Enhancement of Glycine Receptor Function by Ethanol Is Inversely Correlated with Molecular Volume at Position α267*

(Received for publication, September 29, 1997, and in revised form, November 6, 1997)

Qing Ye‡, Vladimir V. Koltchine§, S. John Mihic¶, Maria Paola Mascia‡, Marilee J. Wick§, Suzanne E. Finn‡, Neil L. Harrison‡§, and R. Adron Harris**

*This work was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA 06399, AA 11428, and AA 03527 (all to N. L. H.), and Alcoholism Grants AA 06399, AA 11428, and AA 03527 (all to R. A. H.), AA 11525 (to S. J. M.), and AA 11270 (to N. L. H.) and by University of Chicago Postdoctoral Training Grant DA 07255 (to R. A. H.) and Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637, the Department of Pharmacology and Alcohol Research Center, University of Colorado Health Sciences Center, Denver, Colorado 80262, and the Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

Glycine and γ-aminobutyric acid (GABA) receptors are members of the “superfamily” of ion channels, and are sensitive to allosteric modulation by n-alcohols such as ethanol and butanol. We recently demonstrated that the mutation of Ser-267 to Ile in the α1 subunit abolished ethanol regulation of glycine receptors (Gly-R). In the present study, a pair of chimeric receptors was studied, in which a 45-amino acid domain comprising transmembrane domains 2 and 3 was exchanged between the Gly-R1 and γ-aminobutyric acid α1 subunits. Detailed pharmacologic analysis of these chimeras confirmed that this domain of the Gly-R confers enhancement of receptor function by ethanol and butanol. An extensive series of mutations at Ser-267 in the Gly-R1 subunit was also prepared, and the resulting homomeric receptors were expressed and tested for sensitivity to glycine, and allosteric modulation by alcohols. All of the mutant receptors expressed successfully in Xenopus oocytes. Mutation of Ser-267 to small amino acid residues such as Gly or Ala produced receptors in which glycine responses were potentiated by ethanol. As we have reported previously, the mutant Gly-R1 (Ser-267 → Ile) was completely insensitive to ethanol; mutation of Ser-267 to Val had a similar effect. Mutation of Ser-267 to large residues such as His, Cys, or Tyr resulted in inhibition of Gly-R function by ethanol. These results demonstrate that the size of the amino acid residue at position α267 plays a crucial role in determining the functional consequences of allosteric modulation of the Gly-R by alcohols.

Depression of central nervous system function by ethanol is a complex phenomenon (1, 2), which may involve several neurotransmitter systems. Ligand-gated ion channels play an important role in the regulation of neuronal excitability and are sensitive to pharmacologically relevant concentrations of ethanol. Several such channels are thought to be involved in the behavioral effects of ethanol. Intracerebroventricular administration of glycine augments ethanol-induced loss of righting reflex in the mouse (8), and this effect is blocked by the glycine antagonist strychnine. Enhancement of Gly-R function would be consistent with the behavioral actions of ethanol in vivo. Electrophysiological studies have shown that ethanol enhances Gly-R function in spinal cord neurons from mouse and chick (9, 10), and increases glycine-mediated Cl− uptake into rat brain synaptosomes (11). Recently, Mascia et al. (12, 13) demonstrated ethanol enhancement of the function of Gly-R of defined composition expressed in Xenopus oocytes, with concentrations of ethanol as low as 10 mM being effective in potentiating the actions of low concentrations of glycine. Gly-Rs are members of the “superfamily” of related ligand-gated ion channels (14–16). These receptors are pentameric complexes, with the five subunits arranged around a central pore through which permeant ions pass. The composition of most native neuronal glycine receptors is believed to be α1α1β1β2, but receptors can also be efficiently assembled from α1 subunits alone. Each subunit has four putative transmembrane (TM) domains, with the second (TM2) believed to form the lining of the pore. We have recently demonstrated that ethanol enhancement of Gly-R function involves a critical domain encompassing TM2 and TM3 of the α subunit, and that a specific point mutation within TM2 (Ser-267 → Ile) could completely remove ethanol enhancement of Gly-R function (17). In the present study, we extended our original observations on the critical TM2/TM3 domain by studying in detail alcohol modulation of a complementary pair of receptor chimeras created by domain exchange between Gly-R1 and the GABA α1 subunit, another member of the ligand-gated chloride channel “superfamily,” which can readily form homomeric receptors. We also studied an extensive series of single amino acid replacements at Ser-267 in the Gly-R1 subunit, and have investigated the effects of each mutation on allosteric modulation by ethanol.

EXPERIMENTAL PROCEDURES
Chimeric Receptors and Site-directed Mutagenesis—A pair of chimeric receptors, denoted C1 and C2, were first constructed by methods described previously (17). The C1 chimera consists mostly of Gly-R1, except the large intracellular loop and TM4, which are from GABA α1. C2 is the converse chimera, consisting mostly of GABA α1, except for the

*Correspondence should be addressed: Dept. of Pharmacology and Alcohol Research Center, University of Colorado Health Sciences Center, 4200 E, 9th St., Denver, CO. Fax: 303-315-7499.

‡This work was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA 06399, AA 11428, and AA 03527 (all to R. A. H.), AA 11525 (to S. J. M.), and AA 11270 (to N. L. H.) and by University of Chicago Postdoctoral Training Grant DA 07255 (to V. V. K.). The costs of publication of this article were defrayed in part by advertisement solely to indicate this fact.

**To whom correspondence should be addressed: Dept. of Pharmacology and Alcohol Research Center, University of Colorado Health Sciences Center, 4200 E, 9th St., Denver, CO. Fax: 303-315-7499.

1 The abbreviations used are: NMDA, N-methyl-D-aspartate-prefering; GABA, γ-aminobutyric acid; Gly-R, glycine receptor; TM, transmembrane; HEK, human embryonic kidney.
large intracellular loop and TM4 from Gly-R1. Using site-directed mutagenesis methods described below, a unique BssHII restriction site was then introduced into the cDNA sequence encoding the conserved amino acid sequence "PAR" at the beginning of TM2 in both C1 and C2 chimera. Utilizing a unique SspI restriction site existing on the vector pCIS2, the BssHII/SspI fragments were exchanged, resulting in chimeric receptors C6 and C7 (Fig. 1). All chimeric receptors were confirmed by double-stranded sequencing (Sequenase 2.0, U.S. Biochemical Corp.). To create the point mutant series at Gly-R1, Ser-267, mutations were introduced into the cDNA encoding the human Gly-R1 subunit at bases 883–885, with simultaneous loss of a SacI restriction site. Oligonucleotides 24–30 bases in length were obtained from Operon Technologies (Alameda, CA), 5'-phosphorylated using polynucleotide kinase, and used to create mutations using the unique site elimination method (USE kit; Pharmacia Biotech; Ref. 18). SspI digestion was then used to select in favor of mutants, and clones were screened for the appearance of the desired mutation by digestion with SacI. All restriction enzymes and polynucleotide kinase were obtained from New England Biolabs (Beverly, MA). In addition, a few mutations were created using a Pfu-based polymerase chain reaction method (QuikChange; Stratagene). The sequences of both 5' and 3'-cDNA termini and the sequences through the mutation site were confirmed by sequencing.

Oocyte Expression and Electrophysiology—The methods used for oocyte preparation and cDNA nuclear injection, and the electrophysiological assay of receptor function using Xenopus oocytes have been described previously (19). Drugs were applied for 0.5–3 min (depending on the GABA or glycine concentration), by which time the peak current response was obtained. A 5-min washout period was allowed between applications when low GABA or glycine concentrations were used, increasing to 10 min for higher concentrations. Modulatory drugs were always tested against an EC50 concentration of glycine or GABA, i.e. a concentration of agonist giving 10% of the maximal response obtained in that egg. Data were always obtained from 4–13 oocytes taken from at least two different frogs.

RESULTS

Alcohol Pharmacology of Wild-type Glycine Receptors—Ethanol potentiation of Gly-R1 receptor function has previously been described in receptors expressed in Xenopus oocytes (12, 17). In the present study, we extend these observations to the longer chain alcohol, n-butanol, and to Gly-R1 expressed in the HEK 293 cell line. Fig. 2A illustrates the potentiation of glycine responses by ethanol (100–200 mM) in a cell expressing wild-type Gly-R1. Concentration-response analysis revealed a leftward shift of the entire glycine dose-response curve in the presence of 100 mM ethanol, with no significant change in the maximal current amplitude elicited by glycine (Fig. 2C), indicating that the alcohol causes an increase in the apparent affinity of the receptor for glycine. A similar potentiating action and a leftward shift of the glycine concentration-response curve was seen with butanol (Fig. 2, B and D), although, as expected, butanol was more potent than ethanol.

FIG. 1. Chimeric glycine/GABA ρ receptors C6 and C7. The chimera "C6" consists of the GABA ρ1 subunit (depicted by the thick lines), with 45 amino acid residues, roughly including TM2 and TM3, replaced by the analogous regions from the Gly-R1 subunit (thin lines). The chimera "C7" consists of the Gly-R1 subunit with 45 amino acid residues (thin lines), roughly including TM2 and TM3, replaced by the analogous regions from the GABA ρ1 subunit (thick lines).

FIG. 2. Alcohol pharmacology of wild-type glycine ρ1 receptors. A, enhancement of submaximal glycine responses by ethanol in a HEK 293 cell expressing wild-type Gly-R1. The current traces shown illustrate control responses to a low (30 μM) concentration of glycine, after which 30 μM glycine is reapplied in the presence of 100–200 mM ethanol. Bars over current traces indicate glycine applications for 4 s and ethanol applications for 6 s (2-s pretreatment before glycine application). B, a similar experiment, illustrating enhancement of submaximal glycine responses by 10–20 mM butanol in a HEK 293 cell expressing wild-type Gly-R1. C, concentration-response curves for glycine obtained in the presence and absence of 100 mM ethanol in 293 cells expressing wild-type Gly-R1. Concentration-response data for glycine were normalized to the maximal current in each cell, and fitted according to the logistic equation of the form: I = Imin + (I0 − Imin)/(1 + [A]/EC50 + [A]), where I is current, I0 is the maximal current recorded in a given cell, [A] is the glycine concentration, n is the Hill coefficient, and EC50 is the concentration of glycine eliciting 50% of the maximal current. Each point represents the mean ± S.E. of the normalized current from six individual experiments. The best fit values of the glycine EC50 values and Hill coefficients (in parentheses) are as follows: control, 83 μM (n = 2.7); ethanol, 67 μM (n = 2.1). D, concentration-response curves for glycine obtained in the presence and absence of 20 mM butanol in 293 cells expressing Gly-R1. The best fit values of the glycine EC50 values and Hill coefficients from six experiments are as follows: control, 83 μM (n = 2.7); butanol, 41 μM (n = 1.8).
**Ethanol and Ser-267 in Glycine Receptor α1 Subunit**

**A** C7 Glycine  
**B** GABA  
**C** C7  
**D** C6

**Fig. 3. Pharmacology of chimeric glycine/GABA ρ receptors: gating by agonists.** A, gating of the chimera C7 by 20–1000 μM glycine. Note that both activation and deactivation of this receptor are very rapid. B, gating of the chimera C6 by 0.2–10 μM GABA. Note that both activation and deactivation of this receptor are extremely slow. C, concentration-response curve for glycine activation of the chimera C7. Each point represents the mean ± S.E. of the normalized current from four experiments. Glycine EC_{50}, 293 μM; n = 1.3. D, concentration-response curve for GABA activation of the chimera C6. Each point represents the mean ± S.E. of the normalized current from four experiments. GABA EC_{50}, 1.3 μM; n = 2.3.

**Pharmacology of Chimeric Glycine/GABA ρ Receptors: Gating by Agonists—**A complementary pair of chimeric receptors was tested, in which a 45-amino acid domain had been exchanged between Gly-Rα1 (the function of which is enhanced by ethanol, as shown above) and GABA-Rα1 (the function of which is inhibited by ethanol; Ref. 22). The first chimera tested was denoted “C7” (nomenclature from Ref. 17), consisting almost entirely of Gly-Rα1, with the 45-amino acid TM2-TM3 domain provided by GABA-Rα1 (Fig. 1). This chimera expressed relatively poorly in HEK 293 cells, relative to wild-type Gly-Rα1, but could be gated by glycine. Interestingly, although the TM2 domain was contributed by GABA-Rα1, the kinetics of both activation and deactivation were rapid, resembling the gating behavior of the wild-type Gly-Rα1 (Fig. 3A), with an EC_{50} of 293 μM and a Hill coefficient of 1.3 (Fig. 3C). The converse chimera, denoted C6 (see Fig. 1), consisting almost entirely of GABA-Rα1, with the 45 amino acid domain (comprising TM2, TM3, and the intervening short extracellular loop) provided by Gly-Rα1 (17), expressed quite efficiently in HEK 293 cells (Fig. 3B), and was gated by GABA with an EC_{50} of 1.3 μM and a Hill coefficient of 2.3 (Fig. 3D). Again, although the TM2 domain was contributed entirely by Gly-Rα1, the kinetics of both activation and deactivation were extremely slow, strongly resembling the gating behavior of the wild-type GABA-Rα1 (Fig. 3D).

**Chimeric Glycine/GABA ρ Receptors: Regulation by Alcohols—**The regulation of these chimeric receptors by alcohols was examined in detail and compared with those of the corresponding wild-type receptors. GABA ρ1 subunit function is inhibited by ethanol in Xenopus oocytes (22); in the present study, we expressed this subunit in HEK 293 cells and also observed concentration-dependent inhibition by both ethanol and butanol (Fig. 4A). We then studied the chimera C6, in which GABA responses were potentiated by both ethanol and butanol (Fig. 4B). The concentration-dependence and efficacy of these enhancing effects were similar to those exhibited by the wild-type Gly-Rα1 (cf. Fig. 2). Chimera C7 showed very weak inhibition of glycine currents by both ethanol and butanol (Fig. 4C). The efficacy of these effects were smaller than those seen in the wild-type GABA-Rα1 (cf. Fig. 4A).

These findings strongly confirmed and extended our original findings from experiments in Xenopus oocytes that suggested the pre-eminent importance of the TM2 and TM3 domains in the regulation of the Gly-R by alcohols (17). We next examined the effects of ethanol and butanol on the mutant S267I receptor in HEK 293 cells. The mutant S267I receptor expressed well in HEK cells, at levels comparable to those of the wild-type Gly-Rα1. Gating by glycine was efficient, with normal rapid activation and deactivation kinetics, an EC_{50} of 57 μM and a Hill coefficient of 2.2 (Fig. 5A). Desensitization at high glycine concentrations appeared to be reduced in Gly-R(S267I) relative to wild-type Gly-Rα1. In HEK 293 cells expressing the mutant Gly-Rα1(S267I) subunit, up to 200 mM ethanol had no effect on the response to submaximal glycine (Fig. 5B, cf. Fig. 2A); similar observations were made with 20 mM butanol (Fig. 5C, cf. Fig. 2B). Neither ethanol (100 mM) nor butanol (20 mM) had any significant effect on the concentration-response curve for glycine in the S267I mutant (Table I).

**Gating of Other Serine 267 Mutants by Glycine—**The results obtained with the S267I mutant strongly suggested an important role for Ser-267 in the regulation of Gly-Rα1 by alcohols. Because the side chain of isoleucine is larger and more hydrophobic than that of serine, we were curious to determine which amino acid residues at this position allowed for receptor function, and also to determine whether size, hydrophilicity or some other physical parameter of these residues dictated the abilities of the alcohols to potentiate Gly-Rα1 function. We therefore mutated Ser-267 to the remaining 18 amino acid residues commonly found in mammalian proteins. Due to the large number of mutants, we chose to express these in Xenopus oocytes, in which rapid sampling and screening of multiple constructs was facilitated by the use of a robotic system (23). A few selected mutants were also examined in HEK 293 cells. All of the mutant receptors expressed successfully in the oocytes. Replacement of Ser-267 with His also produced a functional Gly-R in HEK 293 cells, which was gated by glycine with an EC_{50} of 251 μM and a Hill coefficient of 1.5 (Table I). Replacement of Ser-267 with Tyr also produced a functional receptor gated by glycine, with an EC_{50} of 226 μM, Hill slope of 1.3 (Table I). Even the introduction of charged residues at position 267 produced functional Gly-R in Xenopus oocytes.

**Modulation by Alcohols of Multiple Mutants at Ser-267—**We studied the regulation of each of these mutant receptors by ethanol in the Xenopus oocyte preparation. Each was tested by applying 200 mM ethanol and the results expressed in terms of potentiation of the response to a test concentration of glycine. In each case, we standardized the experiment by using an EC_{10} concentration of glycine, i.e., a concentration of glycine that gave 10% of the maximal response obtained in that mutant receptor, the maximal response being usually assessed as the

---

**V. V. Koltchine, unpublished observations.**
response to 10 mM glycine. Some mutant receptors demonstrated enhancement of glycine by ethanol; for example, the S267A and S267N mutants showed potentiation of glycine. Another group of mutants, including S267I and S267V, demonstrated almost no detectable effects of 200 mM ethanol. Unexpectedly, and perhaps most interestingly, another group of mutants, including S267Y, S267H, and S267F, showed quite strong inhibitory effects of ethanol. The results are summarized in the histogram in Fig. 6, in which the amino acids substituted at residue 267 are arranged in descending order of potentiation of Gly-R function by ethanol, from Ser-267, which gave the largest degree of potentiation, down to Tyr-267, which showed the largest degree of inhibition of Gly-R function by ethanol.

**DISCUSSION**

Earlier studies showed that ethanol and other alcohols enhance the action of agonist on homomeric Gly-R consisting only of α1 or α2 subunits (12, 13), but inhibit the function of GABA-R composed of γ1 subunits (22). This raises the question of whether these two opposite actions of alcohols are due to their actions on homologous regions of these two receptors, with different functional consequences, or whether they are due to drug interactions with distinct, non-conserved sequences. Our initial studies with chimeric and mutant receptors suggested that the 45-amino acid domain (comprising TM2, TM3, and the intervening short extracellular loop) acts as a recognition domain for alcohols. The results show that ethanol and Ser-267 in Glycine Receptor α1 Subunit
Table I

| Gly-R mutant | EC_{50} (mM) Control | EC_{50} (mM) + Ethanol (100 mM) | EC_{50} (mM) + Butanol (20 mM) | Hill coefficient |
|--------------|----------------------|--------------------------------|--------------------------------|------------------|
| Wild Type    | 83 (2.7)             | 67 (2.1)                       | 41 (1.8)                       | 1.3              |
| a1(S267I)    | 57 (2.2)             | 57 (1.4)                       | 53 (1.7)                       | 1.3              |
| a1(S267H)    | 251 (1.5)            |                                |                                |                  |
| a1(S267Y)    | 228 (1.2)            |                                |                                |                  |

**FIG. 6. Modulation by alcohols of multiple mutants at Ser-267.**

The modulation of glycine responses in 19 mutants at Ser-267, all expressed in *Xenopus* oocytes, and all tested for modulation by 200 mM ethanol of the response to a EC_{50} test concentration of glycine. Each point represents the mean ± S.E. of the normalized modulation from 4–13 individual oocytes.

counted for both enhancing and inhibitory actions of the alcohols (17), and this finding is confirmed and extended in the present study. The testing of “mini-chimeras” demonstrated that a 45-amino acid sequence in the Gly-R1 and GABA receptor subunits appears to be the prime determinant of both the enhancing and inhibitory actions of alcohols on this subfamily of receptors. One caveat here is that the alcohols do not show their full efficacy as inhibitory modulators at the C7 chimera, when compared with their effects on the wild-type GABA receptor (Fig. 4), suggesting that for the inhibitory actions of the alcohols, there may well be an additional contribution to this effect from receptor structures outside TM2 and TM3.

Another interesting issue with respect to the minichimeras is that the receptor gating kinetics are apparently not a property of TM2, since the 45 amino acid domain (comprising TM2, TM3, and the intervening short extracellular loop) from GABA receptor could be inserted into a Gly-R skeleton to create C7 without significantly slowing channel opening or closing, while the minichimera C6 retains the extremely slow gating behavior of the parent GABA receptor (Fig. 3). These observations suggest that the rate-limiting steps for the opening of these channels might be in the binding of agonist, or might otherwise be controlled in other parts of the molecule not represented within TM2, TM3, or the TM2-TM3 loop.

In our earlier study, we showed that mutation of Ser-267 to Ile abolished the action of ethanol (17), and we therefore selected the Ser-267 residue for extensive mutagenesis. The resulting 19 mutant receptors with different amino acid residues at α267 showed a remarkable gradation of ethanol actions, ranging from 88% potentiation (Ser-267) to 55% inhibition (Tyr-267) of glycine-induced currents. The nature of this distribution (Fig. 6) suggested that a continuous property of the amino acids might determine the action of ethanol. Correlation analysis (Fig. 7) demonstrated that the molecular volume of ethanol was associated with more than 60% of the variation in ethanol activity among the mutant receptors. On the other hand, there was no relationship between ethanol action and hydrophilicity, hydrophobicity, or charge (Fig. 7). These data suggest that the physical size of the residue at α267 is a key regulator of the effects of alcohols on the Gly-R.

It is believed that Ser-270 in the GABA_{A}-R1 subunit (a homolog of Ser-267 in Gly-R1) does not form part of the channel lining, but instead is likely to be sequestered within the interior of the protein (25). Our observations on Ser-267 mutants are consistent with the idea that the binding of small molecules such as alcohols to the receptor protein could alter the subtle thermodynamic equilibria associated with channel gating in the Gly-R. However, it appears unlikely that Ser-267 itself is involved in the primary alcohol binding site, although this may be in the vicinity of residue 267.

An obvious question to be considered here is how mutation of a single amino acid residue can alter the modulatory action of ethanol on Gly-R function so dramatically, from enhancement of receptor function (wild-type, S267G), through no effect (S267I, S267V), to inhibition (S267F, S267Y)? One possible explanation is based on the idea that, in the wild type Gly-R1 receptor, ethanol preferentially binds to and thereby stabilizes the inactivated state of the receptor; conversely, in the wild-type GABA receptor, ethanol would bind to and stabilize the closed state of the receptor. Selective binding to active or inactive states of receptors has been proposed as the basis of the actions of agonists, antagonists, and “inverse agonists” on many types of receptors (26). Such an action of alcohols would be analogous to the preferential binding of local anesthetics to the inactivated state of the voltage-activated sodium channels.
evaluate the hypothesis that ethanol preferentially stabilizes kinetic studies of several of these mutant Gly-R would help to.

ied here should help determine whether Ser-267 controls the canol (13); cut-off analysis on the chimeras and mutants stud-
molecular size of such cavities within a given protein (30, 31). The so-called “cut-off” phenomenon is thought to be due to the lack of effect of long chain alcohols may be a useful tool. The distinction between these two possibilities. In this respect, the lack of effect of long chain alcohols may be a useful tool. The so-called “cut-off” phenomenon is thought to be due to the exclusion of larger alcohols from an amphiphilic binding cavity or “pocket,” and the cut-off may serve as an indicator of the molecular size of such cavities within a given protein (30, 31). Studies of the Gly-R suggest a distinct cut-off at around dode-
canol (13); cut-off analysis on the chimeras and mutants studied here should help determine whether Ser-267 controls the size of such a binding pocket in Gly-R. In addition, single-channel kinetic studies of several of these mutant Gly-R would help to evaluate the hypothesis that ethanol preferentially stabilizes open or closed states of the channel operated by glycine.

Acknowledgments—We are indebted to Prof. Heinrich Betz, Garry Cutting, and Peter Schofield, who supplied cDNAs used in this study, and to Dr. Gareth Tibbs for helpful discussion.

REFERENCES

1. Deitrich, R. A., Dunwiddie, T. V., Harris, R. A., and Erwin, V. G. (1989) Pharmacol. Rev. 41, 491–537
2. Samson, H. H., and Harris, R. A. (1992) Trends Pharmacol. Sci. 13, 206
3. Lovinger, D. M., White, G., and Weight, F. F. (1989) Science 243, 1721–1724
4. Dildy-Mayfield, J. E., and Harris, R. A. (1995) J. Neurosci. 15, 3162–3171
5. Lovinger, D. M. (1993) Neurosci. Lett. 159, 83–87
6. Mascia, M. P., Machu, T. K., and Harris, R. A. (1996) in Pharmacological Effects of Ethanol on the Nervous System (Deitrich, R. A., and Erwin, V. G., eds) pp. 51–72, CRC Press, Boca Raton, FL
7. Betz, H. (1991) Trends Neurosci. 14, 458–461
8. Williams, K. L., Perko, A. P., Barbara, E. J., and DiGregorio, G. J. (1995) Pharmacol. Biochem. Behav. 50, 199–205
9. Celentano, J. J., Gibbs, T. T., and Farb, D. H. (1988) Brain Res. 455, 377–380
10. Aguayo, L. G., and Pancetti, F. C. (1994) J. Pharmacol. Exp. Ther. 270, 61–69
11. Engblom, A. C., and Åkerman, K. E. O. (1991) J. Neurochem. 57, 384–390
12. Mascia, M. P., Mich, S. J., Schofield, P., and Harris, R. A. (1996) Mol. Pharmacol. 50, 402–406
13. Mascia, M. P., Machu, T. K., and Harris, R. A. (1996) Br. J. Pharmacol. 119, 1331–1336
14. Betz, H. (1990) Neuron 5, 383–392
15. Ortel, M. O., and Lant, G. G. (1995) Trends Neurosci. 18, 121–127
16. Vandenberg, R. J., and Schofield, P. R. (1994) Handbook of Membrane Channels, pp. 317–332, Academic Press, Orlando, FL
17. Mich, S. J., Ye, Q., Wick, M., Koltsch, V. V., Finn, S. E., Krasowski, M. D., Mascia, M. P., Valenzuela, C. F., Hansson, K. K., Greenblatt, E. P., Harris, R. A., and Harrison, N. L. (1997) Nature 389, 385–389
18. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 206, 81
19. Mich, S. J., Whiting, P. J., and Harris, R. A. (1994) Eur J. Pharmacol. 268, 209–214
20. Deleted in proof
21. Koltsch, V. V., Ye, Q., Finn, S. E., and Harrison, N. L. (1996) Neuropharmacology 35, 1445–1456
22. Mich, S. J., and Harris, R. A. (1996) J. Pharmacol. Exp. Ther. 277, 411–416
23. Mich, S. J., and Harris, R. A. (1996) BioTechniques 20, 802–804
24. Zamytanian, A. A. (1972) Prog. Biophys. Mol. Biol. 24, 107–123
25. Xu, M., and Akabas, M. H. (1996) J. Gen. Physiol. 107, 195–205
26. Leff, P. (1995) Trends Physiol. Sci. 16, 89–97
27. Streichart, G. R. (1973) J. Gen. Physiol. 62, 37–57
28. Courtney, K. R. (1975) J. Pharmacol. Exp. Ther. 195, 225–236
29. Greedy, J. E., Ranganathan, S., Schofield, P. R., Matsuo, Y., and Nishikawa, K. (1997) Protein Sci. 6, 893–900
30. Li, C., Peoples, R. W., and Weight, F. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8200–8204
31. Curry, S., Moss, G. W. J., Dickinsen, R., Lieb, W. R., and Franks, N. P. (1991) Br. J. Pharmacol. 102, 167–173
32. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
33. Hopp, T. P., and Woods, K. R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3824–3829
34. Zimmermann, J. M., Eliezer, N., and Simha, R. (1968) J. Theor. Biol. 21, 170–201
Enhancement of Glycine Receptor Function by Ethanol Is Inversely Correlated with Molecular Volume at Position α267
Qing Ye, Vladimir V. Koltchine, S. John Mihic, Maria Paola Mascia, Marilee J. Wick, Suzanne E. Finn, Neil L. Harrison and R. Adron Harris

J. Biol. Chem. 1998, 273:3314-3319.
doi: 10.1074/jbc.273.6.3314

Access the most updated version of this article at http://www.jbc.org/content/273/6/3314

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 31 references, 10 of which can be accessed free at http://www.jbc.org/content/273/6/3314.full.html#ref-list-1