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Guanidinoethyl sulphonate is a glycine receptor antagonist in striatum

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1 Guanidinoethyl sulphonate (GES) is an analogue of taurine and an inhibitor of taurine transport. Interactions of GES with $GABA_A$ and glycine receptors are studied by whole cell recording and fast drug application in isolated striatal neurons of the mouse.

2 We confirm that GES is a weak agonist at $GABA_A$ receptors, and is able to antagonize GABA-evoked responses. GES did not gate GlyR.

3 GES antagonized glycine responses in a concentration-dependent and surmountable manner. Glycine dose–response curves were shifted to the right by GES (0.5 mM), yielding EC_{50}s and Hill coefficients of 62 μM and 2.5 in control, 154 μM and 1.3 in the presence of GES.

4 GlyR-mediated taurine responses were competitively antagonized by GES. Taurine dose–response curves, in contrast to the glycine dose–response curves were shifted by GES to the right in a parallel manner.

5 The GlyR-block by GES was not voltage-dependent.

6 In contrast to our findings in the mouse, in rat striatal neurons which lack expression of the α3 GlyR subunit, GES shifted the glycine dose–response curve to the right in a parallel way without affecting the maximal response. Subtype-specificity of the GES action at GlyR must await further investigation in artificial expression systems.

7 We conclude that GES is a competitive antagonist at GlyR. The antagonistic action of GES at inhibitory ionotropic receptors can explain its epileptogenic action. Care must be taken with the interpretation of data on GES evoked taurine release.
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Abbreviations: $GABA_AR$, $GABA_A$ receptor; GAN, giant aspiny neuron; GES, guanidinoethyl sulfonate; GlyR, glycine receptor; MSN, medium spiny neuron

Introduction

Guanidinoethyl sulphonate (GES) is a taurine analogue that competitively inhibits taurine uptake and is widely used for manipulating taurine levels in the brain (Huxtable, 1982; Quesada *et al*., 1984). Taurine is not only a major organic osmolyte in the brain but also a potent modulator of neuronal activity (Oja & Saransaari, 1996; Huxtable, 1989). A selective action of GES on taurine transport has recently been questioned: inhibition of creatine transport (Dai *et al*., 1999) as well as agonistic (Mellor *et al*., 2000) and antagonistic (Li & Lombardini, 1990; Herranz *et al*., 1990) actions at $GABA_A$ receptors ($GABA_AR$) were found. Antagonistic properties of GES at GlyR have never been studied. We have previously demonstrated that all striatal neurons respond to glycine in a strychnine-sensitive manner (Sergeeva, 1998) and determined the expression of GlyR subunits in striatal cholinergic neurons (Sergeeva & Haas, 2001). Since GES is an analogue of taurine and taurine is a partial agonist at GlyRs in the striatum (Sergeeva & Haas, 2001), we have investigated the interactions of GES with GlyR. We report that GES does not gate striatal glycine receptors but competitively antagonizes them.

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Methods

Animals and cell preparation

Housing of mice and all procedures were carried out in accordance with the Animal Protection Law of the Federal Republic of Germany. All efforts were made to minimize animal suffering or discomfort and to reduce the number of animals used. From the brains of 4–10-week-old male C57/Bl6 mice (or 4–6-week-old Wistar rats in the last series of experiments) 400 μm thick horizontal striatal slices were cut and incubated in the following solution (in mM): NaCl 124, KCl 3.7, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.0, $NaHCO_3$ 25.6, glucose 20, saturated with a 95% O_2/5% CO_2 mixture. For whole-cell recording single slices were transferred to a recording chamber. The solution for dissociation and recording had the following composition: NaCl 150, KCl 3.7, $CaCl_2$ 2.0, $MgCl_2$ 2.0, HEPES 10, glucose 20, pH adjusted to 7.4 with NaOH. Single cells were isolated from the striatum by vertical vibration (200 Hz, 0.2 mm amplitude) of a glass sphere, 0.5 mm in diameter, placed close to the surface of the slice (Vorobjev *et al*., 2000). Manipulations and cell identification were performed using an inverted microscope. Acutely isolated giant aspiny striatal cholinergic interneurons (GAN) were distinguished from GABAergic principal neurons (MSN) based on their morphological features (Sergeeva & Haas, 2001).

Whole-cell current recording

Membrane currents were recorded using the standard whole-cell configuration of the patch-clamp technique with borosilicate glass pipettes (4–6 MΩ resistance) connected to an EPC-9 amplifier. Data acquisition, storage and analysis were done with Tida for Windows (HEKA, Lambrecht, Germany). The holding potential was −70 mV. An 80–90% correction for the voltage drop across the electrode series resistance was performed on-line with the use of the series resistance compensation of the amplifier. Only cells with no leak current and series resistance (Rs) lower than 15 MΩ were used. The electrode solution, adjusted to pH 7.2 was (in mM): CsCl 140, $MgCl_2$ 2, $CaCl_2$ 0.5, EGTA 5, HEPES/KOH 10. Membrane potentials were not corrected for the liquid junction potential (5.6 mV). A fast perfusion technique was used for the drug application (Vorobjev *et al*., 1996). Antagonists were applied together with agonists; in the case of strychnine, a 30 second-long preincubation with strychnine preceded the common application. Taurine–GES and glycine–GES interaction at the GlyR was studied in the presence of $GABA_A$R antagonist gabazine (10 μM) in all solutions. All data are expressed as mean±s.e.mean and compared using Student's *t*-test.

Drugs and chemicals

Gabazine (SR-95531, 2-(3-Carboxypropyl)-3-amino-6-(4-methoxyphenyl) pyridazinium bromide), glycine, taurine, GABA (γ-aminobutyric acid), strychnine hemisulphate were purchased from Sigma/RBI (Deisenhofen, Germany) and GES (guanidinoethyl sulphonate) from Toronto Research Chemicals (Canada).

Results

Interaction of GES with striatal $GABA_A$ receptors

Fast application of GES (10 mM) to acutely isolated mouse striatal medium spiny neurons and cholinergic interneurons evoked whole-cell currents reaching only 9.9±2.3% ($n=6$) and 5.6±1.1% ($n=7$), respectively, of maximal GABA (500 μM)-evoked currents. The action of GES was dose-dependent (Figure 1b), the average half maximal concentration and Hill coefficient determined from the concentration–response plots were 534±65 μM and 0.93±0.09 ($n=5$, Figure 1c). Gabazine, a selective $GABA_A$R-antagonist, at a concentration sufficient to block GABA-responses (5 and 10 μM) either completely ($n=4$, GAN) or partially (by 60±8%, $n=8$, 4 GAN and 4 MSN) blocked GES-responses (1 mM, Figure 2a). Although the nature of the residual current remains unclear (Mellor *et al*., 2000), we did not attempt to investigate it further. When applied together with GABA (10 μM), GES inhibited GABA-responses to 26.3±3.34% of control ($n=4$, Figure 2b).

GES is an antagonist at striatal GlyR

Strychnine at a concentration sufficient to block GlyR-mediated responses (500 nM) (Sergeeva & Haas, 2001) did not affect GES (1 mM)-evoked responses ($n=11$), neither in the cells where gabazine completely blocked GES-evoked responses ($n=4$, GAN) nor in the cells where a residual current remained under gabazine ($n=5$, 2 GAN, 3 MSN) (Figure 2c). This indicates that GlyRs are not gated by GES. Glycine (100 μM)-evoked responses were reduced by GES (1 mM) to 36±2.7% in giant aspiny neurons (GAN, $n=20$) and to 35±3.9% in medium spiny neurons (MSN, $n=10$). MSN and GAN showed the same sensitivity of glycine-induced currents to GES (*t*-test, $P=0.9$). As we determined previously the expression of GlyR in GAN (Sergeeva & Haas, 2001), we concentrated our further investigations on this group of striatal neurons. A further reason for this

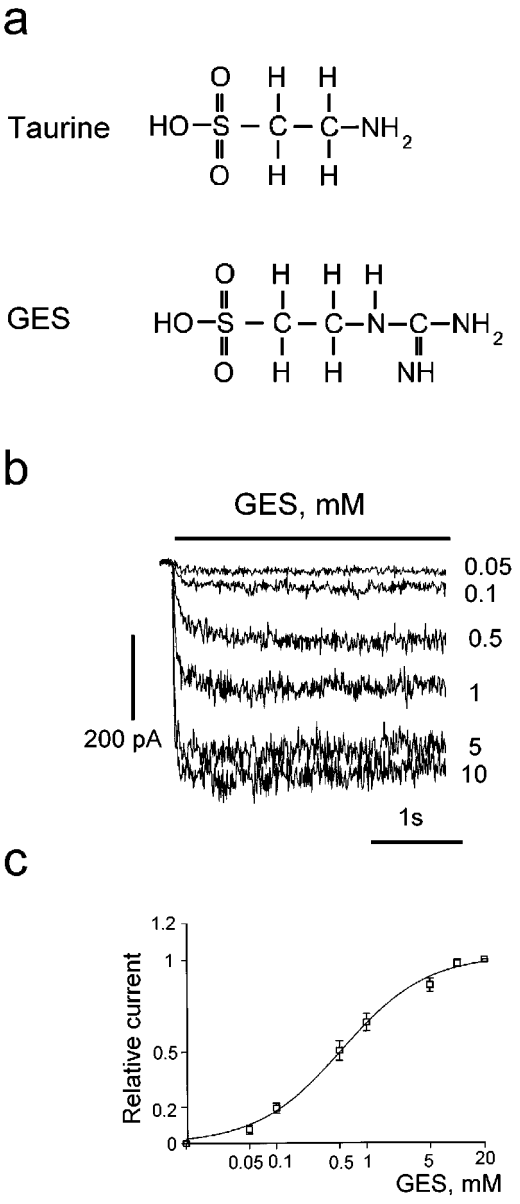

Figure 1 GES dose-dependently induces currents in striatal neurons. (a) GES is a structural analogue of taurine. (b,c) Responses to six different GES concentrations obtained from a giant aspiny neuron (GAN) and dose–response curve, $n=5$, from three medium spiny neurons (MSN) and two GAN, fitted with the equation $R=Rmax/(1+(EC_{50}/[GES,\mu M]n)$, where $EC_{50}=534\pm65$ μM (concentration producing a half-maximal response), $n=0.93\pm0.09$ (Hill slope) and R-relative current.

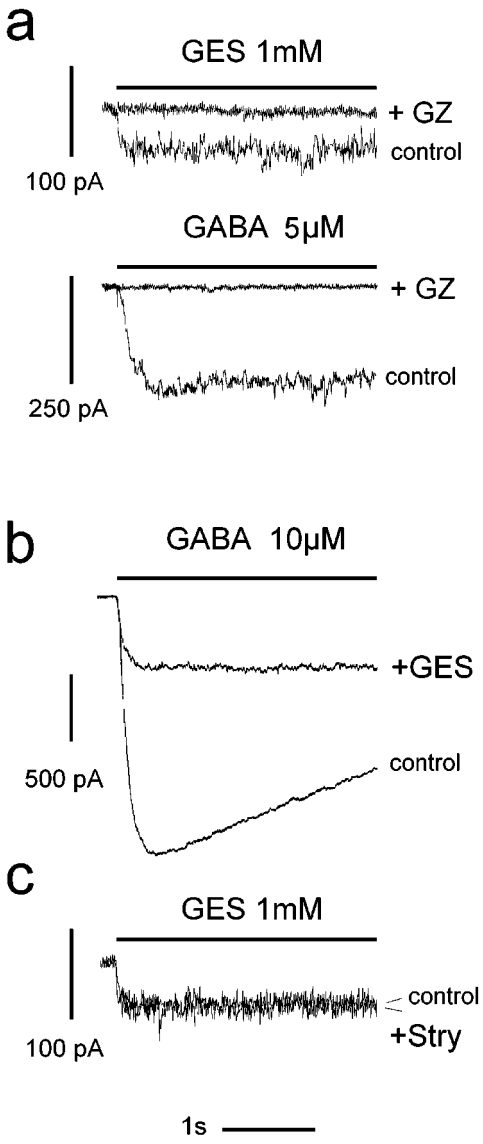

Figure 2 Pharmacological properties of GES. (a) GES- and GABA-evoked responses are blocked by the $GABA_AR$-antagonist gabazine (GZ, 10 μM). (b) GES (1 mM) markedly reduces GABA-response. (c) Strychnine (Stry, 0.5 μM) does not affect the GES response.

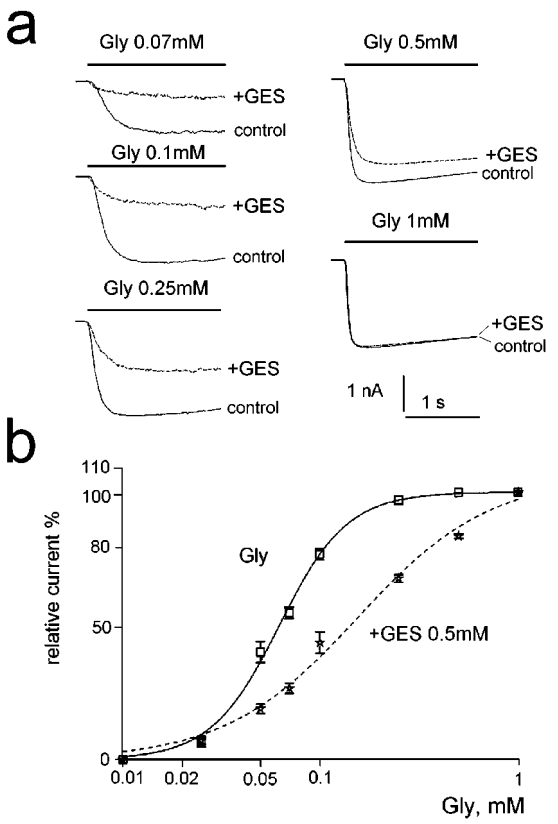

Figure 3 GES competitively inhibits glycine responses. (a) The responses to various concentrations of glycine (solid traces) and glycine plus GES (0.5 mM, dotted traces) illustrate the surmounting of the block by higher concentrations of glycine. (b) Dose–response curves constructed from the experiments conducted on six GANs. Current-amplitudes were normalized to the maximal current recorded with glycine 1 mM in control. Data were fitted with the same equation as in Figure 1.

selection was that the ratio of GES (0.5 mM)-evoked current to the maximal amplitude of the glycine response (1 mM) in MSN was unacceptably large (3–40%, $n=6$), in GAN it was less than 2% (1.4 ± 0.2, $n=6$), which did not disturb dose–response curves, especially in the presence of gabazine. GES (0.5 mM) shifted the dose–response curve for glycine to the right without affecting the maximum response (Figure 3) and changed EC_{50} and Hill coefficient for glycine from 62 ± 1.8 μM and 2.5 ± 0.2 to 154 ± 24 μM and 1.3 ± 0.2, respectively. The change in slope function of the glycine dose–response curve by GES was highly significant ($P<0.01$, t-test). In contrast to glycine, the right shift of the taurine dose–response curve by GES (0.5 mM) was parallel, with the EC_{50} changing from 1.4 ± 0.05 mM to 2.7 ± 0.1 mM (Figure 4) but without any change in the slope function (1.73 ± 0.09 and 1.70 ± 0.09 for the control taurine responses and in the presence of GES, respectively).

When seven different concentrations of GES were applied together with glycine or taurine, the averaged dose–response curves yielded an IC_{50} and a Hill coefficient of 565 ± 38 μM, 1.2 ± 0.07 ($n=10$) and 810 ± 130 μM, 1.1 ± 0.14 ($n=6$) for glycine and taurine, respectively (Figure 5). Responses to glycine (100 μM) or to glycine plus GES (0.5 mM) at holding potentials ranging from -85 to $+50$ mV demonstrated that currents reversed their polarity at 4.7 ± 1.3 mV and 2.5 ± 1.6 mV (for glycine control and glycine plus GES, respectively, Figure 6). The difference between the reversal potentials was not significant. After subtraction of the liquid junction potential (5.6 mV), the resulting reversal potentials were close to the predicted chloride equilibrium potential ($E_{Cl}=-2.7$ mV).

Thus, we have shown here that GES competitively interacts with the taurine binding site of GlyR. Two possibilities exist for the GES interaction with glycine: (1) the glycine site on the GlyR may be different from the taurine binding site; (2) different populations of GlyR ($\alpha2\beta$, $\alpha3\beta$, and possibly $\alpha2$,$\alpha3$ homomeric GlyR) are present in mouse GAN, and their different sensitivity to GES may mask the ideal competitive nature of the block. In order to test the latter hypothesis we performed a series of experiments on rat cholinergic interneurons, which do not express the $\alpha3$ subunit, but $\alpha2\beta$ subunits of GlyR. GES (0.5 mM) shifted the dose–response

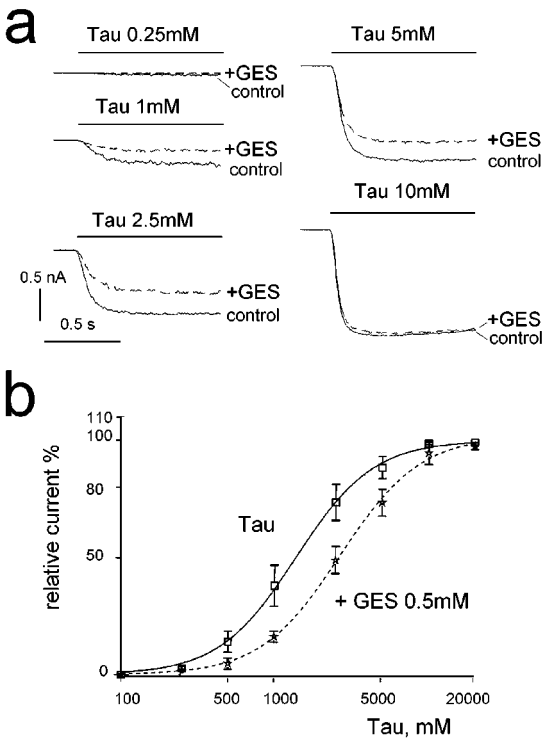

Figure 4 GES competitively inhibits glycine-receptor mediated taurine responses. (a) Representative responses to taurine alone (solid traces) and in the presence of GES (0.5 mM, dotted traces). (b) Averaged dose–response curves. Each point represents the mean value ($n=4$) of peak current amplitudes normalized on the maximal current amplitude, induced by taurine in control. Vertical bars show s.e.mean. Data were fitted with the same equation as in Figure 1.

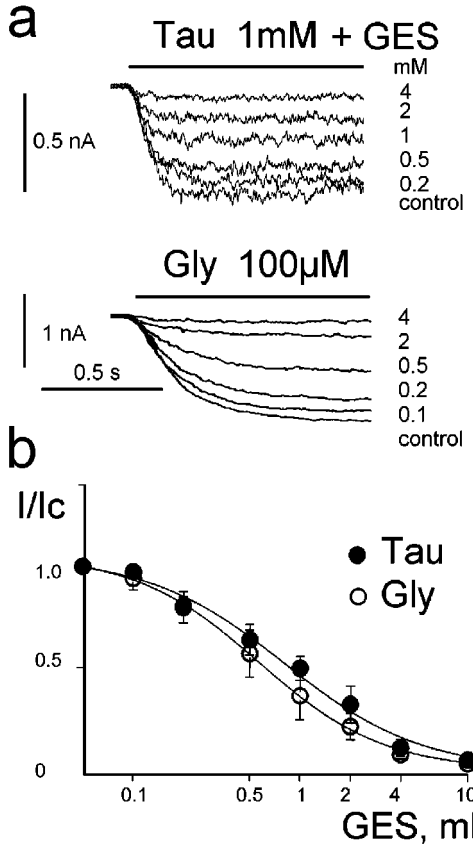

Figure 5 Dose-dependent block of glycine and taurine-evoked currents by GES. (a) Taurine (Tau) and glycine (Gly) responses in the presence of different GES concentrations. (b) The inhibition of glycine (100 μM) and taurine (1 mM) currents by GES was fitted with the following equation: $R = Rmax/(1+(IC_{50}/[L])n)$, where Rmax is the maximal degree of block of the glycine-mediated response achieved by GES, IC_{50} is the concentration of GES producing a half-maximal block, [L] is the blocker concentration and n is the Hill coefficient. Difference between two averaged dose–response curves was not significant (Student's t-test).

curve for glycine to the right without affecting the maximum response (Figure 7) and changed the EC_{50} for glycine from 114 ± 5 μM to 200 ± 5 μM. The slope function of the glycine dose–response curve was not changed by GES (2.4 ± 0.2 in control and 2.35 ± 0.13 in the presence of GES).

Discussion

We have shown that GES does not gate striatal GlyR but is a weak agonist at $GABA_AR$; the latter finding is in agreement with data obtained on cultured murine cerebellar granular cells (Mellor *et al*., 2000). In contrast, on outside-out patches from neonatal cortical neurons, containing $GABA_AR$ and GlyR, GES did not induce any currents (Flint *et al*., 1998), indicating that agonistic properties of GES at $GABA_AR$ depend on subunit composition. GES activated currents in murine cerebellar granular cells and in striatal neurons with comparable EC_{50}s: 321 μM and 534 μM, respectively.

Antagonistic properties of GES at inhibitory ionotropic receptors may be expected from the observation that GES binds to the $GABA_AR$ and impairs recurrent inhibition in the hippocampus (Herranz *et al*., 1990). We show now that GES is a GlyR antagonist. We find the block of glycine-evoked currents in striatal neurons by GES independent of voltage and competitive: it is surmountable by higher concentrations of glycine. However in GAN from mice this occurs not in the classical way (Rang *et al*., 1999): the shift in the dose–response curve for glycine by GES is not parallel (Hill coefficients 2.5 and 1.3 for control and in the presence of GES, respectively). In contrast, the slope function of the taurine dose–response curve is unchanged by GES. The non-parallel shift of the glycine dose–response curve by GES can be the result of an unusual interaction between different, but overlapping binding sites for glycine (full agonist) and taurine (partial agonist) at the GlyR (Schmieden *et al*., 1992). On the other hand, experiments which we conducted on rat GANs support the more likely explanation that different sensitivities to GES of different receptor populations ($\alpha2\beta$, $\alpha3\beta$, and possibly $\alpha2\alpha3$ homomeric GlyR) are responsible for the non-parallel shift of the glycine dose–response curve in mice. In contrast to glycine taurine may not activate all classes of GlyR; this can explain the parallel shift of its dose response curve in the presence of GES in mice. The different ability of taurine to cross-desensitize glycine responses in striatal neurons lacking and expressing the $\alpha3$ subunit which we described previously (Sergeeva & Haas, 2001) may underlie differences in the GES interaction with the glycine-binding site.

GlyR is a pentamer composed of ligand-binding α- and structural β-subunits, which show different regional- and age-

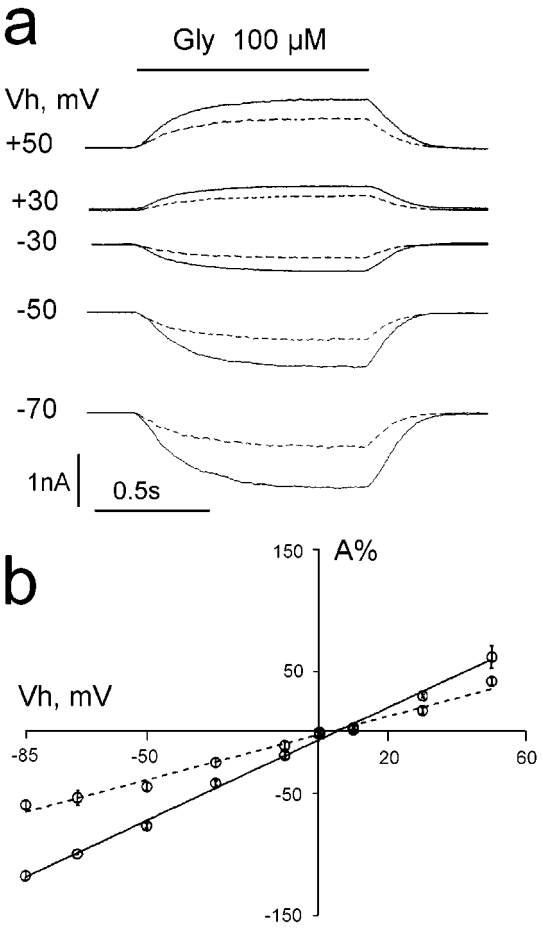

Figure 6 GES antagonism is voltage independent. (a) Representative responses to glycine (solid line) and glycine plus GES (0.5 mM) (dotted line) obtained from a GAN at different holding potentials (Vh). (b) The relationship between peak current–amplitude and holding potential is linear for glycine (solid line) and glycine plus GES (dotted line). The glycine (100 μM) response at Vh −70 mV was taken as 100% in each of the four neurons tested. The lines cross the voltage axis in close proximity to the chloride equilibrium potential.

dependent expression patterns in the CNS (Becker, 1995). In the model proposed by Schmieden *et al.* (1992) on the basis of site-directed mutagenesis of GlyR in oocytes the interaction between binding sites for glycine and taurine at the GlyR depends on its subunit composition: these sites are dissociated in GlyRs composed of only α2-subunits and overlap in those of only α1-subunits.

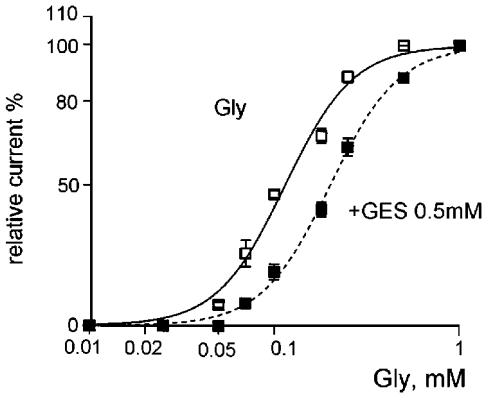

Figure 7 Dose–response curves for glycine in control and in the presence of GES demonstrate competitive antagonism of GES with glycine in rat GANs ($n=4$). Current–amplitudes were normalized to the maximal current recorded with glycine 1 mM in control. Data were fitted with the same equation as in Figure 1.

In the mouse we have previously shown that all GANs express the α2 subunit and a majority of GANs also the α3 and β subunits. In agreement with the above model, strychnine antagonized taurine responses in these neurons non-competitively, while the block of glycine responses was surmountable (Sergeeva & Haas, 2001). Now we find that GES interacts competitively with both taurine and glycine favouring the idea of overlapping binding sites at α2-subunit-containing native GlyRs. Thus GES may be a useful tool to study the molecular organization of GlyR. Further experiments in artificial expression systems would be necessary in the future in order to evaluate subtype-specific antagonism of GES at GlyR.

In conclusion, we have demonstrated direct antagonism of GES at the GlyR. This action is competitive at both taurine and glycine agonist binding sites, suggesting that these sites on GlyRs containing α2, α3 and β subunits are not dissociated but overlapping. This 'side'-effect of the taurine transport antagonist needs to be considered when interpreting taurine depletion studies and the epileptogenic action of GES.

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References

BECKER, C.M. (1995). Glycine receptors: Molecular heterogeneity and implications for disease. *The Neuroscientist,* **1,** 130–141.

DAI, W., VINNAKOTA, S., QIAN, X., KUNZE, D.L. & SARKAR, H.K. (1999). Molecular characterization of the human CRT-1 creatine transporter expressed in Xenopus oocytes. *Arch. Biochem. Biophys.,* **361,** 75–84.

FLINT, A.C., LIU, X. & KRIEGSTEIN, A.R. (1998). Nonsynaptic glycine receptor activation during early neocortical development. *Neuron,* **20,** 43–53.

HERRANZ, A.S., SOLIS, J.M., HERRERAS, O., MENENDEZ, N., AMBROSIO, E., ORENSANZ, L.M. & MARTIN, D.R. (1990). The epileptogenic action of the taurine analogue guanidinoethane sulfonate may be caused by a blockade of GABA receptors. *J. Neurosci. Res.,* **26,** 98–104.

HUXTABLE, R.J.(1982). Guanidinoethane sulfonate and the disposition of dietary taurine in the rat. *J. Nutr.,* **112,** 2293–2300.

HUXTABLE, R.J. (1989). Taurine in the central nervous system and the mammalian actions of taurine. *Prog. Neurobiol.,* **32,** 471–533.

LI, Y. & LOMBARDINI, J.B. (1990). Guanidinoethanesulfonic acid-inhibitor of GABA uptake in rat cortical synaptosomes. *Brain Res.,* **510,** 147–149.

MELLOR, J.R., GUNTHORPE, M.J. & RANDALL, A.D. (2000). The taurine uptake inhibitor guanidinoethyl sulphonate is an agonist at gamma-aminobutyric acid(A) receptors in cultured murine cerebellar granule cells. *Neurosci. Lett.,* **286,** 25–28.

OJA, S.S. & SARANSAARI, P. (1996). Taurine as osmoregulator and neuromodulator in the brain. *Metab. Brain Dis.,* **11,** 153–164.

QUESADA, O., HUXTABLE, R.J. & PASANTES-MORALES, H. (1984). Effect of guanidinoethane sulfonate on taurine uptake by rat retina. *J. Neurosci. Res.,* **11,** 179–186.

RANG, H.P., DALE, M.M. & RITTER, J.M. (1999). How drugs act: general principles. In *Pharmacology* pp. 2–18. Edinburgh: Churchill Livingstone.

SCHMIEDEN, V., KUHSE, J. & BETZ, H. (1992). Agonist pharmacology of neonatal and adult glycine receptor alpha subunits: identification of amino acid residues involved in taurine activation. *EMBO J.,* **11,** 2025–2032.

SERGEEVA, O.A. (1998). Comparison of glycine- and GABA-evoked currents in two types of neurons isolated from the rat striatum. *Neurosci. Lett.,* **243,** 9–12.

SERGEEVA, O.A. & HAAS, H.L. (2001). Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. *Neuroscience,* **104,** 1043–1055.

VOROBJEV, V.S., SHARONOVA, I.N. & HAAS, H.L. (1996). A simple perfusion system for patch-clamp studies. *J. Neurosci. Methods,* **68,** 303–307.

VOROBJEV, V.S., SHARONOVA, I.N., HAAS, H.L. & SERGEEVA, O.A. (2000). Differential modulation of AMPA receptors by cyclothiazide in two types of striatal neurons. *Eur. J. Neurosci.,* **12,** 2871–2880.

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