Comparison of taurine- and glycine-induced conformational changes in the M2-M3 domain of the glycine receptor

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ABSTRACT

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 conformation changes in the glycine receptor chloride channel (GlyR). A substituted cysteine accessibility analysis previously demonstrated that glycine binding induced an increase in surface accessibility of all residues from R271 – K276 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 (MTSET), 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 MTSET 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.
INTRODUCTION

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 4 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 towards the channel gate (7).

Different agonists induce these conformational changes with different efficacies (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 recently been characterized in the ionotropic glutamate receptor cation channel (9-11). These studies show that low efficacy agonists induce a smaller degree of binding site core closure than do high efficacy agonists, and that this smaller degree of closure is translated to the activation gate as a smaller degree of channel opening (11). An alternate 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 (SCAM) 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, sulfhydryl-specific reagents, generally methanethiosulfonate (MTS) 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 6 continuous residues (R271 – K276) 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 they experience a conformational change that is crucial for the activation of GlyRs (14-18), GABA_ARs (19-24) and nAChRs (7,25-27). A structural study of the Torpedo nAChR has recently shown that these residues form an extramembranous extension to the M2 α-helix (4). 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 MTS reaction rates are unlikely to be influenced by steric effects of ligand binding. The aim of the present study is to employ SCAM to determine whether or not glycine and taurine induce identical changes in the surface accessibility of residues R271 – K276.
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, USA). The successful incorporation of the mutations was verified by cDNA 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 EC₅₀ 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 hr, the cells were washed twice with cell culture medium and electrophysiological studies were conducted over the following 24 - 72 hr.

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, USA) and data were directly recorded to disk using pCLAMP 6 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 MΩ 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 hours 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, 3 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 taurine EC₅₀ 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 mean ± standard error of the mean (SEM) of 4 or more independent experiments. The empirical Hill equation, fitted by a non-linear least squares algorithm (Origin, Microcal, Northampton, MA, USA), was used to calculate the 50% effective concentrations for activation (EC₅₀) and inhibition (IC₅₀) and the Hill coefficient (nH) 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 posthoc test for unpaired or paired data as appropriate (Sigmastat, Jandel, San Rafael, CA, USA), with P < 0.05 representing significance.
RESULTS

Characterization of cysteine-substituted mutant GlyRs

It was previously shown that MTSET modification of cysteines introduced into the N-terminal half of the M2-M3 domain (i.e., from R271C – K276C) resulted in irreversible changes in the glycine EC\textsubscript{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\textsubscript{50} and n\textsubscript{H} values for the WT and all cysteine-substituted mutant GlyRs examined in this study are summarized in Table 1. The taurine EC\textsubscript{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\textsubscript{50} measurement in the R271C and K276C mutant GlyRs. The corresponding glycine EC\textsubscript{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\textsubscript{max, tau}/I\textsubscript{max, gly}) for each mutant GlyR is also shown (Table 1). 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 glycine in all mutant receptors examined.

The ability of taurine to inhibit currents activated by an EC\textsubscript{50} glycine concentration was also measured. An example of taurine inhibition of EC\textsubscript{50} (400 µM) glycine currents in the L274C GlyR is shown in Fig. 1B and the inhibitory dose-response for this experiment, averaged from 4 cells, is shown in Fig. 1C. The taurine IC\textsubscript{50} and n\textsubscript{H} values for all tested mutant GlyRs are summarized in Table 1. The maximum extent to which a saturating (50 mM) taurine concentration could inhibit currents activated by an EC\textsubscript{50} glycine concentration is presented in Table 1 as Min I\textsubscript{tau}/I\textsubscript{gly}. The taurine IC\textsubscript{50} was invariably around an order of magnitude lower than its EC\textsubscript{50} in the same mutant GlyR.
Steady-state effects of MTSET

It has previously been demonstrated that MTSET covalently modifies all introduced cysteines from R271C to K276C (14). This modification increased the glycine EC$_{50}$ of the P275C GlyR but decreased the glycine EC$_{50}$ 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 and L274C GlyRs both before and after a 1 min MTSET application. In these and all subsequent experiments, MTSET was applied at a concentration of 100 µM at the A272C, S273C and L274C GlyRs and at 200 µM at the R271C, P275C and K276C GlyRs. These concentrations were chosen to yield reaction time constants in an appropriate range (1 – 10 s) that was neither so rapid as to be influenced by the solution exchange rate nor so slow as to be influenced by receptor desensitization. In the experiments summarized in Fig. 2, MTSET was applied in the presence of a saturating (50 mM) concentration of taurine. MTSET had no significant effect on the magnitude of currents activated by a saturating glycine concentration in any of these mutant GlyRs, consistent with previous findings (14). However, MTSET modification increased the peak magnitude of taurine-gated currents in the L274C mutant GlyR but not in the R271C GlyR (Fig. 2A). The effects of MTSET on the ratio of saturating taurine- to glycine-gated currents ($I_{\text{maxtau}}/I_{\text{maxgly}}$) were investigated in a similar manner for all mutant GlyRs and averaged results are summarized in Fig. 2B. MTSET significantly increased this ratio in the A272C, S273C and L274C GlyRs, significantly decreased it in the P275C GlyR and had no effect on $I_{\text{maxtau}}/I_{\text{maxgly}}$ in the R271C and K276C GlyRs.

Because MTSET functionally modified glycine-gated currents in the R271C and K276C GlyRs, its lack of effect on taurine-gated currents appears to reveal a structural distinction between taurine- and glycine-gated currents. However, a direct comparison requires that MTSET be applied in the presence of a glycine concentration that activates a current similar in magnitude to that activated by a saturating taurine concentration in the same cell. Examples of this experiment carried out in the A272C, S273C and P275C GlyRs are shown in Fig. 3A and averaged results from all tested
mutant GlyRs are summarized in Fig. 3B. Together, these results indicate that taurine- and glycine-gated currents were modified in a similar manner by MTSET in all mutant GlyRs. Therefore, the steady-state effect of MTSET does not depend on the agonist.

In those mutants where MTSET increased $I_{\text{max,tau}}/I_{\text{max,gly}}$ (i.e., A272C, S273C and L274C), MTSET also significantly reduced the taurine EC$_{50}$. 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 EC$_{50}$ 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 EC$_{50}$ 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). Since MTSET causes a 4-fold increase in the glycine EC$_{50}$ (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 IC$_{50}$ for the P275C GlyR (Fig. 5B). The mean taurine IC$_{50}$ and nH values before and after MTSET modification are summarized in Table 2. Similar experiments were carried out on the K276C and R271C GlyRs and the results are also summarized in Table 2. 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 (EC$_{5}$ - EC$_{10}$) 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 EC30 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-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 ± 0.34 s (n = 9 cells) and 2.06 ± 0.43 s (n = 7 cells), respectively. There was no significant difference between these values, indicating that the conformational change experienced by the 274C residue 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 EC20 (200 µM) taurine concentration. This sub-saturating taurine concentration was necessary as higher taurine EC 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 µM (EC10) 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).

The reaction rates of 100 µM MTSET with A272C and P275C were measured at saturating (50 mM) taurine concentrations and glycine concentrations of 200 µM (EC10) and 100 µM (EC10),
respectively. The averaged MTSET reaction rates in the taurine- and glycine-bound states for all
mutant GlyRs examined in this study are also shown in Fig. 6B. Together, the results indicate that the
rate of the MTSET reaction with A272C, S273C, L274C and P275C does not depend on the identity
of the agonist.
DISCUSSION

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 induced 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

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 1, 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 6 discontinuous domains, labelled A - F by Corringer et al. (28). The involvement of these domains in forming the pocket has been confirmed by the crystal structure of AChBP (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 levels (12). Again, this does not necessarily imply that both agonists induce identical structural changes at the gate, because the
conductance of the open pore may be limited by regions other than the gate (e.g., the M3-M4 domain (33)).

Comparison of MTSET reaction rates

MTSET has no effect on the magnitude of current activated by a saturating glycine concentration (14), indicating that it does not alter the GlyR single channel conductance or maximum open probability. In the A272C, S273C and L274C GlyRs, MTSET increased $I_{\text{maxtau}}/I_{\text{maxgly}}$ (Fig. 2B). It also reduced the EC$_{50}$ values of glycine and taurine to a similar extent (Fig. 4B; see also Fig. 5 in (14)). MTSET had exactly the opposite effects on the P275C GlyR. 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 $I = n \cdot i \cdot P_0$, where $I$ is the macroscopic current, $n$ is the total number of channels per cell, $i$ is the single channel conductance and $P_0$ is the open probability. Because MTSET reaction rates were compared in the same cell, $n$ was a controlled variable in our experiments. Furthermore, since the taurine and glycine single channel conductances are identical (e.g., (12)), $i$ is also constant. Indeed, it should be noted that full and partial agonists at all pentameric LGIC members examined to date activate identical unitary conductances. Thus, since the MTSET reaction rate is proportional to the initial value of $I$, it must in turn be proportional to $P_0$. 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 (36).

Since 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 IC50 values were not affected by MTSET in the R271C, P275C or K276C mutant GlyRs (Table 2). Table 1 shows that taurine IC50 values are about 10 times lower than their corresponding EC50 values in those mutants where both can be measured. The later observation is consistent with a model where first taurine binds with a high affinity (equal to the IC50 value), but is unable to activate the channel. At least one additional taurine must bind to a separate low affinity site (equal to the EC50 value) in order to open the channel. Since the homomeric GlyR is likely to contain 5 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 efficaciously 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 reflects the channel open probability and conveys 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.
ACKNOWLEDGMENTS

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REFERENCES

**Table 1.** Taurine activation and inhibition of cysteine-substituted mutant GlyRs

<table>
<thead>
<tr>
<th>GlyR</th>
<th>Glycine EC$_{50}$ (µM)</th>
<th>Taurine activation</th>
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<th>Taurine inhibition</th>
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<td></td>
<td>EC$_{50}$ (mM)</td>
<td>nH</td>
<td>$I_{\text{maxtau}}/I_{\text{maxgly}}$</td>
<td>n</td>
<td>IC$_{50}$ (mM)</td>
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<td>WT</td>
<td>18 ± 2</td>
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<td>1.6 ± 0.1</td>
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<td>R271C</td>
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<td>0.06 ± 0.01*</td>
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<td>A272C</td>
<td>2840 ± 300*</td>
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<td>1.6 ± 0.2</td>
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<tr>
<td>S273C</td>
<td>38 ± 5*</td>
<td>0.71 ± 0.06*</td>
<td>1.6 ± 0.3</td>
<td>0.60 ± 0.04*</td>
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<tr>
<td>L274C</td>
<td>395 ± 10*</td>
<td>6.40 ± 0.31*</td>
<td>1.5 ± 0.1</td>
<td>0.14 ± 0.02*</td>
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<tr>
<td>P275C</td>
<td>1500 ± 300*</td>
<td>15.3 ± 1.20*</td>
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<tr>
<td>K276C</td>
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<td>---</td>
<td>0.08 ± 0.01*</td>
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</table>

* significantly different with respect to WT GlyR

a from Lynch et al. (2001)

b $I_{\text{maxtau}}/I_{\text{maxgly}}$ represents the mean fraction of the saturating glycine-gated current activated by a saturating concentration (50 mM) of taurine.

c Min $I_{\text{tau}}/I_{\text{gly}}$ represents the mean fraction of the half-saturating glycine-gated current remaining in the presence of 50 mM taurine.
Table 2. The effect of MTSET on taurine IC$_{50}$ values for the R271C, P275C and K276C mutant GlyRs.

<table>
<thead>
<tr>
<th>GlyR</th>
<th>Control</th>
<th>After MTSET</th>
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<td></td>
<td>IC$_{50}$ (mM)</td>
<td>$n_H$</td>
<td>IC$_{50}$ (mM)</td>
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<td>R271C</td>
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<tr>
<td>P275C</td>
<td>1.46 ± 0.38</td>
<td>1.0 ± 0.2</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td>K276C</td>
<td>0.59 ± 0.10</td>
<td>0.8 ± 0.2</td>
<td>0.62 ± 0.12</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Fig. 1. Examples of excitatory and inhibitory dose-responses for the WT and L274C GlyRs. A. Averaged glycine and taurine excitatory dose-responses. For each receptor, glycine and taurine dose-responses were measured from the same cell, with the saturating glycine current being set at 1 and the taurine current in the same cell scaled accordingly. All dose-responses are averaged from at least 4 cells and parameters of best fit for taurine dose-responses are given in Table 1. B. An example of taurine inhibition of currents activated by an EC_{50} (400 µM) concentration of glycine. Both ligands were applied for the periods as indicated by the bars. The traces correspond to taurine concentrations of 100, 500 (labeled), 1000, 5000 and 20000 µM. C. Averaged taurine inhibitory dose-response for the L274C GlyR. All points are averaged from 4 cells and the parameters of best fit are given in Table 1.

Fig. 2. Effects of MTSET on the relative peak magnitude of taurine-gated currents. A. Examples of currents activated by saturating concentrations of taurine and glycine in 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 (I_{maxtau}/I_{maxgly}) measured both before and after exposure to MTSET + 50 mM taurine. Each column represents the average of at least 4 cells, and asterisks indicate a statistically significant difference (P < 0.05).

Fig. 3. Similar steady-state effects of MTSET on glycine- and taurine-gated currents. A. In these experiments the glycine concentration was chosen to activate a similar magnitude current as was activated in the same cell by a saturating (50 mM) concentration of taurine. (An exception is that a sub-saturating 200 µM taurine concentration was used in the S273C GlyR to avoid the problem of
current saturation prior to completion of the reaction. This issue is discussed further in the text).

MTSET was then applied in the presence of the same agonist concentration as was used to activate the control current. Sample results are shown for the A272C, S273C and P275C GlyRs. All traces in each row were obtained from the same cell. B. Averaged results for the experiment shown in A. In each case, the percentage current increase was calculated as \( (I_{\text{MTSET}}/I_{\text{control}} - 1) \times 100\% \). MTSET concentrations were the same as in Fig. 2A. The glycine concentrations were as follows: R271C, 200 \( \mu \)M; A272C, 200 \( \mu \)M; S273C, 20 \( \mu \)M; L274C, 100 \( \mu \)M; P275C, 100 \( \mu \)M; K276C, 200 \( \mu \)M. In the WT GlyR, 100 \( \mu \)M MTSET was applied in the presence of EC\(_{100}\) (1 mM) concentrations of glycine or taurine. Using one way ANOVA for paired data (\( P < 0.05 \)), there was no significant difference in the effects of MTSET on taurine- and glycine-gated currents in any tested GlyR.

**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 5 cells expressing L274C GlyRs. C. Mean taurine EC\(_{50}\) values before (unfilled circles) and after (filled circles) MTSET exposure for the A272C, S273C and L274C GlyRs. All points were averaged from at least 4 cells in which control and MTSET-modified EC\(_{50}\) values were both measured.

**Fig. 5.** MTSET does not affect taurine inhibitory potency in the P275C GlyR. A. Examples of taurine inhibition of currents activated by EC\(_{50}\) 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 4 cells both before (unfilled symbols) and after (filled symbols) MTSET modification. Averaged taurine IC\(_{50}\) and \( n_H \) values of best fit are given in Table 2.
Fig. 6. Comparison of MTSET modification rates in the taurine- and glycine-bound states. A. Comparison of the effect of 100 µM MTSET on currents activated by saturating (50 mM) taurine (upper panel) and 100 µM glycine (lower panel) in the same cell expressing L274C GlyRs. MTSET applications were separated by a 2 min DTT wash. Time constants of best fit are shown. B. Averaged MTSET modification rates in the glycine-activated state (unfilled circles) and taurine-activated state (filled circles) for the indicated mutant GlyRs. Modification rates were always compared at a common fraction of the peak current magnitude as shown in A. For the S273C GlyR, taurine was applied at a 200 µM (EC₂₀) concentration and glycine was applied at a 20 µM (EC₁₀) concentration. In the remaining mutant GlyRs, taurine was applied at a saturating (50 mM) concentration and glycine was applied at 200 µM (A272C), 100 µM (L274C) or 2 mM (P275C). All points represent averages of 7 – 10 independent measurements. Using a one way ANOVA for paired data (P < 0.05), there was no significant difference in the MTSET reaction rates in the taurine- and glycine-activated states for any tested mutant.
0.4 mM glycine
2 s
2 nA
taurine
0.5 mM
[agonist] (mM)

A

[agonist] (mM)

B

L274C

0.4 mM glycine

0.5 mM taurine

2 nA

2 s

C

L274C

I/I_{max}

I/I_{max}

[taurine] (mM)
A272C

before MTSET | after MTSET
----------|----------
200 µM gly: | 50 mM tau:

S273C

before MTSET | after MTSET
----------|----------
20 µM gly: | 200 µM tau:

P275C

100 µM gly: | 50 mM tau:

B

![](image)

WT  R271C  A272C  S273C  L274C  P275C  K276C
A. L274C

control

[taurine]: 0.1 0.5 1 5 20 50 mM

after MTSET

[taurine]: 0.1 0.5 1 5 20 50 mM

B. L274C

I_{\text{max, after}} / I_{\text{max, before}} vs [taurine] (mM)

after MTSET

control

C. L274C

taurine EC_{50} (mM)

A272C S273C L274C

control after MTSET
A  

\[ \text{P275C} \]

**control**  
2 mM glycine

![Graph showing current responses to glycine and taurine in control condition.](chart1)

**after MTSET**  
8 mM glycine

![Graph showing current responses to glycine and taurine after MTSET.](chart2)

B  

\[ \text{P275C} \]

\[ \frac{I}{I_{\text{max}}} \]

vs.

\[ \text{[taurine] (mM)} \]

![Graph showing concentration-response curve for taurine with and without MTSET.](chart3)
MTSET: taurine: \[ \tau = 2.78 \text{ s} \]

MTSET: glycine: \[ \tau = 2.66 \text{ s} \]

MTSET reaction rate (M$^{-1}$ s$^{-1}$)

A272C  S273C  L274C  P275C

B

 glycinex
 taurine
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

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