Synthesis and Structure–Activity Studies on Excitatory Amino Acids Structurally Related to Ibotenic Acid

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With use of ibotenic acid as a lead, analogues of (*RS*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and of (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA) were synthesized and tested as excitants of neurons in the cat spinal cord by using microelectrophoretic techniques and as inhibitors of the binding of kainic acid in vitro. Like AMPA and 7-HPCA, (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]-pyridine-5-carboxylic acid (10, 5-HPCA) and (*RS*)-3-hydroxy-5-(bromomethyl)isoxazole-4-propionic acid (11, ABPA) proved to interact potently and selectively with central quisqualic acid receptors, assumed to represent physiological glutamic acid receptors. Analogues of 7-HPCA or 10, in which one or both of the acid groups were masked, were very weak or inactive as neuronal excitants and had no antagonistic effects at excitatory amino acid receptors. The structure of 7-HPCA in the crystalline state was established by X-ray analyses. The preferred conformation of 10 in aqueous solution was determined by ^{1}H NMR spectroscopy. On the basis of these studies, 7-HPCA as well as 10 were shown to adopt preferentially conformations with the carboxylate groups in equatorial positions. It is suggested that AMPA, 7-HPCA, and 10 interact with quisqualic acid receptors in conformations essentially reflecting active conformation(s) of glutamic acid at these receptors.

(*S*)-Glutamic acid (Glu) and (*S*)-aspartic acid (Asp) are putative central excitatory neurotransmitters.[1–4] The possible involvement of these amino acid transmitters in certain neurological diseases[5] has, in recent years, focused much interest on central excitant amino acid receptors. Pharmacological investigations in vivo and receptor binding studies in vitro have disclosed heterogeneity of these receptors, which, at present, are most conveniently subdivided into three classes:[6–9] (1) quisqualic acid (QUIS) receptors at which Glu diethyl ester (GDEE) is a relatively selective antagonist, (2) *N*-methyl-(*R*)-aspartic acid (NMDA) receptors at which (*RS*)-2-amino-5-phosphono-valeric acid (2APV) and various other compounds[7] are selective antagonists, and (3) kainic acid (KA) receptors, which are relatively insensitive to GDEE or 2APV but which can be detected in vitro with use of radioactive KA as a ligand. It is possible that some of these populations of exictant amino acid receptors can be further subdivided.[10,11]

Although the physiological relevance of this receptor classification is unclear, QUIS receptors are assumed to represent the postsynaptic receptors for Glu and NMDA receptors are assumed to represent those for Asp. Consequently, there is a particular interest in selective agonists and antagonists for these classes of receptor. In continuation of previous studies,[12–16] this paper describes the development of some heterocyclic amino acids with very potent and selective activity as agonists at QUIS-preferring receptors.

Like (*S*)-(+)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [(*S*)-AMPA],[15,16] (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA)[14] (Figure 1) is a highly selective agonist at QUIS receptors. While 7-HPCA probably reflects conformations of ibotenic acid recognizable by QUIS receptors[14] (Figure 1), the relatively free rotation of the side chain of AMPA makes predictions of the receptor-active conformation of this ibotenic acid analogue difficult. These aspects prompted us to synthesize (*RS*)-3-hydroxy-4,5,6,7-tetra-

Chart I
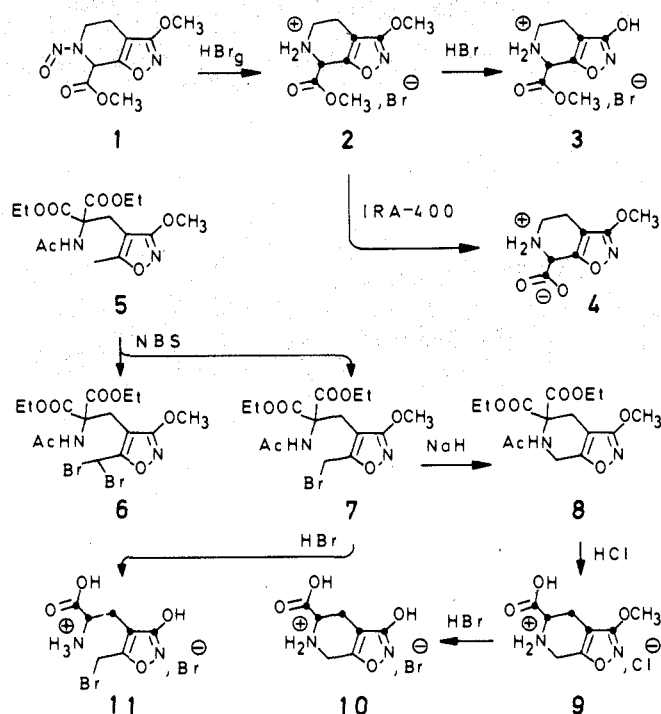
hydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (10, 5-HPCA) (Chart I) as a conformationally restrained analogue

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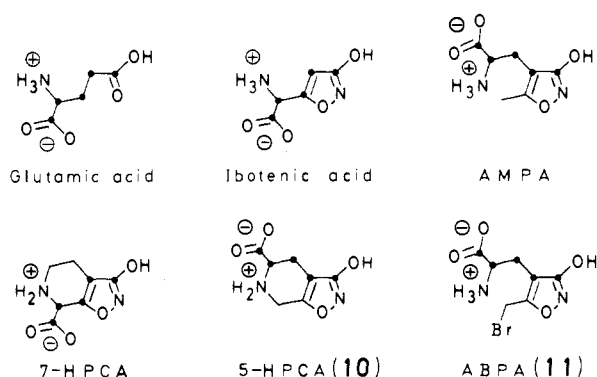(6) Davies, J.; Watkins, J. C. *J. Physiol. (London)* 1979, *297*, 621.

Figure 1. The structure of glutamic acid, ibotenic acid, and some related amino acids.

Figure 2. Numbering of the atoms of the molecule of 7-HPCA monohydrate.

Figure 3. (A–C) Effect of 2APV on the excitation of a spontaneously firing cat spinal interneuron by AMPA, ABPA (11), and NMDA ejected with the indicated currents (nanoamperes) for the times shown by the horizontal bars and symbols: (A) before, (B) during 2APV (20 nA) starting and ceasing as indicated by the horizontal and vertical broken lines, (C) after recovery from 2APV. Ordinates: firing rate, spikes per second. Abscissas: time, minutes. (D–F) Effect of 2APV on the excitation of a spontaneously firing cat dorsal horn interneuron by 7-HPCA, 5-HPCA (10), and NMDA ejected by electrophoretic currents (nanoamperes) and for times indicated by the figures and horizontal bars: (D) before, (E) during ejection of 2APV (20 nA), which commenced 1 min earlier and was termined 1 min after these tracings, (F) 2 min after 2APV. Ordinates: firing rate, spikes per second. Abscissas: time, minutes.

of AMPA (Figure 1). 5-HPCA proved also to be a potent and selective QUIS-receptor agonist, and on the basis of structure–activity studies of AMPA, 7-HPCA, and 5-HPCA, the structural requirements for activation of the QUIS receptors are discussed.

A number of analogues of 7-HPCA, in which one or both of the acid groups are masked, were synthesized and tested biologically. Finally, (RS)-α-amino-3-hydroxy-5-(bromomethyl)isoxazole-4-propionic acid (11, ABPA; Chart I), designed as a QUIS agonist with receptor alkylating properties, was synthesized and studied in vivo and in vitro.

Chemistry. The analogues of 7-HPCA 2–4, were synthesized as outlined in Chart I. The nitroso group of 1[14] was removed by passing a stream of hydrogen bromide gas through a solution of 1 in glacial acetic acid. While rapid evaporation of this solution gave 2, compound 3 was prepared by treatment of 2 with a saturated solution of hydrogen bromide in glacial acetic acid for 16 h. Treatment of 2 with a strongly basic ion-exchange resin gave 4 without detectable decarboxylation of the product. Treatment of 5[12,17] with N-bromosuccinimide (NBS) gave a separable mixture of 6 and 7, whereas the C-4 side chain of 5 was not brominated to any detectable extent. Compound 7 was deprotected under acid conditions to give ABPA·HBr (11). The intramolecular N-alkylation of 7 to give 8 was accomplished at low temperature with use of sodium hydride as a base. At temperatures above 0 °C this reaction gave reaction mixtures containing 8 as a minor product. Attempts to convert 8 directly into 5-HPCA·HBr (10) using aqueous solutions of hydrogen bromide resulted in quite extensive decomposition, whereas stepwise deprotection
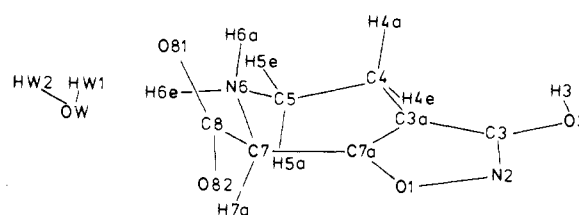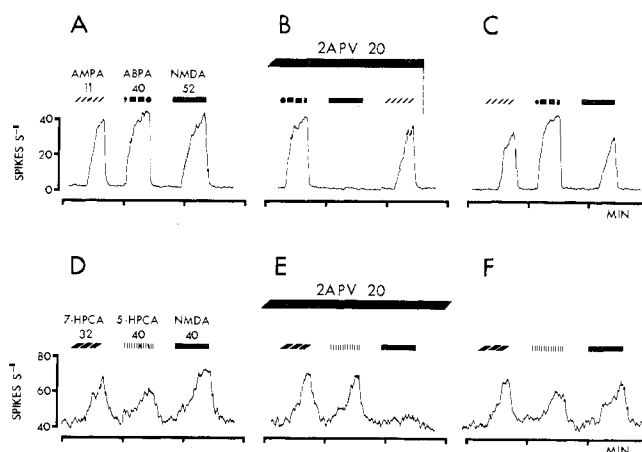
of 8 to give 10 via 9 proceeded smoothly.

For all new compounds the ^{1}H NMR and IR data and the elemental analyses were consistent with the proposed structures. The coupling constants between the two C-4 protons and the C-5 proton ($J_{4a'5a}$ = 10 Hz, $J_{4e'5a}$ = 6 Hz) in the ^{1}H NMR spectrum of the hydrobromide of 5-HPCA (10, Chart I) are in accordance with pseudoaxial–axial and pseudoequatorial–axial configurations, respectively, of these protons. This is consistent with a predominantly equatorial orientation of the carboxylic acid group at C-5. Long-range couplings between the C-7 protons and the pseudoaxial C-4 proton (J = 2 Hz) and the pseudoequatorial C-4 proton (J = 1 Hz) were detected in the ^{1}H NMR spectrum of 10. The ^{1}H NMR spectra of 2–4, recorded in D_2O solutions, disclosed that the C-7 protons in these compounds were to a large extent exchanged for deuterium, in agreement with earlier findings for 7-HPCA.[14] For the products 2–4 and 9–11, which were tested biologically, spectroscopic data are reported.

X-ray Crystallographic Analysis of (RS)-3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-7-carboxylic Acid (7-HPCA) Monohydrate. Bond lengths, valency angles, selected torsion angles, and some nonbonded distances as found by X-ray analysis at 296 and 105 K are listed in Table I. The numbering system and the conformation of the molecule are illustrated in Figures 2 and 4. The isoxazole ring is planar within the limits of

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Table I. Molecular Dimensions of 7-HPCA Monohydrate Obtained from the X-ray Analyses at 296 and 105 K[a]

	296 K	105 K		296 K	105 K
		Intramolecular	Distances, Å		
O1–N2	1.431 (2)	1.421 (1)	C5–H5e	0.99 (2)	0.96 (2)
N2–C3	1.313 (2)	1.320 (2)	N6–H6a	0.89 (2)	0.91 (2)
C3–C3a	1.455 (2)	1.435 (2)	N6–H6e	1.00 (2)	0.96 (2)
C3a–C7a	1.335 (2)	1.347 (2)	C7–H7a	0.97 (2)	0.97 (2)
C7a–O1	1.364 (2)	1.352 (1)	OW–HW1	0.92 (3)	0.86 (3)
C3a–C4	1.509 (2)	1.499 (2)	OW–HW2	0.89 (3)	0.88 (3)
C4–C5	1.531 (2)	1.529 (2)	N6···O1	3.699 (2)	3.653 (1)
C5–N6	1.502 (2)	1.500 (2)	N6···N2	4.632 (3)	4.563 (1)
N6–C7	1.499 (2)	1.490 (2)	N6···O3	5.318 (3)	5.259 (1)
C7–C7a	1.504 (2)	1.495 (2)	N6···O81	2.637 (2)	2.633 (1)
C7–C8	1.545 (2)	1.544 (2)	N6···O82	3.612 (2)	3.618 (1)
C8–O81	1.255 (2)	1.256 (1)	O81···O1	3.919 (2)	3.878 (1)
C8–O82	1.235 (2)	1.246 (1)	O81···N2	5.117 (3)	5.062 (1)
C3–O3	1.328 (2)	1.326 (1)	O81···O3	6.485 (3)	6.456 (1)
O3–H3	0.95 (2)	0.85 (2)	O82···O1	2.875 (2)	2.857 (1)
C4–H4a	1.00 (2)	1.00 (2)	O82···N2	4.267 (2)	4.240 (1)
C4–H4e	1.01 (2)	0.97 (2)	O82···O3	6.193 (2)	6.194 (1)
C5–H5a	0.97 (2)	0.94 (2)			
		Valency	Angles, deg		
C7a–O1–N2	108.0 (1)	106.92 (9)	C7a–C7–C8	111.4 (1)	111.21 (9)
O1–N2–C3	104.8 (1)	105.96 (9)	C3a–C7a–C7	127.1 (1)	126.9 (1)
N2–C3–C3a	112.4 (1)	111.7 (1)	C3a–C7a–O1	111.7 (1)	112.4 (1)
N2–C3–O3	116.4 (1)	117.5 (1)	O1–C7a–C7	121.2 (1)	120.6 (1)
C3a–C3–O3	131.1 (1)	130.7 (1)	C7–C8–O81	115.9 (1)	115.7 (1)
C3–C3a–C7a	103.2 (1)	102.97 (9)	C7–C8–O82	115.2 (1)	115.3 (1)
C3–C3a–C4	135.1 (1)	134.4 (1)	O81–C8–O82	128.8 (1)	128.9 (1)
C7a–C3a–C4	121.8 (1)	122.6 (1)	C3–O3–H3	111 (2)	109 (2)
C3a–C4–C5	110.0 (1)	109.07 (9)	H6a–N6–H6e	102 (2)	105 (2)
C4–C5–N6	110.9 (1)	110.82 (9)	⟨C–N–H⟩	110 (1)	109 (1)
C5–N6–C7	113.0 (1)	113.59 (9)	⟨C(N)–C–H⟩	109 (1)	110 (1)
N6–C7–C7a	107.5 (1)	106.37 (9)	⟨H–C–H⟩	109 (1)	108 (1)
N6–C7–C8	110.3 (1)	110.55 (9)	HW1–OW–HW2	109 (2)	109 (2)
		Torsion	Angles, deg		
C7a–O1–N2–C3	±0.3 (2)	±0.3 (1)	C3a–C4–C5–N6	±45.1 (2)	±44.7 (1)
O1–N2–C3–C3a	∓0.2 (2)	∓0.1 (2)	C4–C5–N6–C7	∓65.1 (2)	∓66.5 (1)
N2–C3–C3a–C7a	±0.1 (2)	∓0.2 (1)	C5–N6–C7–C7a	±46.6 (2)	±48.0 (1)
C3–C3a–C7a–O1	±0.1 (2)	±0.4 (1)	N6–C7–C7a–C3a	∓15.0 (2)	∓15.8 (1)
C3a–C7a–O1–N2	∓0.3 (2)	∓0.5 (1)	C5–N6–C7–C8	±168.2 (1)	±168.8 (1)
C7–C7a–C3a–C4	∓0.4 (2)	∓0.2 (2)	N6–C7–C8–O81	∓10.1 (2)	∓9.4 (1)
C7a–C3a–C4–C5	∓14.6 (2)	∓14.2 (1)	C7a–C7–C8–O82	∓69.0 (2)	∓69.8 (1)
				O1–N2–C3–O3	∓177.9 (1)	∓177.5 (1)
			C3a–C3–O3–H3	±0.2 (9)	±3 (2)

[a] Estimated standard deviations are in parentheses.

experimental error. The six-membered ring adopts a half-chair conformation with the carboxylate group in an equatorial position.

The crystal structure is stabilized by hydrogen bonds. All of the five available hydrogen atoms are utilized in the formation of hydrogen bonds. Hydrogen bond dimensions are given in Table II.

Effects on Kainic Acid Binding. The affinities of 2–4 and 9–11 for the KA binding sites on purified synaptic membranes prepared from rat brains were studied as described earlier[13] on the basis of a published procedure.[18] While Glu ($IC_{50} = 0.35 \, \mu M$) and KA ($IC_{50} = 0.010 \, \mu M$) were inhibitors of [³H]KA binding, the compounds under study were inactive or very weak ($IC_{50} > 100 \, \mu M$).

Single-Cell Pharmacology. Microelectrophoretic techniques[19,20] were used to compare the effects on single neurons (Renshaw cells and interneurons) in the cat spinal cord of AMPA, 7-HPCA, QUIS, NMDA, 2–4, and 9–11. In agreement with earlier findings, AMPA[13] (Figure 3A–C)

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Table II. Hydrogen Bond Distances (Angstroms) and Angles (Degrees) of 7-HPCA Monohydrate Obtained from the X-ray Analyses at 296 K (Upper Entries) and at 105 K (Lower Entries)[a]

	hydrogen bond distances			
A–H···B	A–H	A···B	H···B	∠AHB
OW–HW1···O82[ii b]	0.92 (3)	2.723 (2)	1.83 (3)	164 (2)
	0.86 (2)	2.708 (2)	1.86 (2)	165 (2)
OW–HW2···N2[iii]	0.89 (3)	2.823 (2)	1.95 (2)	165 (2)
	0.88 (3)	2.794 (1)	1.94 (2)	165 (2)
O3–H3···O81[iv]	0.95 (2)	2.544 (2)	1.62 (2)	166 (2)
	0.85 (2)	2.542 (1)	1.72 (2)	164 (2)
N6–H6e···OW[i]	1.00 (2)	2.664 (2)	1.68 (2)	171 (2)
	0.96 (2)	2.635 (1)	1.68 (2)	173 (2)
N6–H6a···O82[v]	0.89 (2)	2.932 (2)	2.41 (2)	118 (2)
	0.91 (2)	2.887 (1)	2.37 (2)	116 (1)

[a] Estimated standard deviations are given in parentheses. [b] Symmetry code: (i) x, y, z, (ii) $1 - x, \frac{1}{2} + y, \frac{1}{2} - z$, (iii) $1 + x, \frac{1}{2} - y, z - \frac{1}{2}$, (iv) $\bar{x}, \frac{1}{2} + y, \frac{1}{2} - z$, (v) $x, \frac{1}{2} - y, -\frac{1}{2} + z$.

and 7-HPCA[14] (Figure 3D–F) were very potent excitants sensitive to GDEE but not to the NMDA antagonist 2APV (Figure 3). On all neurons tested, NMDA-induced excitation was blocked by 2APV (Figure 3) but not by GDEE.

In an earlier study 11 was shown to be less than half as potent as a GDEE-sensitive excitant as (S)-AMPA, the more potent anantiomer of AMPA,[15] whereas the present

studies indicated approximate equipotency of 11 and (*RS*)-AMPA (Figure 3A–C) as GDEE-sensitive excitants. This may suggest that the moderately potent neuronal excitant (*R*)-AMPA[15] also has weak antagonist properties. In spite of the presence of an alkylating group in the molecule of 11, these in vivo studies did not disclose an ability of 11 to bind covalently to QUIS receptors. The onset of excitations by AMPA and 11 were very similar (Figure 3A–C) and these effects were equally sensitive to GDEE (not illustrated).

While 2 and 3 were totally inactive as neuronal excitants, 4 was capable of enhancing slightly the effects of both QUIS and NMDA. The sensitivity of these very weak effects of 4 to GDEE or 2APV could not be established with certainty. None of the compounds 2–4 significantly reduced excitations induced by NMDA or QUIS on the cells studied.

In Figure 3D–F the excitatory effect of 10 is compared with those of 7-HPCA and NMDA. On all cells studied the potency and pharmacological profile of 10 was very similar to that of 7-HPCA. The excitation of neither compound was affected by simultaneously administered 2APV in amounts sufficient to block NMDA-induced excitation (Figure 3D–F), whereas the effects of both 10 and 7-HPCA were reduced by concentrations of GDEE, which also reduced AMPA- and QUIS-induced excitations (not illustrated). On the basis of the present studies, 5-HPCA is estimated slightly weaker than AMPA and about a quarter as effective as NMDA as a neuronal excitant.

While the *O*-methylated analogue of 7-HPCA, 4, was a very weak excitant, the corresponding analogue of 5-HPCA, 9, did not significantly excite cat spinal neurons, and it neither enhanced nor reduced excitations by NMDA, QUIS, 7-HPCA, or 5-HPCA.

Discussion

In continuation of earlier studies,[13–16] the aim of the present project is to elucidate the structural requirements for activation of the QUIS receptors, assumed to represent the physiological postsynaptic receptors for Glu.[1–4] Like AMPA,[13,15,16] 7-HPCA[14] is a potent and highly selective agonist at central QUIS-preferring receptors, at which the diethyl ester (GDEE) of Glu is a selective antagonist. Since masking of the acid groups of Glu results in an antagonist, we synthesized and tested microelectrophoretically the compounds 2–4, in which one or both of the acid groups of 7-HPCA have been masked. None of these compounds, however, significantly reduced excitations by QUIS, 7-HPCA, or 5-HPCA, and with the exception of 4, which was a very weak excitant, these compounds did not affect the activity of cat spinal neurons. Similarly, the *O*-methylated analogue 9 of 5-HPCA was shown to be completely inactive as an agonist or antagonist at receptors for excitatory amino acids. Thus, within the present class of conformationally restricted Glu analogues, both acid groups appear to be essential for effective receptor interaction.

Compound 11 (Figure 1) was designed as an agonist at QUIS receptors capable of alkylating the recognition sites of these receptors. The present in vivo studies did, however, not disclose an ability of 11 to interact irreversibly with QUIS receptors, the onset and offset of excitations by 11 and AMPA being very similar (Figure 3A–C) and the excitations by both of these compounds being reversibly antagonized by GDEE. These observations, supported by the similar potency of AMPA and 11 as excitants and the fact that the methyl group of AMPA can be replaced by the bulky *tert*-butyl group without significant loss of activity,[15] suggest that the substituents at the 5-position of these compounds to QUIS-preferring receptors.

AMPA

⊜ Oxygen

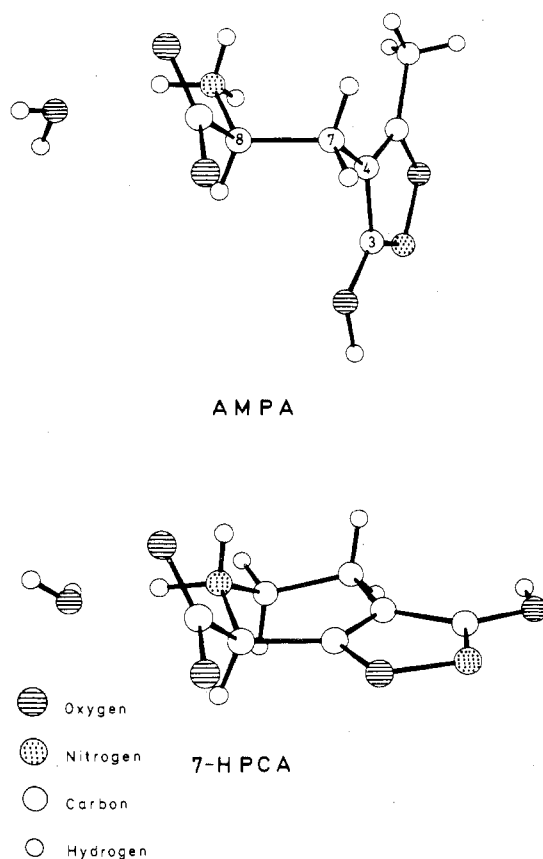⊛ Nitrogen   7-HPCA

◯ Carbon

◯ Hydrogen

Figure 4. The conformations of 7-HPCA and AMPA monohydrates in the crystalline states as established by X-ray analyses.

On the other hand, these substituents seem to affect the conformations of the Glu structure elements of these compounds assumed to be recognized by the receptors.[13,15,21]

Since 7-HPCA is a highly selective agonist at QUIS receptors, it is reasonable to assume that this compound reflects conformations of the selective NMDA agonist ibotenic acid, which are recognizable by the QUIS receptors.[14] Figure 4 illustrates the conformations of AMPA[22] and 7-HPCA in the crystalline states. In the molecule of 7-HPCA, the isoxazole ring is planar, and the six-membered ring is in a half-chain conformation with the carboxylate group in an equatorial position. Molecular mechanics calculations using the MM2 program[23–25] indicate an energy barrier of 31 kJ/mol for conversion of the low-energy conformation of 7-HPCA found in the crystalline state (Figure 4) into an energetically equivalent low-energy conformation with the carboxylate group in an axial orientation. Although the energies of agonist–QUIS receptor interactions, including the ability of the receptor macromolecule(s) to bind agonists in conformations different from those of lowest energy, are unknown, it is likely that the depicted conformation of 7-HPCA (Figure 4) and/or the conformation with the carboxylate group in the axial

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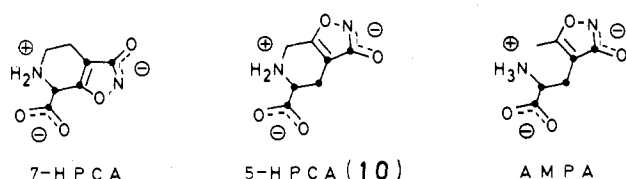(25) Allinger, N. L.; Yuh, Y. M. "Molecular Mechanics Program MM2"; updated Jan 1980.

Figure 5. Structures of the fully ionized molecules of 7-HPCA, 5-HPCA (10), and AMPA.

position essentially reflect the QUIS receptor-active conformations of this compound.

The preferred conformation of the hydrobromide of 5-HPCA (10) was studied by using ^{1}H NMR spectroscopy. In agreement with the findings for 7-HPCA, derived from X-ray analyses, the ^{1}H NMR analysis of 10 indicated that this compound preferentially adopts a conformation with the carboxylate group in an equatorial position. The similarity of the Glu structural elements of 7-HPCA and 10 can be recognized by superimposing the charged groups of the fully ionized molecules of these compounds (Figure 5).

The molecule of AMPA is relatively flexible, and molecular mechanics calculations using the MM2 program[23-25] indicate energy barriers less than 29 kJ/mol[21] for rotation around the C4–C7 bond. By altering the torsion angle C3–C4–C7–C8, it is possible to convert the conformation of AMPA, observed in the crystalline state[22] (Figure 4), into a conformation very similar to that of 10 determined in aqueous solution. In light of the relatively small energies required for this conformational change of AMPA,[21] it is likely that the accessible conformations of 10, the carboxylate group being either equatorial or axial, largely reflect the receptor-active conformations of AMPA.

In general, the present structure–activity analyses are based on the assumption that the conformations of the fully ionized molecules of the Glu analogues under study are similar to those of the compounds, which have been subjected to X-ray or ^{1}H NMR analyses or molecular mechanics calculations (Figure 5). On the basis of these structure analyses and considerations, it is understandable that the pharmacological profiles of 7-HPCA, 10, and AMPA as agonists at QUIS-preferring receptors on single neurons are very similar. It is proposed that the Glu structure elements of these compounds as indicated in Figure 5 are recognized by QUIS receptors, which, in turn, may explain why the methyl group of AMPA can be replaced by other groups, such as bromomethyl (compound 11) or *tert*-butyl groups,[15] without significant loss of activity.

Experimental Section

Chemistry. General Procedures. Melting points are corrected and were determined in capillary tubes. Elemental analyses were performed by P. Hansen, Chemical Laboratory II, University of Copenhagen. IR spectra, obtained on a Perkin-Elmer grating infrared spectrophotometer, Model 247, were recorded in KBr pellets. ^{1}H NMR spectra were recorded on a Varian T60 spectrometer or a JEOL FX 90Q instrument (compound 10). Me$_4$Si was used as an internal standard except for the compounds dissolved in D$_2$O, where sodium 3-(trimethylsilyl)propanesulfonate was used. Thin-layer chromatography (TLC) and gravity column chromatography (CC) were performed with silica gel F$_{254}$ plates (Merck) and silica gel (Woelm, 0.063–0.200 mm), respectively. Compounds containing the 3-isoxazolol unit were visualized on TLC plates with UV light and a FeCl$_3$ spraying reagent (yellow color). Compounds containing amino groups were visualized with a ninhydrin spraying agent, and all compounds under study were detected on TLC plates with a KMnO$_4$ spraying reagent. All evaporations were performed at ca. 15 mmHg with a rotatory evaporator.

(RS)-Methyl 3-Methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-7-carboxylate Hydrobromide (2). Through a solution of 1[14] (130 mg, 0.54 mmol) in glacial acetic acid (8 mL) kept at 5 °C was passed a stream of hydrogen bromide gas, until the color of the solution became pale yellow (30 s). The solution was immediately evaporated and this residue recrystallized (methanol–ether) to give 2 (132 mg, 84%): mp 182 °C dec; IR 3080 (m), 3000–2900 (several bands, m-s), 2740–2390 (several bands, ms), 1745 (s), 1675 (m), 1635 (w), 1545 (s), 1525 (s) cm^{-1}; ^{1}H NMR (D$_2$O) δ 5.3 (ca. 0.1 H, s), 3.83 (3 H, s), 3.75 (3 H, s), 3.6–3.3 (2 H, br t), 2.58 (2 H, t). Anal. (C$_9$H$_{13}$N$_2$O$_4$Br) C, H, N; Br: calcd, 27.26; found, 26.75.

(RS)-Methyl 3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-7-carboxylate Hydrobromide (3). A solution of 2 (84 mg, 0.29 mmol) in a solution of hydrogen bromide in glacial acetic acid (4 mL, 33%) was kept at 25 °C for 16 h. The solution was evaporated and the residue recrystallized (methanol–ethyl acetate) to give 3 (67 mg, 83%): mp 174 °C dec; IR 3100–2830 (several bands, ms), 2780–2300 (several bands, ms), 1735 (s), 1665 (m), 1635 (w), 1545 (s) cm^{-1}; ^{1}H NMR (D$_2$O) δ 5.4 (ca. 0.15 H, s), 3.78 (3 H, s), 3.49 (2 H, t, J = 5 Hz), 2.60 (2 H, t, J = 5 Hz). Anal. (C$_8$H$_{11}$N$_2$O$_4$Br) C, H, N, Br.

(RS)-3-Methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridine-7-carboxylic Acid (4). Compound 2 (53 mg, 0.18 mmol), dissolved in water (3 mL), was transferred to a column containing an ion-exchange resin [Amberlite IRA-400 (OH), 15 mL]. Evaporation of appropriate fractions and recrystallization (water–ethanol) of crude 4 gave 4 (11 mg, 29%): mp 187 °C dec; IR 3150–2250 (several bands, wm), 1660 (s), 1630 (m), 1525 (s), 1350 (s) cm^{-1}; ^{1}H NMR (D$_2$O) δ 5.0 (ca. 0.25 H, s), 3.78 (3 H, s), 3.37 (2 H, t, J = 5 Hz), 2.53 (2 H, t, J = 5 Hz). Anal. (C$_8$H$_{10}$N$_2$O$_3$·^{2}/$_3$ H$_2$O) C, H, N.

Ethyl α-(Ethoxycarbonyl)-α-acetamido-3-methoxy-5-(dibromomethyl)isoxazole-4-propionate (6) and Ethyl α-(Ethoxycarbonyl)-α-acetamido-5-(bromomethyl)isoxazole-4-propionate (7). A solution of 5[12] (1.65 g, 4.8 mmol), NBS (0.86 g, 4.8 mmol), and benzoyl peroxide (20 mg) in tetrachloromethane (15 mL) was refluxed for 6 h and then filtered and evaporated to give an oil. CC [silica gel, 225 g; eluents, dichloromethane containing butanone (6–15%)] gave 6 (203 mg, 8%): mp 81–83 °C (ethyl acetate–hexane). Anal. (C$_{15}$H$_{20}$N$_2$O$_7$Br$_2$) H, N, C: calcd, 36.02; found, 37.38; Br: calcd, 31.95; found, 30.12. Furthermore, 7 (1.28 g, 63%), mp 143.5–146.0 °C (ethyl acetate–hexane) (lit.[17] mp 147.0–147.5 °C), was obtained. The IR spectrum of 7 was identical with that of an authentic sample.[17]

Ethyl 3-Methoxy-5-(ethoxycarbonyl)-6-acetyl-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-5-carboxylate (8). To a suspension of sodium hydride (45 mg, 1.5 mmol) in dry N,N-dimethylformamide (2 mL), kept at ca. 0 °C, was added, during a period of 1 min, 7 (316 mg, 0.75 mmol). After the mixture was stirred for 30 min at 0 °C, a clear solution was obtained, and after an additional 30 min at 25 °C glacial acetic acid (0.1 mL) was added and the solution evaporated. Upon addition of water (10 mL), the mixture was extracted with chloroform (3 × 15 mL). The combined chloroform phases were dried (MgSO$_4$) and evaporated to give an oil. CC [silica gel, 15 g; eluent, dichloromethane–butanone (10:1)] afforded 8 (134 mg, 53%), mp 97.5–99.0 °C (ethyl acetate–hexane). Anal. (C$_{15}$H$_{20}$N$_2$O$_7$) C, H, N.

(RS)-3-Methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridine-5-carboxylic Acid Hydrochloride (9). A solution of 8 (152 mg, 0.45 mmol) in hydrochloric acid (10 mL, 1 M) was heated to reflux for 2.5 h. After cooling to 25 °C, the solution was extracted with chloroform (2 × 10 mL). The aqueous phase was evaporated to dryness and the residue recrystallized (methanol–ethyl acetate) to give 9 (85 mg, 81%): mp 216 °C dec; IR 3050–2300 (several bands, ms), 1740 (s), 1680 (m), 1525 (s), 1490 (m) cm^{-1}; ^{1}H NMR (D$_2$O) δ 4.4–4.1 (3 H, m), 3.83 (3 H, s), 3.3–2.7 (2 H, m). Anal. (C$_8$H$_{10}$N$_2$O$_4$·HCl) C, H, N, Cl.

(RS)-3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridine-5-carboxylic Acid (5-HPCA) Hydrobromide (10). A solution of 9 (40 mg, 0.17 mmol) in a solution of hydrogen bromide in glacial acetic acid (2 mL, 33%) was kept at 25 °C for 16 h and then evaporated. The residue was recrystallized (ethanol–ethyl acetate) to give 10 (26 mg, 57%): mp 235 °C dec; IR 3100–2400 (several bands, ms), 1730 (s), 1675 (m), 1635 (w), 1560 (m), 1535 (s), 1510 (m) cm^{-1}; ^{1}H NMR (D$_2$O) δ 4.4–4.3 (2

H, m), 4.27–4.10 (1 H, dd, J = 10 and 6 Hz), 3.17–2.56 (2 H, m, J = –15, 10, 6, 2, and 1 Hz). Anal. $(C_7H_8N_2O_4 \cdot HBr)$ H, N, C: calcd, 31.72; found, 32.43; Br: calcd, 30.15; found, 29.62.

(***RS***)-α-**Amino-3-hydroxy-5-(bromomethyl)isoxazole-4-propionic Acid (ABPA) Hydrobromide (11).** A solution of 7 (150 mg, 0.36 mmol) in hydrobromic acid (2 mL, 48%) was heated to reflux for 25 min and then evaporated. The residue was recrystallized (glacial acetic acid–ethyl acetate) to give 11 (75 mg, 60%): mp 196 °C dec; IR 3200–2400 (several bands, ms), 1740 (s), 1675 (s), 1640 (s), 1535 (s), 1490 (s) cm^{-1}; ^{1}H NMR (D_2O) δ 4.56 (2 H, s), 4.34 (1 H, t, J = 6 Hz), 3.12 (2 H, d, J = 6 Hz). Anal. $(C_7H_9N_2O_4Br \cdot HBr \cdot {}^1/_3H_2O)$ C, H, N, Br.

Crystal Structure of (*RS***)-3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic Acid (7-HPCA) Monohydrate.** Crystal data are as follows: $C_7H_8N_2O_4 \cdot H_2O$, M_r 202.17. Monoclinic, space group $P2_1/c$ (no. 14), Z = 4, $F(000)$ = 424, a = 7.614 (3) Å, b = 11.515 (4) Å, c = 9.729 (4) Å, β = 90.87 (3)°, V = 853.0 Å^3, D_c = 1.574 g cm^{-3}, D_m = 1.55 g cm^{-3}, μ(Mo K_α) = 1.27 cm^{-1} (296 K); a = 7.5053 (7) Å, b = 11.445 (3) Å, c = 9.686 (2) Å, β = 90.08 (1)°, V = 832.0 Å^3, D_c = 1.614 g cm^{-3}, μ(Mo K_α) = 1.30 cm^{-1} (105 K).

A single crystal of the size 0.09 × 0.45 × 0.25 mm was used for the determination of the unit cell parameters and for the collection of two sets of intensity data. The measurements were performed at 296 K with a Picker FACS-1 diffractometer and at ca. 105 K with an Enraf-Nonius CAD-4 diffractometer. The crystal was cooled in a stream of nitrogen gas provided by an Enraf-Nonius low-temperature device. The temperature was kept constant within 0.5 K during the experiment. Graphite monochromated Mo K_α radiation (λ = 0.7107 Å) was used in the θ–2θ scan mode to a maximum θ of 30.4° (296 K) and 35.0° (105 K). Of the 2488 (296 K) and 3653 (105 K) independent reflections measured, 1885 ($I_o > 2.0\sigma(I_o)$, 296 K) and 2560 ($I_o > 2.0\sigma'(I_o)$, $\sigma'(I_o) = [\sigma^2(I_o) + (0.08I_o)^2]^{1/2}$, 105 K) were regarded as observed reflections and used in the refinement procedure. The $\sigma(I_o)$ was calculated from counting statistics.

The structure was easily solved by direct methods, and the quantity minimized was $\sum w(|F_o| - k|F_c|)^2$, where the weights were initially taken as unity. The positions of the hydrogen atoms were obtained from difference maps.

In subsequent full-matrix least-squares calculations an overall scale factor, atomic coordinates for all atoms, anisotropic thermal parameters for the non-hydrogen atoms, and isotropic thermal parameters for the hydrogen atoms were refined. The refinements converged at R = 0.046, R_w = 0.048 with w^{-1} = $(2\sigma^2(F_o) + 0.0003F_o^2)$ for the 296 K data set and at R = 0.041, R_w = 0.054 with w^{-1} = $(\sigma^2(F_o) + 0.02 F_o^2)$ for the 105 K data.

Table III lists the final positional and equivalent isotropic thermal parameters. Lists of structure factors and anisotropic thermal parameters of the non-hydrogen atoms are available as supplementary material.

Calculations using the 296 K data were carried out by using the X-RAY 76 program system.[27] Calculations using the 105 K data were carried out by using the Enraf-Nonius Structure Determination Package.[26]

Molecular Mechanics Calculations on 7-HPCA. Since the present version of the MM2 program[25] is unable to handle charged species properly, the calculations have been performed on the unchanged molecule. Besides the parameters already included in the program, the following parameters, primarily for the isoxazole moiety, were used. Stretching parameters: O(1)–N2, K_s = 6.00, bond length = 1.427 Å, N2–C3, K_s = 9.60, bond lengths = 1.317 Å. Bending parameters: C7a–O1–N2, K_B = 0.77, bond angle = 106.9°; O1–N2–C3, K_B = 0.70, bond angle = 105.7°; N2–C3–C3a, K_B = 0.43, bond angle 112.0°; N2–C3–O3, K_B = 0.70, bond angle = 117.3°; C3–O3–H3, K_B = 0.35, bond angle = 110.0°; N2–O1–lone pair, K_B = 0.35, angle 103.26°. Torsional parameters: O1–N2–C3–C3a, V_1 = 0.0, V_2 = 16.25, V_3 = 0.0; O1–N2–C3–O3, V_1 = –2.00, V_2 = 16.25, V_3 = 0.0; N2–C3–C3a–C7a, V_1 = –0.93,

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Table III. Atomic Positions and Equivalent Isotropic Temperature Factors (×10^2 Å^2) Obtained from the X-ray Analyses of 7-HPCA Monohydrate at 296 K (Upper Entries) and at 105 K (Lower Entries)[a]

atom	x	y	z	U_{eq}^b/U_{iso}
O1	0.0243 (1)	0.2505 (1)	0.4831 (1)	3.71
	0.0249 (1)	0.24948 (8)	0.48471 (9)	1.32
N2	–0.1487 (2)	0.2941 (1)	0.5067 (2)	4.01
	–0.1499 (1)	0.29284 (9)	0.5077 (1)	1.49
C3	–0.1624 (2)	0.3863 (1)	0.4279 (1)	2.99
	–0.1656 (2)	0.3866 (1)	0.4293 (1)	1.06
C3a	–0.0034 (2)	0.4081 (1)	0.3508 (1)	2.64
	–0.0056 (1)	0.40924 (9)	0.3528 (1)	0.96
C4	0.0523 (2)	0.4974 (1)	0.2467 (2)	3.15
	0.0487 (2)	0.4994 (1)	0.2487 (1)	1.19
C5	0.2513 (2)	0.4917 (1)	0.2272 (2)	2.97
	0.2505 (2)	0.4935 (1)	0.2283 (1)	1.14
N6	0.3118 (2)	0.3681 (1)	0.2136 (1)	2.80
	0.3116 (1)	0.36926 (8)	0.2150 (1)	1.08
C7	0.2859 (2)	0.2982 (1)	0.3417 (1)	2.63
	0.2889 (1)	0.29957 (9)	0.3438 (1)	0.98
C7a	0.1029 (2)	0.3218 (1)	0.3906 (1)	2.68
	0.1033 (1)	0.32211 (9)	0.3930 (1)	1.00
C8	0.3137 (2)	0.1678 (1)	0.3119 (2)	3.02
	0.3189 (1)	0.1684 (1)	0.3147 (1)	1.11
O81	0.3261 (2)	0.1404 (1)	0.1875 (1)	4.10
	0.3318 (1)	0.14055 (8)	0.18969 (9)	1.51
O82	0.3159 (2)	0.1041 (1)	0.4141 (1)	4.49
	0.3226 (1)	0.10439 (8)	0.41869 (9)	1.58
O3	–0.3118 (1)	0.4453 (1)	0.4348 (1)	3.82
	–0.3168 (1)	0.44612 (8)	0.43673 (9)	1.29
OW	0.6486 (2)	0.3740 (1)	0.1437 (2)	4.79
	0.6520 (1)	0.37546 (8)	0.14920 (9)	1.12
HW1	0.668 (3)	0.447 (2)	0.109 (2)	7.9 (8)
	0.668 (3)	0.444 (2)	0.114 (2)	5.3 (7)
HW2	0.698 (3)	0.322 (2)	0.088 (2)	8.4 (8)
	0.698 (3)	0.322 (2)	0.094 (2)	4.3 (6)
H4a	–0.010 (2)	0.479 (2)	0.158 (2)	4.5 (5)
	–0.015 (2)	0.482 (2)	0.160 (2)	2.2 (5)
H4e	0.023 (2)	0.578 (2)	0.279 (2)	4.2 (5)
	0.016 (2)	0.577 (2)	0.281 (2)	1.8 (4)
H5a	0.311 (2)	0.526 (2)	0.305 (2)	3.5 (4)
	0.313 (2)	0.529 (2)	0.302 (2)	2.2 (5)
H5e	0.294 (2)	0.533 (1)	0.145 (2)	3.3 (4)
	0.291 (2)	0.531 (1)	0.146 (2)	1.2 (4)
H6a	0.254 (3)	0.333 (2)	0.144 (2)	5.2 (6)
	0.251 (2)	0.334 (1)	0.146 (2)	1.5 (4)
H6e	0.436 (3)	0.362 (2)	0.184 (2)	6.2 (6)
	0.435 (3)	0.366 (2)	0.188 (2)	2.9 (5)
H7a	0.374 (2)	0.325 (1)	0.408 (2)	3.4 (4)
	0.382 (2)	0.323 (2)	0.407 (2)	1.2 (4)
H3	–0.308 (3)	0.512 (2)	0.378 (2)	7.8 (7)
	–0.312 (3)	0.504 (2)	0.382 (2)	4.0 (6)

[a] Estimated standard deviations are given in parentheses. [b] For the non-hydrogen atoms, $U_{eq} = {}^1/_3(U_{11} + U_{22} + U_{33} + 2U_{13}\cos\beta)$.

V_2 = 15.00, V_3 = 0.0; N2–C3–O3–H3, V_1 = 1.00, V_2 = 1.65, V_3 = 0.0; N2–C3–C3a–C4, V_1 = –0.27, V_2 = 15.00, V_3 = 0.0; N6–C7–C8–O81, V_1 = 0.40, V_2 = –0.30, V_3 = –0.07; O1–C7a–C7–N6, O1–C7a–C7–C8, N2–O1–C7a–C3a, N2–O1–C7a–C7, N2–C3–O3–lone pair, C3–N2–O1–lone pair, and C3–N2–O1–C7a, V_1 = V_2 = V_3 = 0.0.

Full-energy minimization was performed with respect to all internal coordinates. The starting coordinates were taken from the X-ray structure analyses of 7-HPCA monohydrate.

Microelectrophoretic Studies. Experiments were performed on lumbar dorsal horn interneurons and Renshaw cells of cats anesthetized with pentobarbitone sodium (35 mg/kg intraperitoneally initially, supplemented intravenously when required). Extracellular action potentials were recorded by means of the center barrel of seven-barrel micropipets, which contained 3.6 M NaCl. The compounds were administered electrophoretically from the outer barrels of the micropipets,[19] which contained aqueous solutions: GDEE (0.2 M, pH 3.5), 2APV (0.05 M in 0.15 M NaCl, pH 7.6), NMDA (0.05 M in 0.15 M NaCl, pH 7.6), AMPA (0.1 M, pH 7.3), 7-HPCA (0.1 M, pH 7.3), QUIS (0.005 M in 0.15 M NaCl, pH 7.5), 2 (0.1 M, pH 3.5), 3 (0.1 M, pH 7.3), 4 (0.1 M, pH 7.3), 9 (0.1 M, pH 7.3), 10 (0.1 M, pH 7.3), and 11 (0.1 M, pH 7.3).

The excitatory amino acids were administered for times sufficient to obtain maximal effects at the particular rate of ejection. The relative potencies of the compounds were determined from a comparison of electrophoretic currents required to produce equal and submaximal excitation of the cells, making allowance for the dilution of some amino acids in 0.15 M NaCl. Antagonism was apparent from a slower onset and reduced degree of excitation.

Kainic Acid Binding Studies. The effects of the compounds on kainic acid binding were studied as described earlier[13] on the basis of a published procedure.[18] The membrane preparation was frozen rapidly at $-70\ ^\circ$C and kept at $-20\ ^\circ$C for at least 18 h before use in the receptor-binding assay. For the [^{3}H]kainic acid binding assay procedures, aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate at 4 °C for 5 min in 2 mL of 0.05 M Tris-citrate buffer (pH 7.1) containing 0.005 μM [^{3}H]kainic acid, and the IC_{50} values of the agents tested were determined by using conventional procedures.

Notes

Resolution of 5,6-Dihydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin in Relation to the Structural and Stereochemical Requirements for Centrally Acting Dopamine Agonists

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The enantiomers of 5,6-dimethoxy-2-(*N*,*N*-dipropylamino)tetralin were prepared with use of (+)- and (−)-dibenzoyltartaric acid as the resolving agent. Ether cleavage with BBr$_3$ gave the enantiomers of the dihydroxy compound 5,6-dihydroxy-2-(*N*,*N*-dipropylamino)tetralin (5,6-(OH)$_2$-DPATN). The in vitro activities of (+)- and (−)-5,6-(OH)$_2$-DPATN were evaluated in binding studies with rat striatal tissue with use of [^{3}H]-*N*-*n*-propylnorapomorphine (NPA) as the ligand. IC_{50} (nM) values for (−)- and (+)-5,6-(OH)$_2$-DPATN were 2.5 and 400, respectively. The in vivo efficacy of the enantiomers was evaluated by examining their effects on the metabolism of dopamine in rat striatum. After a 0.5 μmol/kg ip injection of the (−) enantiomer, the concentrations of the metabolites HVA and DOPAC were reduced to 50% of control values, whereas at this dose the (+) isomer was inactive. On the basis of these findings together with the stereochemical data of previously described DA agonists, a dopamine-receptor model has been developed which consists of two binding sites for the amine nitrogen of DA agonists in addition to a major binding site for the *m*-hydroxy group. The relevance of this model with its accessory features is discussed in relation to the structure and pharmacological data of different DA agonists.

In a previous report on dopamine (DA) receptor topography[1] we proposed a model of the DA receptor with the nitrogen and the catechol group of DA as essential sites of interaction with the receptor. From MO calculations of DA and comparison of N–OH distances in active and inactive rigid DA analogues, it was found that in order to exhibit activity, these key distances should be about 7.8 Å for the N–OH$_{para}$ and about 6.4 Å for the N–OH$_{meta}$ distances, respectively. In addition, we suggested the presence of a steric barrier at the receptor site to account for the inactivity of isoapomorphine as a DA agonist. Independently a comparable model was developed by McDermed[2] based upon the absolute configuration of 5-hydroxy-2-(*N*,*N*-dipropylamino)tetralin (5-OH-DPATN) and 6,7-dihydroxy-2-(*N*,*N*-dipropylamino)tetralin (6,7-OH$_2$-DPATN). As 5,6-(OH)$_2$-DPATN is a closer analogue of dopamine than the 5-monohydroxy analogue, we resolved 5,6-(OH)$_2$-DPATN into its optical stereoisomers and tested their activities in vitro as well as in vivo as DA agonists.

The availability of the stereoisomers of one of the most potent DA agonists can be extremely useful for receptor-binding assays of DA agonists, which have become a powerful tool in investigations of DA receptors. In contrast to the ^{3}H-labeled antagonist binding results, data from the ^{3}H-labeled agonist studies are, however, still controversial. Despite the introduction of several binding methods, optimal assay conditions are still being investigated.[3-5] One of the reasons stems from the different definitions of specific binding that have been used. In our opinion, one

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