Kinetic Analysis of Glycine Receptor Currents in Ventral Cochlear Nucleus

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Harty, T. Patrick and Paul B. Manis. Kinetic analysis of glycine receptor currents in ventral cochlear nucleus. J. Neurophysiol. 79: 1891-1901, 1998. Glycine plays an important role as an inhibitory neurotransmitter in the ventral cochlear nucleus. However, little is known about the kinetic behavior of glycine receptors. The present study examines the kinetics of the native inhibitory glycine receptors in neurons of the ventral cochlear nucleus, using outside-out patches from acutely dissociated cells and a fast flow system. Steps into 1 mM glycine revealed fast phases of desensitization with time constants of 13 and 129 ms, that together produced a 40% reduction in current from the peak response. Slower desensitization phases also were observed. After removal of glycine, currents deactivated with two time constants of 15 and 68 ms, and these rates were independent of the glycine concentration between 0.2 and 1 mM. Recovery from desensitization was slow relative to desensitization itself. These results demonstrate that glycine receptors can exhibit faster rates of desensitization and deactivation than previously reported.

INTRODUCTION

In the central nervous system (CNS), fast inhibitory postsynaptic potentials typically result from an increase in membrane permeability to chloride and other monovalent anions (Araki et al. 1961; Coombs et al. 1955; Ito et al. 1962). In the spinal cord and brain stem, the primary ligand responsible for gating these anion-selective membrane channels is the amino acid glycine (Curtis et al. 1968; Werman et al. 1968). Together with γ-aminobutyric acid (GABA), serotonin, and acetylcholine, glycine-gated chloride conductances define a receptor superfamily based on common features of their protein structure (Betz 1990; Unwin 1989). These features include polypeptide subunits arranged in pentameric structures to form ligand-gated, ion-conducting channels.

Purification of glycine receptors has resulted in the identification of two main subunits, α (48 kDa) and β (58 kDa) with similar structure. A third protein, gephyrin, is thought to play a role in anchoring glycine receptors in the plasma membrane (Kirsch et al. 1991; Pfeiffer et al. 1982). Like other receptors in the superfamily, glycine subunits are composed of four membrane-spanning regions, extracellularly localized N and C termini and an intracellular hydrophilic loop between transmembrane segments 3 and 4 containing potential phosphorylation sites (Grenningloh et al. 1987, 1990). The properties of glycine receptor subunits have been investigated using protein expression in oocytes as well as mammalian cell lines. When expressed homomerically in oocytes (Schmieden et al. 1989), the α subunit produces glycine-gated currents similar to those observed after the expression of whole brain DNA. Strychnine and picrotoxin block the chloride-dependent conductance produced by glycine. Expressed homomerically, the β subunit is inactive, whereas heteromeric expression of both α and β subunits results in receptors that are relatively insensitive to the chloride channel blocker, picrotoxin (Pribilla et al. 1992).

Although the pharmacological and structural properties of glycine receptors have been well characterized, the kinetics that govern receptor gating have not been investigated in detail. For example, very little has been reported on activation and deactivation kinetics. In the continued presence of glycine, desensitization of whole cell currents occurs with time constants that range from 0.5 s in hypothalamic neurons (Akaiki and Kaneda 1989) to 350 s in oocytes expressing the α subunit (Schmieden et al. 1989). However, the methods used in these studies were not optimal for measurement of rapid receptor kinetics.

Glycine is a major inhibitory neurotransmitter in cochlear nucleus (Caspar et al. 1979; Godfrey et al. 1977; Harty and Manis 1996; Kakemata et al. 1992). Glycinergic projections to the ventral cochlear nucleus (VCN) originate in the ipsilateral dorsal cochlear nucleus (DCN), the superior olivary complex, the trapezoid body, and the contralateral VCN (Benson and Potashner 1990; Saint Marie et al. 1991; Wenthold 1987; Wickesberg and Oertel 1988, 1990). Glycine receptors can be found on most of the cell types within the VCN, including bushy, stellate, and granule cells (Altschuler et al. 1986; Frostholm and Rotter 1986; van den Pol and Gorcs 1988; Wenthold et al. 1988), where they mediate fast, disynaptic inhibitory postsynaptic potentials (IPSPs) after auditory nerve stimulation (Oertel 1983; Wu and Oertel 1986). The responses to iontophoresis of glycine are voltage dependent and mediated by a chloride permeable conductance (Harty and Manis 1996). The existence of unusually rapid desensitization kinetics of the glutamate receptor in this nucleus (Raman and Trussell 1992) motivated us to investigate the kinetics of the glycine receptors because the IPSPs are brief in comparison with glycine-mediated responses relationships or for evaluation of receptor kinetics. The experiments described in this paper were designed to provide this information with the use of outside-out patches from acutely isolated guinea pig VCN neurons and a fast-perfusion system.

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METHODS

Tissue preparation

The methods used to dissociate neurons of the VCN have been described in detail elsewhere (Harty and Manis 1996). Briefly, guinea pigs of either sex weighing 150–300 g were anesthetized with 35–40 mg/kg pentobarbital injected intraperitoneally. The brain stem posterior to the superior colliculus was removed, hemisected, and blocked to expose the cochlear nucleus on the dorsolateral surface of the brain stem. Using a vibrating tissue slicer, 400-μm slices were cut in a parasagittal plane of section. The VCN was microdissected from these slices and placed in a spinner flask (Belco) with 15 ml of a piperezine-N,N’-bis(2-ethanesulfonic acid) (PIPES)-buffered dissection solution containing (in mM) 145 NaCl, 5 KCl, 26 glucose, 2 CaCl₂, 2 MgCl₂, 10 PIPES, 1 kynurenic acid, and 0.5 mg/ml bovine serum albumin (pH 7.0, osmolarity 305–310 mosM). The spinner flask was placed in a water bath at 30°C with a stream of 100% O₂ (150 ml/min) directed at the surface of the solution. After allowing 10–15 min for the tissue to reach the water bath temperature, trypsin (10 mg) was added for 30 min. Slices were then rinsed two to three times with dissection solution and the spinner flask was filled (25–30 ml) with fresh dissection solution and removed from the water bath. The trypsin-treated tissue was incubated at room temperature (with 100% O₂ and constant stirring) for ≥30 min before electrophysiological recording was begun.

Electrophysiology

To separate VCN neurons from the tissue slices, a slice was dissociated mechanically using fire-polished Pasteur pipettes with successively smaller openings at the tip. The resulting cell suspension was placed in a 35-mm petri dish that had been imprinted with a 1-cm diam well (BB Press) and coated with poly-L-lysine. Dissociated neurons were permitted to settle and attach to the dish for ~10–15 min, at which time the extracellular recording solution was perfused at ~0.5 ml/min. This solution contained (in mM) 145 NaCl, 5 KCl, 25 glucose, 2 CaCl₂, 2 MgCl₂, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES; pH 7.35, osmolarity 305–310 mosM).

Electrodes used for outside-out patch recordings were pulled from TW150 glass blanks (WPI) and fire polished to a final resistance of 2–5 MΩ. Recording electrodes were filled with a solution containing (in mM) 145 mM CsCl, 4 mM NaCl, 10 ethylene glycol-bis-(β-aminoethoxy) ether)-N,N',N’-tetraacetic acid, 1 CaCl₂, 10 HEPES, and 2 NaATP (pH 7.2–7.3, osmolarity 290–295 mosM). All recordings were made in voltage-clamp mode with an Axopatch 200 (Axon Instruments). Membrane potential and drug application, as well as data acquisition were controlled by a version of the data acquisition and analysis program, DATAC (Bertrand and Bader 1986). Glycine-evoked currents were low-pass filtered at 1 or 2 kHz, digitized at 10 kHz, and stored on a personal computer for analysis.

Glycine application

Glycine was applied to outside-out patches using a rapid perfusion system similar to that described by Franke et al. (1987), which consisted of a perfusion pipette pulled from theta glass tubing (Sutter Instruments) to a tip diameter of 250–300 μm and attached to a piezoelectric bimorph bender element (PTZ5H, Vermontron). Glycine-free and glycine-containing extracellular recording solutions were fed by gravity to each side of the perfusion pipette, resulting in a steady flow consisting of the two solutions separated by a well-defined interface. After obtaining a stable outside-out patch recording, the tip of the recording electrode was positioned on the glycine-free side close to the solution interface and 100–200 μm in front of the tip of the perfusion pipette. Voltage applied to the bimorph element produced a rapid lateral displacement of the perfusion pipette tip (~40 μm), thereby rapidly changing the solution perfusing the cell or patch to one containing glycine. To minimize overshoots and subsequent oscillations in the displacement, the voltage applied to the bimorph element was conditioned to have a sigmoidal onset and offset (Corey and Hudspeth 1980).

The onset and offset times for the application system were determined using two methods. The first method involved measuring changes in the tip potential of a patch pipette filled with intracellular recording solution as the perfusion solution was switched between normal and a 1:2 dilution of the extracellular recording solution. This method was used after recording glycine responses from a patch to assess the timing of the application system. The time-to-peak of the test response was influenced by a number of factors including the velocity of the perfusing solution and the distance of the recording electrode from the tip of the perfusion pipette. For most experiments involving patches, the recording electrode was positioned 100 μm from the perfusion pipette tip. At this distance, the change in potential at the tip of an open recording electrode switched to dilute extracellular recording solution had a 10–90% rise time of 1.6 ± 0.7 (SD) ms (Fig. 1A). The current returned to baseline with a similar time course after switching back to full-strength extracellular solution. For distances further from the perfusion pipette, the average 10–90% rise time increased to 1.8 ± 0.7 ms (200 μm) and 2.2 ± 0.8 ms (300 μm). Recording the change in tip potential of the recording electrode at measured distances from the tip of the perfusion pipette also provided an estimate of the velocity of the solution, which averaged 48 ± 20 μm/ms.

The second method for testing the speed of application involved pulling a patch (from cultured hippocampal neurons), and switching from a low sodium (20 mM) solution containing kainate (1 mM) to a high sodium solution (140 mM) containing the same concentration of kainate. The resulting change in inward current, primarily through nondesensitized non-N-methyl-D-aspartate receptors, reflects the minimum transition time between two solutions that the system is capable of with a patch present on the recording electrode. As shown in Fig. 1B, the time course of the increase in inward current across a patch was similar to the change in tip potential of an open-tipped recording pipette in diluted recording solution. For three patches, the 10–90 rise time of the inward current averaged 1.5 ± 0.3 ms.

Data analysis

To generate a concentration-response relationship for VCN glycine receptors, all patches were exposed to 1 mM glycine and two to three lower concentrations (0.01–0.5 mM). For each concentration, four to six responses were averaged and the peak current and the current amplitude after a 1-s application were determined. Current amplitudes were normalized by the values obtained at 1 mM and averaged with values from three to five patches at the same concentration. These averages were then fit using a Simplex algorithm (SigmaPlot, Jandel Scientific) with the equation

\[
I = I_{\text{max}} \frac{[\text{glycine}]^n}{[\text{EC}_{50}]^n + [\text{glycine}]^n} \tag{1}
\]

Where \( I \) is the glycine-evoked current (at the peak of the response or at 1 s) relative to the current obtained at 1 mM glycine, \( I_{\text{max}} \) is the maximum obtainable current (initially set to 1 but allowed to vary during the fitting procedure), \( \text{EC}_{50} \) is the concentration of glycine that produces a 50% response, and \( n \) is the Hill coefficient.

Curv fitting of activation, desensitization and offset of glycine-
evoked currents was performed with Levenberg-Marquardt algorithms in Origin (Microcal) or Sigmaplot (Jandel Scientific). For glycine concentrations <0.5 mM, fits were performed to 1-s glycine applications sampled at 2 kHz. For glycine concentrations of 0.5 and 1 mM, fits were performed to shorter glycine applications (20 ms) sampled at 50 kHz because the current onsets were faster. Onset data were fit with a first-order exponentially increasing function taken to a power of 1–6. A power of 1 was adequate to fit onsets at glycine concentrations of 0.01–0.2 mM. At higher concentrations, the initial component of the onset became sigmoidal and was better fit by higher powers. Desensitization and offset currents were fit with one, two, or three exponentially decaying functions. Goodness of fit was determined by comparison of χ² values.

RESULTS

Glycine responses from outside-out patches

Results are based on recordings from 48 outside-out patches, each from a different cell. The majority of the patches were exposed to a standard glycine application, which consisted of 1 mM glycine applied for 1 s to patches held at −60 mV. As shown in Fig. 2, A–C, patches responded to a 1-s application with an inward current having a rapid onset and a rapid offset when the patch was returned to glycine-free solution. At high concentrations (Fig. 2A), the current decayed with an exponential time course during glycine application, suggesting that VCN glycine receptors are subject to desensitization.

The amplitudes of the peak current and the current at the end of a 1-s application were analyzed in the presence of different concentrations of glycine (Fig. 2). Peak inward currents increased sigmoidally with increasing glycine concentrations of 0.01–1 mM. Fitting the data with Eq. 1 (see METHODS) produced an EC$_{50}$ of 113 μM and a Hill coefficient of 1.20. The amplitude of the inward current at the end of a 1-s application also increased sigmoidally with increasing glycine concentration. The dose-response curve was shifted slightly to the left relative to the curve for the peak current, with an EC$_{50}$ of 63 μM and a Hill coefficient of 1.44.

Activation

The onset of the inward current in response to application of 1 mM glycine showed a sigmoidal rise and was as fast as our solution exchange (Fig. 3, A1 and B1). To estimate the activation rate, these responses were fit with an exponential function. Activation time constants at this concentration of glycine varied from 0.8 to 4.6 ms, with a mean of 2.0 ± 1.2 ms. For glycine concentrations of 1 and 0.5 mM, the majority of time constants fell within a range (1–3 ms), which was similar to the transition time for the application system. These activation time constants were not used in subsequent data analysis because the fits were poor. With lower glycine concentrations, the activation of the glycine receptor slowed considerably (Fig. 3, A2 and B), requiring the use of long (1 s) pulses to study activation kinetics. For example, at 0.1 mM (Fig. 3A2), the activation time constant was 13 ± 6 ms (n = 5).

As illustrated in Fig. 3C, there was a linear relationship between glycine concentration and the reciprocal of the activation time constant. The following simple model was assumed for activation of a receptor

\[
\text{Gly} + R \rightleftharpoons \text{GlyR}
\]  

(2)

Then \(1/\tau_{on} = K_{on} [\text{Gly}] + K_{off}\), where \(K_{on}\) = slope of the relationship between \(1/\tau_{on}\) and \([\text{Gly}]\) (Fig. 3C). A linear regression was performed on the activation time constants. Only glycine concentrations between 0.01 and 0.2 mM were included because higher concentrations had activation kinetics close to the exchange time constant for fast perfusion system. Linear regression produced a slope of 1.06 ± 0.12 (ms × mM)$^{-1}$ and a y intercept of 0.0032 ± 0.0036 ms$^{-1}$. The correlation coefficient for the fit was 0.98 ± 0.34 (n = 5, P < 0.003). The y intercept could be used to determine the offset time constant of the binding reaction, but the large standard deviation of the experimental estimate prevents this value from being useful.

Deactivation

When glycine was removed from the patch, the currents decayed rapidly back to baseline. For 31 of 32 patches,
(Fig. 4A) was $2.72 \pm 5.88 \times 10^{-5}$; for double exponential fits (Fig. 4B), the mean $\chi^2$ value was an order of magnitude smaller ($2.70 \pm 3.90 \times 10^{-6}$, $t = 2.49, P < 0.05$).

The distribution of all $\tau_f$ and $\tau_s$ values (Fig. 4C) revealed a well-defined group in the range of 0–30 ms. Although fitting the decay with the sum of two exponentials produced a lower $\chi^2$ value (Fig. 4) than fits with a single exponential. The mean $\chi^2$ value for single exponential fits

![Diagram](image)
GLYCINE RECEPTOR KINETICS IN VENTRAL COCHLEAR NUCLEUS

Receptors, each with a characteristic dissociation constant. Receptors with a fast off rate would have a lower affinity for glycine than receptors with a slower off rate. If this is the case, then the relative number of channels closing with either of the two dissociation constants should change with glycine concentration (i.e., the lower affinity receptor with the faster off rate should make a smaller contribution at lower glycine concentrations). To examine this possibility, the offsets of the current evoked by different concentrations of glycine (0.05–1 mM) were fit with two exponentials (Fig. 5A). The time constants for the fast and slow components of decay did not change significantly with decreasing glycine concentration. For 1 mM glycine, \( t_f = 23.3 \pm 8.7 \) ms and \( t_s = 91.9 \pm 18 \) ms, whereas for 0.05 mM, \( t_f = 21.8 \pm 5.2 \) ms and \( t_s = 94.0 \pm 10.2 \) ms. The relative contribution of \( t_f \) to the current offset also did not diminish with decreasing glycine concentration. The fast component accounted for 69 ± 16% and 58 ± 7% of the current offset decay at glycine concentrations of 1 and 0.01 mM, respectively. It thus appears that subtypes of glycine receptors with different dissociation rates probably do not account for the two exponential decays of the offset current.

A second possibility for biexponential decay of glycine-evoked currents is that the simple model presented above, which predicts a single exponential decay, is not adequate to represent the glycine receptor. Several factors might account for the improved fit obtained with two exponentials. First, there could be two or more subtypes of glycine receptors, each with a characteristic dissociation constant. Receptors with a fast off rate would have a lower affinity for glycine than receptors with a slower off rate. If this is the case, then the relative number of channels closing with either of the two dissociation constants should change with glycine concentration (i.e., the lower affinity receptor with the faster off rate should make a smaller contribution at lower glycine concentrations). To examine this possibility, the offsets of the current evoked by different concentrations of glycine (0.05–1 mM) were fit with two exponentials (Fig. 5A). The time constants for the fast and slow components of decay did not change significantly with decreasing glycine concentration. For 1 mM glycine, \( t_f = 23.3 \pm 8.7 \) ms and \( t_s = 91.9 \pm 18 \) ms, whereas for 0.05 mM, \( t_f = 21.8 \pm 5.2 \) ms and \( t_s = 94.0 \pm 10.2 \) ms. The relative contribution of \( t_f \) to the current offset also did not diminish with decreasing glycine concentration. The fast component accounted for 69 ± 16% and 58 ± 7% of the current offset decay at glycine concentrations of 1 and 0.01 mM, respectively. It thus appears that subtypes of glycine receptors with different dissociation rates probably do not account for the two exponential decays of the offset current.

A second possibility for biexponential decay of glycine-
evoked currents is that desensitization of the receptor contributes to deactivation. Experiments with GABA<sub>A</sub> receptors have led to the proposal that receptors enter prolonged desensitized states (Jones and Westbrook 1995). Transitions out of the desensitized state into a conducting state after removal of GABA lead to delayed openings. With increasing amounts of desensitization, transitions from the desensitized state to an open state should contribute to a larger fraction of the current decay that can be evident as a shift in the relative proportions of the fast and slow off-rates. This can be evaluated by comparing the rates and amplitudes of the current decay after short (20 ms) glycine applications, when little desensitization has occurred, with the decay following long (1 s) applications, when greater desensitization has occurred. For a group of 27 patches, both 20 ms and 1 s responses were obtained. The shorter time constant was similar for 20 ms responses (τ<sub>f</sub> = 10 ± 3 ms) and 1 s responses (τ<sub>f</sub> = 14 ± 5 ms) and accounted for a similar proportion of the current offset (57 ± 14% for 20 ms responses, 61 ± 17% for 1 s responses, t = 1.04, P > 0.05). This hypothesis also predicts that the deactivation time constants are independent of the duration of glycine application. However, we observed the opposite result: τ<sub>d</sub> was significantly longer for 1 s responses (60 ± 17 ms) than for 20 ms responses (38 ± 14 ms, t = 6.60, P < 0.001).

A third possibility is that biexponential decay of current could result from the presence of two or more distinct open states of the receptor, each with a characteristic rate of transition to the closed state, or conversely with transitions from one open state to multiple, energetically distinct closed states. These possibilities could be addressed with single channel recording but our patches always contained multiple channels and only macroscopic current kinetics could be studied. However, single-channel analysis has shown that glycine receptors in the mammalian nervous system have multiple discrete conductance states (McNiven and Martin 1993). These may correspond to multiple open states that could have different kinetic behavior.

**Desensitization**

As shown in Fig. 2A, currents evoked by 1 mM glycine exhibited a marked decrease in amplitude in the continued presence of glycine, indicating that receptors were desensitizing. The average amplitude of the peak inward current for 40 patches was 0.40 ± 0.39 nA, with a range of 0.08–1.44 nA. The percent decline of the glycine-evoked current for a 1-s application ranged from 19 to 85% of the peak current, with a mean of 49 ± 17%. To characterize the kinetic properties of desensitization in more detail, current decay during glycine application was fit with one, two, or three exponentials (see METHODS). In the majority of patches (26 of 38), the goodness-of-fit parameter, χ², for 1 s responses was the smallest when the sum of three exponentials was used for the fit (t = 2.35, P < 0.05). For responses best fit by the sum of three exponentials (Fig. 6A), the fastest time constant (τ<sub>1</sub>) was 13 ± 7 ms, the intermediate time constant (τ<sub>2</sub>) was 129 ± 107 ms, and the slowest time constant (τ<sub>3</sub>) was 1,721 ± 1,114 ms. The amplitude of the τ<sub>3</sub> component was 0.211 ± 0.173 nA, which represented the largest portion (64 ± 22%) of the total decay. The other two time constants, τ<sub>1</sub> and τ<sub>2</sub>, accounted for 19 ± 14% and 17 ± 11%, respectively, of the remaining decay. Current decay during 1–s
glycine applications in 10 of the other 12 patches was best fit with the sum of two exponentials, whereas a single exponential fit was best for 2 patches. For the two exponential fits (Fig. 6B), \( \tau_1 \) was 61 ± 42 ms and \( \tau_2 \) was 1,652 ± 1,067. The average amplitude of \( \tau_2 \) was 0.189 ± 0.162 nA, which represented 87 ± 8% of the total decay. The time constants for the two single exponential fits were 1,390 and 460 ms (data not shown).

The large range of desensitization rates prompted a closer look at the distribution of all time constants (Fig. 6C). The distribution of time constants did not appear to be uniform when the distribution is constructed with 100-ms bins (the figure does not include 5 time constants that were between 3,000 and 6,000 ms). It appears that there are three groups of time constants: <100 ms, 400–1,400 ms, and 1,600–2,600 ms. To determine if desensitization also occurred on a longer time scale, glycine was applied to a subset of patches for 9 s (n = 12). The glycine response in these patches desensitized by an average of 84.1 ± 11.8%. Because the sampling rate was slow and the data were low-pass filtered, only the slow phases of desensitization could be adequately examined. The fastest phase of desensitization was not visible in the data. For 9 of 12 patches, the decay of a 9 s glycine response was best fit with two exponential components (Fig. 7). The faster component, \( \tau_1 \), was 315 ± 296 ms and accounted for 11.6 ± 6.3% of the desensitization. The time constant of the slower component, \( \tau_2 \), was 3,470 ± 1,632 ms. For three patches, a single exponential was sufficient to fit the data; the mean value of the time constant was 3,796 ± 2,007 ms.

Recovery from desensitization

To determine the rate at which glycine receptors recovered from desensitization, glycine was applied to patches using a paired-pulse protocol. The duration of the desensitizing pulse was 1 s and was followed at various intervals (0.1–11 s) by a 100-ms test pulse. An example of responses produced by this protocol in a single patch is shown in Fig. 8A. A summary of the results for eight patches is shown in Fig. 8B. The best fit of the data with a single exponential function had a time constant of 1,505 ms (Fig. 8B, --). However, the data were better fit using a double exponential function with a \( \tau_1 \) = 305 ms and a \( \tau_2 \) = 3,452 ms (Fig. 8B, ----). The shorter time constant accounted for 39% of the amplitude of the recovery function.

**DISCUSSION**

Glycine receptors in the VCN are similar in some respects to glycine receptors found in other regions of the CNS. The EC\(_{50}\) values of 113 \( \mu M \) for peak responses and 63 \( \mu M \) for response amplitudes determined 1 s after glycine application fell within the range of EC\(_{50}\) concentrations reported for glycine receptors in other cell types, the lowest being 30 \( \mu M \) for embryonic spinal cord cultures (Laube et al. 1995) and the highest being 500 \( \mu M \) for oocytes injected with human mRNA (Gundersen et al. 1984). The EC\(_{50}\) values for peak responses determined 1 s after glycine application fall within the range of EC\(_{50}\) concentrations reported for glycine receptors in other cell types, the lowest being 30 \( \mu M \) for embryonic spinal cord cultures (Laube et al. 1995) and the highest being 500 \( \mu M \) for oocytes injected with human mRNA (Gundersen et al. 1984). The EC\(_{50}\) values correspond reasonably well with those reported for acutely isolated cells from the hypothalamus (90 \( \mu M \)) (Akaike and Kaneda 1989) and for postnatally cultured hippocampal neurons (61 \( \mu M \)) (Fatima-shad and Barry 1992). A change in the EC\(_{50}\) as the receptors undergo desensitization suggests an increase in affinity with increasing desensitization. Although these results were not statistically significant, desensitization is not complete at 1 s and changes in affinity might be revealed with longer applications of glycine.

**Activation**

The activation rate of glycine receptors in the VCN was concentration dependent, at least over the range of concentrations where the responses were slower than our fluid exchange time (0.01–0.2 mM). Current activation was adequately fit by a single exponential for concentrations <0.5 mM and was characterized by a time constant that increased linearly with increasing glycine concentration. These properties are consistent with the onset properties of other ligand-dependent receptors such as GABA (Celentano and Wong 1994; Jones and Westbrook 1995).
Desensitization and deactivation

One of the main findings of these experiments is that glycine-mediated responses in the VCN exhibit two rapidly desensitizing components that were not reported in previous studies of glycine receptor kinetics. Time constants for the desensitization of glycine-mediated currents in other preparations range from 0.5 to 350 s (Agopyan et al. 1993; Akaike and Kaneda 1989; Fatima-shad and Barry 1992; Ito and Cherubini 1991; Lewis et al. 1991; Melnick and Baev 1993; Schmieden et al. 1989). The absence of reports of rapid desensitization of glycine receptors may be because of limitations in the speed of application used in previous work.

The fast desensitization that we observe has time constants of 13 and 129 ms that account for a significant portions of current decay (19 and 17%, respectively) in 1 mM glycine. Although the peak concentration of glycine achieved in the cleft during synaptic transmission is not known, the best estimate for another amino acid transmitter, glutamate, is \(~1 \text{mM}\) (Clements et al. 1992). Rough calculations based on existing models (Faber et al. 1992; Titmus et al. 1996) suggest that concentrations in the millimolar range also might be achieved at glycnergic synapses. If such concentrations are achieved and if glycine persists in the synaptic cleft long enough, then desensitization could play a role in synaptic responses. Although incomplete, desensitization on these shorter time scales is more likely to contribute to physiological responses mediated by glycine receptors than the slower desensitization rates previously reported. On the other hand, a recent study on GABA receptors in cortex (Galarreta and Hestrin 1997) suggests that brief pulses of low concentrations of GABA have activation and deactivation time courses that are appropriate to explain the shapes of IPSCs. If glycine achieves only low concentrations (\(~10 \mu\text{M}\)) in the synaptic cleft, then desensitization is unlikely to play a significant role in shaping IPSPs.

Desensitization of glycine receptors in the VCN also occurred on longer time scales. For 1-s applications, the best fits of current decay during desensitization often were obtained with three exponentials. Using long applications of glycine, additional phases of desensitization can be observed. The multiplicity of components associated with desensitization is not unprecedented. For example, desensitization on time scales ranging from tens of milliseconds to minutes has been reported for nicotinic acetylcholine receptors (Scuca and Mozrzymas 1992). Multiphasic desensitization has been reported for macroscopic GABA currents in outside-out patches of hippocampal neurons (Celentano and Wong 1994; Jones and Westbrook 1995). Celentano and Wong (1994) observed triphasic desensitization in 70% of the patches; this is similar to the frequency of patches in the present study that were best fit by a sum of three exponentials. The values of the three exponents characteristic of GABAergic desensitization (15, 200, and 1,400 ms) are also similar to the values observed for VCN glycine receptors. Additional experiments will be necessary to determine if these multiple phases of desensitization are due to multiple desensitized states of the receptor (as proposed by Celentano and Wong (1994)) or separate mechanisms by which glycine receptor function can be modulated, as suggested by recent reports of phosphorylation- and zinc-dependent modulation (Agopyan et al. 1993; Kumamoto and Murata 1996; Laube et al. 1995; Song and Huang 1990; Vaello et al. 1994; Wang and Randic 1996).

The offset of the glycine response after a prolonged application of glycine (20 ms and 1 s) was best fit by two exponentials. We examined the possibility that this biexponential decay could be explained by receptor subpopulations within the patch, each of which had different offset kinetics. However, we did not find a positive correlation between glycine concentration and the contribution of the fast component of decay (presumably associated with lower affinity receptors). We also considered the possibility proposed for GABA receptors (Jones and Westbrook 1995) that desensitization might contribute to deactivation kinetics. Comparison of current offsets after glycine applications of 20 ms or 1 s revealed that the relative amplitudes of fast and slow components of decay did not change with the extent of desensitization of receptors. However, the slow decay time constant increased with increasing duration of glycine application. This finding suggests that desensitization may influence deactivation kinetics but not through the same mechanisms that have been proposed for GABA receptors.

Although desensitization may play important roles in synaptic transmission under certain conditions, the deactivation rates of the receptor-agonist complex also can contribute to determining the time course of synaptic currents. Models of glycine receptors (Faber et al. 1992; Titmus et al. 1996) suggest that glycine may be cleared from the cleft within 1 ± 2 ms; analysis of other synapses suggests that transmitter concentration decays with a time constant of \(~100 \mu\text{s}\) after release (Clements 1996; Wahl et al. 1996). Thus the receptor kinetics themselves will be the main factor that determines the response time course. Because our data suggests that desensitized states do not contribute to late openings after agonist removal (the time course of the fast deactivation is unaffected by prior desensitization), the decay of an IPSP should be dominated by the deactivation rate of the receptors. In our experiments, \(~60\%\) of the off-rate was contributed by a rapid component with a time constant of 13–15 ms. Because comparable kinetic data for the decay time constants for IPSCs in the VCN are not available, we made measurements from published records of individual IPSPs by fitting the decay phase of the IPSPs to a function with a single exponential term. The estimated decay time constants (\(\tau_2\)) at 30–34°C ranged from 1.3 to 5.4 ms [VCN: 1.6 ms (Oertel 1983) and 5.4 ms (Wickesberg and Oertel 1990); LSO: 1.3 ms (Wu and Kelly 1995b), 2.6 ms (Sanes 1990)]. Assuming a \(Q_{10}\) of 2.1 for glycine receptors (Titmus et al. 1996), our measured deactivation rate of 13 ms at 22°C corresponds to 5.3 ms at 34° (or 3.5 ms, assuming a \(Q_{10}\) of 3). It appears that the average deactivation rates in patches are slower by a factor of two to three than the decay of most IPSPs, although there is scatter in both sets of measurements. A few individual patches had decay time constants (4.8 and 6.5 ms at 22°C) consistent with those of the shorter IPSPs. Several factors may contribute to such a disparity, including altered channel kinetics in the patches as a result of mechanical or biochemical disruption, differences in receptor populations between different cell types, or a difference in kinetics between synaptic and extrasynaptic receptors.

Recovery of VCN glycine receptors from desensitization also was described best by multiple time constants (300 and
3,000 ms). This finding supports the existence of multiple desensitized states, perhaps as many as the three states suggested by the desensitization analysis. Multiphasic recovery from desensitization also has been reported in other preparations, most notably for GABA receptors in the hippocampus (Jones and Westbrook 1995). Recovery from desensitization produced by 1-ms applications of GABA was best fit by the sum of two exponentials, with time constants of 35 and 1,000 ms. However, recovery was dependent on duration of GABA application. With 5-s applications, recovery occurred with a single time constant of 13 s. A similar dependence of multiphasic recovery from desensitization on time of agonist application has been reported for acetylcholine receptors (Adams 1975). Based on the experiments reported here, we cannot determine if recovery of glycine receptors demonstrates a similar dependence.

Subunit composition

It is worth considering the hypothesis that glycine receptor kinetics might be different in different cells by virtue of their subunit composition. Based on results of glycine receptor cloning, there are at least four alpha subunit variants, expression of which varies with brain area and developmental state (Kuhse et al. 1995). Additional heterogeneity of receptors can result from alternative splicing of alpha subunits (Kuhse et al. 1991; Malosio et al. 1991). Variations in the levels of expression of each subunit suggest cell specific heterogeneity of subunit expression and possibly receptor assembly. Heterologously expressed glycine receptors composed only of α1 or β3 subunits demonstrate different pharmacological sensitivity to β-alanine and taurine (Langosch et al. 1990), but so far no obvious differences in desensitization have been reported. As discussed earlier, the specific subunit assembly of the receptors may influence equilibrium binding parameters. In the cochlear nucleus, the mature forms of glycine receptor subunits (α1, α3, and β) are expressed in the major cell types (Sato et al. 1995), but the precise stoichiometry and distributions of subtypes are not known.

Functional roles of rapid receptor kinetics

Recent evidence has demonstrated that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on neurons of the chick nucleus magnocellularis (the avian homologue of the VCN) are calcium permeable (Otis et al. 1995; Zhou et al. 1995) and desensitize more rapidly than AMPA receptors in other brain regions (Raman and Trussell 1992). Analysis of subunit expression in similar neurons of the rat medial nucleus of the trapezoid body suggests that these properties arise from a selective expression of AMPA receptor subunits (Geiger et al. 1995). The rapid desensitization and off-rates of glutamate receptors in these nuclei are thought to be important for the retention of the precise temporal information necessary for sound localization.

Although inhibitory inputs in the VCN are thought to be involved in several different functions, a clear correlation between receptor kinetics and their contribution to information processing has not been established. Several hypotheses have been advanced that do not depend on the specific timing of inhibition, including roles in increasing the modulation depth of single-format vowel stimuli (Wang and Sachs 1995), in controlling discharge rate for tonal stimuli (Caspar et al. 1994), or in setting the membrane potential of bushy cells (Rothman et al. 1993). However, two hypotheses have been presented that do depend on the existence of brief IPSPs in VCN neurons. First, it has been observed that stellate cells of the VCN can report the intensity of a complex sound in their discharge rate over a wide range of intensities (Blackburn and Sachs 1990). This integration requires that the cells suppress information from low-threshold (high-spontaneous rate) auditory nerve fibers at the higher stimulus intensities to pass information from the high-threshold (low-spontaneous rate) fibers at high intensities. Lai et al. (1994a,b) investigated the properties of a model that could accomplish this task by using shunting inhibition produced by short-duration IPSPs, as originally proposed by Winslow et al. (1987). A narrow range of IPSP time-to-peak values (α functions with τp of 0.25–2.0 ms) were most effective in allowing the model cells to respond to the high-threshold inputs in the presence of activity in the low-threshold auditory nerve fibers. The duration of effective IPSPs overlaps the values measured at VCN synapses and would be consistent with rapid receptor kinetics. A second hypothesis is based on the observation that VCN cells show a suppressed response to the second click of a pair at very short intervals (Wickesberg 1996), a phenomenon likely related to monaural echo suppression. It is thought that glycincergic interneurons in the dorsal cochlear nucleus that project to the VCN (Wickesberg and Oertel 1988, 1990) are in part responsible for this suppression. Again, the inhibitory conductance changes that occur in the VCN neurons must be brief to account for the narrow range of intervals (interclick intervals < ~4 ms) over which the suppression occurs.

It is likely that short-duration inhibitory conductances play a crucial role in stimulus processing beyond the cochlear nucleus, as the glycincergic IPSPs in several other auditory brain stem nuclei appear to have brief duration (Grothe and Sanes 1994; Robertson 1996; Sanes 1990; Wu and Kelly 1992, 1995a,b). It also has been suggested that brief, timed inhibitory inputs also play a role in binaural processing for interaural time delays (Brughera et al. 1996; Grothe and Sanes 1994; Yin and Chan 1990). Thus the relatively rapid desensitization and deactivation rates that we have reported here may be appropriate and useful for the stringent temporal processing requirements encountered throughout the auditory system.

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