ogenate (0.5 mg of protein), 0.1 mL of ^{3}H-labeled ligand (1.0 nM DTLET or 1.0 nM DSLET for δ, 2.0 nM DAMGO for μ), and 0.1 mL of the test compound in replicates of three. After incubation for 60 min at 37 °C, the reactions were terminated by rapid filtration on Whatman GF/B glass-fiber filters and subsequent 10 mL wash of ice-cold buffer. Filters were prepared for liquid scintillation counting. Specific binding was calculated as the difference in radioactivity bound in the absence and presence of 10 μL of levorphanol. IC_{50} values, the concentration of test compound that inhibited ^{3}H-labeled ligand binding by 50%, were obtained by regression analysis of a log-logit transformation of binding data as described by Limbird.[30]

Affinity for the κ opioid receptor was assessed using a crude membrane homogenate prepared from guinea pig brain in KRH buffer (millimolar: HEPES, 25; NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; and $MgCl_2$, 1.2; pH adjusted to 7.4) according to the method described by Takemori et al.[33] Test tubes containing approximately 1 mg of protein, 4 nM of [^{3}H]-U-69,593 (New England Nuclear), and enough KRH buffer to bring the final volume to 0.5 mL were incubated at 37 °C for 60 min before the contents of the tubes were filtered through Whatman GF/C filters which were presoaked in 0.3% polyethylenimine for 1 h. The filters were washed three times with 4 mL of 5 mM Tris-HCl, pH 7.4. Radioactivity on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was defined as the amount of binding that was not inhibited by 1 μM of (-)ethylketocyclazocine.

Acknowledgment. We thank Dr. B. Pitzele and Dr. D. W. Hansen, Jr., CNS Chemistry, for their encouragement, and Mrs. P. Finnegan for analysis of NMR spectra. The help of Dr. R. H. Mazur in the preparation of this manuscript is gratefully acknowledged.

Registry No. **3**, 123715-02-6; **3** (R = Cbz), 137764-59-1; **4**, 99953-00-1; **5**, 92137-66-1; **6**, 137649-87-7; **7**, 137649-88-8; **7A**·2HCl, 137650-32-9; **7A** (free base), 137650-54-5; **8**, 99952-51-9; **9**, 137649-89-9; **9A**·2HCl, 137650-33-0; **9A** (free base), 137650-55-6; **10**, 137649-90-2; **11**, 137649-91-3; **12**·HCl, 137649-92-4; (*R*)-**12A**·2HCl, 137650-34-1; (*S*)-**12A**·2HCl, 137650-35-2; (*R*)-**12A** (free base), 137650-56-7; (*S*)-**12A** (free base), 137650-57-8; **13**, 109608-86-8; **14**, 137649-93-5; **15**·oxalate, 137649-95-7; **15A**·HCl, 137650-36-3; **15A** (free base), 137650-58-9; **16**, 137649-96-8; **17**, 137649-97-9; **18**, 137649-98-0; **18A**·HCl, 137650-37-4; **18A** (free base), 137650-59-0; **19**, 137649-99-1; **19A**·HCl, 137650-38-5; **19A** (free base), 137650-60-3; **20**, 60479-64-3; **21**, 137650-00-1; **22**, 137650-01-2; **22A**·HCl, 137650-39-6; **22A** (free base), 137650-61-4; **23**, 137650-02-3; **24**, 137650-03-4; **25**, 137650-04-5; (*R*)-**25A**·HCl, 137650-40-9; (*S*)-**25A**·HCl, 137650-41-0; (*R*)-**25A** (free base), 137650-62-5; (*S*)-**25A** (free base), 137650-63-6; **26**, 82770-45-4; **27**, 137650-05-6; **28A**·HCl, 137650-42-1; **28A** (free base), 137650-64-7; **29A**·HCl, 137650-43-2; **29A** (free base), 137650-65-8; **30**, 16387-61-4; **31**, 137650-06-7; **32**, 137650-07-8; **33**, 137650-08-9; **33A**, 137650-44-3; **34**, 18328-11-5; (*E*)-**35**, 137650-09-0; (*Z*)-**35**, 137650-31-8; **36**, 137650-10-3; **37** (R = H); (*R*)-**37**·HCl (R = DMT), 137650-45-4; (*S*)-**37**·HCl (R = DMT), 137650-46-5; (*R*)-**37** (R = DMT, free base), 137650-66-9; (*S*)-**37** (R = DMT, free base), 137650-67-0; **38**, 104-53-0; (*R*)-**39**·HCl (R = DMT), 137650-47-6; (*S*)-**39**·HCl (R = DMT), 137650-48-7; (*R*)-**39** (R = DMT, free base), 137650-68-1; (*S*)-**39** (R = DMT, free base), 137650-69-2; **40**, 58089-70-6; **41**, 137650-13-6; **42** (R = H), 137650-14-7; **42** (R = Cl_3CCO), 137650-12-5; (*R*)-**42**·HCl (R = DMT), 137650-49-8; (*S*)-**42**·HCl (R = DMT), 137650-50-1; (*R*)-**42** (R = DMT, free base), 137650-70-5; (*S*)-**42** (R = DMT, free base), 137650-71-6; **43**, 137650-15-8; **44**, 137650-16-9; **45**, 137650-17-0; **46**·2HCl, 137650-18-1; **46** (free base), 137650-51-2; **47**, 137650-19-2; **48**, 137650-20-5; **49**, 137650-21-6; **50**, 137650-22-7; **51**, 137650-23-8; **52**·HCl, 137650-24-9; **52** (free base), 137650-52-3; **53**, 137650-25-0; **54**, 137650-26-1; **55**, 137650-27-2; **45**·HCl, 137650-28-3; **56** (free base), 137650-53-4; **57**, 137650-29-4; **58**·HCl, 137650-30-7; **58** (free base), 137668-03-2; (*E*)-$Ph(CH_2)_3CH{=}CHCOOMe$, 55283-02-8; $Ph(CH_2)_5COOMe$, 5581-76-0; $Ph(CH_2)_3NH_2$, 2038-57-5; Boc-D-Ser, 6368-20-3; $Ph(CH_2)_4Br$, 13633-25-5; $Ph(CH_2)_3OMs$, 69804-99-5; $PhCH_2CH_2NH_2$, 64-04-0; $(EtO)_2P(O)Ch_2COOMe$, 1067-74-9; $CH_3CH{=}CHBr$, 590-14-7; $Br(CH_2)_3Ph$, 637-59-2; D-Ala-$NH(CH_2)_3Ph$, 99952-51-9; $H_2C{=}C(CH_3)COCl$, 920-46-7; $Ph_3P^+CH_2C{\equiv}CSiMe_3{\cdot}Br^-$, 42134-49-6; 2-pyridinecarboxaldehyde, 1121-60-4; (*S*)-(-)-1-amino-2-(methoxymethyl)pyrrolidine, 59983-39-0; isoxazolidine hydrochloride, 39657-45-9.

Supplementary Material Available: Observed opioid activities of all target compounds in the binding and mouse writhing tests (3 pages). Ordering information is given on any current masthead page.

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β-Proline Analogues as Agonists at the Strychnine-Sensitive Glycine Receptor

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3-Carboxy-3,4-dehydropyrrolidine was found to bind with affinity equal to that of glycine in a [^{3}H]strychnine binding assay. Simple substitution of the 1-, 2-, 4-, or 5-position resulted in marked loss of affinity. A decline in affinity was also found upon enlargement, contraction, or saturation of the 5-membered ring. However, β-proline and azetidine-3-carboxylic acid retained significant binding affinity. Despite its good affinity in [^{3}H]strychnine binding, 3-carboxy-3,4-dehydropyrrolidine showed only weak agonist activity in intracellular recordings of cultured murine spinal cord neurons. This apparent lack of correlation between binding and functional results is discussed in light of the current models of the strychnine-sensitive glycine receptor.

Introduction

The strychnine-sensitive glycine receptor is a member of the ligand-gated ion channel family of receptors.[1] Within this family, it is most closely related to the GABA, receptor.[2] Like the $GABA_A$ receptor, the glycine receptor

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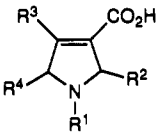
0022-2623/92/1835-0233$03.00/0

Table I. Inhibition of [^{3}H]Strychnine Binding



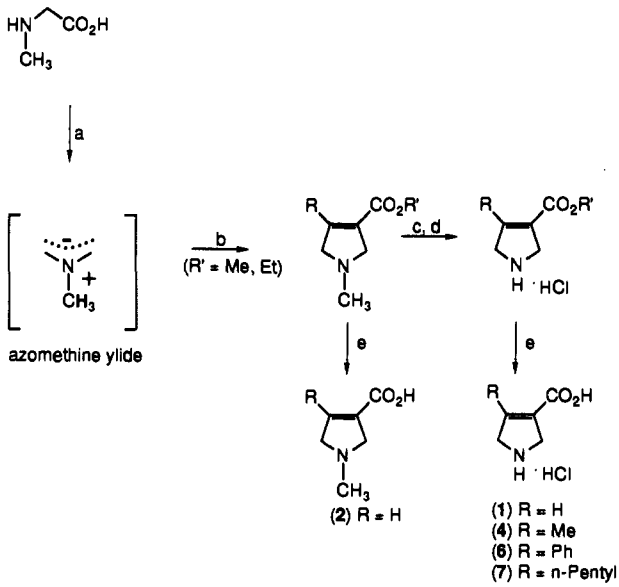
example	R^1	R^2	R^3	R^4	IC_{50} (μM)	% inhib at 100 μM	NaCl shift
		glycine			6.2 ± 0.3		10.5
		β-alanine			11.6 ± 2.1		5.8
		D-alanine			90.9 ± 6.4		
		L-alanine			198.0 ± 36		
		L-proline			414.0 ± 63		
		D-proline				4	
1	H	H	H	H	7.9 ± 0.7		2.8
2	CH_3	H	H	H		31	
3	H	CH_3	H	H		0	
4	H	H	CH_3	H		0	
5	H	H	H	CH_3		40	
6	H	H	Ph	H		0	
7	H	H	$(CH_2)_5CH_3$	H		10	
8	β-proline				17.2 ± 1.1		
9	pyrrole-3-carboxylic acid					46	
10	azetidine-3-carboxylic acid				23.7 ± 1.5		
11	isonipecotic acid				94.1 ± 3.2		3.3
12	D,L-3,4-dehydroproline				242.0 ± 4		
13	L-3,4-dehydroproline					34 (1 mM)	
14	L-azetidine-2-carboxylic acid				399.0 ± 41		
15	isoguvacine				694.0 ± 89		

has an inhibitory role, mediating an increase in chloride conductance. However, in contrast to the $GABA_A$ receptor, the glycine receptor is located mainly in the spinal cord and lower brainstem, where glycine appears to be the major inhibitory neurotransmitter.[3]

Drugs that modulate $GABA_A$ and $GABA_B$ receptors (for instance, diazepam and baclofen, respectively) are used for treatment of spasticity.[4] However, because of the extensive involvement of GABA receptors in higher brain centers, these agents cause sedation, muscle weakness, and other undesired effects. As part of a program to discover novel antispastic agents that would be free of these side effects, we elected to search for novel glycine receptor agonists.

Initial results involving analogues of glycine[5,6] and heterocyclic glycine isosteres[7] were disappointing due to severe restrictions in allowed structural variations of this very simple molecule. Since the higher homologue β-alanine is known to possess good affinity for the glycine receptor,[8] and since this lead appeared to afford greater latitude for structural modification, we turned our attention to β- and γ-amino acids. The present study describes the glycine receptor activity of various β-proline analogues in binding and electrophysiological assays.

Scheme I[a]



[a] (a) $(CH_2O)_x$, $MgSO_4$ in toluene, 90 °C; (b) $RC{\equiv}CCO_2R'$; (c) $ClCO_2CHClCH_3$, CH_2Cl_2, 0 °C; (d) CH_3OH, reflux; (e) 4 N HCl, reflux.

Chemistry

Although the synthesis of 1 has been previously reported,[9-13] known synthetic routes are multistep in nature

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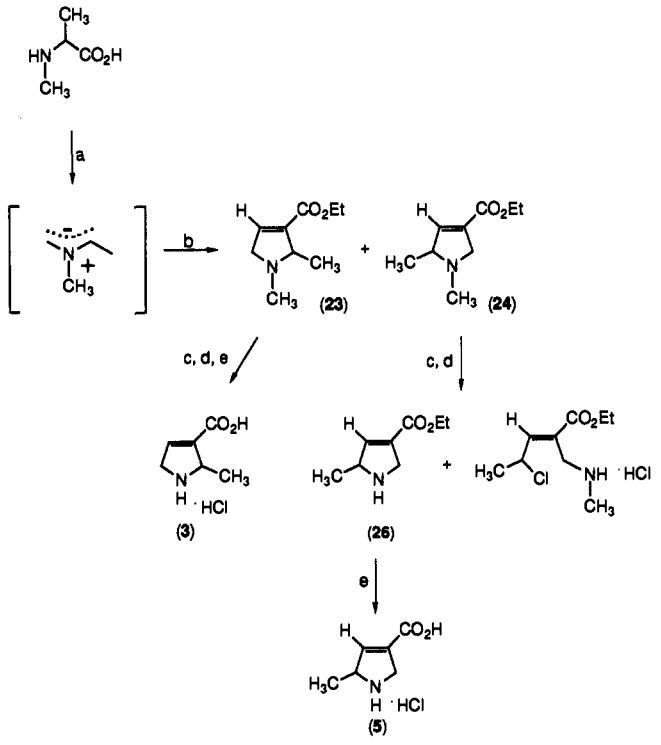
Scheme II[a]



[a] (a) $(CH_2O)_x$, $MgSO_4$ in toluene, 90 °C; (b) $HC{\equiv}CCO_2Et$; (c) $ClCO_2CHClCH_3$, CH_2Cl_2, 0 °C; (d) CH_3OH, reflux; (e) 4 N HCl, reflux.

and are not amenable to systematic ring modification. Synthesis of **1** was initially carried out via the published route, but the analogues reported in this paper were made by a more direct, albeit low-yielding,[14] three-step sequence which allowed access to a variety of substitution patterns on either the dihydro- or tetrahydropyrrole-3-carboxylic acid ring system. This ring-forming procedure was based on a [3 + 2] cycloaddition reaction between a transient azomethine ylide and an unsaturated ester.[15,16] Heating *N*-alkylglycine or alanine with formaldehyde under dehydrating conditions afforded the desired transient azomethine ylides (Schemes I–III). These ylides were trapped with alkynoate esters in low to moderate yields to give the desired dihydropyrrole-3-carboxylate ring systems, or with acrylates to give the corresponding tetrahydropyrrole-3-carboxylates in moderate yields. The cycloaddition products were subsequently dealkylated and hydrolyzed

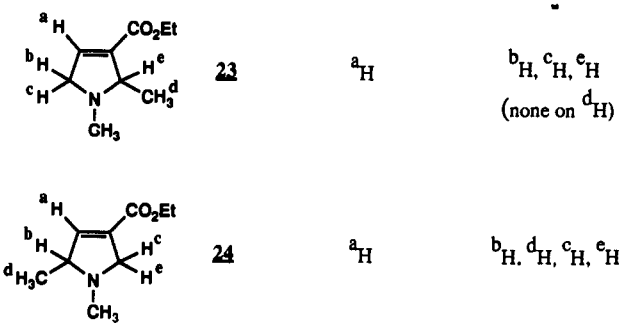


Figure 1. Since NOE experiments were carried out in nondegassed $CDCl_3$, the NOE enhancements reported are of a qualitative nature only.

to give many of the cyclic amino acids shown in Table I.

The route to 1- and 4-substituted dihydropyrrole-3-carboxylates is outlined in Scheme I. Sarcosine (*N*-methylglycine) and paraformaldehyde were heated together in toluene at 90–100 °C. The choice of reaction temperature was found to be critical in that the reactions did not proceed at less than ca. 75 °C but decomposed when heated to reflux. This sensitivity to reaction temperature was particularly pronounced when the 4-position of the cyclic product was unsubstituted. In this case, the generation of higher boiling side products was observed. The *N*-methyl group was cleanly removed by treatment with 1-chloroethyl chloroformate in methylene chloride,[17] followed by treatment with methanol at reflux to decompose the intermediate 1-chloroethyl carbamate. Purification of the amino esters before the final hydrolysis simplified the isolation of the amino acids to that of trituration with an alcohol or ether.

The 2- and 5-methylated dihydropyrrolecarboxylates (**3** and **5**, respectively) were synthesized by trapping the corresponding azomethine ylides, derived from *N*-methyl-DL-alanine, with ethyl propiolate (Scheme II). This reaction yielded a 4:1 mixture of the isomeric 3,4-dehydropyrrolidine products which was separated by column chromatography. The major, 2-methyl-substituted product was readily elaborated to the desired 2-methyl-3,4-dehydropyrrolidine-3-carboxylic acid. However, the 5-methylated analogue underwent ring fragmentation as a competing reaction along with the desired demethylation. The relative isomeric distribution of these products may be rationalized on the basis of electronic effects. That is, the methyl group derived from alanine is electron releasing by hyperconjugation, and hence would destabilize the transient carbanion generated by decarboxylation. The major 2-methyl isomer is derived from the more stable tautomeric form of the azomethine ylide where electron density is localized on the unsubstituted methylene unit. Assignment of the isomeric location of the methyl group was made on the basis of difference NOE experiments; the vinylic proton resonances at the 4-position in both the 2- and 5-methyl isomers were irradiated and the signals saturated. All of the dihydropyrroline ring protons showed a positive NOE enhancement in both compounds (Figure 1), but a positive NOE enhancement was seen only for the ring-substituted methyl of compound **24** (the 5-methyl isomer). Because the NOE relaxation is predominantly a through-space effect, we have correlated the strong NOE

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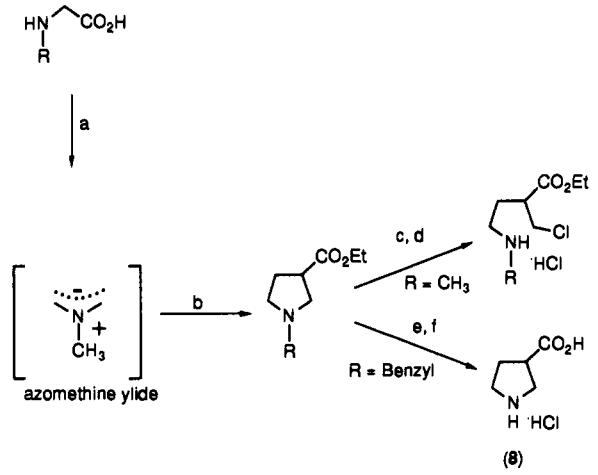
Scheme III[a]



(8)

[a] (a) $(CH_2O)_x$, $MgSO_4$ in toluene, 90 °C; (b) $H_2C{=}CCO_2Et$; (c) $ClCO_2CHClCH_3$, CH_2Cl_2, 0 °C; (d) CH_3OH, reflux; (e) H_2, Pd/C; (f) 4 N HCl, reflux.

with a methyl group proximal to the irradiated 4-position and, therefore, assigned the minor isomer (**24**) to be 5-methyl. In addition to the NOE, the structural assignment of compounds **23** and **24** was also supported by proton–proton coupling in the NMR. Here, the vinyl proton (^{a}H) (Figure 1) appeared as a doublet of doublets in compound **23** and as a broad doublet in compound **24**.

Tetrahydropyrrole-3-carboxylic acid (β-proline, **8**)[9] was also prepared using a [3 + 2] cycloaddition reaction to generate the desired ring system (Scheme III). However, in this case the chloroformate-mediated demethylation was unsuccessful because the 1-chloroethyl carbamate intermediate underwent ring fragmentation in preference to N-demethylation. This problem was overcome by using *N*-benzylglycine in place of sarcosine. The benzylic group could be removed by catalytic hydrogenation, and this procedure provided a successful alternative to the chloroformate deprotection technique.

Pyrrole-3-carboxylic acid[18] and 3-carboxyazetidine[19] were prepared as previously reported. L-Azetidine-2-carboxylic acid, isonipecotic acid, isoguvacine, and D,L- and L-3,4-dehydroproline were obtained from commercial sources.

Results

[^{3}H]Strychnine binding to rat spinal cord membranes was used to assess affinities of compounds at the strychnine-sensitive glycine receptor. Glycine inhibited [^{3}H]-strychnine with an IC_{50} of 6.2 μM (Table I), while the higher homologue β-alanine had only slightly less affinity (IC_{50} = 11.6 μM). The good affinity of β-alanine prompted us to examine other higher homologues of glycine, including various cyclic derivatives. Among the commercially available derivatives, the cyclic GABA analogue isonipecotic acid (piperidine-4-carboxylic acid, **11**) was found to have measurable affinity for the glycine receptor.

Based on the results with **11**, the cyclic β-alanine analogue **1** was evaluated in [^{3}H]strychnine binding. In this assay **1** had an affinity equal to glycine (IC_{50} = 7.9 μM, Table I) and possessed agonist activity (see below). Unfortunately, except for compounds **2** and **5**, simple substitution of **1** led to total loss of receptor binding affinity (compounds **2–7**, Table I). Although **5** displayed significant binding affinity (40% inhibition at 100 μM) as its racemate, this level of activity was deemed insufficient to undertake the separation of its component enantiomers. Clearly, compound **1** achieves a close fit to a very restricted receptor site. Despite the lack of activity of the 4-methyl derivative **4**, we elected to place pentyl (**7**) and phenyl (**6**) substituents at this position on the chance that a more distant auxiliary binding site might be present at this position. No improvement in affinity was detected (Table I).

The fully saturated β-proline **8** showed activity almost equal to **1**. However, pyrrole-3-carboxylic acid (**9**), the aromatic derivative of **1**, was inactive, presumably because the amine was no longer sufficiently basic to possess a positive charge at physiological pH.

Movement of the carboxy residue of **8** to afford proline resulted in more than 20-fold loss of affinity (Table I). The binding of D- and L-proline showed stereoselectivity, with only the L isomer being active. This stereopreference is opposite to that observed for alanine (Table I). D,L-3,4-Dehydroproline (**12**) bound with greater affinity than the pure L isomer (**13**), suggesting a preference for the D isomer. This preference is the same as alanine but opposite to that of proline.

Azetidine-3-carboxylic acid (**10**) bound to the glycine receptor with an affinity comparable to that of β-alanine and β-proline (Table I). Comparing the distance from the ring nitrogen to the carboxylate carbon atom calculated[20] for energy-minimized nonionized forms of β-proline (3.6 Å) and (**10**) (3.2 Å), the latter clearly represents a more compact cyclized analogue of β-alanine and, therefore, presumably a closer mimic of glycine (amino–carboxy distance of 2.5 Å). L-Azetidine-2-carboxylic acid (**14**), which might be considered a structurally closer analogue of alanine, bound with significantly less affinity. Isoguvacine (**15**), 3,4-dehydroisonipecotic acid, was 6-fold less active than isonipecotic acid (**11**).

Sodium chloride is known to have opposite effects on the affinity of glycine receptor agonists and antagonists in [^{3}H]strychnine binding.[21-23] Since antagonists show increased affinity in the presence of 1 M NaCl, while agonists show decreased affinity, the NaCl shift has been used as a predictor of agonist/antagonist activity.[22,23] For instance, glycine shows a NaCl ratio of 10.5 (Table I), while strychnine shows a ratio of 0.4. However, it is not entirely clear whether the ratio differentiates agonists from antagonists or, alternatively, whether it distinguishes compounds that bind to the glycine site from those that bind to the strychnine site. In one case, several amino acid-like glycine antagonists gave agonist-like NaCl shifts.[23] In the present series, compounds **1** and **11** gave NaCl shifts of 2.8 and 3.3, suggesting the possibility of agonist activity.

Another effect of 1 M NaCl is to alter the slope of the glycine inhibition curve. In the absence of NaCl (Figure 1), glycine inhibits [^{3}H]strychnine binding with a Hill coefficient less than one (0.71 ± 0.02, n = 3); however, in the presence of NaCl, glycine shows a steeper curve, with a Hill coefficient of 1.35 ± 0.03 (n = 3). The other com-

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Table II. Change in Input Resistance of Cultured Spinal Cord Neurons Produced by Application of Putative Glycine Agonists

	concentration-dependent decrease in input resistance, %				
example	100 μM	1 mM	100 μM + 100 μM bicuc + 3 μM TTX	1 mM + 100 μM bicuc + 3 μM TTX	1 mM + 100 μM bicuc + 3 μM TTX + 10 μM strych
1	22.7 ± 13.2 (n = 10)	60.4 ± 12 (n = 7)	5.1 ± 6.3 (n = 6)	37.2 ± 13.8 (n = 7)	4.7 (n = 1)
10	9.4 ± 4.7 (n = 5)	83.1 ± 8.4 (n = 6)	10.7 ± 7.2 (n = 5)	71.7 ± 18.6 (n = 9)	37.7 ± 14.3 (n = 6)
11	19.2 ± 13.2 (n = 3)	73.6 ± 16.7 (n = 3)	3.0 ± 8.1 (n = 3)	0.1 ± 4.6 (n = 4)	

[a] Bicuc = bicuculline, TXX = tetrodotoxin, strych = strychnine.

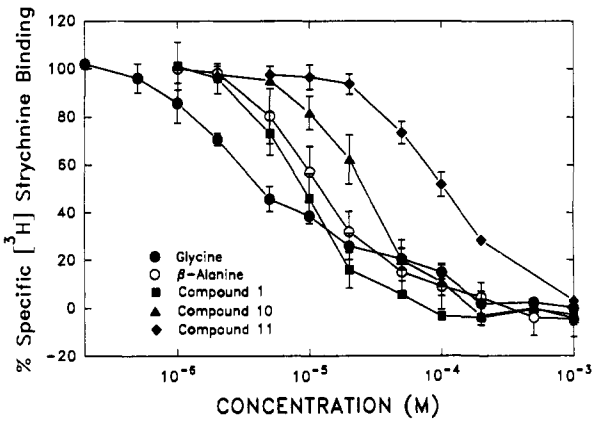

Figure 2. Inhibition of [^{3}H]strychnine binding by glycine, β-alanine, 1, 10, and 11. Data are expressed as the mean ± SEM of three or more independent experiments.

pounds which produced significant inhibition of [^{3}H]-strychnine binding all showed slopes near unity in the absence of NaCl (Figure 2). These results suggest a difference in binding properties between glycine and the other putative agonists.

To resolve the ambiguity of the NaCl shift data, we evaluated 1, 10, and 11 in cultured mouse spinal cord cells and compared their activity to the reference agonists glycine and β-alanine. Pressure ejection of greater than 10 μM glycine produced a depolarization of the membrane potential (recorded with KCl electrodes) and a decrease in the input resistance due to the opening of chloride channels. The concentration–response relationship for glycine was steep, with complete shunting of the input resistance occurring at 100 μM. The Hill coefficient was 4.26 (r = 0.996, Figure 3B). β-Alanine produced a similar decrease in the input resistance. The roughly similar concentration–response curve for β-alanine suggests that channel opening by this close structural analogue also requires binding of more than one molecule. Although the two curves were not strictly parallel, β-alanine was about 2-fold less potent than glycine, in agreement with the relative affinities of the two compounds in displacing [^{3}H]strychnine binding. The above results with glycine and β-alanine are consistent with previous reports.[24-26]

All three of the novel putative glycine agonists produced a concentration–dependent decrease in input resistance

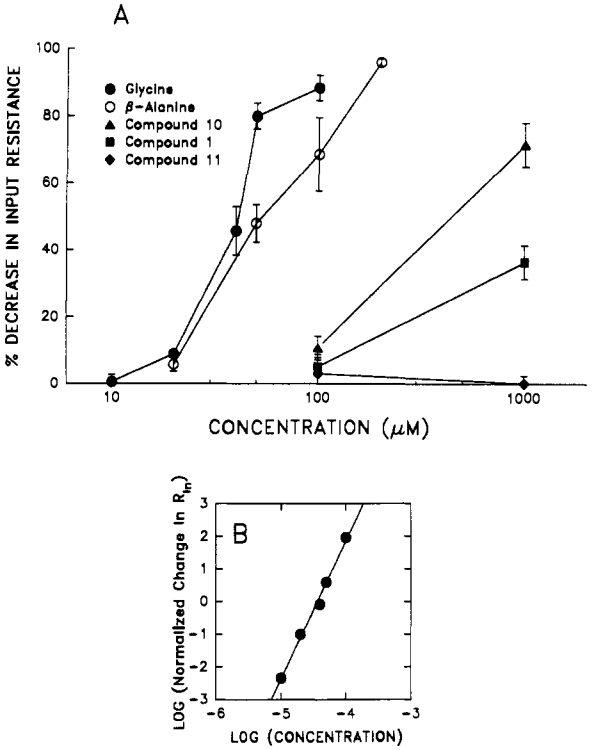

Figure 3. (A) Concentration response curves for the change in input resistance in cultured spinal neurons following pressure ejection of glycine, β-alanine, 1, 10, and 11. The curves for glycine and β-alanine were generated in normal DBPS, while the medium for the other compounds included 100 μM bicuculline and 3 μM TTX to block the contribution of GABA receptor mediated changes in input resistance. Data are expressed as the mean ± SEM change in input resistance of 3–10 cells. (B) Hill plot of glycine data presented in A. The Hill coefficient was 4.26 (r = 0.996).

(Table II). However, both 1 and 11 have been shown previously to exhibit GABA agonist activity.[27] Since a GABA agonist would shunt the membrane in a manner similar to a glycine agonist, it was necessary to distinguish these two agonist effects by measuring the putative glycine agonist effects in the presence of the GABA antagonist bicuculline (100 μM). Tetrodotoxin (3 μM) was also added to the medium to prevent the spontaneous firing that occurs following GABA blockade. Under these conditions, compound 11 had no glycineric activity, while 1 and 10 were only weak glycine agonists (Table II, Figure 3A). To test further whether the remaining activity was mediated by the glycine receptor, strychnine (10 μM) was added to the medium. Under these conditions, all the activity of

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1 was reversed. However, there was still a 40% decrease in input resistance in the cells exposed to compound 10. The cause of this residual activity is not known; possibilities include mediation by the strychnine-insensitive neonatal form of the glycine receptor[28] or by a separate receptor for taurine or β-alanine.[29]

Discussion

From its established localization in lower motor centers of the mammalian central nervous system,[3] the strychnine-sensitive glycine receptor is a promising target for nonsedating therapy of spasticity and other neuromuscular conditions. However, despite this potential, the development of pharmacologically useful glycine agonists has proved elusive. In large measure, the inability to synthesize new glycine agonists results from the extreme steric intolerance of the glycine receptor.[5-7,25] Compounding this problem may be the stoichiometry of channel activation by agonists. The present electrophysiological results indicate that about four molecules of glycine must bind to the receptor complex before the chloride channel is opened (Figure 3B). Binding[30] and patch clamp[31,32] experiments indicate that two or three molecules are required. Although the exact number of glycine molecules needed to open the channel is unclear, the above studies clearly imply the need for binding of more than one molecule of glycine. Furthermore, molecular cloning and biochemical studies on the glycine receptor[33] and the related $GABA_A$ and nicotinic receptors show that each receptor is a pentamer,[33,34] consisting of several different but homologous subunits.[34,35] The glycine receptor contains at least two such subunits, an α-subunit that has a high affinity for glycine and can be blocked by strychnine, and a β-subunit that has a 100-fold lower affinity for both glycine and is not significantly blocked by strychnine. The apparent stoichiometry of the pentameric complex is proposed to be three α-subunits and two β-subunits.[36] To fully mimic glycine, an agonist presumably must bind to and activate at least the three α-subunits (and possibly the β-subunits), a requirement that could be difficult to fulfill. The additional steric requirements of binding to multiple subunits may explain the difficulty in identifying novel agonists or antagonists at this site. Since presumably strychnine does not bind to the β-subunit, the ability to displace [^{3}H]-strychnine binding would be only a partial indicator of affinity for the holoreceptor. Combined, these considerations may explain the poor functional activity of compounds 1 and 11. Whether a compound that selectively activated only one subunit would have useful functional or therapeutic activity is an unexplored question.

In addition to differences between the α- and β-subunits, differences between the adult and neonatal forms of the α-subunit (α_1 and α_2, respectively) may explain the unexpectedly poor activity of 1 and 11 in the electrophysiological studies. Primary cultures of mouse spinal cord mainly express the neonatal isoform of the glycine receptor.[37] There are significant differences in the pharmacology of the adult and neonatal forms of the glycine receptor.[28,38] Most strikingly, the rat (but not human) α_2-subunit is about 500 times less sensitive to strychnine than its adult counterpart.[28] Since the tissue for our binding studies was derived from adult rat spinal cord, this isoform of the receptor may have a higher affinity for these novel agonists than the neonatal isoform expressed in cultured neurons. Additional studies with the two receptor isoforms may shed light on this possibility.

Two models could explain the observed high Hill slope of glycine in the electrophysiological studies (Figure 3B). In the first model, the receptor subunits are allosterically coupled, so that transition to an open-channel state requires a concerted conformational change in all the subunits (or at least the α-subunits). A second model posits several independent subunits, each of which controls a separate sector of the channel. Each of the subunits must be in the activated state before the channel can pass chloride ions. Although both models are consistent with the electrophysiological data, they should be distinguishable in binding experiments. If several subunits were allosterically coupled as in model 1, the binding of glycine should show positive cooperativity, with a Hill coefficient greater than one. Conversely, binding to several dissimilar independent sites as predicted by model 2 should give a Hill slope less than one. Unfortunately, experiments under different NaCl conditions support either alternative (i.e., the Hill coefficient was less than 1 in the absence of NaCl and greater than 1 in the presence of 1 M NaCl). The overall behavior of the receptor may be best explained by a combination of both models, that is, two or more dissimilar glycine binding sites with partial allosteric coupling.

In addition to the strychnine-sensitive inhibitory glycine receptor, a strychnine-insensitive binding site on the excitatory *N*-methyl-D-aspartate (NMDA) receptor has been described.[39] This site interacts allosterically with the NMDA binding site to produce channel opening. Of those compounds discussed above, only azetidine-3-carboxylic acid (compound 10) bound with appreciable affinity to the glycine site of the NMDA receptor (unpublished results). This suggests a divergent structural preference for this new glycine receptor.[39]

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In summary, these results illustrate the severe structural limitations placed on compounds binding at the strychnine-sensitive glycine receptor. Compounds 1, 10, and 11 clearly represent novel leads, but the failure of simple analogues to retain binding activity suggests that further improvements in affinity may be difficult to achieve. The reason for the poor agonist activity of these leads is unclear. Additional ligands are needed to further explore this poorly understood receptor.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet MX-1 FT spectrometer. The ^{1}H NMR spectra were recorded on a Varian EM 390, a IBM W-P100SY NMR spectrometer (100 MHz), a Varian XL 200 NMR spectrometer (200 MHz), or a Varian XL 300 spectrometer equipped with a 5-mm broad-band switchable probe. All spectra were consistent with the proposed structures. The peaks are described in ppm downfield from TMS (internal standard). The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer. Although all intermediates were characterized by ^{1}H NMR, IR, and MS, these data are given only for final products and selected intermediates. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values; values outside the limits are indicated. TLC was carried out with 0.25-mm silica gel F254 (E Merck) glass plates. Some intermediate products were used directly without purification or characterization.

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1,4-dimethyl-, Methyl Ester (16). To a mechanically stirred suspension of *N*-methylglycine (50.7 g, 0.57 mol), powdered paraformaldehyde (18.8 g, 0.57 mol), and magnesium sulfate (140 g) in toluene (1 L) was added methyl 2-butynoate (56.3 g, 0.57 mol), and the reaction mixture was heated 3 h at 95–100 °C. The reaction mixture was allowed to cool, charged with a fresh mixture of *N*-methylglycine (50.7 g) and paraformaldehyde (18.8 g), and then heated an additional 3 h as before. The reaction mixture was filtered, and the product was extracted into 1 N HCl (3 × 250 mL) and the acid washed with toluene. The aqueous phase was adjusted to pH 9.0 with a 50% NaOH solution, extracted with dichloromethane (4 × 200 mL) which was dried over sodium sulfate, and concentrated to a yellow oil (17.0 g, 19%). NMR ($CDCl_3$, 90 MHz): δ 3.6 (s, 3 H), 3.4 (m, 4 H), 2.3 (s, 3 H), 2.0 (s, 3 H).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-methyl-, Methyl Ester, Monohydrochloride (17). A solution of 16 (12.0 g, 77.4 mmol) in dichloromethane (100 mL) was cooled to 5 °C in an ice bath. A solution of 1-chloroethyl chloroformate (11.1 g, 77.4 mmol) was added dropwise over 30 min whereupon the reaction mixture was filtered through a pad of silica (75 g) and the product was eluted with ethyl acetate. The solvents were concentrated to an oil (9.0 g), which was dissolved in methanol (100 mL), refluxed 1 h, and then concentrated to a solid. Precipitation from methanol with diethyl ether afforded a fluffy, crystalline solid (4.0 g, 30%), mp 160–161 °C. Anal. ($C_7H_{11}NO_2$·HCl) C, H, N. NMR (D_2O): δ 4.3 (m, 4 H), 3.8 (s, 3 H), 2.2 (s, 3 H). IR (KBr): 1720, 1668, 1429, 1290, 1077, 763 cm^{-1}. MS: *m/z* 142.0 (37.2), 126.0 (76), 82.0 (100).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-methyl-, Monohydrochloride (4). The ester 17 (3.3 g, 20.2 mmol) was refluxed for 2.5 h in 4 N HCl, concentrated in vacuo at 50 °C, and then reconcentrated from water. The solid was triturated with 2-propanol, suspended in diethyl ether, filtered, and dried at 78 °C in vacuo to give a white solid (2.5 g, 76%), mp 227–9 °C. Anal. ($C_6H_9NO_2$·HCl) C, H, N. NMR (D_2O): δ 4.3 (m, 4 H), 2.2 (s, 3 H). IR (KBr): 1719, 1376, 1281, 1219, 1121 cm^{-1}. MS: *m/z* 170 (55.4), 154.1 (63.5), 82.1 (100).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1-methyl-4-*n*-pentyl-, Ethyl Ester (18). To a mechanically stirred suspension of *N*-methylglycine (26.7 g, 0.30 mol), powdered paraformaldehyde (9.0 g, 0.30 mol), and magnesium sulfate (75 g) in toluene (500 mL) was added ethyl 2-hexynoate (50.0 g, 0.30 mol), and the reaction mixture was heated 3 h at 95–100 °C. The reaction solids were filtered, and the product was extracted into 1 N HCl (3 × 100 mL). The acid was neutralized by adding solid sodium bicarbonate with stirring, and the product was extracted into ethyl acetate (3 × 100 mL), dried (magnesium sulfate), and concentrated to an oil (8.4 g) which was purified by chromatography on silica (toluene to load, 20–50% ethyl acetate in hexane to elute) to give a pure oil (8.0 g, 12%). NMR ($CDCl_3$): δ 4.1 (q, 4 H, *J* = 7 Hz), 3.6 (m, 4 H), 2.5 (t, 2 H), 2.3 (s, 3 H), 1.3 (m, 9 H), 0.9 (t, 3 H, *J* = 7 Hz).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-pentyl-, Ethyl Ester, Monohydrochloride (19). The *N*-methyl precursor 18 (7.55 g, 33.6 mmol) was demethylated exactly as described for compound 16. Ether trituration of the product gave a finely divided, fluffy solid (4.9 g, 59%), mp 76–77 °C. Anal. ($C_{12}H_{21}NO_2$·HCl) C, H, N. NMR (D_2O): δ 4.8 (s, 4 H), 4.3 (q, 2, *J* = 7 Hz), 2.7 (t, 2 H, *J* = 7 Hz), 1.5 (quint, 2 H, *J* = 7 Hz), 1.3 (m, 7 H), 0.9 (t, 3 H, *J* = 7 Hz). IR (KBr): 1766, 1667, 1257, 1106, 1036, 770 cm^{-1}. MS: *m/z* 212.1 (100), 138.1 (25), 68.0 (31.4).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-pentyl-, Monohydrochloride (7). A solution of 19 in 4 N HCl (60 mL) was refluxed 3 h and concentrated to a gray solid which was reconcentrated from tetrahydrofuran. Suspension of the solid in diethyl ether gave, after several hours, a fine white solid (3.6 g, 95%), mp 189–190 °C. Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N. NMR (D_2O): δ 4.34 (s, 4 H), 2.70 (t, 2 H, *J* = 7 Hz), 1.56 (m, 2 H), 1.35 (m, 4 H), 0.92 (t, 3 H, *J* = 7 Hz). IR (KBr): 1741, 1671, 1205, 694 cm^{-1}. MS: *m/z* 184.1 (46.2), 138.1 (36.9), 112.0 (76.9), 68 (100).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1-methyl-4-phenyl-, Ethyl Ester (20). To a magnetically stirred suspension of *N*-methylglycine (13.8 g, 0.15 mol), powdered paraformaldehyde (5.16 g, 0.17 mol), and magnesium sulfate (50 g) in toluene (300 mL) was added ethyl 3-phenylpropynoate (30.0 g, 0.17 mol), and the reaction mixture was heated to 90 °C. After 2 h the reaction was cooled and charged with a fresh mixture of *N*-methylglycine (13.8 g) and paraformaldehyde (5.16 g). The reaction mixture was heated to 90 °C, but became unstirable after 30 min, and the reaction mixture was allowed to cool and the solids filtered. The product was extracted into 1 N HCl (2 × 100 mL) which was washed with toluene, combined with ethyl acetate (100 mL), and neutralized with solid sodium bicarbonate. The aqueous phase was washed with ethyl acetate (2 × 100 mL), and the ethyl acetate layers were combined, dried over magnesium sulfate, and concentrated to an oil (9.2 g) which was chromatographed to give a colorless oil (8.8 g, 20.2%). NMR ($CDCl_3$): δ 7.3 (m, 5 H), 4.1 (q, 2 H, *J* = 7 Hz), 3.9 (s, 4 H), 2.5 (s, 3 H), 1.15 (q, 3 H, *J* = 7 Hz). IR (LF): 1701, 1296, 756, 699 cm^{-1}. MS: *m/z* 232 (64.2), 158 (100).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-phenyl-, Ethyl Ester, Monohydrochloride (21). A solution of 20 (8.0 g, 34.6 mmol) in dichloromethane (100 mL) was stirred under nitrogen in an ice bath and treated with a solution of 1-chloroethyl chloroformate (4.95 g, 34.6 mmol) in dichloromethane (50 mL) over 45 min. The reaction mixture was stirred at room temperature 30 min and filtered through a pad of silica (75 g) which was washed with 1 L of ethyl acetate. The solvents were concentrated, the residue was dissolved in methanol (200 mL), and the reaction mixture was refluxed 3 h. Concentration gave a solid which was precipitated from hot methanol (20 mL) with diethyl ether (150 mL), filtered, washed with ether, and dried in vacuo to give a fluffy white solid (3.82 g, 44%), mp 159–161 °C. Anal. ($C_{13}H_{15}NO_2$·HCl) C, H, N. NMR (D_2O): δ 7.3 (s, 5 H), 4.6 (dt, 4 H, *J* = 14.2, 1.4 Hz), 4.2 (q, 2 H), 1.2 (t, 3 H). IR (KBr): 1695, 1282, 758 cm^{-1}. MS: *m/z* 218.1 (21.8), 144.1 (100), 115.0 (17.5).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-4-phenyl-, Monohydrochloride (6). Ester 21 was dissolved in 4 N HCl (45 mL) and refluxed for 2 h. The solvent was concentrated, the residue was reconcentrated from tetrahydrofuran, and the solid was suspended in 2-propanol, filtered, and washed with diethyl ether. The solid was dried in vacuo at 78 °C overnight (2.25 g, 79%), mp 247–249 °C. Anal. ($C_{11}H_{11}NO_2$·HCl) C, H, N. NMR (D_2O): δ 7.5 (s, 5 H), 4.6 (dt, 4 H). IR (KBr): 1700, 1255, 756, 696 cm^{-1}. MS: *m/z* 188.9 (5.4), 169.9 (4.7), 144.0 (100), 115.0 (28.2).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1-methyl-, Ethyl Ester (22). To a stirred suspension of *N*-methylglycine (15.0 g, 0.17 mol), powdered paraformaldehyde (6.0 g, 0.20 mol), and magnesium sulfate (40 g) in toluene (300 mL) was added ethyl 2-propynoate (20.0 g, 0.20 mol), and the reaction mixture was

heated to 100 °C. After 1.5 h the reaction mixture was allowed to cool, filtered, and extracted with 1 N HCl (2 × 100 mL). The acid was washed with chloroform (2 × 75 mL) and adjusted to pH 9.0 with a 50% NaOH solution, and the aqueous phase was extracted with chloroform (2 × 100 mL). The chloroform was dried over sodium sulfate and concentrated to a crude red oil (6.7 g, 67% purity by GC analysis). The crude ester was unstable to distillation and was hydrolyzed without purification. NMR ($CDCl_3$, 90 MHz): δ 6.7 (s, 1 H), 4.2 (q, 2 H, J = 7 Hz), 3.6 (s, 4 H), 2.4 (s, 3 H), 1.2 (t, 3 H, J = 7 Hz).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1-methyl- (2). The crude ester **22** (5.7 g) was refluxed for 2 h in 4 N HCl and concentrated to a black semisolid, decolorized by warming in methanol with activated carbon. The solids were filtered, and the methanol was concentrated to a light brown solid (0.82 g) which was purified by precipitation from hot methanol with 2-propanol. The solid was washed with cold 2-propanol and then diethyl ether, filtered, and dried in vacuo at 78 °C to give 0.12 g of product, mp 215–216 °C. Anal. ($C_6H_{11}NO_2$·HCl) C, H, N. NMR (D_2O): δ 6.8 (s, 1 H), 4.5–4.8 (m, 2 H), 4.1–4.2 (m, 2 H), 3.1 (s, 3 H). IR (KBr): 1735, 1650, 1236, 736, 709 cm^{-1}. MS: m/z 127.1 (43.9), 82.1 (100), 6.0 (23.5), 42.0 (51).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1,2-dimethyl-, Ethyl Ester (23) and 1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-1,5-dimethyl-, Ethyl Ester (24). In each of two identical reactions, a suspension of *N*-methylalanine (10.0 g, 97.1 mmol), powdered paraformaldehyde (3.5 g, 116 mmol), and magnesium sulfate (22 g) in toluene (175 mL) was prepared. Ethyl propiolate (11.4 g, 116 mmol) was added, and the reaction mixture was heated to 90–100 °C for 3.5 h. The reaction mixtures were cooled, filtered, and combined before extraction with 1 N HCl (2 × 250 mL). The acid was washed with chloroform (3 × 100 mL), combined with ethyl acetate (200 mL), and neutralized with stirring by adding solid sodium bicarbonate. The layers were separated, and the aqueous phase was extracted with ethyl acetate (2 × 200 mL). The ethyl acetate layers were combined, dried over magnesium sulfate, and concentrated to a yellow oil (16.7 g, 51%) as a mixture of the 2- and 5-methylpyrrolines. Chromatography on silica (200 g) using a gradient of 50–100% ethyl acetate in heptane afforded the less polar 5-methyl isomer (3.4 g pure material, 10%) plus the more polar 2-methyl isomer (13.3 g, 41% but contaminated with the 5-methyl derivative). **23.** NMR ($CDCl_3$): δ 6.65 (s, 1 H), 4.1 (m, 2 H), 3.8 (m, 1 H), 3.5 (m, 1 H), 3.25 (m, 1 H), 2.4 (s, 3 H), 1.2 (m, 6 H). IR (LF): 1719, 1456, 1281, 1172, 1121, 746 cm^{-1}. MS: m/z 170.1 (55.4), 154.1 (63.5), 96.1 (34.4), 82.1 (100). **24.** NMR ($CDCl_3$): δ 6.5 (s, 1 H), 4.1 (m, 2 H), 3.9 (m, 1 H), 3.45 (m, 1 H), 3.35 (m, 1 H), 2.4 (s, 3 H), 1.2 (m, 6 H). IR (LF): 1720, 1456, 1278, 1169, 1116, 738 cm^{-1}. MS: m/z 170.1 (50.7), 154.1 (62), 96.1 (38.3), 82.0 (100).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-2-methyl-, Ethyl Ester (25). A solution of **23** (1.75 g, 10.4 mmol) in methylene chloride (30 mL) was cooled under nitrogen in an ice bath. A solution of 1-chloroethyl chloroformate (1.5 g, 10.4 mmol) in methylene chloride (15 mL) was added dropwise over 20 min, and the reaction mixture was removed from the bath and stirred for 45 min. The crude reaction mixture was poured onto silica (10 g), and the intermediate amide was eluted with ethyl acetate (100 mL) to give an oil (1.8 g). The amide was deprotected by refluxing 2 h in methanol (50 mL), but the amine salt could not be readily crystallized from methanol/ether. The ether solution was extracted with water (2 × 5 mL), neutralized with solid sodium bicarbonate, and extracted repeatedly with ethyl acetate (5 mL), and the organic phases were combined and dried over magnesium sulfate then concentrated. Chromatography of the residue on silica (10% methanol in ethyl acetate) afforded the free base as an oil (0.17 g, 10.6%) which was characterized by NMR and directly hydrolyzed to the amino acid (**3**). NMR ($CDCl_3$, 90 MHz): δ 6.8 (m, 1 H), 4.5 (q, 3 H), 3.8 (m, 2 H), 2.5 (bs, 1 H), 1.3 (m, 6 H).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-2-methyl-, Monohydrochloride (3). The ester **25** (0.17 g, 1.1 mmol) was dissolved in 4 N HCl (3 mL), refluxed 2.5 h, then concentrated in vacuo to a light brown solid which was triturated with 2-propanol and then diethyl ether, and dried in vacuo overnight at 78 °C to give a solid (0.11 g, 64%), mp 229–230 °C. Anal. ($C_6H_9NO_2$·HCl) C, H, N. NMR (D_2O): δ 6.9 (s, 1 H), 4.8 (q, 3 H, J = 7 Hz), 1.56 (d, 2 H, J = 7 Hz). IR (KBr): 1711, 1639, 1585, 1201, 717 cm^{-1}. MS: m/z 128 (M + 1, 10.7), 112 (100), 68 (88.5).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-5-methyl-, Ethyl Ester (26). The 1-methyl precursor **24** (2.5 g, 14.8 mmol) was dissolved in methylene chloride (25 mL) and cooled under nitrogen to −15 °C before a solution of 1-chloroethyl chloroformate (2.1 g, 14.8 mmol) in methylene chloride (25 mL) was added dropwise over 20 min. After warming to room temperature, the reaction mixture was refluxed for 45 min, cooled, and poured onto 20 g of silica, and the product was eluted with ethyl acetate and concentrated. The residue was refluxed for 3 h in methanol (100 mL), concentrated, and triturated with ethyl acetate to give a white solid which was recrystallized from 2-propanol/diethyl ether to give white crystals (1.3 g, 46%) but contaminated with a ring opened side product. The free base (0.10 g, 9.5%) was isolated by chromatography as described for the 2-isomer (**25**) and directly hydrolyzed. NMR ($CDCl_3$, 90 MHz): δ 6.7 (m, 1 H), 4.2 (q, 3 H), 3.9 (m, 2 H), 2.85 (bs, 1 H), 1.3 (t, 6 H).

1*H*-Pyrrole-3-carboxylic Acid, 2,5-Dihydro-5-methyl-, Monohydrochloride (5). Hydrolysis of **26** (0.10 g, 0.65 mmol) was carried out as described for **3** to yield a light brown solid (0.068 g, 60%), mp 224–227 °C. Anal. ($C_6H_9NO_2$·HCl) C, H, N. NMR (D_2O): δ 6.73 (m, 1 H), 4.83 (m, 3 H), 1.5 (d, 2 H, J = 7 Hz). IR (KBr): 1706, 1657, 1225, 749, 706 cm^{-1}. MS: m/z 127 (8.9), 112 (48), 82 (53), 68 (100).

Receptor Binding. [^{3}H]Strychnine binding to rat spinal cord membranes was carried out in 50 mM Tris-citrate (without NaCl) as previously described.[7] In experiments evaluating the effects of NaCl, the assay was performed in parallel in 50 mM Tris-citrate with or without 1 M NaCl.[22] The NaCl shift is expressed as the ratio of the IC_{50} values in the presence and absence of 1 M NaCl. IC_{50} values in structure–activity studies were calculated by nonlinear curve fitting to a Langmuir isotherm, and IC_{50} values in NaCl shift experiments were fit to a logistic equation.

Electrophysiological Studies. Cultures of spinal-cord neurons were kindly supplied by Dr. Robert L. MacDonald, University of Michigan, Ann Arbor, MI. Details of the culturing procedures used in this study can be found elsewhere.[40] Briefly, spinal cords and dorsal-root ganglia were dissected from 12- to 14-day-old fetal-mouse embryos. Following dissociation and trituration, they were suspended in Eagle's minimal essential medium (supplemented with serum and glucose) in collagen-coated, 35-mm dishes. Cultures were maintained in an incubator at 35–37 °C in an atmosphere of 10% CO_2. Cultures were used for recording at 3–6 weeks in vitro in Dulbecco's phosphate buffer saline (DPBS) (composition in mM: $CaCl_2$, 1.0; $MgCl_2$, 8–10; NaCl, 143.4; KCl 4.2; and glucose 5.6 in 9.5 mM sodium phosphate buffer at pH 7.4). All experiments were performed at room temperature (20–25 °C).

Standard intracellular recordings were made with glass micropipettes filled with 3 M KCl (20–40 MΩ). An active bridge circuit was used to simultaneously record membrane potential and pass current. Some experiments were performed while passing constant hyperpolarizing current (<1 nA) to prevent spontaneous depolarizing activity from generating action potentials. When a stable membrane potential was obtained, input resistance measurements were obtained by passing hyperpolarizing current (40–200-ms duration) and recording the voltage change on a Gould chart recorder. Drugs were administered by micropipette with a tip diameter of 5–10 μm (puffer pipette) that was positioned 10–40 μm from the cell body. Using a timed pressure regulator, drug was ejected from the barrel at 0.5–1 psi for 10–30 s. All drugs were dissolved in DPBS, or if insoluble, in DMSO and diluted in buffer to a concentration of no greater than 0.1% DMSO.

For putative glycine agonists, the principle measure was the change in input resistance produced by pressure ejection of the test compound. Hyperpolarizing current pulses were delivered throughout at a frequency of approximately 1 Hz. Data were utilized only when five consecutive measurements during drug application were within 15% of each other. To calculate the change in input resistance, the mean of five voltage measurements

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during the drug delivery was calculated and divided by the mean of measurements made before and after the drug pipette was brought next to the cell.

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Registry No. 1, 72519-12-1; 2, 137569-65-4; 3·HCl, 137569-66-5; 3 (free base), 137569-80-3; 4·HCl, 137569-67-6; 4 (free base), 137569-81-4; 5·HCl, 137569-68-7; 5 (free base), 137569-82-5; 6·HCl, 137569-69-8; 6 (free base), 137569-83-6; 7·HCl, 137569-70-1; 7 (free base), 137569-84-7; 8, 59378-87-9; 9, 931-03-3; 10, 36476-78-5; 11, 498-94-2; 12, 3395-35-5; 13, 4043-88-3; 14, 2133-34-8; 15, 64603-90-3; 16, 137569-71-2; 17, 137569-72-3; 18, 137569-73-4; 19, 137569-74-5; 20, 137569-75-6; 21, 137569-76-7; 22, 35516-94-0; 23, 68384-73-6; 24, 137569-77-8; 25, 137569-78-9; 26, 137569-79-0; H-Sar-OH, 107-97-1; H-Gly-OH, 56-40-6; Me-Ala-OH, 3913-67-5; MeC≡CCOOMe, 23326-27-4; PrC≡CCOOEt, 16205-90-6; PhC≡CCOOEt, 2216-94-6; HC≡CCOOEt, 623-47-2; strychnine, 57-24-9.

Nucleosides and Nucleotides. 103. 2-Alkynyladenosines: A Novel Class of Selective Adenosine A_2 Receptor Agonists with Potent Antihypertensive Effects[†,1]

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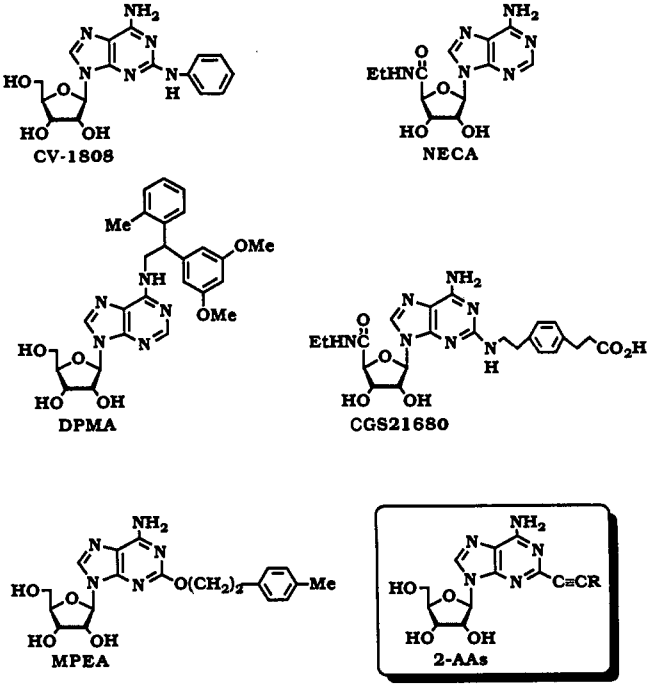
The synthesis and receptor-binding activities at A_1 and A_2 adenosine receptors for a series of 2-alkynyladenosines are described. The palladium-catalyzed cross-coupling reaction of 2-iodoadenosine (**4a**) with various terminal alkynes in the presence of bis(triphenylphosphine)palladium dichloride and cuprous iodide in *N,N*-dimethylformamide containing triethylamine gives 2-alkynyladenosines (**5a–r**). An economical synthetic method for the preparation of 9-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-6-chloro-2-iodopurine (**2**), which is a precursor of **4a**, is also included. Several transformation reactions of 2-(1-octyn-1-yl)adenosine (**5e**) and 2-(1-ethyn-1-yl)adenosine (**9**) and a similar cross-coupling reaction of 6-chloropurine derivative **11** and 8-bromoadenosine (**13**) with 1-octyne are also reported. Many of these 2-alkynyladenosines tested for A_1 and A_2 adenosine receptor binding activities in rat brain are selective for the A_2 adenosine receptor. Among them, 2-(1-hexyn-1-yl)adenosine (**5c**) has the highest affinity for both A_1 and A_2 receptors with K_i values of 126.5 and 2.8 nM, respectively. The structure–activity relationship of this series of compounds including 6- or 8-alkynylpurine nucleosides and 2-alkyl- and 2-alkenyladenosines is discussed in terms of potency at both receptor subtypes. Additionally, we describe how hypotensive activity and heart rate decrease brought on by **5** and some other compounds with spontaneously hypertensive rats are proportional to the order of the potency to both A_1 and A_2 binding affinities. Thus, 2-alkynyladenosines are interesting and promising as antihypertensive agents that should be considered for further detailed preclinical evaluation.

Adenosine has long been recognized as a physiologically important material to mediate a wide variety of physiological effects including decreased locomotor activity,[2] inhibition of neurotransmitter release,[3] hypothermia,[4] negative inotropic and chronotropic action,[5] inhibition of lipolysis,[6] inhibition of renin release,[7] inhibition of platelet aggregation,[8] and peripheral and cerebral vasodilation.[9] However, the therapeutic potential of adenosine is considered to be low, since extracellular adenosine is known to be unstable due to its rapid uptake into red blood cells,[10] deamination to inosine by adenosine deaminase,[11] and phosphorylation to 5′-AMP by adenosine kinase. Moreover, such a wide variety of actions is thought to have some detrimental effects in therapeutic use.

Adenosine-induced effects can be attributed to the action via interactions with two cell-surface adenosine receptor subtypes, A_1 and A_2, that either inhibit (A_1 receptor) or stimulate (A_2 receptor) the activity of adenylate cyclase. On the basis of the receptor binding–pharmacological relationship of several adenosine analogues, adenosine analogues that have preferential affinity for A_1 receptors produce depression in heart rate and cardiac contractility,

Chart I



on the other hand those analogues having high affinity for A_2 receptors produce vasodilation. Adenosine agonists that

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