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Research Report

Allosteric modulation of the glycine receptor activated by agonists differing in efficacy

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ABSTRACT

The glycine receptor (GlyR) is the predominant inhibitory neurotransmitter receptor in the brainstem and spinal cord but is also found in higher brain regions. GlyR function is affected by a variety of allosteric modulators including drugs of abuse, such as ethanol and inhalants and the ubiquitous divalent cation zinc. Two-electrode voltage-clamp experiments were conducted on *Xenopus laevis* oocytes expressing wild-type α1 homomeric glycine receptors to compare the degree of enhancement produced by zinc on GlyR activated by two agonists (glycine vs. taurine) that vary markedly in their efficacies. Zinc potentiation of both glycine- and taurine-evoked currents was the same at the concentrations of agonists that produced the same currents, corresponding to 6% of the maximal effect of glycine compared to 23% of the maximal effect of taurine. Similar results were seen with 50 and 200 mM ethanol. A direct comparison of agonist concentration–response curves showed that zinc enhancement was greater, overall, for taurine-activated than glycine-activated receptors. In addition, zinc only enhanced taurine- but not glycine-activated GlyR when agonists were applied at saturating concentrations. These data suggest that zinc affects taurine affinity, as well as the probability of channel opening at sub-maximal taurine concentrations, and that the magnitude of allosteric modulation at the GlyR depends on the efficacy of the agonist tested. This has implications for mutagenesis studies in which changes in the degree of allosteric modulation observed may result from mutation-induced changes in agonist efficacy.

1. Introduction

The glycine receptor (GlyR) is a member of the Cys-loop family of ligand-gated ion channels. It is the primary inhibitory receptor in the brainstem and spinal cord but also plays important roles in higher brain regions, including the hippocampus, nucleus accumbens and prefrontal cortex (Baer et al., 2009; Jonsson et al., 2012, 2009; Lynch, 2004). GlyRs are pentameric in structure with the five subunits arranged around a central anion-conducting channel. Thus far, four alpha subunits and one beta subunit

Abbreviations: EC_x, effective concentration producing x% of maximal agonist effect; GlyR, glycine receptor; MBS, modified Barth's saline; Po, channel open-state probability

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have been identified, of which α1–3 and β are found in humans. GlyRs can express either as homomeric receptors composed solely of α subunits, or as αβ heteromeric receptors with a stoichiometry of 2α:3β (Betz et al., 1993; Bowery and Smart, 2006; Lynch, 2004). In the spinal cord a developmental switch occurs from prenatal α2 homomers to α1β receptors in the adult. However, in the forebrain it appears that the α2 subunit continues to be expressed into adulthood, along with the β subunit (Jonsson et al., 2012).

GlyR activity is affected by a large variety of allosteric modulators including zinc, alcohols, anesthetics and inhaled drugs of abuse (Beckstead et al., 2000; Harvey et al., 1999; McCracken et al., 2010, 2013; Mihic et al., 1997), making it a promising clinical target for the treatment of alcohol and drug addiction (Söderpalm and Ericson, 2013; Tipps et al., 2010). Zinc is present endogenously at nanomolar concentrations known to enhance GlyR function. It exhibits biphasic actions at GlyRs, potentiating currents at concentrations <10 μM, while higher concentrations produce inhibition (Harvey et al., 1999; Laube et al., 2000).

Taurine is a partial agonist of the GlyR, with approximately 5% the efficacy of glycine (Lape et al., 2008), and is believed to be an important GlyR agonist in a number of brain regions (Albrecht and Schousboe, 2005). For example, Mori et al. (2002) showed that an uptake inhibitor of taurine induced a strychnine-sensitive chloride current in hippocampal organotypic slice cultures. Previous research has largely focused on allosteric modulation at glycine-activated receptors. Modulators shift glycine concentration–response curves either to the left or to the right but have no effects at maximally-effective glycine concentrations. However, Kirson et al. (2013, 2012) showed that ethanol, volatile anesthetics, inhaled drugs of abuse and zinc are able to enhance currents elicited by maximally-effective concentrations of taurine, but not glycine. This suggested that these modulators had an effect on the probability of taurine-activated channel opening (Po), which would already be near maximum when a saturating concentration of glycine was tested. Most studies of allosteric modulation are performed using concentrations of agonists that are low on their concentration–response curves, since it is at these agonist concentrations that the greatest modulatory effects are seen. In this study we investigated whether agonist efficacy also affects the magnitude of zinc and ethanol enhancement of GlyR function seen under those conditions.

2. Results

Zinc was tested for its enhancing effects of α1 homomeric GlyR currents elicited by submaximal concentrations of glycine or taurine. Concentrations of each agonist were first identified that produced equal currents, corresponding to 5–10% of the maximally-effective glycine response ($EC_{5–10}$ glycine). In order to do so the $EC_{5–10}$ concentration of glycine was first identified in each oocyte and then the concentration of taurine producing a similar current was determined (Fig. 1A). Where that concentration of taurine fell on the taurine concentration–response curve was next determined, relative to a maximally-effective concentration of taurine (100 mM). Concentrations of 84±4 μM glycine had EC values of 6.28±0.70 relative to 10 mM glycine, while concentrations of 1.2±0.2 mM taurine, producing currents of the same magnitude in each oocyte as glycine, had EC values of 22.69±3.98 relative to 100 mM taurine. The concentrations of taurine used thus fell significantly higher on their concentration–response curves than the concentrations of glycine did on theirs [$t(4)=4.06$, $p<0.005$] (Fig. 1B).

We next compared the enhancing effects of 100 nM zinc on currents produced by a low concentration of glycine with zinc effects on the same currents produced by the partial agonist taurine. In this experiment, zinc was co-applied with concentrations of glycine or taurine producing 5–10% of the maximally effective glycine response; i.e., the absolute currents produced by glycine and taurine were similar (Fig. 2A). Zinc-potentiated currents were compared to those produced by agonist alone as shown in Fig. 2B. Co-application with zinc resulted in a significant enhancement of both glycine- and taurine-mediated currents [$F(1,23)=24.12$, $p<0.001$]. There was, however, no difference in the degree of zinc enhancement seen between glycine and taurine [$F(1,23)=1.19$, $p>0.28$].

When data from individual oocytes were plotted (Fig. 2C), there was a large degree of variation in zinc enhancement seen. Previous studies revealed that the buffers used in our studies contain nanomolar levels of contaminating zinc, sufficient

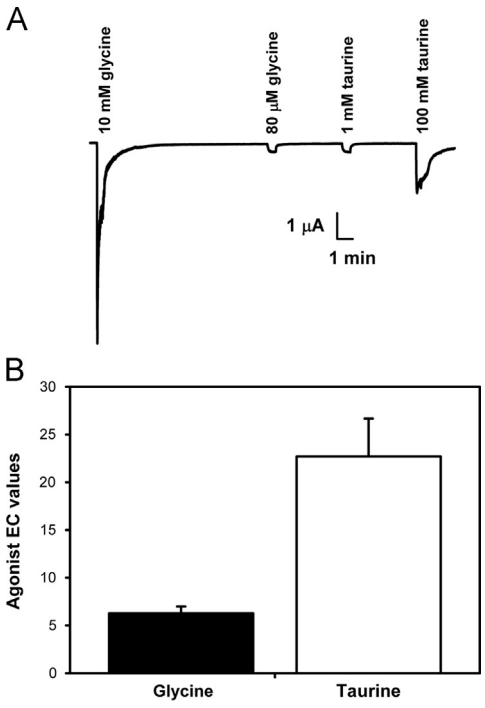

Fig. 1 – Low concentrations of glycine and taurine, eliciting similar currents, correspond to different effective concentrations on their respective concentration–response curves. (A) Sample tracings showing the identification of concentrations of glycine and taurine that elicited similar currents, between 5% and 10% of the current produced by a saturating (10 mM) concentration of glycine. The glycine and taurine concentrations were chosen so as to elicit similar absolute currents. (B) Despite producing similar absolute currents the concentrations of glycine and taurine identified fell at markedly different points on their respective concentration–response curves. Data are shown as mean+S.E.M. of 5 oocytes.