnine on the glycine current because this compound is a highly selective and potent (IC_{50} = 5–10 nM) glycine receptor antagonist (Young and Snyder, 1973, 1974). Surprisingly, glycine currents were strongly antagonized only with high strychnine concentrations (10–100 μM) (Fig. 7A), whereas 1 μM strychnine, coapplied with glycine decreased the mean glycine current amplitudes by only 18 ± 1.8% (n = 8; Fig. 7B), an equivalent strychnine-insensitivity was found in three neurons where strychnine was applied before glycine. The apparent strychnine IC_{50} was 12 μM. We also examined the effects of picrotoxin, an antagonist of both GABA_A and glycine receptors in some preparations (Rajendra et al., 1997) on glycine currents at 0 mV. A 1-min application of 50 μM picrotoxin moderately decreased glycine current amplitudes (Fig. 8A). At a greater picrotoxin concentration (200 μM) glycine current amplitudes were slightly increased (data not shown). On average, 50 μM picrotoxin decreased mean glycine current amplitudes by 15 ± 4%, whereas 200 μM increased it by 22 ± 6% (Fig. 8C). We also examined the effects of Zn^{2+}, known to potentiate and to block glycine currents at a low (1 μM) and high (10 μM) concentrations, respectively (Bloomenthal et al., 1994; Laube et al., 1995); we found that 1-min preincubation with 1 and 10 μM zinc only slightly decreased glycine current amplitudes, by 8 ± 2 and 6 ± 2%, respectively (Fig. 8C).

What Is the Endogenous Ligand for NAcc Glycine-Like Receptors? Because glycine-immunoreactive cells and fibers have not been identified to date in forebrain structures such as NAcc (Rampon et al., 1996), we further examined the possibility that GABA could be the endogenous transmitter for the glycine-like receptor. GABA applications (500 μM) onto class II NAcc neurons evoked currents with strong similarities to the glycine-evoked currents in that they displayed a strong desensitization (Fig. 9A) and were also strongly inactivated by a conditioning GABA application (data not shown). Bicuculline (40 μM) moderately decreased the GABA currents and altered the time to peak of the current, with a recovery on washout (Fig. 9A). Averaged over four neurons, bicuculline decreased mean GABA current amplitude by only 17 ± 6% (Fig. 9B). Because of the surprising ineffectiveness of bicuculline we also tested picrotoxin (200 μM), another potent GABA_A receptor antagonist. As with bicuculline, picrotoxin markedly altered the kinetics of the currents, with recovery on washout (Fig. 9C). However, picrotoxin also failed to fully antagonize the response (mean GABA_A current amplitudes decreased by 66 ± 12%; Fig. 9D). The persistence of a sizable GABA current in the presence of a high concentration of picrotoxin suggests that GABA may activate a conductance different from that of GABA_A receptors, although we cannot totally rule out that the concentration of GABA used in our experiments may also partially account for this effect due to competitive agonist/antagonist interactions. This current did not involve GABA_B receptors because it was not altered by the presence in the recording solution of a GABA_B receptor antagonist (CGP 55845) or Ca^{2+}, known to block potassium currents. Despite the fact that the glycine current was strongly inactivated by a conditioning glycine application, it was still completely antagonized by 100 nM strychnine.
pulse, currents evoked by 500 μM GABA did not alter glycine current amplitudes at any interapplication interval (Fig. 9E), suggesting little interaction between GABA and glycine-like receptors in NAcc.

Because taurine, an amino acid with a high affinity for glycine receptors, has been found in striatum and NAcc (see Discussion), we tested its effects on NAcc neurons. Class I neurons devoid of glycine-like receptors did not respond to taurine applications (n = 4). In contrast, class II NAcc neurons and interneurons responded dose dependently to taurine as well as to glycine applications (Fig. 10A); taurine-evoked currents were maximal at 10 mM. To determine whether taurine and glycine activate the same receptor, we first examined the ability of taurine to inactivate glycine currents. Based on the results of the previous experiments (Fig. 6, C and D), we elicited taurine currents followed by glycine currents at critical intervals (1, 2, 4, and 20 s). As with the glycine/glycine inactivation experiments (Fig. 6, C and D), 1 mM taurine strongly inactivated glycine currents at the 1-s interval and the inactivation disappeared with a 20-s interval (Fig. 10B; control glycine current not shown), suggesting that both agonists interacted on the same receptor. The mean glycine current amplitude was 53 ± 6 and 67 ± 4% of control for interapplication intervals of 1 and 2 s, respectively (n = 6; Fig. 10C). To further test the ability of glycine and taurine to interact, we showed that 10 mM taurine-mediated currents were almost totally occluded by concurrent glycine applications (1 mM) (Fig. 10D), the amplitude of the mean current evoked by coapplication of glycine and taurine was 5 ± 4% of control taurine current (n = 6).

**Discussion**

In these experiments on acutely isolated NAcc neurons we found that all NAcc interneurons and 20% of medium spiny cells expressed glycine-like receptors. Although their biophysical properties are similar to those of other glycine receptors, their pharmacological properties set them apart. In addition, our data support the possibility that taurine is an endogenous transmitter for NAcc glycine-like receptors.
Identity of NAcc Glycine-Like Receptors. These newly described NAcc receptors exhibited a set of properties reminiscent of strychnine-sensitive glycine receptors. Thus, NAcc glycine-evoked currents rectified outwardly in both the hyperpolarizing and depolarizing range, a phenomenon observed at voltages negative to $-50 \text{ mV}$ in cultured (Bormann et al., 1987; Fatima-Shad and Barry, 1992, 1993) and acutely isolated hypothalamic (Akaike and Kaneda, 1989) neurons, as well as in recombinant glycine receptors in expression systems (Gundersen et al., 1984; Akagi et al., 1991; Morales et al., 1994). The affinity of glycine for NAcc glycine-like receptors was $110 \mu M$, a value similar to those obtained from glycine receptors in hypothalamic, cerebellar, and ventral cochlear neurons (Akaike and Kaneda, 1989; Virginio and Cherubini, 1997; Harty and Manis, 1998). NAcc glycine currents desensitized rapidly with a biphasic course, in agreement with glycine receptors in other studies (Akaike and Kaneda, 1989; Lewis et al., 1991). However, in contrast to neurons in hypothalamus (Akaike and Kaneda, 1989), only the slow desensitization was clearly voltage-dependent in NAcc neurons. NAcc glycine currents also showed a strong inactivation, as reported for other glycine receptors (Akaike and Kaneda, 1989; Virginio and Cherubini, 1997; Harty and Manis, 1998).

Fig. 7. Dose-response relationship for glycine-evoked currents in the presence of increasing concentrations of strychnine. A, representative responses evoked at $0 \text{ mV}$ holding potential by application of glycine (1 mM; solid column) and strychnine (0.01–100 $\mu M$; gray column). B, mean amplitudes averaged from eight cells of peak glycine currents in the presence of different strychnine concentrations. Strychnine IC$_{50} = 12 \mu M$.

Fig. 8. Neither picrotoxin nor zinc blocked the glycine currents. A, $50 \mu M$ picrotoxin only slightly decreased the amplitude of the current evoked by $100 \mu M$ glycine. B, in another NAcc cell, $10 \mu M$ zinc failed to block glycine currents. C, mean effects of picrotoxin (50 and $200 \mu M$; $n = 5$), and zinc ($1$ and $10 \mu M$; $n = 5$) on glycine-evoked peak current amplitudes. All these antagonists failed to markedly alter glycine current amplitudes. All currents were recorded at $0 \text{ mV}$ holding potential. Neurons were preexposed to zinc.
For example, glycine binding sites are extremely abundant in spinal cord and to a lesser extent in the midbrain, hypothalamus, cerebellum, and thalamus and are almost absent in forebrain structures such as cortex, NAcc, or dorsal striatum (Rajendra et al., 1997). An in situ hybridization study by Sato et al. (1991), showing that forebrain regions lack α1 subunits, supported the absence of glycine receptors in the forebrain. However, the same authors (Sato et al., 1992) showed that α2 subunits were expressed, although at moderate levels, in the NAcc. Interestingly, a recent study by Racca et al. (1998) confirmed the presence of α2 subunits in the forebrain (striatum) but also found the expression of α1 subunit mRNA. It would be interesting to extend such immunohistochemical study to the NAcc, to determine the kind of glycine receptor subunits expressed there and whether a similar expression in a subset of neurons mirrors our electrophysiological data and the Racca et al. (1998) findings in the dorsal striatum. Although these findings do not directly demonstrate the presence of functional glycine receptors, they may explain the ability of glycine to evoke currents in some NAcc neurons. Indeed, α subunits, known to carry glycine binding sites, are usually associated with the presence of functional glycine receptors. The study by Racca et al. (1998) also may help explain the fact that glycine currents were partly restricted in our study to interneurons, which represent only 5 to 10%
of the NAcc neuronal population. Although Racca et al. (1998) reported only weak-to-moderate levels of α1 mRNAs in striatum, they found a few scattered, highly positive cells that could represent NAcc interneurons and/or the fraction of medium spiny neurons that did show glycine currents. The expression of α2 mRNA and more specifically the presence of α2* subunit, if confirmed, may explain the surprisingly weak strychnine effect we observed as these subunits are usually considered to be less sensitive to strychnine (Vanderberg et al., 1992a,b). The method of coapplication of strychnine and glycine cannot account for the relative ineffectiveness of strychnine compared with published data (Young and Snyder, 1973, 1974), as preincubation of a subset of cells with 1 μM strychnine (data not shown) also failed to block glycine currents as reported in other studies. We obtained a similar result when testing the effects of 10 μM zinc (Fig. 8B). Finally, although there are no data that would support the idea that heteromeric glycine receptors composed of α1 and α2 subunits may be combined to form functional NAcc glycine receptors, this hypothesis cannot be ruled out given the extraordinary ability of subunits that do not belong to the same class of receptors to form new entities (see below).

An alternative to the necessity for α1 and/or α2 proteins was recently formulated by Betz et al. (1999), who proposed that β subunits, widely expressed in the forebrain (Fujita et al., 1991; Malosio et al., 1991; Kirsch et al., 1993; Racca et al., 1997), along with a protein yet to be identified, may form a novel complex that retains only some of the properties of glycine receptors. Recent studies indicate that receptors of different families can interact to alter their individual properties. Thus, dopamine D2 and somatostatin SST5 receptors, two structurally related G protein-coupled receptors, can form a novel hetero-oligomer with pharmacological properties different from those receptors alone (Rocheville et al., 2000). A similar heterodimerization was also reported for δ- and κ-opioid receptors (Jordan and Devi, 1999). With respect to ionotropic receptors, the ρ subunit of GABA<sub>C</sub> receptors interacts with a novel splice variant of the glycine transporter GLYT-1 (Hanley et al., 2000) that is also found in NAcc (Zafra et al., 1995). These findings challenge the dogma of strict boundaries between receptors. Several studies (for review, see Rajendra et al., 1997) have established unequivocally that the primary sequence of glycine receptor α and β subunits share a considerable homology with subunits of other multimeric receptors such as nicotinic (Noda et al., 1982), serotonin type 3 (Maricq et al., 1991), and GABA<sub>Δ</sub> receptors (Schoffelmeier et al., 1987). Such similarities could support the presence of receptors displaying distinct properties such as described herein.

**Endogenous Agonist of NAcc Glycine-Like Receptors.** Regardless of the exact identity of the subunits forming the NAcc glycine-like receptors, the nature of their endogenous agonist remains to be determined. Although glycine evoked the ionic conductance, this amino acid may not be the endogenous ligand for the NAcc receptors because no glycine-containing terminals have been detected in this region (Rampon et al., 1996). Because the primary structure of GABA<sub>Δ</sub> and glycine receptors shares significant sequence homologies, we examined the possibility that GABA could activate the glycine-like receptors in NAcc. We found that picrotoxin, a potent GABA receptor antagonist, did not totally block the GABA-mediated currents, suggesting the involvement of either a GABA receptor resistant to picrotoxin or a glycine receptor insensitive to picrotoxin. The latter choice has some credibility, because the ability of picrotoxin to block glycine receptors varies dramatically with the subunit composition of the receptor. Thus, α homomeric glycine receptors are much more sensitive to picrotoxin block than heteromeric α plus β glycine receptors (Pribilla et al., 1992). In addition, Fucile et al. (1999) reported that GABA activates homomeric α1 glycine receptors from zebrafish. Similarly, in rat olfactory bulb neurons both glycine and GABA can bind to either glycine or GABA<sub>Δ</sub> receptors (Trombley et al., 1999). However, the fact that GABA failed to inactivate or occlude glycine currents supports the idea that GABA does not bind to the glycine-like receptors.

If neither GABA nor glycine prove to be the endogenous ligand for this novel NAcc glycine-like receptor, it is possible that another amino acid with a high affinity for glycine receptors could serve that function. Early studies on putative glycine receptor agonists identified β-alanine and taurine as having a strong affinity for glycine receptors (Curtis et al., 1968; Young and Snyder, 1973). Taurine is a good candidate because it is the most abundant amino acid, after glutamate, and is widely, although unevenly, distributed in the brain (Palkovits et al., 1986), including the NAcc (Madsen et al., 1987). Both taurine (Ottersen and Storm-Mathisen, 1986; Madsen et al., 1987) and cysteine sulfinate decarboxylase, the enzyme responsible for taurine biosynthesis (Legay et al., 1987a,b), have been found predominantly in forebrain structures, including the striatum.

The presence of taurine in NAcc medium spiny neurons and its apparent absence in terminals pose the question of its release. An interesting parallel can be drawn between GABA and taurine in the NAcc. GABA, also a nonvesicular transmitter, can be released into the extracellular space from the soma by prolonged activation of excitatory amino acid receptors (for review, see Attwell et al., 1993), presumably by an influx of Na<sup>+</sup> through NMDA receptor channels in neurons of striatum and NAcc (Schoffelmeier et al., 2000). This would explain why, in NAcc slices, we rarely observed GABA<sub>Δ</sub> receptor antagonist-insensitive inhibitory postynaptic potentials, because we evoked synaptic events only with single stimulations; a train of stimuli would have been required for nonvesicular release. It might also suggest that glycine receptors may be silent under normal conditions. Furthermore, it is likely that these glycine- and taurine-like receptors are the same entity because the existence of a specific taurine receptor has yet to be demonstrated. This idea is supported by the recent findings of Hussy et al. (2001) who reported that taurine-mediated osmotic regulation in the neurohypophysis is exerted through glycine receptors. To date, no protein that could unequivocally account for this receptor has been identified and characterized. In addition, our occlusion results indicating that taurine acts on the same receptor as glycine further strengthen the idea that taurine is an endogenous glycine receptor agonist. It seems unlikely that taurine-mediated currents are the result of GABA<sub>Δ</sub> receptor activation, because the affinity of taurine for GABA<sub>Δ</sub> receptors is low. In addition, if the response evoked by taurine reflected an effect on GABA<sub>Δ</sub> receptors, the likelihood of taurine-occluding glycine responses would be very small.

Furthermore, the presence of glycine β receptor subunits as well as the newly reported α subunits, further strengthens...
the case for existence of functional glycine receptors in the NAc. Finally, these NAc glycine receptors present no apparent similarities to a newly described excitatory glycine receptor (Chatterton et al., 2002).

In conclusion, glycine elicits a current in some medium spiny neurons and in all interneurons in the rat NAc. Although many of the biophysical properties of this current are similar to those of glycine currents found in spinal cord and brain stem, its pharmacological characteristics set it apart. The activation of this receptor, as with GABA receptors in most medium spiny neurons, should selectively inhibit a subset of NAC neurons.

Acknowledgments

We are indebted to Dr. J. Surmeier for considerable assistance in setting up the acutely isolated neuron preparation. We also thank Drs. N. Hussy, P. Schweitzer, and M. Tallent for helpful comments.

References


Bormann J, Hamill OP, and Sakmann B (1987) Mechanism of anion permeation setting up the acutely isolated neuron preparation. We also thank

Drs. N Hussy, P Schweitzer, and M Tallent for helpful comments.

**References**


Bormann J, Hamill OP, and Sakmann B (1987) Mechanism of anion permeation setting up the acutely isolated neuron preparation. We also thank

Drs. N Hussy, P Schweitzer, and M Tallent for helpful comments.

**References**


Bormann J, Hamill OP, and Sakmann B (1987) Mechanism of anion permeation setting up the acutely isolated neuron preparation. We also thank

Drs. N Hussy, P Schweitzer, and M Tallent for helpful comments.

**References**


Bormann J, Hamill OP, and Sakmann B (1987) Mechanism of anion permeation setting up the acutely isolated neuron preparation. We also thank

Drs. N Hussy, P Schweitzer, and M Tallent for helpful comments.

**References**


Bormann J, Hamill OP, and Sakmann B (1987) Mechanism of anion permeation setting up the acutely isolated neuron preparation. We also thank

Drs. N Hussy, P Schweitzer, and M Tallent for helpful comments.


Young AB and Snyder SH (1973) Strychnine binding associated with glycine receptors of the central nervous system. *Proc Natl Acad Sci USA* **70**:2832–2836.


**Address correspondence to:** Dr. Gilles Martin, University of Massachusetts School of Medicine, Department of Neurobiology, 364 Plantation St., Worcester, MA 01605-2324. E-mail: gilles.martin@umassmed.edu