Voltage Dependence of the Glycine Receptor–Channel Kinetics in the Zebrafish Hindbrain

PASCAL LEGENDRE
Institut des Neurosciences, Université Pierre et Marie Curie, 75252 Paris Cedex 05, France

INTRODUCTION

Multiple voltage-dependent postsynaptic mechanisms modulate the activity of ligand-gated channels responsible for excitatory and inhibitory postsynaptic currents. Such mechanisms may function to control the postsynaptic efficacy of synaptic events (Faber and Korn 1987) or may operate to prevent cell damage due to excessive depolarization, as for excitatory glutamatergic synaptic events (Collingridge and Lester 1989; Rothman and Choi 1990). In most cases, voltage dependence results from open channel blockade by ions such as Mg$^{2+}$ for N-methyl-D-aspartate (NMDA) receptors (Mayer et al. 1984; Nowak et al. 1984) or by the neurotransmitter itself, as shown for the nicotinic acetylcholine receptor (Liu and Dilger 1991; Ogden and Colquhoun 1985; Sine et al. 1990, Sine and Steinbach 1984). It can also be due to voltage-dependent changes in ion permeation, as for some kainate receptor subtypes (Curtin et al. 1992), central nicotinic receptors (Mulle and Changeux 1990) or 5-HT3 receptors (Derkach et al. 1989). But this can also be related to intrinsic voltage-dependent gating behavior of the receptor channel itself as, for example, the voltage-dependent desensitization described for GABA$_A$ and glycine receptors (Akaike and Kaneda 1989; Borrmann et al. 1987; Burgard et al. 1996, Dominguez-Perrot et al. 1996; Gunderson et al. 1984, 1986; Mellor and Randall 1998).

Voltage dependence of glycineric inhibitory postsynaptic currents (IPSCs) duration was first described in the Mauthner cell (M-cell) of the goldfish (Faber and Korn 1987). A similar property of glycine responses was reported in larva and adult zebrafish M-cell (Hatta and Korn 1998; Legendre and Korn 1995) in and mammalian neurons in slices (Otis and Mody 1992; Stuart and Redman 1990). However, the GlyRs gating properties involved have not yet been elucidated.

In the zebrafish hindbrain an increase in miniature IPSC (mIPSC) duration with membrane depolarization is correlated with the increase in GlyRs opening burst duration (Legendre and Korn 1995), suggesting that fast GlyRs kinetics can be voltage sensitive. Recent analysis of the gating behavior of GlyRs using fast-flow application techniques on outside-out patches had revealed a complex behavior of the zebrafish glycine-operated channels (Legendre 1998). In the zebrafish hindbrain, the decay time of mIPSCs is controlled by gating modes (a reluctant and a willing gating mode) closely similar to those described for the bullfrog N-type calcium channel (Bean 1989; Boland and Bean 1993; Elmslie et al. 1990; Elmslie and Jones 1994).

The interconversion between these two gating modes is voltage dependent for the bullfrog N-type calcium channel (Boland and Bean 1993). This might also be the case for GlyRs as the GlyRs Markov model predicts that an increase of the rate constant from the doubly liganded closed state to the reluctant closed state can greatly enhance the duration of mIPSCs (Legendre 1998). However, changes in the time course of the deactivation phase of a mIPSC might also result from a change in the closing rate constant and/or the dissociation rate constant (Legendre 1998).

To address this issue I took advantage of the M-cell of the 50-h-old zebrafish (Danio rerio) brain preparation (Legendre and Korn 1994). I analyzed the voltage-dependent gating properties of the glycine receptors (GlyR) using fast-flow application techniques (Franke et al. 1987; Lester et al. 1990) and
outside-out recordings to unravel the voltage-dependent channel gating reactions. By comparing my experimental data to simulated traces obtained from GlyRs Markov model, I demonstrate that changes in glycineric mIPSCs duration with membrane potential are likely to result from a voltage dependence of closing rate constants for the GlyR channel. The origins of the voltage dependence of closing rate constants are discussed with respect to anion permeation versus charged moieties in receptor subunits that can move with respect to the electrical field when the channel gates.

METHODS

Isolated intact brain preparation

The isolated intact zebrafish brain was prepared as previously described (Legendre and Korn 1994). Briefly, the brains of 50-h-old larvae were dissected out and glued to a coverslip using a plasmathrombin embedding procedure. Before starting the experiments, brain preparations were stored for 15 min in an oxygenated (95% O2–5% CO2) bathing solution containing (in mM) 145 NaCl, 1.5 KCl, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, and 10 glucose, with the osmolarity adjusted to 330 mOsm.

Outside-out patch-clamp recordings

Standard outside-out recordings (Hamill et al. 1981) were achieved under direct visualization (Nikon Optiphot microscope) on the M-cell located in the fourth hindbrain rhombomere (Metcalfe et al. 1986) as previously described (Legendre 1998). The isolated brain was continuously perfused at room temperature (20°C) with the oxygenated bathing solution (2 ml/min) in the recording chamber (0.5 ml). Patch-clamp electrodes were pulled from thick-wall (10–15 MΩ) borosilicate glass. They were fire-polished and filled with (in mM) 135 CsCl, 2 MgCl2, 4 Na3ATP, 10 EGTA, 10 HEPES, pH 7.2. The osmolarity was adjusted to 290 mOsm. Outside-out patches were obtained by slowly pulling the pipettes out of the brain. The resistance of outside-out patches ranged from 2 to 10 GΩ.

Currents were recorded using an Axopatch 1D amplifier (Axon instruments), filtered at 10 kHz, and stored using a digital tape recorder (DAT DTR 1201, SONY).

Drug delivery

Outside-out single-channel currents were evoked using a fast-flow application system (Franke et al. 1987; Legendre 1998; Lester et al. 1990). Drugs were dissolved in a control solution containing (in mM) 145 NaCl, 1.5 KCl, 2 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES, pH 7.2, osmolarity 330 mOsm. Control and drug solutions were gravity fed into the two channels of a thin-wall glass theta tube (2 mm OD, Hilgenberg, Germany) pulled and broken to obtain a tip diameter of 200 μm. One lumen of the tube was connected to reservoirs filled with solutions containing different glycine concentrations. The solution exchange was performed by rapidly moving the solution interface across the tip of the patch pipette, using a piezoelectric translator (Physics Instrument, model P245.30). Concentration steps of glycine lasting 1–200 ms were applied every 5–10 s. The exchange time (0.08 ms) was determined after rupturing the seal by monitoring the change in the liquid junction evoked by the application of a control solution diluted by 10% to the open tip of the patch pipette (Legendre 1998). As the absolute exchange on the patch partially results from an unstirred layer around the patch, the theoretical limit to the speed of solution change was estimated using the method published by Magonochie and Knight (1989) (see Legendre 1998, for detailed analysis). Assuming that the patch has a spherical geometry with a diameter of 0.5 μm (patch electrode resistance >10 MΩ) and a diffusion coefficient for glycine close to 0.5–1 × 10−5 cm2 s−1, the estimated absolute exchange time was found to be ≤0.1 ms.

Outside-out patch current analysis

Single-channel currents were filtered at 10 kHz using an eight-pole Bessel filter (Frequency Devices), sampled at 50 kHz (Digidata 1200 interface, Axon Instruments), stored on an IBM AT compatible computer using Pclamp software 6.03 (Axon Instruments) and analyzed off-line with Axograph 3.5 software (Axon Instruments).

The time courses of outside-out responses was analyzed by averaging 10–15 single events using Axograph 3.5 (Axon Instruments; filter cutoff frequency: 10 kHz). The activation time constants of currents evoked by a low concentration of glycine 0.1–0.03 mM glycine applications (100–200 ms) were estimated by fitting the onset of the responses with a sum of two sigmoidal curves (Legendre 1998) using Axograph 4 software (filter cutoff frequency: 10 kHz). To fit the rise time of these responses, their onset was determined from that of the chloride currents evoked by the application of a saturating concentration (3–10 mM) of glycine (Legendre 1998). The first 150 ms of the decay phase of the outside-out currents evoked by a brief (1 ms) application of 3–10 mM glycine was fitted with a sum of two exponential curves to determine their decay time constants (Legendre 1998).

Kinetic modeling programs

The kinetic model for GlyR behavior we used was previously determined for M-cell GlyRs (Legendre 1998). Glycine-evoked currents were analyzed off-line using chemical kinetic modeling programs (Axograph 4, Axon Instruments) on a Power Macintosh (7600/132) to adjust the rate constants to obtain theoretical responses with time course similar to the experimental data. This program first calculated the evolution of the number of channels in each given state for given rate constants. Simulated traces were obtained using Axograph 4 software by varying one rate constant with voltage according to the experimental measurements.

Patch currents represent the average of ≥10 traces as specified in the figure legends or the text. Results are presented as means ± SD throughout unless otherwise noted.

RESULTS

I examined the voltage dependence of the activation and deactivation kinetics of native glycine receptors obtained from the Mauthner cell (M-cell) using fastflow application techniques. Two types of glycineric receptors have been functionally characterized on the zebrafish M-cell (Legendre 1997). They represent the expression of homomeric-like α1 and heteromeric-like α/β receptors (Legendre 1997). These two receptors can be discriminated by their mean conductance states and the number of their subconductance levels. In the present study, I focused my analysis on heteromeric-like receptors characterized by a single conductance state of 40–46 pS because their general kinetic properties have been previously determined (Legendre 1998). Patches containing channels with a main conductance state of 80–86 pS and multiple subconductance levels were therefore omitted.

Time course of 3 mM evoked outside-out currents with voltage

Transient outside-out currents evoked by a short step into a saturating concentration of glycine have closely similar time course to that of mIPSCs recorded in the zebrafish M-cell.
(Legendre 1998). The deactivation phase of these currents could be fitted by the sum of two exponential curves with decay time constants of \(\approx 5\) ms and \(\approx 30\) ms \((V_h = -50\) mV\). The double exponential deactivation results from the complex gating behavior of GlyRs (Legendre 1998) (see Fig. 5A). Several mechanisms might underlie the voltage-dependent increase in mIPSC decay time and the single-channel opening burst durations (Legendre and Korn 1995). It can result from a decrease in the closing rate constant, or a decrease in the dissociation rate constant.

Analysis of the time course of glycine-evoked responses with voltage would thus give information about the possible voltage-dependent gating reactions (Legendre 1998). The basis of gating modes, or a decrease in the dissociation rate constant.

Increasing the holding potential \((V_h)\) from \(-60\) to \(+20\) mV did not change significantly the maximum chloride conductance measured at the peak of the responses evoked by 1-ms applications of 3 mM glycine. A linear current-voltage relationship was obtained in all patches tested \((n = 10)\) when \(V_h\) was increased from \(-50\) to \(+20\) mV (Fig. 1). For \(V_h\) less than \(-50\) mV, a small deviation of the recorded current amplitude from the linear regression line was observed (Fig. 1B). This slight decrease in the maximum macroscopic conductance with low voltages is likely to be due to rectification of GlyRs microscopic conductance, as previously described on patches pulled from the zebrafish M-cell (Legnendre and Korn 1994).

The deactivation phase of outside-out currents evoked by a saturating concentration of glycine is voltage dependent. When \(V_h\) was increased, the deactivation phase of these responses was prolonged (Fig. 2A). It remained biphasic at all membrane potentials tested and could be fitted by the sum of two exponential curves (Figs. 2 and 3). Short and long decay time constants were \(\tau_{fast} = 5.1 \pm 0.53\) ms and \(\tau_{slow} = 41.4 \pm 7.8\) ms \((n = 10)\) at \(V_h = -50\) mV. When the patches were depolarized to \(+20\) mV, \(\tau_{fast}\) and \(\tau_{slow}\) significantly increased to reach \(9.8 \pm 0.97\) ms and \(67.7 \pm 11.5\) ms \((mean \pm SD, n = 8)\), respectively (paired \(t\)-test, \(P = 0.01\)). \(\tau_{fast}\) increased progressively with voltage (Fig. 2B). The relationship between \(\tau_{fast}\) and the holding potential can be fitted by a single exponential function between \(-50\) and \(+20\) mV given an increase in \(\tau_{fast}\) with a limiting slope of \(e\)-fold/95 mV (Fig. 2C). \(\tau_{slow}\) also increased progressively with voltage (Fig. 3A). Assuming that \(\tau_{slow}\) also changed exponentially when membrane potential was increased, we found that \(\tau_{slow}\) increased \(e\)-fold per 111 mV/between \(-50\) and \(+10\) mV. This is closely similar to that obtained for \(\tau_{fast}\).

In contrast to the decay time constants, the relative amplitude of these two decay components was not significantly voltage dependent (paired \(t\)-test, \(P = 0.1\)). At \(V_h = -50\) mV, the fast decay component represented \(64.2 \pm 2.8\%\) \((n = 10)\) of the total current while depolarizing the patch to \(+20\) mV slightly decreased its relative amplitude to \(54.1 \pm 10\%\) \((n = 8)\) (Fig. 3B).

The voltage sensitivity of the two decay time constants with voltage and the lack of voltage dependence of their relative proportion might result from voltage-dependent opening rate constants. To test this hypothesis, we analyzed the activation phase of the transient current evoked by 1-ms application of a saturating concentration of glycine (3 mM). When a saturating concentration of agonist is applied, the limiting factor for the rise time of the evoked currents becomes the opening rate and the closing rate constants (\(\beta1\) and \(\alpha1\) for GlyRs, respectively) linking the open state and the doubly liganded closed state. According to the GlyR Markov model (Legendre 1998) shown in Fig. 5A, changes in the rise time constant \(\tau_{on} = 1/\beta1 + \alpha1\) with voltage can give information on the voltage sensitivity of these rate constants. But, as the opening rate constant \(\beta1\) of GlyRs is \(>10\) times faster than the closing rate constant \(\alpha1\) \((\beta1 \approx 9,000 \text{ s}^{-1}; \alpha1 \approx 600–700 \text{ s}^{-1})\) (Legendre 1998), any modifications in the onset duration will mainly reflect fluctuations in the opening rate constant \(\beta1\). For example a two time change in \(\alpha1\) will modify \(\tau_{on}\) by \(\approx 4\%\) only.

To estimate the voltage dependence of the opening rate constant \(\beta1\), I measured the 20–80% rise time of the responses evoked by 1-ms application of 3 mM glycine. As shown in Fig. 3, C and D, the 20–80% rise time did not change with voltage. The 20–80% rise time measured at \(V_h = -70\) mV \((0.2 \pm 0.012\) ms; \(n = 6)\) or \(V_h = -50\) mV \((0.19 \pm 0.01\) ms; \(n = 6)\) was not significantly modified (paired \(t\)-test, \(P = 0.1\)) when the
be due to voltage sensitivity of the dissociation rate constant $k_{\text{off}}$. As these rate constants also control the rising phase of responses evoked by a nonsaturating concentration of the agonist (Legendre 1998), I analyzed the activation phase of the outside-out currents evoked by 0.1 mM glycine applications at membrane potentials ranging from $-60$ mV to $+20$ mV. The activation phase of 0.1 mM glycine-evoked responses has a sigmoidal onset corresponding to the presence of two binding sites. The biphasic time course reflects equilibration between the two opening gating modes of GlyRs (Legendre 1998).

The activation phases of these responses was better fitted with a sum of two sigmoidal functions of the form

$$f(t) = a + \frac{b - a}{1 + e^{-\frac{t - t_{\text{half}}}{\tau_{\text{on1}}}}}$$

where $a$ and $b$ are the relative amplitudes of the two components and $\tau_{\text{on1}}$ and $\tau_{\text{on2}}$ are the corresponding time constants (Fig. 4A) (Legendre 1998). Activation time constants were analyzed from responses evoked by 100- to 200-ms pulse applications of 0.1 mM glycine to ensure glycine-binding equilibrium at the peak of the responses. Increasing the holding potential from $-60$ to $20$ mV did not significantly change the fast ($\tau_{\text{on1}}$) and the slow ($\tau_{\text{on2}}$) time constants of the two components of the activation phase (paired $t$-test, $P = 0.1$). $\tau_{\text{on2}}$ showed a small tendency to increase when $V_h$ was increased (Fig. 4C). When the outside-out patches were held at $-50$ mV, $\tau_{\text{on1}}$ and $\tau_{\text{on2}}$ had a value of 2.45 ± 0.31 ms and 8.1 ± 1.99 ms ($n = 5$), respectively. Changing $V_h$ from $-50$ to $20$ mV gave $\tau_{\text{on1}}$ and $\tau_{\text{on2}}$ values of 2.18 ± 0.5 ms and 10.5 ± 2.32 ms ($n = 5$), respectively.

The relative amplitude of these two components was concentration dependent (Legendre 1998) but did not change significantly with voltage (paired $t$-test, $P = 0.1$; Fig. 4D). For example, $\tau_{\text{on1}}$ had a relative amplitude of 0.619 ± 0.13 at $V_h = -50$ mV and 0.624 ± 0.15 at $V_h = +20$ mV ($n = 5$). These observations imply that transitions between gating reactions linking the willing and reluctant states of the GlyR are not voltage sensitive. They also suggest that the dissociation rate constant $k_{\text{off}}$ shows relatively little voltage dependence.

Closed rate constants are likely to be voltage dependent

My experimental data therefore suggests that the closing rate constants $\alpha_1$ and $\alpha_2$ (Fig. 5A) are most likely to be voltage sensitive. But the mean open times cannot be directly estimated from classical stationary analysis of the glycine-gated channel activity due to unresolved short closures (<0.1 ms), which correspond to the fast opening rate constants of GlyRs (Legendre 1998). To determine the voltage sensitivity of these closing rate constants, experimental data, described herein, were therefore compared with simulated outside-out currents using the Markov model previously proposed for zebrafish GlyRs (Legendre 1998) (Fig. 5A). The rate constants were adjusted to construct simulated traces with time courses similar to experimental measurements performed at $V_h = -50$ mV (see Fig. 5).

Two different kinetic models were tested: in one closing rate constants and in the other dissociation rate constants were given a voltage dependence. This comparison was done because the two times change in the decay time constants of outside-out current between $-50$ and $+20$ mV might also result from a slight voltage sensitivity of the dissociation rate

Responses evoked by a nonsaturating concentration of glycine

Changes in the deactivation time constants with voltage can also reflect voltage-dependent interconversion between the two GlyR gating modes (i.e., the rate constants linking the willing state A2C and the reluctant state A2C* of Fig. 5A). It can also

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constant $k_{off}$ as predicted by the GlyR Markov model shown in Fig. 5A (Legendre 1998). Moreover, this Markov model predicts that a two times change in the $k_{off}$ value will have a limited effect on the activation time course of responses evoked by the application of a low concentration of glycine (Legendre 1998). The complex deactivation phase of transient currents evoked by a short pulse of glycine corresponds to clusters of bursts of channel openings arising from the two open states O1 and O2 linked to the willing state A2C and the reluctant state A2C*, respectively (Legendre 1998). The number of openings per burst arising from O1 can be influenced by changes in the $k_{off}$ value with respect to the opening rate constant $\beta$ and the rate constant $d$ between A2C and A2C* ($N = 1 + (\beta_d / d + k_{off})$). Decreasing $k_{off}$ will also decrease the probability of escape from the reluctant gating mode, which will, in turn, increase the duration of the clusters of bursts of openings arising from O2 while burst duration remains unchanged. This results because the number of openings per burst ($N$) arising from O2 is not modified because it depends primarily on the opening rate constant $\beta_2$ and the reverse rate constant $r$ between A2C* and A2C ($N = 1 + (\beta_2 / r)$). Finally, a model in which the opening rate constants $\beta$ were given a voltage dependence is unlikely. Such a model predicts a $\approx 40\%$ decrease in the 20–80% rise time of responses evoked by 3 mM glycine applications when the patch is depolarized from $-50$ mV to $+20$ mV. This was not experimentally observed (Fig. 3, C and D).

The first model had two voltage-dependent closing rate constants ($\alpha_1$ and $\alpha_2$) with similar voltage sensitivities, as the two deactivation components $\tau_{fast}$ and $\tau_{slow}$ were increased to the same extent by depolarizing the patch to $+20$ mV (Figs. 2 and 3). Changes in the rate constants $\alpha$ with voltage were calculated using the relation of the form

$$\alpha = \alpha_{-50 \text{ mV}} \exp\left[\left(-V_h - 50 \text{ mV}\right)/95 \text{ mV}\right]$$

where $\alpha_{-50 \text{ mV}}$ is the closing rate constant estimated at $-50$ mV ($\alpha_1 = 620 \text{ s}^{-1}$ and $\alpha_2 = 1,300 \text{ s}^{-1}$).

The second model supposes that the dissociation rate constant $k_{off}$ is voltage dependent. Changes in the dissociation rate constant $k_{off}$ with voltage were calculated using the following equation

$$k_{off} = k_{off,-50 \text{ mV}} \exp\left[\left(-V_h - 50 \text{ mV}\right)/95 \text{ mV}\right]$$

where $k_{off,-50 \text{ mV}}$ is the dissociation rate constant estimated at $-50$ mV ($k_{off} = 1,550 \text{ s}^{-1}$).

We first compared the time course of responses to 1-ms application of 3 mM glycine with data from simulations based on these theoretical models. Theoretical and experimental data were compared at $V_h$ from $-60$ to $+20$ mV. As shown in Fig. 5B, changes in $k_{off}$ with voltage (model 2) cannot properly describe the voltage-dependent increase of the fast decay component when the membrane is depolarized. In contrast a good agreement between experimental and simulated data was obtained when $\alpha$ was made to be voltage dependent. Changes in $\alpha$ with voltage can also account for the increase of the slow decay component with membrane depolarization (Fig. 5C). Furthermore this model predicts that the relative amplitudes of the two decay components has a little voltage dependence (Fig. 5D). This is not the case when $k_{off}$ was made to be voltage sensitive. An increase in $k_{off}$ with voltage will evoke a decrease in the relative proportion of the fast decay component, which is not the case in my experimental conditions (Fig. 5D). To obtain a similar increase in the decay time constant of the fast deactivation component with $V_h$ depolarized to $+20$ mV, $k_{off}$ must decrease $e$-fold/32 mV. In this case, the model predicts that the time constant of the second decay component will increase by four times (160 ms) at $V_h = +20$ mV, whereas the relative proportion of the fast decay component will decrease to 23%. This was not experimentally observed.

The accuracy of model 1 was confirmed when experimental responses evoked by a low concentration of glycine were
compared with theoretical traces obtained from models 1 and 2. As previously mentioned, setting $\alpha$ or $k_{\text{off}}$ as voltage-dependent rate constants did not strongly modify the fast activation time constant of responses evoked by the application of 0.1 mM glycine (Fig. 6B). When $V_h$ was depolarized from $-60$ to $+20$ mV, the two models predict that the relative proportion of the two activation phase components will be little affected by voltage, as observed experimentally (Fig. 6C). However, model 2 ($k_{\text{off}}$ being voltage sensitive) predicts a decrease in the slow time constant of the activation phase component when $V_h$ was increased, which is not the case in my experimental conditions (Fig. 6B). Altogether these results suggest that the change in glycine-evoked transient outside-out current duration with membrane potential may be explained by voltage-dependent closing rate constants $\alpha 1$ and $\alpha 2$, the other gating reactions being voltage insensitive.

**DISCUSSION**

The present study shows that the voltage dependence of the duration of glycine-evoked transient outside-out current can result from an increase in the mean open times of GlyRs. This is consistent with my previous work showing that the deactivation time course of glycine-gated mIPSCs depends primarily on the glycine-gated channel kinetics (Legendre 1998). It seems unlikely that this property reflects the presence of postsynaptic immature GlyRs because voltage-dependent IPSCs duration can be observed in the M-cell of the adult zebrafish (Hatta and Korn 1998) and of the adult goldfish (Faber and Korn 1987).

**Voltage dependence of glycine-evoked outside-out currents**

The peak current evoked by a brief application of a saturating concentration of glycine varied linearly between $-60$ and $+20$ mV as do evoked glycine-evoked synaptic current recorded in the adult goldfish M-cell (Faber and Korn 1987) and in spinal motoneurons of the cat (Stuart and Redman 1990). This is consistent with the lack of voltage sensitivity of the opening rate constants of GlyRs and the maximum open probability ($0.9$) of glycine-gated channels measured at $V_h = -50$ mV (Legendre 1998). The fast decay time constant increased $e$-fold per $95$ mV. This is closely similar to that reported for evoked IPSCs in the cat motoneurons ($e$-fold/91) (Stuart and Redman 1990), which suggests that hindbrain zebrafish GlyRs share some functional characteristics with mammalian spinal cord receptors. The voltage dependence of the decay time constants is, however, two to three times less than that for glycine-gated channel activity ($e$-fold/35 mV) recorded under stationary conditions (Legendre and Korn 1995). This is likely to be due to two independent mechanisms (Legendre and Korn 1995), one involving voltage-dependent closing rate constant and the other reflecting voltage-dependent slow desensitization (Akaike and Kaneda 1989). These observations also imply that desensitization should show a stronger voltage dependence than the opening rate constant. Voltage-dependent desensitization cannot, however, account for the change in duration of the postsynaptic responses with voltage. It develops too slowly, and it cannot shape the time course of mIPSCs or transient outside-out currents evoked by a short application ($<50$ ms) of glycine (Legendre 1998).

A voltage dependence of IPSC duration mediated by changes in channels kinetics is also observed at GABAergic synapses. The deactivation phase of GABA-evoked transient current and the amount of GABA$_A$ desensitization are also voltage dependent (Mellor and Randall 1998; Yoon 1994). Although it appears that the increase in the proportion of the
fast desensitized GABA-evoked currents and the increase in GABA-evoked response duration are, as for GlyRs, independent (Mellor and Randall 1998), it seems likely that distinct mechanisms operate at these two receptors. The biphasic deactivation of GABAergic responses is controlled by a fast desensitization mechanism (Jones and Westbrook 1995) that increases at depolarized potentials in cerebellar granule cells (Mellor and Randall 1998). To the contrary, the amount of desensitized current evoked by glycine application is decreased when the membrane is depolarized (Akaike and Kaneda 1989; Legendre and Korn 1995). Moreover, changes with voltage of GABAergic IPSCs duration are characterized by a modification of the relative amplitude of the two decay components, whereas their decay time constants remain unchanged (Mellor and Randall 1998). This is the opposite for GlyRs.

Voltage-dependent kinetics of glycine-gated channels

A voltage dependence of transition rate constants for GABA A receptors, which might underlie changes in GABAergic mIPSC duration, has not yet been demonstrated (Mellor and Randall 1998), but the deactivation time course of GABA-evoked responses and the GABA A receptors desensitization depend crucially on GABA A subunits combination (McClellan and Twyman 1999). This renders kinetic analysis with Markov model approximations much more difficult. However, the number of potential GlyR subunit combinations is much less than for GABA. By focusing my analysis on one type of GlyRs, presumably α1/β-like GlyRs (Legendre 1997), I was able to determine a Markov model describing GlyRs activation kinetics (Legendre 1998) and so could determine which GlyR gating reaction possessed a voltage dependence. The gating scheme I used provides good approximations of the activation and deactivation behavior of GlyRs receptors activated by short glycine applications over a wide range of agonist concentration, although no desensitized states were included (Legendre 1998). The desensitized states were not incorporated because they are too slow to influence the mIPSCs time courses at all voltages tested.

Changes in the mean open time with voltage might, however, reflect open channel block mechanisms. But this cannot account for the change in glycine-evoked responses duration with voltage. The current-voltage (I-V) curve is linear in the voltage range over which changes in decay time duration occurs. Moreover, single-channel conductance is insensitive to voltage for V kh between −50 and +20 mV (Legendre and Korn 1994). Finally it is unlikely that the agonist itself can block the glycine-gated channel as proposed for acetylcholine on the nicotinic receptors (Liu and Dilger 1991; Ogden and Colquhoun 1985; Sine et al. 1990; Sine and Steinbach 1984) because glycine is weakly charged at neutral pH. A decrease in the closing rate constant implies that opening of the GlyRs chloride channels is dependent on the membrane voltage only. This differs from most voltage-gated channels where typically all gating rates depend on voltage (Chen and Hess 1990; Horn and Vandenberg 1984; Keynes 1994; Kuo and Bean 1994), whereas closing rates can be voltage independent as for the Shaker potassium channel, the squid sodium channel or the N-type calcium channel (Aldrich and Stevens 1987; Boland and Bean 1993; Cota and Armstrong 1989; Miller 1990; Vandenberg and Bezanilla 1991).

Mechanisms underlying the voltage dependence of the GlyRs closing rate constant

Changes in closing rate constants with voltage have also been reported for acetylcholine receptors (ACHRs) (Ascher et al. 1978; Auerbach et al. 1996; Colquhoun and Sakmann 1985; Magleby and Stevens 1972; Marchais and Marty 1979; Neher and Sakmann 1976; Sheridan and Lester 1977; Sine et al. 1990). Two types of mechanisms have been proposed to explain this voltage dependence for AChRs (Auerbach et al. 1996; Marchais and Marty 1979). The first mechanism is related to ions permeation through the pore of the channel. It implies that favored binding of permeant cations on its binding
0.1 mM glycine

A

-50 mV:
\( \tau_{on1} = 2.15 \text{ ms (62\%)} \)
\( \tau_{on2} = 9.1 \text{ ms} \)

\( \bigcirc +20 \text{ mV} : \)
\( \tau_{on1} = 2.6 \text{ ms (55\%)} \)
\( \tau_{on2} = 9.81 \text{ ms} \)

10 ms

B

\( \alpha_{efold/95 \text{ mV}} \)
\( \alpha_{refold/95 \text{ mV}} \)

\( \tau_{on} (\text{ms}) \)

\( \alpha_{efold/95 \text{ mV}} \)
\( \alpha_{refold/95 \text{ mV}} \)

C

\( \text{Relative amplitude} \)

\( \text{Data} \)

\( \alpha_{efold/95 \text{ mV}} \)
\( \alpha_{refold/95 \text{ mV}} \)

\( \text{Holding potential (mV)} \)

site at more hyperpolarized potential will hinder channel closing and therefore increase the mean open time of the channel (Marchais and Marty 1979). This hypothesis supposes that the channel cannot close until the ion dissociates from its binding sites. A second mechanism, not entirely incompatible with the first, is related to voltage-sensitive charge movements in the protein during gating and has been proposed to explain changes in closing rate constants of mouse AChRs (Auerbach et al. 1996). It supposes that charged moieties in the AChRs protein change their disposition after agonist binding and move with respect to the electrical field when the channel gates (Auerbach et al. 1996). Both models could explain the voltage dependence of the GlyRs channel closing rate constants. The GlyRs channel pore has at least two anion binding sites (Bormann et al. 1987), and this binding could be favored at depolarized membrane potential, which will in turn hinder channel closing. But if the anion binding hypothesis is true, receptor channels with closely identical pore should have similar voltage-dependent properties. This is not the case for GlyRs and GABA<sub>A</sub> receptors (GABA<sub>A</sub>R). Both these receptors have highly conserved M2 domains that form the pore of the channel (Betz 1992; David-Watine et al. 1999), and the walls of the channels have very similar electrical properties (Bormann et al. 1987). Although responses evoked by fast applications of GABA increase in duration with voltage, even when fast desensitization processes are not involved, this is not due to a change in the deactivation time constant with voltage but to an increase in the relative proportion of the slow deactivation component (Mellor and Randall 1998). This is the opposite to what I observed for GlyRs, suggesting that changes in anion binding with voltage may not significantly modify the opening duration of the GlyR and GABA<sub>A</sub>R anionic channel. It is the therefore tempting to speculate that the decrease in the closing rate constant of GlyRs at depolarized potential results largely from charge movement with respect to the electrical field during channel gating. Studies using recombinant GlyRs having mutations in the pore region of the channel are needed to address this issue definitively (Auerbach et al. 1996).

**Physiological significance**

A slow desensitization process of GlyRs cannot play a significant role after release of a single vesicle, although it might modulate glycinergic synaptic efficacy when a long-lasting (2–4 s) depolarization of postsynaptic membrane is coupled with high-frequency inhibitory cell activity. In contrast, changes in the decay time of glycine evoked responses with voltage can enhance the efficacy of single inhibitory responses in the face of an increased excitation (Faber and Korn 1987). This implies that the increase of glycinergic inhibitory postsynaptic potential (IPSP) duration with membrane depolarization will significantly favor their summation or prolong the membrane hyperpolarization. This will, however, depend on the membrane time constant of the cell. Effectively, a membrane time constant larger than the deactivation time constant of the synaptic current will tend to reduce the effect of changes in the current decay time with voltage on IPSP duration but will increased its efficacy to control IPSP duration (Singer et al. 1998). Glycinergic synapses can also inhibit cell activity by shunting electrotonic transmission due to the evoked decrease in the cell input resistance. In this case, a depolarization of the membrane will also enhance the effect of glycinergic synapses on the input resistance of the cell, which...
REFERENCES


