0026-895X/95/050919-09$3.00/0

MOLECULAR PHARMACOLOGY, 48:919–927 (1995).

Pharmacology of the Inhibitory Glycine Receptor: Agonist and Antagonist Actions of Amino Acids and Piperidine Carboxylic Acid Compounds

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Received June 5, 1995; Accepted August 4, 1995

SUMMARY

To define structure-activity relations for ligands binding to the inhibitory glycine receptor (GlyR), the agonistic and antagonistic properties of α- and β-amino acids were analyzed at the recombinant human α_1 GlyR expressed in *Xenopus* oocytes. The agonistic activity of α-amino acids exhibited a marked stereoselectivity and was highly susceptible to substitutions at the C_α-atom. In contrast, α-amino acid antagonism was not enantiomer dependent and was influenced little by C_α-atom substitutions. The β-amino acids taurine, β-aminobutyric acid (β-ABA), and β-aminoisobutyric acid (β-AIBA) are partial agonists at the GlyR. Low concentrations of these compounds competitively inhibited glycine responses, whereas higher concentrations elicited a significant membrane current. Nipecotic acid, which contains a *trans*-β-amino acid configuration, behaved as purely competitive GlyR antagonist. Our data are consistent with the existence of a common binding site for all amino acid agonists and antagonists, at which the functional consequences of binding depend on the particular conformation a given ligand adopts within the binding pocket. In the case of β-amino acids, the *trans* conformation appears to mediate antagonistic receptor binding, and the *cis* conformation appears to mediate agonistic receptor binding. This led us to propose that the partial agonist activity of a given β-amino acid is determined by the relative mole fractions of the respective *cis/trans* conformers.

Glycine and GABA are the major inhibitory neurotransmitters in the central nervous system; both amino acids produce an increase in postsynaptic chloride conductance by binding to specific plasma membrane receptors (1). In addition, the endogenous amino acids β-alanine and taurine display inhibitory activity when applied to neurons. Taurine gates both inhibitory GlyR and $GABA_A$ receptor channels (2), whereas β-alanine is a more selective GlyR agonist (3). Interestingly, low concentrations of these amino acids have also been found to antagonize the response to the natural agonist glycine (4–6). These findings raise the questions of how amino acid ligands are discriminated at their respective receptor binding sites and what determines their agonistic and antagonistic potencies. Site-directed mutagenesis has shown that minor amino acid substitutions within the ligand binding region of the GlyR have dramatic effects on agonist selection (6–10). The structural features distinguishing agonistic from antagonistic amino acids are, however, not clear.

Biochemical and molecular studies of the GlyR indicate a pentameric structure of this receptor protein, which is composed of ligand-binding α and structural β subunits (1, 11). The α subunits exist in various developmentally and regionally regulated isoforms (α_1 to α_4) (12–17), whereas only a single β subunit gene (18) has been described so far. Heterologous expression of the α_1, α_2, or α_3 subunits in *Xenopus laevis* oocytes (6, 14) or mammalian cells (19) generates functional homo-oligomeric receptor proteins of pharmacological properties similar to those of the native GlyR, showing that these proteins harbor the agonist and antagonist binding sites of this receptor.

Site-directed mutagenesis identified three distinct domains within the extracellular amino-terminal region that contribute to ligand binding. Domain I corresponding to amino acid position 111 of the α_1 subunit is important for taurine activation (6). Domain II is characterized by two aromatic residues at positions 159 and 161, which determine the affinity and rank order of potency of amino acid agonists (8, 9, 20). Domain III preceding the first transmembrane segment M1 has been proposed to confer agonist-antagonist discrimination (9, 10). In addition, point mutations in a region connecting transmembrane segments M2 and M3 of the α_1 subunit cause hyperekplexia in humans (21) by reducing

This work was supported by Deutsche Forschungsgemeinschaft (Leibniz-Programm), Human Capital and Mobility Program (Contract ERB-CHRXCT930167), and Fonds der Chemischen Industrie.

ABBREVIATIONS: β-ABA, β-aminoisobutyric acid; β-AIBA, β-aminoisobutyric acid; ACPC, 1-aminopropane-1-carboxylic acid; ACHC, 1-aminohexane-1-carboxylic acid; GABA, γ-aminobutyric acid; GlyR, glycine receptor; isoTHAO, 5,6,7,8-tetrahydro-4*H*-isoxazole[4,3-c]azepin-3-ol.

the glycine affinity of the GlyR by >100-fold (22, 23) without altering its sensitivity to β-alanine and taurine antagonism (7, 24). Finally, an amino acid substitution at position 56 of the α_1 subunit is known to cause a 4-fold decrease in agonist affinity in the mouse mutant *spasmodic* (25, 26).

We report a detailed analysis of the agonist and antagonist potencies of different amino acids at the recombinant human α_1 GlyR and propose a model how β-amino acids might function as partial agonists. In addition, we show that piperidine carboxylic acids act exclusively as competitive antagonists. Our data support the idea that different binding conformations of β-amino acids determine their agonistic and antagonistic potencies.

Materials and Methods

Experimental procedures. *X. laevis* oocytes were removed from frogs anesthetized with urethane (Sigma Chemical Co.), dissected by collagenase (Sigma) treatment, and injected with human GlyR α_1 cRNA (10–20 ng/oocyte) synthesized by *in vitro* transcription (Invitrogen mCAP kit) as described previously (22). Voltage-clamp recording of whole-cell currents was performed 24 hr after injection at a holding potential of −70 mV as described previously (5).

Drugs. All drugs used in the present study were purchased from Aldrich, with the exception of guavacine and isoguavacine, which were ordered from Research Biochemicals International. Drugs were dissolved in frog Ringers' solution and adjusted to pH 7.2. Inhibition curves were obtained by coapplication of the agonist at a concentration corresponding to its EC_{50} value with increasing concentrations of antagonist. Wash-out times between each application were 3–5 min.

Statistical analysis. Experimental values are presented as the mean ± standard error. For evaluation of the EC_{50} from dose-response curves, data from different oocytes were fitted by a least-squares procedure (eq. 1): $I/I_{max} = C^h/(C^h + EC_{50}{}^h)$, where I is current, I_{max} is the maximal response, C is the agonist concentration, and h is the Hill coefficient. Whenever possible, the equation (eq. 2): $I/I_{max} = IC_{50}{}^h/(A^h + IC_{50}{}^h)$ was used to fit concentration inhibition curves, where IC_{50} corresponds to the concentration of antagonist (A) producing half-maximal inhibition.

Results

For the present study, recombinant homo-oligomeric GlyR was generated by injecting human GlyR α_1 subunit cRNA into *Xenopus* oocytes. After an incubation time of 1–3 days, superfusion with increasing glycine concentrations induced inward directed membrane currents, which ranged from 0.2 to 5 μA (Fig. 1). The corresponding peak amplitudes could be fitted to a single sigmoidal curve (eq. 1), with an agonist concentration eliciting a half-maximal response (EC_{50}) of ~200 μM and a Hill coefficient (h) of 2.4 (Table 1). We used these recombinant α_1 GlyRs to determine both the agonist and antagonist activities of different amino acid and piperidine derivatives.

α-Amino acid derivatives. We analyzed the pharmacology of different methylated glycine derivatives (Fig. 1A). Only sarcosine, a molecule with a secondary amino group, was able to induce significant membrane currents with an EC_{50} of ~13 mM and a maximal amplitude (I_{max}) of 50% compared with that obtained with saturating concentrations of glycine (Fig. 1B, Table 1). The Hill coefficient (h = 2.1 ± 0.2) determined from the resulting dose-response curve (Fig. 1C) was close to that obtained with glycine. *N',N*-Dimethylglycine and betaine, which carry two and three methyl substitutions (Fig. 1A), respectively, did not evoke current responses at concentrations up to 100 mM but reduced glycine-induced currents with half-maximal inhibition values (IC_{50}) of ~20–30 mM (Fig. 1D, Table 1). Increasing glycine concentrations (up to 10 mM) abolished *N',N*-dimethylglycine or betaine inhibition (not shown), which characterizes these molecules as competitive antagonists. No significant inhibition was seen with sarcosine (Fig. 1, B and D). These data imply that a free amino group is required for efficient gating of the GlyR channel, whereas double- or triple-methylated derivatives act as low-affinity antagonists.

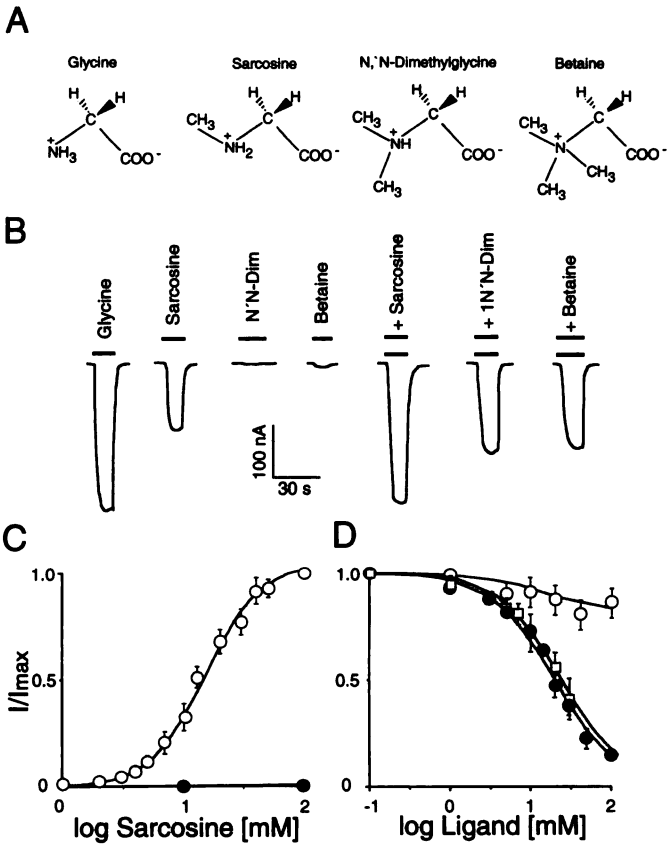

Fig. 1. Current responses elicited by glycine and *N*-methylated glycine derivatives. *Xenopus* oocytes were injected with α_1 cRNA, and voltage-clamp recording was performed as detailed in Materials and Methods. A, Molecular structures of glycine, sarcosine, *N',N*-dimethylglycine, and betaine. B, *Left*, membrane currents elicited by superfusion of 140 μM glycine and 10 mM sarcosine, *N',N*-dimethylglycine (*N'N'-Dim*), and betaine, each. *Right*, coapplication of glycine (140 μM) with the same compounds (10 mM). C, Agonist dose-response relation for sarcosine (○) and *N',N*-dimethylglycine (●). Data points represent the mean ± standard error of experiments with five oocytes. D, Inhibition of glycine current obtained on coapplication of sarcosine (○), *N',N*-dimethylglycine (●), and betaine (□). Current values were normalized to the responses obtained with 100 mM sarcosine (C) and 0.2 mM glycine (D), respectively.

Next, we analyzed ligands with C_α-atom substitutions for current activation and glycine competition (Fig. 2A). As reported previously (8, 27), α-amino acid derivatives with hydrophobic or hydrophilic groups at the C_α-atom showed a stereospecific agonist activity. L-α-Alanine was 3-fold more potent in current induction than D-α-alanine (Table 1). However, in competition experiments with glycine used at a concentration corresponding to its EC_{50} value both L- and D-α-

TABLE 1

Pharmacology of α- and β-amino acids

Amino acid responses were recorded from oocytes injected with α1 cRNA as described under Materials and Methods. The Hill coefficients (*h*) represents the slope of the respective activation and inhibition dose response curve of *n* experiments. I_{max} values of α- and β-amino-acids are given as a percentage of the maximal glycine current. IC_{50} values were determined using glycine concentrations of about 0.2 mM.

Ligand	EC_{50}	*h*	I_{max}	*n*	IC_{50}	*h*	*n*
	mM		%		mM		
α-amino acids							
Glycine	0.20 ± 0.03	2.4 ± 0.1	100	5	[a]		5
Sarcosine	13.2 ± 2.1	2.1 ± 0.2	50.1 ± 9.9	5	[a]		5
N,'N-dimethylglycine	[a]			4	30.1 ± 9.7	1.2 ± 0.04	4
Betaine	[a]			4	21.7 ± 2.3	1.4 ± 0.20	4
L-α-alanine	3.1 ± 0.8	2.0 ± 0.1	80.5 ± 5.6	5	[a]		6
D-α-alanine	9.0 ± 1.3	1.8 ± 0.1	69.0 ± 6.5	6	[a]		4
L-Serine	5.1 ± 0.7	1.9 ± 0.2	86.7 ± 8.6	5	[a]		5
D-Serine	[a]			4	8.5 ± 1.5	1.1 ± 0.03	4
Aminoisobutyric acid	[a]			3	2.9 ± 0.5	1.6 ± 0.20	3
L-α-aminobutyric acid	[a]			7	6.0 ± 0.8	1.5 ± 0.07	7
D-α-aminobutyric acid	[a]			4	3.6 ± 0.5	1.4 ± 0.05	4
ACPC	[a]			3	17.0 ± 2.5	1.5 ± 0.20	3
ACHC	[a]			3	15.0 ± 4.0	1.3 ± 0.20	3
β-amino acids							
Taurine	1.7 ± 0.1	1.7 ± 0.3	31.9 ± 2.8	18	0.7 ± 0.12	[b]	4
β-ABA	5.6 ± 0.8	1.4 ± 0.1	7.1 ± 1.9	8	0.3 ± 0.02	1.3 ± 0.09	7
β-AIBA	8.7 ± 0.8	1.5 ± 0.1	6.5 ± 2.8	5	0.8 ± 0.10	1.4 ± 0.10	3

[a] No agonistic or antagonistic function detectable.
[b] Not determined.

alanine potentiated the glycine-evoked membrane currents up to the amplitude elicited by these agonists alone, i.e., to 69 ± 6.5% (D-alanine) and 80 ± 5.6% (L-alanine) (Fig. 2B; Table 1). L-Serine, carrying a polar $C_α$-substitution, exhibited an EC_{50} value of 5.1 ± 0.7 mM and a Hill coefficient of 1.9 ± 0.2 when applied alone and enhanced the glycine response as described for D-alanine (Fig. 2B). In contrast, D-serine was inactive as an agonist up to concentrations of 50 mM but inhibited glycine-evoked currents with an IC_{50} value of ~8 mM (Fig. 2B, Table 1).

Both D- and L-α-aminobutyric acid, which carry an ethyl substitution at the $C_α$-atom, as well as α-aminoisobutyric acid (Fig. 2A) did not act as agonists but fully antagonized the glycine response with similar affinities (IC_{50} values of 3–6 mM; Hill coefficients of 1.4–1.6) (Fig. 2C; Table 1). The parallel shift of the glycine dose-response curve seen in the presence of 3 mM α-aminoisobutyric acid (Fig. 2D) or D- and L-α-aminobutyric acid (not shown) to the right indicates that the inhibition produced by these molecules is competitive.

Derivatives with cyclic $C_α$-substitutions like ACPC and ACHC inhibited the glycine response poorly (Fig. 2C; Table 1). Amino acids with two carboxylic groups like L-glutamic acid and L-aspartic acid were inactive in GlyR activation and inhibition (data not shown).

These results demonstrate that the agonistic activity of α-amino acids was reduced even by minor substitutions at the $C_α$-atom and dependent on the respective stereoisomer. In contrast, the antagonistic potency of α-amino acids was less sensitive to the size of $C_α$-atom substitutions and not stereoselective.

β-Amino acid derivatives. Taurine and β-alanine are known to be partial agonists of the GlyR with affinities lower than that of glycine (6). We analyzed the effects of DL-β-ABA and DL-β-AIBA (for structures, see Fig. 3A) on GlyR function. Application of 10–100 mM taurine, β-ABA, and β-AIBA, respectively, induced maximal currents of 31.9 ± 2.8%, 7.1 ± 1.9%, and 6.5 ± 2.8% compared with that obtained with saturated glycine concentrations (Fig. 3B). No significant response (<1 nA) was seen on superfusion of noninjected oocytes with 100 mM concentrations of these β-amino acids. The I/V curves of glycine and β-ABA reversed at a holding potential of −25 to −30 mV, indicating that the low-efficiency channel gating properties of β-amino acids are not dependent on membrane potential (data not shown). The dose-response curves of β-ABA and β-AIBA saturated at ~50 mM (Fig. 3C), with EC_{50} values of 6–9 mM and Hill coefficients of ~1.5 for both (Table 1).

Taurine is known to antagonize glycine responses with an IC_{50} value that is significantly lower than its EC_{50} value for the GlyR (6). This "dualistic" action of taurine prevented an accurate determination of its IC_{50} value by the approach we describe. Therefore, we performed a competition experiment in which 2 mM taurine was coapplied with increasing concentrations of glycine to show the competitive behavior of this β-amino acid. The resulting dose-response curve was not sigmoidal; a weak current potentiation was seen at glycine concentrations of ≤0.1 mM, current inhibition at a glycine concentration of <1 mM, and recovery of maximal responses at saturating doses of glycine (Fig. 3D).

We also analyzed the antagonistic potency of β-ABA and β-AIBA. Both ligands inhibited the glycine response with IC_{50} values of 0.3 ± 0.02 mM and 0.8 ± 0.1 mM, respectively, and Hill coefficients of ~1.4 (Fig. 3E, Table 1). Again, this inhibition was competitive as revealed by parallel shifts of the glycine dose-response curve to the right (Fig. 3E, *inset*; data not shown).

In conclusion, all β-amino acid tested behaved as partial agonists of the GlyR, with a rank order of potency of taurine > β-ABA > β-AIBA, but also showed competitive antagonism with IC_{50} values for β-ABA < taurine ≤ β-AIBA (Table 1).

Heterocyclic carboxylic acids. The molecular basis for the dualistic agonistic-antagonistic action of β-amino acids is presently unclear. To reveal whether the GlyR binds these compounds in different molecular conformations, we tested

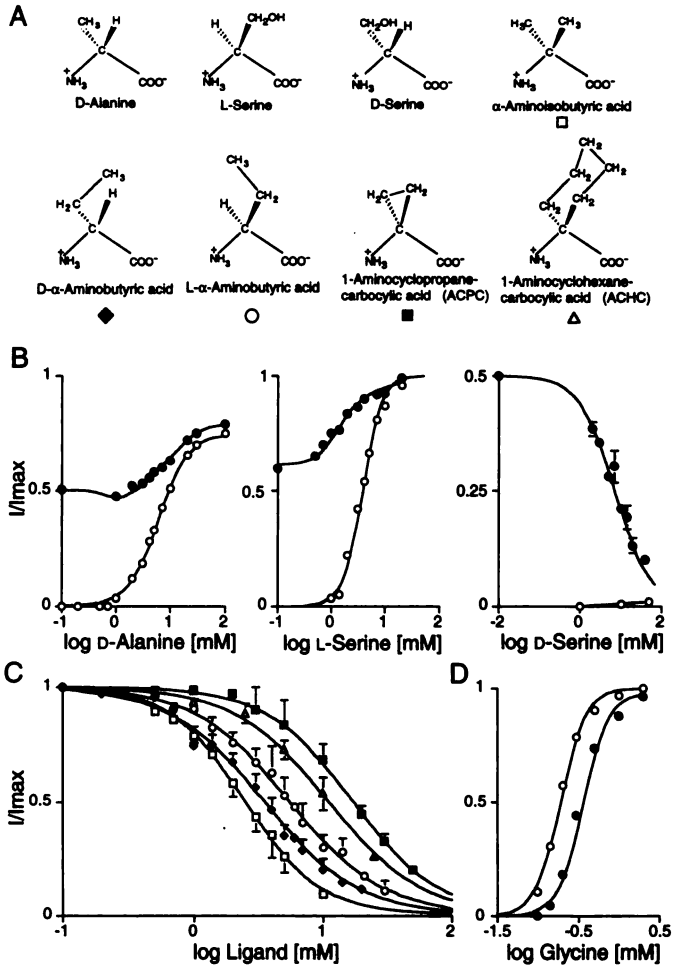

Fig. 2. Agonist and antagonist dose-response relations of α-amino acids. A, Molecular structures of the α-amino acids tested. B, Representative dose-response curves of D-alanine (*left*) and L-serine (*middle*) from an individual α_1 cRNA-injected oocyte and of D-serine (*right*) from four injected oocytes in the absence (○) and presence (●) of 0.2 mM glycine. Peak amplitudes of agonist-induced currents were plotted as current relative to that obtained with saturating glycine concentrations and were fitted by the Hill equation and by eye, respectively. C, *Left*, inhibition of glycine induced currents by α-aminoisobutyric acid, L-α-aminobutyric acid, D-α-aminobutyric acid, ACPC, and ACHC (for symbols, see A). Data points were normalized to the response obtained with 170 μM glycine and represent the mean ± standard error of experiments with four or five oocytes. *Right*, Representative and normalized glycine dose-response curves of an individual oocyte obtained in the absence (○) and presence (●) of 3 mM α-aminoisobutyric acid.

various molecules in which free rotation around the C—C atoms of the β-amino acid is restricted by heterocyclic ring formation, i.e., piperidine carboxylic acid derivatives. None of the six-membered heterocycles tested elicited membrane currents up to concentrations of 10–50 mM (not shown). However, carboxyl-group substitution at the C3 (nipecotic acid) and C4 (isonipecotic acid) ring carbon atoms (Fig. 4A) generated antagonists with IC_{50} values of 0.8 mM and 0.23 mM, respectively (Fig. 4B, Table 2). The Hill coefficients calculated from the inhibition curves of both derivatives were 1.5 (Table 2), which indicates some cooperativity of glycine displacement. Piperidine-4-sulfonic acid displayed an IC_{50} value of 0.40 mM, whereas guavacine and isoguavacine had 2–3-fold lower antagonistic potencies than their hydrated analogues (Table 2).

Analysis of the glycine dose-response relations showed that nipecotic acid (Fig. 4C) and isonipecotic acid (not shown) caused a parallel shift of the response curve without appreciably altering its slope or maximal response. Similar results were obtained when inhibition curves for nipecotic acid were performed at different glycine concentrations (Fig. 4D).

The lack of a negative charge due to carbamidation or hydroxylation, as given in nipecotamid and 3- or 4-hydroxypiperidine, respectively, resulted in poor antagonists with IC_{50} values 15–20-fold higher than that of their carboxylated analogues (Fig. 4B, Table 2).

All active piperidine derivatives carry substitutions at the C3 or C4 ring atoms. However, L- or D-pipecolinic acid, piperidine analogues with a carboxylic group at the C2 atom, produced no detectable inhibition of glycine currents up to a concentration of 10 mM for both. Piperidine-2,3-dicarboxylic acid, piperidine-2,4-dicarboxylic acid, and piperidine-2,5-dicarboxylic acid also failed to interact with the GlyR (data not shown). Thus, acidic piperidine derivatives in which the distance between amino and carboxyl group is >2.4 Å competed glycine with comparable affinities but were inactive as GlyR agonists. Furthermore, all inhibition experiments described above indicate a competitive behavior and thus reflect antagonistic binding within a common ligand binding pocket.

The antagonistic potency of nipecotic acid was also tested on taurine and β-ABA responses. Fig. 5A demonstrates again the efficient reduction of the glycine response by 2 mM nipecotic acid, whereas taurine and, in particular, β-ABA currents were poorly antagonized. The corresponding inhibition curve of nipecotic acid on coapplication with taurine revealed an IC_{50} value of 2.44 ± 0.21 mM (eight experiments) and a Hill coefficient of 1.36 ± 0.05 (Fig. 5B). For β-ABA, full inhibition by this piperidine derivative could not be achieved due to its low apparent affinity. Again, nipecotic acid produced a shift of the taurine dose-response curve to the right (Fig. 5C), indicating a largely competitive mechanism of action. Interestingly, however, antagonism by strychnine also appeared to differ for this particular agonist. Previous studies have shown that strychnine is a high affinity competitive antagonist of both glycine (IC_{50} value of 30 ± 0.5 nM; see Fig. 5D) and taurine gating of the α_1 GlyR (6). However, to antagonize β-ABA elicited currents, an alkaloid concentration of 220 ± 42 nM was required for half-maximal inhibition (Fig. 5D). This might suggest a different site of action of β-ABA; however, we provide evidence that the inefficient strychnine antagonism of the β-ABA response is due to the partial agonist activity of this β-amino acid.

Simulation of partial agonist function. The data have presented above provide evidence that a unitary binding site at the GlyR mediates both amino acid agonism and piperidine or β-amino acid inhibition. Furthermore, because taurine-elicited currents were competitively inhibited by strychnine and nipecotic acid, a common site appears to exist for all of these GlyR ligands. At the latter, taurine, and most likely also β-ABA and β-AIBA, exhibit both agonist and antagonist activity as evident from their respective EC_{50} and IC_{50} values. As the latter differed ~2-fold for taurine and 10–20-fold for β-ABA and β-AIBA (compare Table 1 with Ref. 6), the partial agonist activity of these compounds may reflect "self-inhibition" of channel gating.

To evaluate whether the weak current responses and low Hill coefficients (Table 1) of these β-amino acids might result

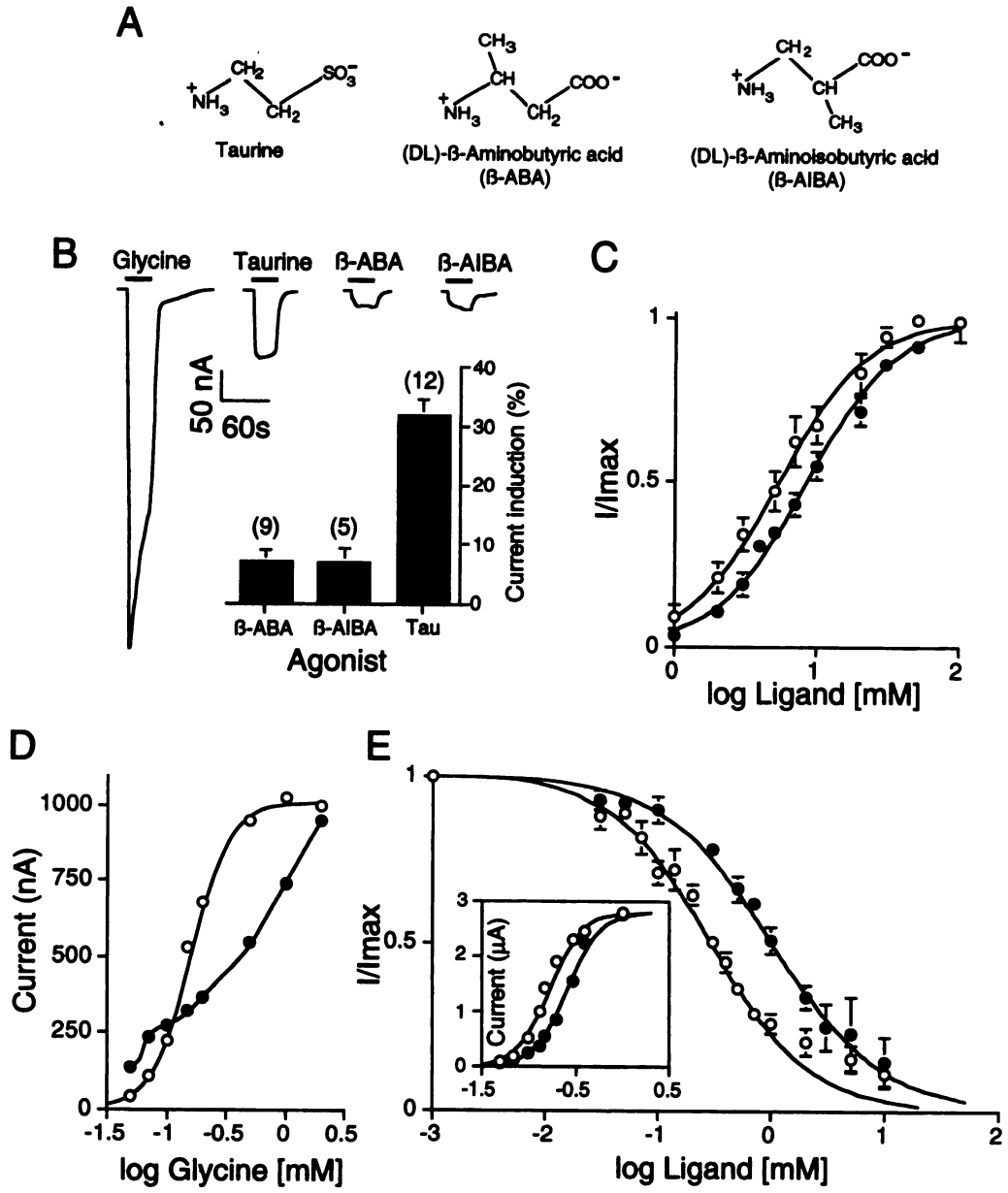

Fig. 3. Agonist and antagonist dose-response relations of β-amino acids. A, Molecular structures of taurine, DL-β-ABA, and DL-β-AIBA. B, Membrane currents elicited on superfusion of 1 mM glycine, 10 mM taurine, and 100 mM β-ABA or β-AIBA, respectively. The histogram represents the mean ± standard error of the respective maximal currents normalized to the response seen with 1 mM glycine. The number of experiments are given in parentheses. C, Dose-response curves for β-ABA (○) and β-AIBA (●) obtained from experiments with four to seven oocytes. D, Representative dose-response curves of glycine in the absence (○) and presence (●) of 2 mM taurine from an individual oocyte. Data points were fitted by a sigmoidal curve and by eye, respectively. E, Inhibition by β-ABA (○) and β-AIBA (●) of the current obtained with 0.17 mM glycine; current values are normalized to the response seen with glycine alone. The sigmoidal curves represent fits of the responses obtained at β-ABA or β-AIBA concentrations of <1 mM; note deviation of these curves at higher concentrations, which reflects the agonistic activity of these compounds. *Inset*, glycine dose-response curves of a representative oocyte obtained in the absence (○) and presence (●) of 0.3 mM β-ABA.

from self-inhibition, we performed competition experiments in which nipecotic acid mimicked the antagonistic and glycine mimicked the agonistic activities of β-amino acids. Coapplication of glycine and nipecotic acid at concentration ratios of 1:3.3, 1:6.6, or 1:10 produced sigmoidal dose-response curves that were saturated at amplitudes of 76 ± 2%, 45 ± 5%, and 32 ± 9%, respectively, compared with the response obtained with 2 mM glycine alone (Fig. 6A). Moreover, the corresponding Hill coefficients decreased from 2.5 ± 0.2 for glycine to 2.1 ± 0.14, 1.7 ± 0.16, and 1.5 ± 0.1, respectively, for the different glycine/nipecotic acid mixtures (Fig. 6B), whereas the apparent EC_{50} values increased only slightly (compare Fig. 6B with Fig. 6A). Thus, the reduced maximal currents and Hill coefficients of β-amino acid agonists can be interpreted as a result of simultaneous agonistic and antagonistic receptor occupation.

Strychnine was shown to inhibit the current responses elicited by glycine more efficiently than those produced by β-ABA (Fig. 5D). Because inhibition was determined at the EC_{50} value of β-ABA, i.e., a concentration ~20-fold higher than its IC_{50} value for glycine displacement, strychnine inhibition required competition not only of the agonistically but

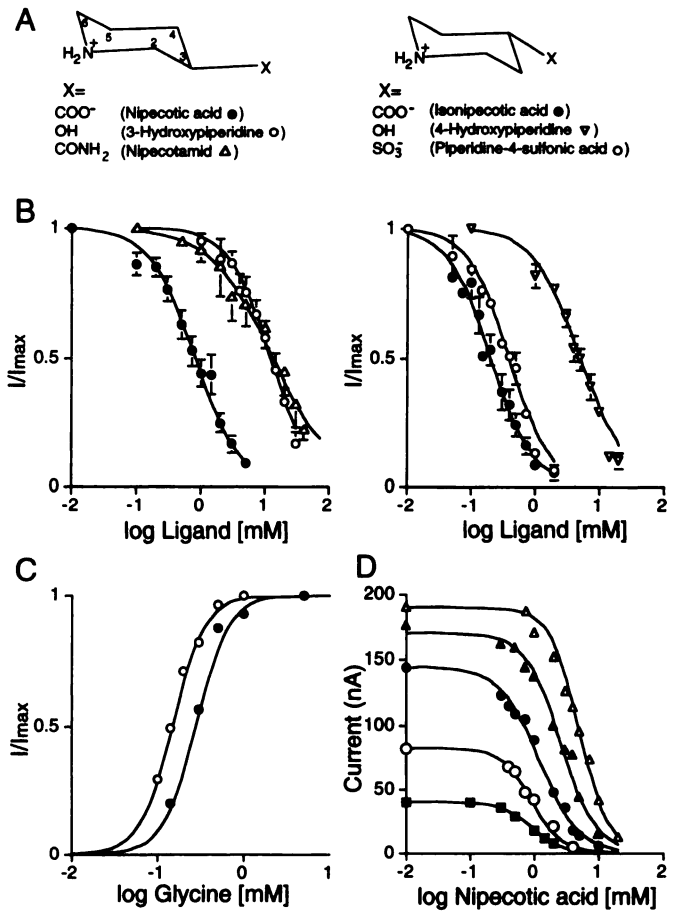

Fig. 4. Pharmacological characteristics of piperidine derivatives. A, Structures of the piperidine compounds used; substitutents at positions 3 and 4 (×) are indicated below the ring structures. B, Inhibition curves of piperidine derivatives with side group substitutions at the C3 (*left*) and C4 (*right*) ring atoms. Glycine was applied at a concentration of 0.2 mM (for symbols, see A). C, Representative dose-response curve of glycine in the absence (○) and presence (●) of 1 mM nipecotic acid derived from experiments with two oocytes (error bars were smaller than the symbols used for the different data points). D, Inhibition of glycine responses obtained at agonist concentrations of 0.1 (■), 0.17 (○), 0.2 (●), 0.3 (▲), and 0.5 (△) mM by increasing concentrations of nipecotic acid; data are from one representative oocyte.

TABLE 2

Pharmacology of piperidine derivatives

Inhibition of glycine currents by piperidine derivatives was analyzed in oocytes injected with α_1 cRNA. IC_{50} values and Hill coefficients (*h*) were determined using glycine at a concentration of about 0.2 mM.

Ligand	IC_{50}	*h*	*n*
	mM		
Isonipecotic acid	0.23 ± 0.03	1.53 ± 0.08	5
Piperidine-4-sulfonic acid	0.40 ± 0.05	1.54 ± 0.05	3
Nipecotic acid	0.82 ± 0.09	1.56 ± 0.10	13
Isoguavacine	0.46 ± 0.05	1.16 ± 0.17	4
Guavacine	2.20 ± 0.42	1.13 ± 0.10	3
4-Hydroxypiperidine	4.66 ± 0.38	1.30 ± 0.05	3
3-Hydroxypiperidine	11.82 ± 1.70	1.30 ± 0.30	4
Nipecotamid	13.20 ± 2.44	1.32 ± 0.03	6

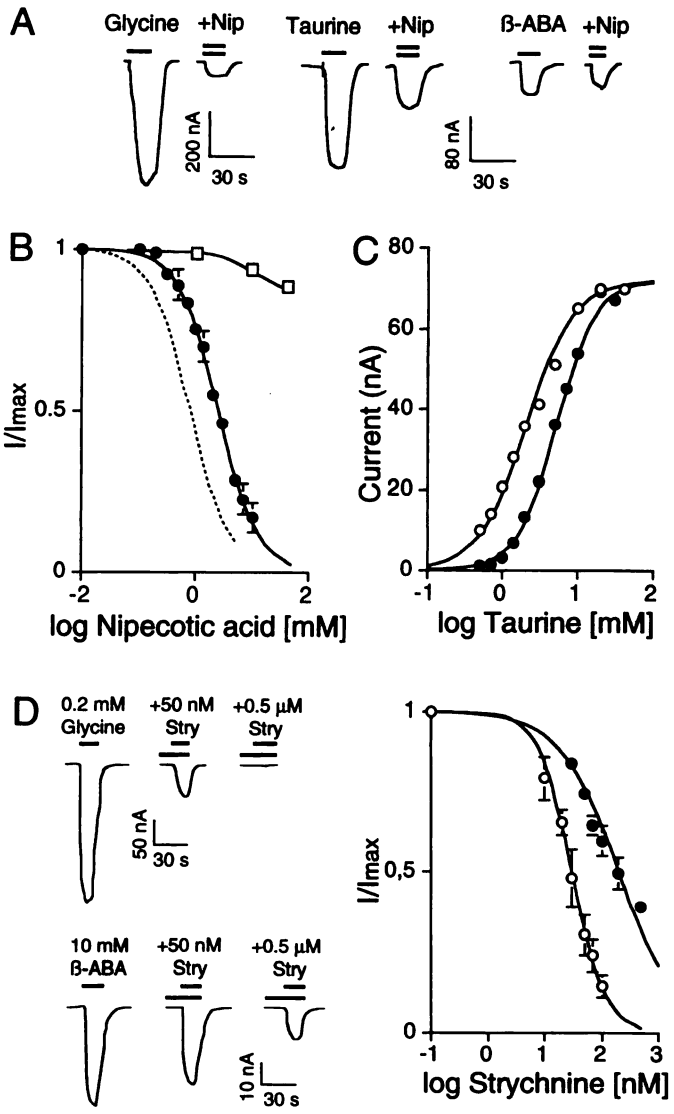

Fig. 5. Inhibition of agonist-induced currents by nipecotic acid and strychnine. A, Current responses obtained with 200 µM glycine, 2 mM taurine, and 10 mM β-ABA in the absence and presence of 2 mM nipecotic acid (*+Nip*). Bars above the recordings indicate durations of drug application. Note different current scale for the glycine response. B, Inhibition of taurine- (●) and β-ABA- (□) elicited membrane currents by nipecotic acid. *Dotted line*, for comparison, glycine inhibition by nipecotic acid (see Fig. 4) is indicated. C, Dose-response relations of taurine in the absence (○) and presence (●) of 2 mM nipecotic acid obtained from an individual oocyte. D, *Left*, inhibition of glycine (*top traces*) and β-ABA (*bottom traces*) elicited current responses by 50 and 500 nM strychnine. Both agonists were applied at concentrations eliciting a half-maximal response. *Right*, strychnine inhibition curves for glycine (○) and β-ABA (●) evoked currents (four experiments).

also of the antagonistically occupied β-ABA sites, a situation that could explain the apparently lower efficacy of the alkaloid in blocking the β-amino acid current. To examine this hypothesis, we mimicked strychnine inhibition of β-amino acid-induced currents by coapplying the alkaloid with a 1:10 glycine/nipecotic acid mixture. When 0.7 mM glycine and 7 mM nipecotic acid, which together elicited a half-maximal response (see Fig. 6A), were coapplied with 50 or 100 nM strychnine, only a weak inhibition (25% and 58%, respectively) of the glycine/nipecotic acid current was seen. In contrast, the same strychnine concentration produced 68% and 90% inhibition of the glycine control response (Fig. 6C). The corresponding strychnine inhibition curve of glycine in the presence of nipecotic acid revealed an IC_{50} value of 108 ± 10 nM (Fig. 6C) compared with 30 ± 0.5 nM for glycine alone (Fig. 5D). The low affinity of nipecotic acid did not allow a full

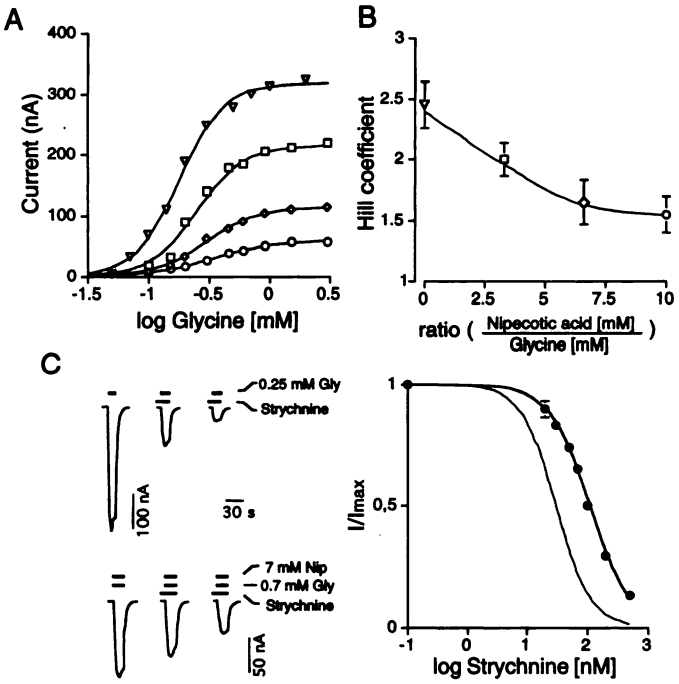

Fig. 6. Simulation of β-amino acid function by coapplication of glycine and nipecotic acid. A, Glycine dose-response curves in the absence (▽) and presence (□, ◇, ○) of nipecotic acid. Data points represent current responses elicited by coapplication of increasing glycine and increasing nipecotic acid concentrations used at ratios of 1:3.3 (□), 1:6.6 (◇), and 1:10 (○), respectively. The corresponding EC_{50} values and Hill coefficients were 0.18 mM and 2.3, 0.25 mM and 2.2, 0.3 mM and 2, and 0.33 mM and 1.7. B, Plot of the Hill coefficient determined from three or four experiments as shown in A versus the concentration ratios of nipecotic/glycine acid. Symbols correspond to the ratios used in A. C, *Top traces*, inhibition of glycine-induced currents by 50 nM (*middle trace*) and 100 nM (*right trace*) strychnine. *Bottom traces*, inhibition by strychnine (concentrations as above) of currents generated on coapplication of 0.7 mM glycine and 7 mM nipecotic acid. *Right*, corresponding strychnine inhibition curve for the nipecotic/glycine acid current derived from experiments with two oocytes. *Dotted line*, for comparison, strychnine inhibition of glycine currents (see Fig. 5D) is indicated.

simulation of current induction by β-ABA; however, the data obtained are consistent with partial agonist function being a consequence of self-inhibition of β-amino acids.

Discussion

In the present study, we analyzed the pharmacological properties of several amino acids and piperidine derivatives by voltage-clamp recording from α_1 GlyR-expressing oocytes. Our results show that α- and β-amino acids can display agonist and/or antagonist activity. From comparison of their molecular structures, we deduce a model of partial agonist activity for compounds active at the GlyR.

Structural determinants of amino acid agonism and antagonism. Our recordings show that the pharmacological properties of α-amino acids at the GlyR are critically determined by the size and polarity of C_α-atom substitutions. Accordingly, the agonistic efficacy of α-amino acids was strongly reduced on introduction of a single methyl (alanine) or hydroxymethyl (serine) group at the C_α-atom. Furthermore, the L enantiomers of alanine and serine were better agonists than the D compounds (8). Extended substitutions at the C_α-atom as found in isobutyric acid, ACHC, or ACPC caused a complete loss of agonist activity; however, all of these compounds behaved as competitive antagonists, whose efficacy appeared largely independent of chirality and C_α-atom side-chain extension. The reason for the lack of agonist activity of the extended α-amino acids is presently unclear and may include reduced efficacies in channel gating. The findings discussed, however, strengthen the view that all α-amino acids interact with a common binding site within the ligand pocket of the GlyR.

In contrast to α-amino acids, β-amino acids usually displayed both agonist and antagonist activity. Even large molecules like β-ABA and β-AIBA, which contain methyl groups at their C_α or C_β atoms, respectively, still were able to elicit significant current responses. However, there was an inverse correlation between the agonistic and antagonistic potencies of β-amino acids. For the most potent agonist β-alanine, antagonism was barely detectable (7), whereas taurine, β-ABA, and β-AIBA exhibited IC_{50} values that were 2–20-fold lower than their respective EC_{50} values. This classifies the latter β-amino acids as partial agonists.

Antagonistic α- and β-amino acids inhibited membrane currents evoked by glycine in a competitive fashion, as did the classic GlyR antagonist strychnine (28) and nipecotic acid (present study). Strychnine and nipecotic acid were also shown to act as competitive blockers of α- and β-amino acid-induced currents. Together, these data indicate the existence of a common binding site for all GlyR agonists and antagonists tested and suggest that the functional consequences of binding may be determined by the particular conformation a given ligand adopts within the receptor's ligand binding pocket.

To reasonably explain the dual agonistic and antagonistic properties of β-amino acids, their molecular structures must be considered. Because saturated carbon atoms allow free rotation around C—C atom bonds, several molecular conformations of β-amino acids are possible. In solution, 33% of the β-alanine and 25% of the taurine molecules have been shown to be in a *trans* conformation (29). A similar *trans* conformation is found in nipecotic acid, where it is restricted by heterocyclic ring formation (Fig. 7). In the present study, nipecotic acid behaved as a pure antagonist whose IC_{50} value was similar to that of β-amino acids. This supports the view that the *trans* orientation of β-amino acids mediates antagonistic binding to the GlyR. The *cis* conformation of β-amino acids, in which the distance between the amino and carboxylic groups is comparable to that in glycine (Fig. 7), consequently should have agonistic activity. Conformation-dependent drug activities have also been documented for the $GABA_A$ receptor (30) and the GABA transporter (31) proteins.

The potency of piperidine compounds at the GlyR was strongly dependent on the presence of an acidic substitution at the third or fourth carbon atom. Correspondingly, nipecotic acid, isonipecotic acid, guavacine, isoguavacine, and piperidine-4-sulfonic acid all behaved as GlyR antagonists. Polar substitutions as found in nipecotamid and hydroxypiperidines enhanced the potency of these compounds, a finding that excludes hydrophobic ligand/protein interaction as a major determinant of piperidine carboxylic acid binding. Interestingly, piperidine-2,3- and piperidine-2,4-dicarboxylic acids were inactive despite the presence of carboxylic groups at the C3 and C4 atoms, respectively. These data suggest that small uncharged groups near the nitrogen atom are

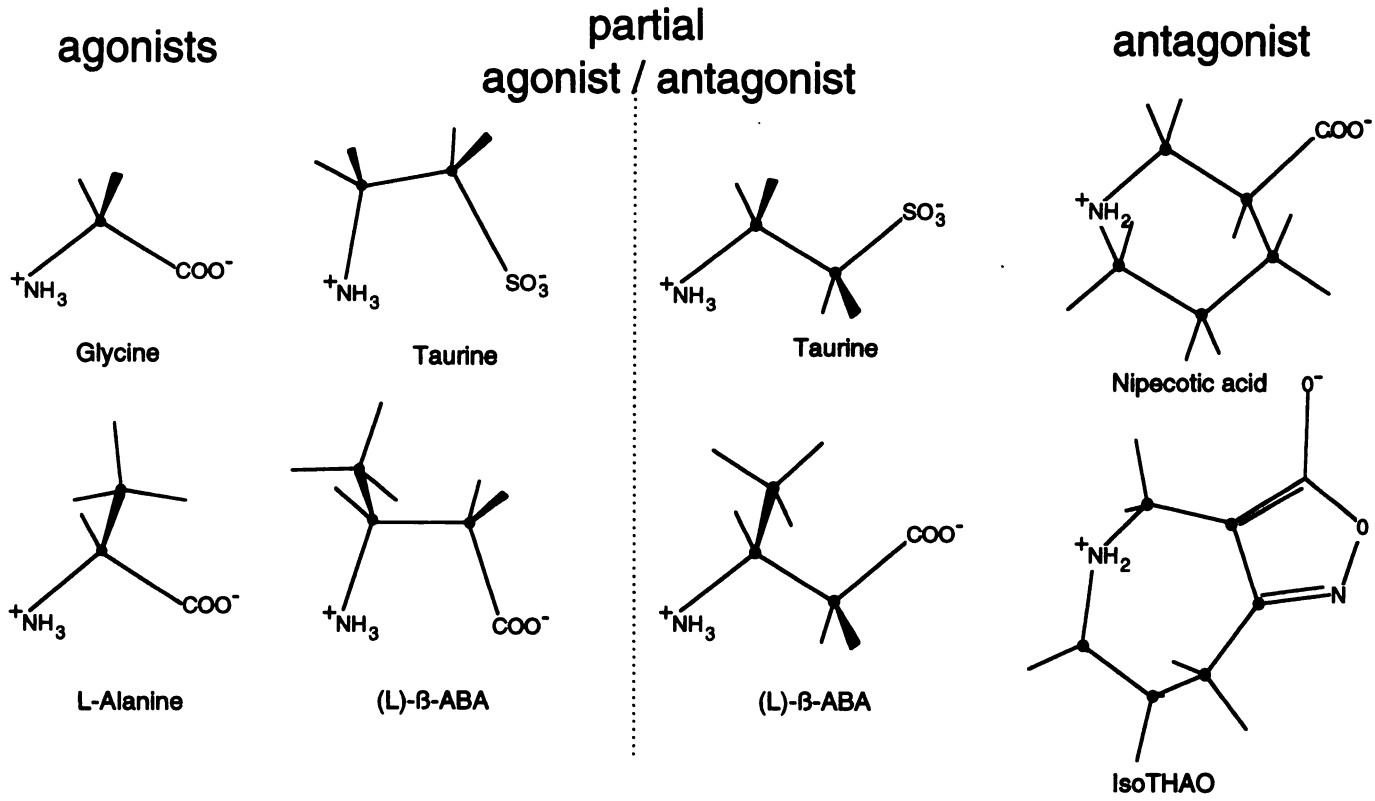

Fig. 7. Structural comparison of GlyR agonists and antagonists. *Left*, glycine and L-alanine are shown as examples of GlyR agonists. The distance of the amino and the acidic groups in these amino acids is similar to that found for taurine and (L)-β-ABA in their respective *cis* conformations; both ligands display partial agonist activity. *Right*, in their *trans* conformation, taurine and β-ABA are believed to act as antagonists because nipecotic acid, which exists only in a ring-immobilized quasi-*trans* form, is an exclusive antagonist of the GlyR. Note that the structure of nipecotic acid resembles that of isoTHAO, another established GlyR antagonist.

important for efficient receptor binding, a conclusion that extends our results obtained with different α-amino acids.

In the structures of different GlyR active compounds, including isoTHAO (32) and strychnine (33), a glycine-related motif has been found (34). However, none of these molecules acts as an agonist. Comparison to the three-dimensional structure of nipecotic acid reveals similarities of isoTHAO with respect to both the orientation and distance of amino and acidic groups (Fig. 7). Thus, the aforementioned antagonists of the GlyR appear to be structurally related to piperidine-3-carboxylic acids and β-amino acid compounds. In contrast, the antagonists isonipecotic acid, isoguavacine, and 5,6,7,8-tetrahydro-4*H*-isoxazole[4,5-d]azepin-3-ol (35) are structurally related to the inactive GABA molecule. Furthermore, TACA and CACA, GABA analogues with a C—C atom double bond, are restricted in stretched and folded conformations, respectively (36). CACA is structurally related to isonipecotic acid and also behaves as a GlyR antagonist, whereas TACA is inactive.[1] These findings strengthen the view that with increasing distance between the nitrogen and the acidic group, GlyR agonists are transformed into antagonists.

Partial agonist function of β-amino acids. The maximal currents induced by β-amino acids are significantly lower than the maximal glycine response. Our data suggest that the I_{max} value of these compounds depends on their relative agonistic and antagonistic properties, i.e., the respective EC_{50} and IC_{50} values. Following the argument given above, we interpret the partial agonist function of β-amino acids as a reflection of the ratio of agonistic to antagonistic conformers. This is consistent with EC_{50} and IC_{50} values being similar for β-alanine (7) but differing ~2-fold for taurine and 10–20-fold for β-ABA and β-AIBA, respectively.

The reduced maximal current response of β-amino acids at the α_1 GlyR expressed in oocytes was mimicked in our simulation experiments, in which nipecotic acid was used to generate the antagonistic and glycine was used to generate the agonistic components of β-amino acid binding. Increases in relative nipecotic acid content resulted in sigmoidal dose-response relations that showed strongly reduced I_{max} values. At a concentration ratio of glycine and nipecotic acid of 1:3.3 (this concentration ratio corresponds to an EC_{50}/IC_{50} ratio of 1:1), maximal currents similar to those elicited by β-alanine were obtained. Increasing relative nipecotic acid concentrations generated I_{max} values comparable to those obtained for taurine and β-ABA, respectively. Furthermore, Hill coefficients decreased with increasing agonist/antagonist ratios, a result that resembles the different Hill coefficients seen with glycine and taurine. These differences in apparent agonist cooperativity were previously interpreted as distinct mechanisms of GlyR channel gating but now can be concluded to simply reflect the partial agonist function of β-amino acids.

As a consequence of self-inhibition, a selective increase in the agonist, but not antagonist, affinity of the GlyR should result in higher I_{max} values for β-amino acids. This has been seen with different GlyR α_1 subunit mutants (8, 26) and

[1] V. Schmieden, unpublished observations.

GlyRs expressed in various cell lines (24). There, taurine and β-alanine showed lower EC_{50} values and thus behaved as full agonists. In contrast, hyperekplexia α_1 mutants display rather low glycine affinities and no detectable β-amino acid response, whereas the antagonistic potency of these ligands was unchanged (7, 24). This demonstrates that distinct subdomains of the GlyR's ligand pocket are involved in the binding of agonists and antagonists. Their molecular identification in conjunction with the results described for the present study may help to design potent GlyR agonists that could provide a lead for the development of novel muscle-relaxant and anticonvulsive compounds.

Acknowledgments

We thank J. Kuhse for helpful discussions, P. Schloβ for critical reading of the manuscript, and M. Baier and H. Reitz for secretarial assistance.

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