In the ionotropic glutamate receptor, the global conformational changes induced by partial agonists are smaller than those induced by full agonists. However, in the pentameric ligand-gated ion channel receptor family, the structural basis of partial agonism is not understood. This study investigated whether full and partial agonists induce different conformational changes in the glycine receptor chloride channel (GlyR). A substituted cysteine accessibility analysis demonstrated previously that glycine binding induced an increase in surface accessibility of all residues from Arg271 to Lys276 in the M2-M3 domain of the homomeric α1 GlyR. Here we compare the surface accessibility changes induced by the full agonist, glycine, and the partial agonist, taurine. In GlyRs incorporating the A272C, S273C, L274C, or P275C mutation, the reaction rate of the cysteine-specific compound, methanethiosulfonate ethyltrimethylammonium, depended on how strongly the receptors were activated but was agonist-independent. Reaction rates could not be compared in the R271C and K276C mutant GlyRs because methanethiosulfonate ethyltrimethylammonium did not modify the extremely small currents induced by saturating taurine or equivalent low glycine concentrations. The results indicate that bound taurine and glycine molecules impose identical conformational changes to the M2-M3 domain. We therefore conclude that the higher efficacy of glycine is due to an increased ability to stabilize a common activated configuration.

The glycine receptor chloride channel (GlyR) mediates fast inhibitory neurotransmission in the vertebrate central nervous system (1, 2). It belongs to the family of pentameric ligand-gated ion channels (LGICs) that includes the nicotinic acetylcholine receptor (nAChR) as its prototypical member (3). Each subunit incorporates a large N-terminal extracellular domain and four α-helical membrane-spanning domains. The second membrane-spanning (M2) domains curve radially so as to form a tapering, water-filled pore with a hydrophobic barrier (or channel gate) at either its mid-point (4) or intracellular boundary (5). The N-terminal domains contain the agonist-binding sites and a disulfide loop that is an invariant feature of LGIC receptors (6). Agonists binding in the N-terminal domain initiate conformational changes that propagate as a wave toward the channel gate (7). Different agonists induce these conformational changes with different efficiencies (where efficacy is the ability of an agonist to open the channel once bound to the receptor). If the efficacy of an agonist is sufficiently low, it will behave as a partial agonist (8). The structural basis of differential agonist efficacy is not yet understood for any member of the LGIC family.

Partial agonism could be caused by one, or a combination, of the following two sharply contrasting mechanisms. First, it is possible that different agonists induce different structural changes throughout the protein. A clear example of this has been characterized recently (9–11) in the ionotropic glutamate receptor cation channel. These studies show that low efficacy agonists induce a smaller degree of binding site core closure than do high efficacy agonists, and show that this smaller degree of closure is translated to the activation gate as a smaller degree of channel opening (11). An alternative possibility is that high and low efficacy agonists induce identical global conformational changes but that the activated state is more stable for the more efficacious agonist.

Glycine and taurine activate the GlyR with different efficacies; taurine has a low efficacy and generally behaves as a partial agonist at this receptor, whereas glycine is a full agonist (12). The present study seeks to establish whether or not taurine and glycine induce identical conformational changes in a domain that links the ligand-binding site to the activation gate. This may provide insights into the structural basis of partial agonist action at the GlyR.

The substituted cysteine accessibility method can quantitate changes in protein conformation. The method entails introducing cysteine residues one at a time into a protein domain of interest. The reactivity of these cysteines is then assayed by exposure to highly soluble, sulphydryl-specific reagents, generally methanethiosulfonate derivatives (13). If a functional property of the channel is irreversibly modified upon exposure to such a reagent, the cysteine is assumed to be exposed at the water-accessible protein surface. Differences in the cysteine modification rate between the closed and open states may provide information about the associated structural changes. Our laboratory previously used this approach to demonstrate a glycine-induced increase in the surface exposure of six continuous residues (Arg271-Lys276) in the GlyR M2-M3 linker domain (14). These residues lie mid-way between the binding site and the activation gate (7), and it is now well established that

Received for publication, January 18, 2004, and in revised form, February 19, 2004
Published, JBC Papers in Press, February 23, 2004, DOI 10.1074/jbc.M400548200

Nian-Lin R. Han‡§, John D. Clements®, and Joseph W. Lynch‡**
From the ‡School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072 and ¶Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia
they experience a conformational change that is crucial for the activation of GlyRs (14–18), γ-aminobutyric acid, type A receptors (19–24), and nACHRs (7, 25–27). A structural study of the Torpedo nACHR has shown recently (4) that these residues form an extramembranous extension to the M2 α-helix. This domain is likely to interact closely with loops 2 and 5 of the ligand-binding domain (4, 17, 18, 23, 24). A particular advantage of investigating this domain is that it lies outside the ligand-binding pocket (6), so changes in methanethiosulfonate reaction rates are unlikely to be influenced by steric effects of ligand binding. The aim of the present study is to employ substituted cysteine accessibility method to determine whether or not glycine and taurine induce identical changes in the surface accessibility of residues Arg271–Lys278.

**MATERIALS AND METHODS**

*Mutagenesis and Expression of Human GlyR α1 Subunit cDNAs—* Site-directed mutations were incorporated into the human GlyR α1 subunit cDNA in the pCIS2 vector using the QuickChange mutagenesis kit (Stratagene, La Jolla, CA). The successful incorporation of the mutations was verified by DNA sequencing. The WT and all cysteine mutant GlyRs investigated in this study incorporated the C41A mutation, which eliminated the only free external sulfhydryl group. This mutation had no significant effect on GlyR EC_{50} values or peak current magnitudes (14). The WT and mutant plasmid constructs were transiently transfected into HEK293 cells using a modified calcium phosphate precipitation method. After transfection for 24 h, the cells were washed twice with cell culture medium, and electrophysiological studies were conducted over the following 24–72 h.

*Patch Clamp Electrophysiology—* Glycine- and taurine-gated currents were measured using whole-cell recording at a holding potential of −50 mV using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA), and data were directly recorded to disk using pCLAMP6 software (Axon Instruments). Cells were continually perfused at 2 ml/min with the standard bathing solution containing (in mM): 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, pH 7.4. Patch pipettes were heat-polished and had tip resistances of 2–4 megohms when filled with the standard intracellular solution containing (in mM): 145 CsCl, 2 CaCl2, 2 MgCl2, 10 HEPES, 10 EGTA, pH 7.4. At least 50% of full series resistance compensation was applied in all recordings.

MTSET, obtained from Toronto Research Chemicals (Toronto, Canada), was prepared as a stock solution of 10 mM in distilled water and maintained on ice for up to 3 h until used. It was applied to cells within 30 s of being dissolved into room temperature bathing solution. The disulfide-reducing agent, dithiothreitol (DTT), was prepared daily as a 1 mM solution in the standard bathing solution. This DTT-containing solution had no irreversible effect on the magnitude of currents in the WT GlyR. Solutions were applied to cells via a parallel system of gravity-fed tubes, and solution exchange was effected with a time constant of about 100 ms. Experiments were performed at room temperature (19–22 °C).

The effects of MTSET on glycine- and taurine-gated currents were tested using the following procedure. Prior to MTSET application, cells were bathed in 1 mM DTT for 1 min to ensure that exposed sulfhydryl groups were fully reduced. Then the agonist dose response was measured by applying increasing agonist concentrations at 1-min intervals. The relative magnitude of currents activated by saturating concentrations of taurine and glycine was also measured. Following this, three consecutive brief applications of a constant glycine concentration were applied at 1-min intervals to establish that the current magnitude was invariant (±5%) prior to the application of MTSET. Following application of the MTSET-containing solution, cells were washed in control solution for at least 2 min before the maximum current magnitudes and tauine EC_{50} values were measured again. If an irreversible effect was observed, the MTSET concentration was adjusted so that the time constant of the current response was between 0.3 and 20 s. The receptor desensitization rate was low (<0.005 s^{-1}) for all mutant GlyRs used in this study and as such did not impact significantly on the measurement of MTSET reactivity rates. It is estimated that a 10% irreversible change in current over 1 min would have been reliably detected.

Data Analysis—Results are expressed as means ± S.E. of four or more independent experiments. The empirical Hill equation, fitted by a non-linear least squares algorithm (Origin, Microcal, Northampton, MA), was used to calculate the 50% effective concentrations for activation (EC_{50}) and inhibition (IC_{50}) and the Hill coefficient (n_H) values. Exponential fits were performed using the same non-linear least squares algorithm. Statistical significance was determined by one-way ANOVA using the Student-Newman-Keuls post hoc test for unpaired or paired data as appropriate (SigmaStat, Jandel, San Rafael, CA), with p < 0.05 representing significance.

**RESULTS**

*Characterization of Cysteine-substituted Mutant GlyRs—* It was shown previously that MTSET modification of cysteines introduced into the N-terminal half of the M2-M3 domain (i.e. from R271C to K276C) resulted in irreversible changes in the glycine EC_{50} values without affecting the saturating current magnitudes (14, 17). The same study also showed that the MTSET reaction rate of all these cysteines was relatively slow in the resting closed state but was increased dramatically in the glycine-bound state. The aim of the present study is to compare the effects of MTSET on the taurine-activated receptor with its effects on the glycine-activated receptor.

The taurine agonist EC_{50} and n_H values for the WT and all cysteine-substituted mutant GlyRs examined in this study are summarized in Table I. The taurine EC_{50} values for the A272C, S273C, L274C, and P275C mutant GlyRs are significantly increased relative to the WT value. Averaged glycine and taurine dose responses for the WT and L274C mutant GlyRs are shown in Fig. 1A. The peak magnitudes of taurine-gated currents were too small to permit reliable EC_{50} measurement in the R271C and K276C mutant GlyRs. The corresponding glycine EC_{50} values for the WT and all mutant GlyRs, reproduced from Lynch et al. (14), are included for comparison. The ratio of the maximum current activated by taurine relative to that activated by glycine (I_{MAX,TAU}/I_{MAX,Gly}) for each mutant GlyR is also shown (Table I). In calculating these ratios, the glycine- and taurine-gated currents were both recorded from the same cell. The cysteine substitution mutations invariably caused a significant reduction in this ratio. Thus, taurine behaves as a full agonist in the WT GlyR but as a partial agonist relative to

---

**Table I**

<table>
<thead>
<tr>
<th>GlyR</th>
<th>Glycine EC_{50}</th>
<th>Taurine activation</th>
<th>Taurine inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>n_H</td>
<td>I_{max,tau}/I_{max,gly}</td>
</tr>
<tr>
<td>WT</td>
<td>18 ± 2</td>
<td>0.12 ± 0.03</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>R271C</td>
<td>2500 ± 0.30</td>
<td>0.06 ± 0.01</td>
<td>10.2 ± 0.40</td>
</tr>
<tr>
<td>A272C</td>
<td>2840 ± 0.20</td>
<td>0.07 ± 0.01</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>S273C</td>
<td>35 ± 5.60</td>
<td>1.0 ± 0.64</td>
<td>15.3 ± 1.20</td>
</tr>
<tr>
<td>L274C</td>
<td>395 ± 10.1</td>
<td>6.40 ± 0.31</td>
<td>15.3 ± 1.20</td>
</tr>
<tr>
<td>P275C</td>
<td>1500 ± 30.0</td>
<td>15.3 ± 1.20</td>
<td>15.3 ± 1.20</td>
</tr>
<tr>
<td>K276C</td>
<td>2714 ± 48.2</td>
<td>0.08 ± 0.01</td>
<td>15.3 ± 1.20</td>
</tr>
</tbody>
</table>

* From Lynch et al. (14)

* I_{max,tau}/I_{max,gly} represents the mean fraction of the half-saturating glycine-gated current remaining in the presence of 50 mM taurine.

---

*ANOVA using the Student-Newman-Keuls post hoc test for unpaired or paired data as appropriate (SigmaStat, Jandel, San Rafael, CA), with p < 0.05 representing significance.*
glycine in all mutant receptors examined.

The ability of taurine to inhibit currents activated by an EC50 glycine concentration was also measured. An example of taurine inhibition of EC50 (400 μM) glycine currents in the L274C GlyR is shown in Fig. 1B, and the inhibitory dose response for this experiment, averaged from four cells, is shown in Fig. 1C. The taurine IC50 and nH values for all tested mutant GlyRs are summarized in Table I. The maximum extent to which a saturating (50 mM) taurine concentration could inhibit currents activated by an EC50 glycine concentration is presented in Table I as Min Itau/Igly. The taurine IC50 was invariably around an order of magnitude lower than its EC50 in the same mutant GlyR.

Steady-state Effects of MTSET—It has been demonstrated previously that MTSET covalently modifies all introduced cysteines from R271C to K276C (14). This modification increased the glycine EC50 of the P275C GlyR but decreased the glycine EC50 of the remaining mutant GlyRs, all without affecting the saturating glycine-gated current magnitude (14).

Fig. 2A shows examples of currents activated by saturating concentrations of taurine and glycine in the R271C (upper panel) and L274C (lower panel). Sample currents are shown both before (left panel) and after (right panel) a 1-min application of MTSET + 50 mM taurine. MTSET was applied at a concentration of 100 μM in the WT, A272C, S273C, and L274C GlyRs and 200 μM in the R271C, P275C, and K276C GlyRs. Taurine and glycine were both applied at concentrations of 50 mM. B, the averaged ratio of peak taurine-gated currents to peak glycine-gated currents (Imaxtau/Imaxgly) measured both before and after exposure to MTSET + 50 mM taurine. Each column represents the average of at least four cells, and asterisks indicate a statistically significant difference (p < 0.05).
sensitivity in the A272C and S273C GlyRs (Fig. 4C). To determine whether MTSET affected the taurine EC50 values, after MTSET modification, it was not possible to directly determine whether MTSET affected the taurine EC50 values. Examples of taurine dose responses before and after a 1-min application of 100 μM MTSET for the L274C mutant GlyR are shown in Fig. 4A, and averaged results are shown in Fig. 4B. MTSET caused a similar increase in taurine sensitivity in the A272C and S273C GlyRs (Fig. 4C).

Because the peak magnitude of taurine-gated currents in the P275C, K276C, and R271C GlyRs remained extremely low after MTSET modification, it was not possible to directly determine whether MTSET affected the taurine EC50 values. However, it was possible to measure the effects of MTSET modification on the potency of taurine inhibition of glycine-gated currents. Progressively increasing concentrations of taurine were applied to the P275C GlyR in the presence of an EC50 glycine concentration (Fig. 5A, upper panel). When this procedure was repeated following a 1-min application of 100 μM MTSET + 50 mM glycine, there was no apparent change in the taurine inhibitory potency (Fig. 5A, lower panel). Because MTSET causes a 4-fold increase in the glycine EC50 (14), it was necessary to increase the glycine concentration from 2 to 8 mM to maintain half-maximum current activation. The results indicate that MTSET modification had no effect on the taurine IC50 for the P275C GlyR (Fig. 5B). The mean taurine IC50 and nH values before and after MTSET modification are summarized in Table II. Similar experiments were carried out on the K276C and R271C GlyRs, and the results are also summarized in Table II. Together, they suggest that MTSET modification does not alter the affinity of these receptors for taurine.

The remainder of this study relies on the comparison of MTSET reaction rates in the glycine- and taurine-activated states. Such a comparison was not possible in the R271C and K276C GlyRs as MTSET did not modify currents induced by either saturating taurine or equivalent (EC50 to EC10) glycine concentrations.

MTSET Reactivity Rates with A272C, S273C, L274C, and P275C—Because differences in MTSET reaction rate can be indicative of differences in receptor conformation, we compared the MTSET reaction rates in the taurine- and glycine-bound states in the A272C, S273C, L274C, and P275C mutant GlyRs. It is important to note that the MTSET reaction rate in the closed state is approximately an order of magnitude slower than it is in the presence of EC50 glycine in all mutant GlyRs tested here (10). An example of an experiment on the L274C mutant GlyR is shown in Fig. 6A. Both panels in this figure were recorded from the same cell. In Fig. 6A, taurine was applied at a saturating concentration (50 mM), and MTSET was applied at 100 μM. The MTSET-induced current increase was adequately described by a single exponential with a time constant of 2.78 s. Currents recorded 2 min later confirm that covalent modification has taken place (Fig. 6A, upper panel). Following a 1-min exposure to 1 mM DTT, the experiment was repeated using a glycine concentration (100 μM) that activated a current of similar magnitude to that activated by saturating taurine (Fig. 6A, lower panel). MTSET modification of the Glycine Receptor Activation
glycine-gated current proceeded with a time constant of 2.66 s. The averaged time constants in the taurine- and glycine-activated states were $2.26 \pm 0.34$ s ($n = 9$ cells) and $2.06 \pm 0.43$ s ($n = 7$ cells), respectively. There was no significant difference between these values, indicating that the conformational change experienced by Cys-274 depends on the fraction of peak current activated, rather than the identity of the agonist.

In the S273C GlyR, the MTSET reactivity rate was examined at the EC$_{50}$ (200 $\mu M$) taurine concentration. This sub-saturating taurine concentration was necessary as higher taurine EC$_{50}$ values would have resulted in current saturation prior to completion of the MTSET reaction, which in turn would have distorted the apparent reaction rate. A glycine concentration of 20 $\mu M$ (EC$_{10}$) was used to activate currents of similar magnitude in the same cell. Again, there was no significant difference in MTSET reactivity between the taurine- and glycine-activated states (Fig. 6B).

![Fig. 4. Effects of MTSET on the taurine EC$_{50}$ of the A272C, S273C, and L274C GlyRs. A, examples of taurine dose responses in the L274C GlyR before and after exposure to 100 $\mu$M MTSET. Both sets of traces were from the same cell. B, taurine dose responses, measured before and after MTSET exposure, averaged from five cells expressing L274C GlyRs. C, mean taurine EC$_{50}$ values before (open circles) and after (filled circles) MTSET exposure for the A272C, S273C, and L274C GlyRs. All points were averaged from at least four cells in which control and MTSET-modified EC$_{50}$ values were both measured.](https://www.jbc.org/)

<table>
<thead>
<tr>
<th>GlyR</th>
<th>Control</th>
<th>After MTSET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$</td>
<td>nH</td>
</tr>
<tr>
<td>R271C</td>
<td>0.59 ± 0.18</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>P275C</td>
<td>1.46 ± 0.38</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>K276C</td>
<td>0.59 ± 0.10</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

Fig. 5. MTSET does not affect taurine inhibitory potency in the P275C GlyR. A, examples of taurine inhibition of currents activated by EC$_{10}$ glycine in the P275C GlyR both before (upper panel) and after (lower panel) MTSET modification. Both sets of traces were from the same cell. MTSET was applied at a concentration of 200 $\mu$M for 1 min, and taurine was applied at concentrations of 0.1, 1 (labeled), 5, and 50 mM. B, taurine inhibitory dose responses averaged from four cells both before (open symbols) and after (filled symbols) MTSET modification. Averaged taurine IC$_{50}$ and nH values of best fit are given in Table II.
applied at 200 M concentration rates were always compared at a common fraction of the peak activated state (filled circles) for the indicated mutant GlyRs. Modification/H9262/L274C GlyRs. MTSET applications were separated by a 2-min DTT wash. Time constants of best fit are shown.

Two Models for Partial Agonism—Two sharply contrasting models have been developed to describe the activation of oligomeric proteins, the coupled Monod-Wyman-Changeux (MWC) model (34) and the uncoupled or sequential Koshland-Nemethy-Filmer (KNF) model (35). In the simplest version of the MWC model, all the subunits change conformation simultaneously, and in consequence the receptor can exist in only the closed or entirely activated states. In contrast, the KNF model proposes that each subunit can independently adopt a specific conformation change depending on the number and species of bound agonist molecules, leading to a series of intermediate protein conformational states.

The structural basis of partial agonism in the ionotropic glutamate receptor has been investigated using a combination of crystallographic and electrophysiological techniques (11). This study revealed that a series of partial agonists promoted a range of conformational changes at the ligand-binding site, with the magnitude of this structural change being directly correlated with the probability of entering higher subconductance states. Thus, increasing agonist efficacy was manifested by an increased ability to open the channel to its maximal extent. This is a classic KNF-type mechanism.

Alternatively, partial agonism could also result from an MWC-type model whereby high and low efficacy agonists induce identical structural changes throughout the receptor. In such a mechanism, higher efficacy would be manifested by an increased ability to stabilize the open state. Because extended MWC models can explain many characteristics of LGIC behavior (34), this model is the favored hypothesis for the present study.

Taurine as a Low Efficacy Agonist—By using classical receptor theory (8), it can be shown that \( F_{\text{max}} = E/(1 + E) \), where \( F_{\text{max}} \) is the maximum fraction of receptors that can be activated by a saturating agonist concentration, and \( E \) is the equilibrium constant for gating (or efficacy). A combination of rapid agonist application techniques and equilibrium single channel kinetic analysis was used by Lewis et al. (12) to estimate \( E \) values of 16 and 3.4 for glycine and taurine, respectively, at the WT \( \alpha \) GlyR. According to these figures, taurine should activate around 77% of the peak glycine current in the WT GlyR, although we find taurine to be a full agonist of the WT GlyR. As summarized in Table I, all mutant GlyRs examined in this study significantly reduced this percentage. The above equation indicates that variations in \( E \) have no measurable effect on peak current magnitude unless they occur within a limited range of around 0.1 to 10. This means that a moderate reduction may not significantly reduce the glycine \( F_{\text{max}} \) whereas any reduction in the \( E \) would yield a reduction in the \( F_{\text{max}} \) for taurine. Thus, although the mutations examined in the present study reduced the \( F_{\text{max}} \) of taurine relative to glycine, they did not necessarily exert a selective effect on taurine efficacy.

The fact that mutations converted taurine into a partial agonist was convenient as it facilitated the comparison between the taurine- and glycine-activated states. For the purposes of analyzing the structural basis of partial agonism, it does not matter whether partial agonism was achieved by appropriate choice of agonist, receptor mutagenesis, or a combination of the two.

Prior to the present study little, if any, experimental evidence was available concerning the structural basis of partial agonism in pentameric LGICs. A variety of biochemical and electrophysiological approaches have shown that the agonist-binding pocket of LGIC receptors is formed by six discontinuous domains, labeled A–F by Corringer et al. (28). The involvement of these domains in forming the pocket has been confirmed by the crystal structure of acetyl/choline binding protein (6). In the GlyR, taurine and glycine both bind in this cavity but appear to interact differently with at least one of the molecular groups lining its wall (29–32). However, even if the respective agonists induce different local structural changes at the binding site, this does not necessarily imply that they induce different global conformational changes (8). Similarly, single channel kinetic analysis has not yet permitted an unequivocal resolution of this issue. In the WT \( \alpha \) GlyR,
the difference in $E$ values between glycine and taurine was explained by a difference in the channel opening rates, with the closing rates being similar for both agonists (12). Although these results suggest that glycine and taurine differ in their abilities to stabilize the open state, they provide no information about the underlying structural changes. In addition, taurine and glycine have been shown to activate similar unitary conductance ($i$) and glycine-gated currents. In the A272C, S273C, L274C, and P275C GlyRs, MTSET increased the GlyR single channel conductance or maximum open probability. In the A272C, S273C, L274C, and P275C GlyRs, the reaction rates of MTSET with A272C, S273C, L274C, and P275C were not significantly different for saturating taurine-gated currents and partially activating glycine-gated currents, provided that the same current magnitude was activated in the same cell (Fig. 6B). To interpret this result, it is useful to consider the equation: $I = n \times i \times P_o$, where $i$ is the macroscopic current, $n$ is the total number of channels per cell; $i$ is the single channel conductance, and $P_o$ is the open probability. Because MTSET reaction rates were compared in the same cell, $n$ was a controlled variable in our experiments. Furthermore, because the taurine and glycine single channel conductances are identical (e.g. Ref. 12), $i$ is also constant. Indeed, it should be noted that full and partial agonists at all pentameric LGIC members examined to date identify unitary conductances. Thus, because the MTSET reaction rate is proportional to the initial value of $I$, it must in turn be proportional to $P_o$. Therefore, the MTSET reaction rate simply reflects the fraction of channels that are open and not the number of occupied binding sites. This agrees with our recent observation that the MTSET reaction rate with S273C did not change significantly when the number of agonist-binding sites per GlyR was reduced from 5 to 3 (6).

Because the M2-M3 domains are positioned mid-way along the conformational “wave” that proceeds from the ligand-binding site to the channel gate (7), it must therefore be concluded that binding site information is integrated prior to reaching the M2-M3 domain. Hence, the ligand-induced inter-subunit cooperative interactions take place in the ligand-binding domain, in accordance with the MWC model of receptor activation (34).

Mechanism of Taurine Inhibition—The taurine IC$_{50}$ values were not affected by MTSET in the R271C, P275C, or K276C mutant GlyRs (Table II). Table I shows that taurine IC$_{50}$ values are about 10 times lower than their corresponding EC$_{50}$ values in those mutants where both can be measured. The latter observation is consistent with a model where the first taurine binds with a high affinity (equal to the IC$_{50}$ value) but is unable to activate the channel. At least one additional taurine must bind to a separate low affinity site (equal to the EC$_{50}$ value) in order to open the channel. Because the homomeric GlyR is likely to contain five identical taurine-binding sites (6), this situation is most likely caused by negative cooperativity among agonist-binding sites. It is likely that the cysteine substitutions create or enhance this negative cooperativity. A high affinity for taurine inhibition would result if a single taurine molecule can prevent the channel opening efficiently when the other binding sites are occupied by glycine. We propose that MTSET does not affect the affinity of the first taurine-binding reaction but increases the either the affinity of subsequent agonist binding steps or the receptor gating efficacy.

CONCLUSIONS
This study compared the effects of MTSET on taurine- and glycine-gated currents. In the A272C, S273C, L274C, and P275C GlyRs, the MTSET reaction rate was a function of the degree of receptor activation. The conformation and accessibility of this domain thus reflect the channel open probability and convey no information about the identity or number of bound agonist molecules. Therefore, partial agonism in the GlyR is conferred by an MWC-like mechanism whereby partial and full agonists induce similar conformational changes, with the higher efficacy being due to an increased ability to stabilize the open state. This is at variance with the mechanism of partial agonism recently proposed for the glutamate receptor ion channel.

REFERENCES
Comparison of Taurine- and Glycine-induced Conformational Changes in the M2-M3 Domain of the Glycine Receptor

Nian-Lin R. Han, John D. Clements and Joseph W. Lynch

doi: 10.1074/jbc.M400548200 originally published online February 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M400548200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 36 references, 12 of which can be accessed free at http://www.jbc.org/content/279/19/19559.full.html#ref-list-1