Affinity and Selectivity of the Optical Isomers of 3-Quinuclidinyl Benzilate and Related Muscarinic Antagonists

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All of the optical isomers of the muscarinic antagonists 3-(1-azabicyclo[2.2.2]octyl) α-hydroxy-α,α-diphenylacetate (3-quinuclidinyl benzilate, QNB, **1**), 3-(1-azabicyclo[2.2.2]octyl) xanthene-9-carboxylate (3-quinuclidinyl xanthene-9-carboxylate, QNX, **2**), and 3-(1-azabicyclo[2.2.2]ocytl) α-hydroxy-α-phenylpropionate (3-quinuclidinyl atrolactate, QNA, **3**) were prepared and studied in binding and functional assays. In all instances the esters of (*R*)-1-azabicyclo[2.2.2]octan-3-ol (3-quinuclidinol) had greater affinity for the M_1 and M_2 subpopulations of muscarinic acetylcholine receptors (M-AChRs) than did their *S* counterparts. The enantiomers of QNB (**1**), QNX (**2**), and QNA (**3**) in which the alcoholic portion of the muscarinic antagonists had the *S* absolute stereochemistry were more selective for the M_1-AChRs. This selectivity was modulated by the nature and, in the case of QNA, the chirality of the acid portion. The most potent isomer in the series was (*R*)-QNB. In the QNA series the diastereoisomer with the absolute *R* configuration of the alcohol (a) and the *R* configuration of the acid (b) was the most potent in both binding and functional assays whereas (*Sa,Rb*)-QNA was the most selective for the M_1 subtype of M-AChRs. In fact, the latter diastereomer was as potent and selective as pirenzepine for M_1-AChRs.

The relationship between the stereochemistry and potency of muscarinic acetylcholine receptor (M-AChR) antagonists has been studied thoroughly in the past.[1] Synthesis of 3-(1-azabicyclo[2.2.2]octyl) α-hydroxy-α,α-diphenylacetate (QNB, **1**)[2,3] provided one of the most potent M-AChR antagonists and led us to investigate some of its analogues.[4,5]

It has been reported[3] and confirmed[6] that the *R*-(−) enantiomer of 1-azabicyclo[2.2.2]octan-3-ol (3-quinuclidinol) leads to esters possessing greater anticholinergic activity than those obtained from the *S*-(+) enantiomer. Sternbach and Kaiser[2] were unable to obtain the *S*-(+) enantiomer of the alcohol in an optically pure form but did resolve some of its derivatives. A method permitting resolution of both enantiomeric alcohols was published 27 years later.[7] The role of chirality of the acid part was addressed when Inch et al.[8] reported the *R,R* isomer of 3-(1-azabicyclo[2.2.2]octyl) α-hydroxy-α-cyclohexyl-α-phenylacetate to be the most potent member of a series of QNB-related compounds.

The heterogeneity of M-AChRs first proposed by Goyal and Rattan[9] was previously based on the affinity of the selective muscarinic antagonist pirenzepine.[10,11] High-affinity pirenzepine binding sites (M_1) that are localized in brain and ganglia were associated with phosphoinositide (PI) breakdown, whereas low-affinity pirenzepine binding sites (M_2) that predominate in peripheral tissues were linked to inhibition of adenylate cyclase (AC).[12,13] More recently, however, both M_1 and M_2 receptor subtypes have been linked to PI breakdown in brain and peripheral tissues.[14-17] We have examined the effect of the enantiomers on PI breakdown in rat cerebral cortex and AC inhibition in rat heart, since robust responses were previously reported in these tissues, which are enriched in M_1 and M_2 receptors, respectively.[18,19]

Apparent affinity constants (K_i's) were determined by using [^{3}H]pirenzepine and the nonselective [^{3}H]QNB as ligands. M_1-AChR (bovine striatal membrane) and M_2-AChR (rat myocardial membranes) rich tissues were used as a source of receptor. The racemic forms of QNX (**2**)[20] and QNA (**3**)[4] showed substantial selectivity for M_1-AChRs. In order to determine the role of chirality of these compounds on their selectivity and potency we prepared all of the isomers of **1–3** and studied them in binding and functional assays. The results of these studies are described (Table I) and discussed in this paper.

Chemistry. Synthesis of the isomers of **1–3** was accomplished by resolution of racemic 3-quinuclidinol[7] and subsequent transesterification of ethyl esters in the previously described manner (method A).[4] Although the specific rotations of the enantiomers of 3-quinuclidinol were in agreement with those reported in Ringdahl et al.,[7] the specific rotations of isomers of QNB prepared in this study were lower than those reported by Meyerhoffer.[6] To prove that the alkaline transesterification did not lead to

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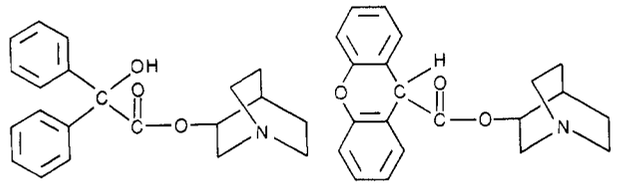

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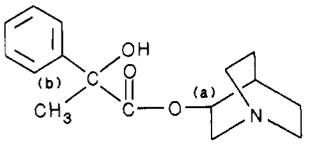

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racemization of 3-quinuclidinol, we also synthesized the enantiomers of QNX (2) by the mixed-anhydride procedure (method B). The difference in the observed specific rotation was negligible. The resolution of racemic atrolactic acid was accomplished by the modification of the classical McKenzie and Clough[21] approach. The enantiomeric purity of the isomeric ethyl atrolactates was determined by chiral HPLC.

Biological Evaluation. The results of biological studies are summarized in Table I. All of the compounds were examined in the receptor-binding and enzyme-linked functional assays. In the binding studies, the affinities for M_1- and M_2-AChRs were measured by the ability of the compounds to displace [^{3}H]pirenzepine and [^{3}H]QNB, respectively. In the enzyme-linked functional assays the reversal of carbachol-stimulated PI hydrolysis[18] and oxotremorine-induced inhibition of adenylate cyclase (AC)[19,22] were measured.

Results and Discussion

Binding studies confirmed the high affinity of *R* isomers of QNB (1) and QNX (2) for both M_1- and M_2-AChRs. Both compounds are therefore nonselective. The difference in the affinity between the enantiomers is more pronounced in the [^{3}H]QNB binding test where (*R*)-1 is 25-fold more potent than (*S*)-1 and (*R*)-2 is 190-fold more potent than (*S*)-2. In the [^{3}H]pirenzepine binding assay, the difference between the affinity of the enantiomers of 1 and 2 is less pronounced. Here, the (*R*)-1 enantiomer is only 3-fold more potent than (*S*)-1 and (*R*)-2 is 26-fold more potent than (*S*)-2. It is the *S* enantiomers of QNB (1) and QNX (2) that show selectivity toward M_1-AChRs. Thus (*S*)-1 and (*S*)-2 show 10.2-fold and 14.0-fold greater affinity for the M_1- vs the M_2-AChR, respectively.

The reduction in size of the acid fragment by replacement of benzilic or xanthene-9-carboxylic acid with atrolactic acid renders all stereoisomers M_1 selective. The most potent stereoisomer of QNA, i.e., (*R*,*R*)-3 is essentially equipotent with (*R*)-1 and (*R*)-2 in displacement of [^{3}H]-pirenzepine binding. Given the reduced size of the lipophilic acid fragment that could indicate either the absence of a hydrophobic bonding region accommodating the second phenyl group of 1, or the second benzo-fused ring of 2, or a better fit of the methyl group of the atrolactic acid fragment into a hydrophobic cavity. The chirality of the acid fragment seems to affect not only the affinity but the selectivity as well. Thus (*R*a,*R*b)-3 is 23.8-fold less potent in displacing [^{3}H]QNB vs [^{3}H]pirenzepine binding. The replacement of the *R* atrolactate fragment with its *S* enantiomer leads to lower affinity for both receptors. In (*S*a,*R*b)-3 the additive effects of 3-quinuclidinol and atrolactate chiralities are observed. The compound is nearly 2400-fold less potent than (*R*)-2 in [^{3}H]QNB binding and 95-fold less potent in [^{3}H]pirenzepine binding. (*S*a,*R*b)-3 is also the most selective isomer in the series.

In functional studies the potency pattern is retained, and the selectivity pattern is generally amplified. In those studies (*R*a,*R*b)-3 is the most potent at the M_1-AChR. The most selective compound, (*S*a,*R*b)-3, is as potent and as selective as pirenzepine.

In conclusion, the structural requirements for potent binding of 3-(1-azabicyclo[2.2.2]octyl) esters to M-AChRs resides in the *R* enantiomers of the alcoholic portion. The selectivity toward M_1-AChRs appears to be vested by the *S* enantiomer when the acid fragment lacks a chiral center. In the case of the QNA (3) the chirality of the atrolactic acid fragment (the *R*a enantiomer) controls the binding potency. Introduction of either antipode of the atrolactic acid fragment renders all of the esters of 3-quinuclidinol selective for the M_1 subpopulation of AChRs. These results suggest that the hydrophobic binding site for M_1-AChRs is less restrictive than is the corresponding location in M_2-AChRs.

Experimental Section

Melting points were determined on microscope slides with a Bristoline apparatus and are uncorrected. IR spectra were recorded with a Beckman FT 1300 spectrophotometer. NMR spectra were recorded with either a Varian EM-360A-60 MHz or a General Electric QE-300 MHz spectrometer. Me_4Si was used as internal standard. Optical rotations were measured with a Perkin-Elmer (241 MC) micropolarimeter.

HPLC was executed with Du Pont Model 8800 and Waters Model 6000A chromatographs by using differential UV detectors set at 254 nm. Chiral Pirkle 1-A columns were provided by Regis Chemical Co. and consisted of a spherical 5 μm α-aminopropyl packing modified with the *N*-(3,5-dinitrobenzoyl)-D-phenylglycine. The columns used had 10-mm i.d. and 250-mm length. The racemic mixture of ethyl atrolactate was resolved in 0.5% 2-propanol/hexane at second pass (recycle) with the flow rate set at 3 mL/min. At second pass α = 1.15 was observed. The resolved ethyl atrolactate enantiomers showed only one peak despite recycle.

Chemistry. Method A. (*R*)-3-(1-Azabicyclo[2.2.2]octyl) α-Hydroxy-α,α-diphenylacetate [(*R*)-1]. (*R*)-(-)-3-Quinuclidinol (5.14 g, 40.4 mmol) was dissolved in 70 mL of benzene and refluxed for 30 min; a Dean–Stark reflux head was used to remove traces of water. A clean piece of sodium metal (ca. 1.0 g, ca. 0.04 g-atom) was added, and the suspension was refluxed for 30 min. The unreacted sodium metal was removed, and the quinuclidinol solution was transferred to a flask that contained ethyl benzilate (5.02 g, 19.6 mmol) in 70 mL of benzene, which also had been refluxed for 30 min under a Dean–Stark reflux head to remove traces of water. The solution was refluxed for 36 h, and the benzene was removed under vacuum. The residue was taken up in ethyl acetate (200 mL) and washed with water (600 mL). The ethyl acetate layer was dried over sodium sulfate and was evaporated to dryness under vacuum. This afforded a pale yellow solid residue. The residue was dissolved in chloroform and chromatographed on silica with chloroform followed by a 7:3 chloroform/methanol solution. The isolated solid was recrystallized from acetonitrile to afford white needlelike crystals (1.1 g, 16.6%), mp 193–196 °C: ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 10 H), 5.1–4.7 (m, 1 H), 3.0–2.3 (m, 6 H), 2.0–1.2 (m, 6 H); IR (KBr) 3433, 3050, 1728, 1232 cm^{-1}; HPLC (RP ODS-2, 6:4:1 MeOH/H_2O/THF) with 5 mM heptanesulfonic acid (pH 3), t_R 6.4 min; TLC (silica, 4:1:1 BuOH/AcOH/H_2O), R_f 0.58; $[\alpha]^{25}_D$ -26.96° (*c* 0.011, 0.2 N HCl). Anal. ($C_{21}H_{23}NO_3$) C, H, N.

(*S*)-(+)-3-(1-Azabicyclo[2.2.2]octyl) α-Hydroxy-α,α-diphenylacetate [(*S*)-1]. The synthesis was carried out as above to yield white needlelike crystals (0.72 g, 8.5% yield), mp 192–194 °C: ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 10 H), 5.1–4.8 (m, 1 H), 3.1–2.4 (m, 6 H), 2.0–1.3 (m, 6 H); IR (KBr) 3438, 3050, 1728, 1232 cm^{-1}; HPLC (RP ODS-2, 60:40:10 MeOH/H_2O/THF) with 5 mM heptanesulfonic acid (pH 3), t_R 6.6 min; TLC (silica, 4:1:1

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Table I. Receptor Binding and Functional Studies of Optical Isomers

compound	binding studies: K_i,[a] nM [^{3}H]pirenzepine (M_1) bovine striatum	[^{3}H]QNB (M_2) rat myocardium	M_2/M_1	functional studies: K_i, nM phosphatidyl inositol turnover (M_1) rat cortex	inhibition of adenylate cyclase (M_2) rat heart	M_2/M_1
(*R*)-**1**	0.19 ± 0.02[b] (1.86)	0.23 (0.99)[c]	1.2	8.4 ± 3.4	2.2 ± 0.6	0.3
(*S*)-**1**	0.57 (1.06)	5.83 (1.08)	10.2	15.4 ± 7.2	123 ± 92	8.0
(*R*)-**2**	0.20 ± 0.08 (1.47)	0.39 ± 0.16 (1.24)	1.9	0.9 ± 0.3	6.9 ± 2.3	7.7
(*S*)-**2**	5.16 ± 1.3 (1.04)	74. ± 17 (1.17)	14.0	27 ± 4.5	1660 ± 1313	61
(*Ra*,*Rb*)-**3**	0.29 ± 0.14 (1.24)	6.9 ± 0.8 (1.18)	23.8	0.8 ± 0.3	153 ± 6	190
(*Ra*,*Sb*)-**3**	2.9 ± 1.2 (0.98)	37 ± 7 (1.06)	13.0	4.3 ± 1.8	1460 ± 622	340
(*Sa*,*Rb*)-**3**	19 ± 5 (1.03	934 ± 259 (1.09)	49.0	11.3 ± 5.3	26700	2362
(*Sa*,*Sb*)-**3**	156 ± 17 (0.98)	1372 ± 154 (1.06)	8.8	87.5 ± 2.3	13700 ± 7156	157
atropine	0.2	0.7	3.5	0.5 ± 0.1	7.5 ± 2.5	12
pirenzepine	3.6	377.0	105	8.1 ± 1.1	18300 ± 410	2316

[a] See the Experimental Section for method of determination and calculation; estimated K_i values are reported. [b] Standard deviations are given following ± sign. [c] All numbers in parentheses are pseudo-Hill coefficients.

BuOH/AcOH/H_2O), R_f 0.59; $[\alpha]^{25}_D$ +27.55° (*c* 0.013, 0.2 N HCl). Anal. ($C_{21}H_{23}NO_3$) C, H, N.

(*R*)-(−)-3-(1-Azabicyclo[2.2.2]octyl) xanthene-9-carboxylate oxalate [(*R*)-2] was prepared from (*R*)-(−)-3-quinuclidinol and methyl xanthene-9-carboxylate by the same procedure used for preparation of (*R*)-**1**. Treatment of the product with oxalic acid in EtOH/Et_2O afforded pale yellow crystals of the oxalate (1.69 g, 16.9%), mp 143–145 °C (from EtOH/Et_2O): ^{1}H NMR ($CDCl_3$) δ 11.3 (m), 7.5–6.9 (m, 8 H), 4.9 (s, 1 H), 5.0–4.7 (m, 1 H), 3.8–2.7 (m, 7 H), 2.2–1.2 (m, 6 H); IR (KBr) 3440, 1741, 1160 cm^{-1}; TLC (RP-C18, methanol:0.5 M ammonium acetate (8:2)) R_f 0.46; $[\alpha]^{25}_D$ −20.84° (*c* 0.0216, 1.0 N HCl). Anal. ($C_{23}H_{23}NO_7$) C, H, N.

(*S*)-(+)-3-(1-Azabicyclo[2.2.2]octyl) xanthene-9-carboxylate oxalate [(*S*)-2] was prepared as described in the preceding experimental to yield pale yellow crystals as the oxalate (1.68 g, 16.1%), mp 141–143 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 8.0 (m), 7.7–7.0 (m, 8 H), 5.2 (s, 1 H), 5.1–4.7 (m, 1 H), 3.7–3.0 (m, 7 H), 2.1–1.4 (m, 5 H); IR (KBr) 3438, 1734, 1162 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.5 M ammonium acetate) R_f 0.53; $[\alpha]^{25}_D$ +20.65° (*c* 0.0335, 1 N HCl). Anal. ($C_{23}H_{23}NO_7 \cdot 0.5H_2O$) C, H, N.

(*R*)-3-(1-Azabicyclo[2.2.2]octyl) (*R*)-atrolactate oxalate [(*Ra*,*Rb*)-3] was prepared from *R*-(−)-3-quinuclidinol and ethyl (*R*)-atrolactate by the same procedure described for preparation of (*R*)-**1**. The product was purified by chromatography on silica with chloroform followed by 9:1 and 7:3 chloroform/methanol solutions. Conversion to an oxalate afforded white crystals (2.80 g, 26.9%), mp 186–188 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 7.6–7.2 (m, 5 H), 5.0 (br, 1 H), 3.7 (m, 1 H), 3.3–2.8 (m, 5 H), 2.2–1.2 (m, 5 H), 1.7 (s, 3 H); IR (KBr) 3361, 1725, 1661, 1255 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.05 M ammonium acetate) R_f 0.46 $[\alpha]^{25}_D$ −47.04° (*c* 0.0111, 1 N HCl). Anal. ($C_{18}H_{23}NO_7 \cdot 0.5H_2O$) C, H, N.

(*S*)-3-(1-Azabicyclo[2.2.2]octyl) (*S*)-atrolactate oxalate [(*Sa*,*Sb*)-3] was prepared as above by using (*S*)-3-quinuclidinol and ethyl (*S*)-atrolactate to afford a white solid oxalate (1.92 g, 18.5%), mp 184–186 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 7.6–7.2 (m, 5 H), 5.0 (br, 1 H), 3.6 (m, 1 H), 3.3–2.9 (m, 5 H), 2.2–1.4 (m, 6 H), 1.7 (s, 3 H); IR (KBr) 3407, 1725, 1638, 1252 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.5 M ammonium acetate) R_f 0.41; $[\alpha]^{25}_D$ +45.16° (*c* 0.0058, 1 N HCl). Anal. ($C_{18}H_{23}NO_7$) C, H, N.

(*S*)-3-(1-Azabicyclo[2.2.2]octyl) (*R*)-atrolactate oxalate [(*Sa*,*Rb*)-3] was prepared as described in the preceding experiment by using (*S*)-3-quinuclidinol and ethyl (*R*)-atrolactate to afford a white solid oxalate (1.64 g, 15.2%), mp 74–76 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 7.6–7.2 (m, 5 H), 5.0 (br, 1 H), 3.6 (m, 1 H), 3.3–2.9 (m, 5 H), 2.1–1.4 (m, 6 H), 1.7 (s, 3 H); IR (KBr) 3407, 1730, 1620, 1239 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.5 M ammonium acetate) R_f 0.51; $[\alpha]^{25}_D$ +6.76° (*c* 0.0314, 1 N HCl). Anal. ($C_{18}H_{23}NO_7 \cdot 0.5H_2O$) C, H, N.

(*R*)-3-(1-Azabicyclo[2.2.2]octyl) (*S*)-Atrolactate Oxalate [(*Ra*,*Sb*)-3]. With (*R*)-3-quinuclidinol and ethyl (*S*)-atrolactate as the starting materials, the synthesis was carried out as detailed previously to afford the oxalate as a white solid (1.98 g, 18.8%), mp 78–80 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 7.6–7.2 (m, 5 H), 5.0 (br, 1 H), 3.6 (m, 1 H), 3.3–2.8 (m, 5 H), 2.2–1.4 (m, 6 H), 1.7 (s, 3 H); IR (KBr) 3394, 1730, 1630, 1239 cm^{-1}; TLC (RP-C18 8:2 methanol/0.5 M ammonium acetate) R_f 0.51; $[\alpha]^{25}_D$ −10.87° (*c* 0.0379, 1 N HCl). Anal. ($C_{18}H_{23}NO_7$) C, H, N.

Method B. (*R*)-(−)-3-(1-Azabicyclo[2.2.2]octyl) Xanthene-9-carboxylate Oxalate [(*R*)-2]. Xanthene-9-carboxylic acid (3.54 g, 15.6 mmol) was added to 45 mL of methylene chloride under argon. The solution was cooled to about 5 °C in an ice bath, and trifluoroacetic anhydride (15 mL, 0.11 mol) was added. The mixture was stirred for 1 h and then evaporated to dryness under vacuum at room temperature. The residue was taken up in 40 mL of methylene chloride and cooled to about 5 °C in an ice bath. (*R*)-(−)-3-Quinuclidinol (2.35 g, 18.5 mmol) was added with 10% aqueous potassium carbonate solution. The methylene chloride layer was dried and evaporated to dryness under vacuum to give an orange-brown oil. The product was taken into chloroform and chromatographed on silica with chloroform followed by a 7:3 chloroform/methanol solution. This afforded, after conversion to an oxalate, white crystals (1.1 g, 16.0%), mp 142–145 °C (from EtOH/Et_2O): ^{1}H NMR ($CDCl_3$) δ 11.3 (s), 7.5–6.9 (m, 8 H), 4.9 (s, 1 H), 5.0–4.7 (m, 1 H), 3.8–2.7 (m, 6 H), 2.2–1.2 (m, 5 H); IR (KBr) 3433, 1736, 1160 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.5 M ammonium acetate) R_f 0.43; $[\alpha]^{25}_D$ −21.77° (*c* 0.0173, 1 N HCl). Anal. ($C_{23}H_{23}NO_7$) C, H, N.

(*S*)-(+)-3-(1-Azabicyclo[2.2.2]octyl) xanthene-9-carboxylate oxalate [(*S*)-2] was prepared as detailed for (*R*)-**2** to yield pale yellow crystaline oxalate (0.76 g, 11.5%), mp 143–145 °C (from EtOH/Et_2O): ^{1}H NMR (DMSO-d_6) δ 9.0 (s), 7.5–6.9 (m, 8 H), 5.2 (s, 1 H), 5.0–4.6 (m, 1 H), 3.7–2.8 (m, 6 H), 2.1–1.4 (m, 5 H); IR (KBr) 3488, 1738, 1160 cm^{-1}; TLC (RP-C18, 8:2 methanol/0.5 M ammonium acetate) R_f 0.52; $[\alpha]^{25}_D$ +20.53° (*c* 0.0271, 1 N HCl). Anal. ($C_{23}H_{23}NO_7 \cdot 0.25H_2O$) C, H, N.

Pharmacology. Receptor Binding Studies. The potency of the isomers **1–3** to inhibit ligand binding to muscarinic cholinergic receptors in rat myocardium (M_2) or bovine striatal membranes (M_1) was determined as follows: male Sprague–Dawley rats (200–250 g) were sacrificed by decapitation, and the myocardium was removed, exsanguinated, weighed, and minced with scissors. The tissue was homogenized (Brinkmann Polytron; setting 5.5; 3 × 20 s) in Tris·HCl buffer (pH 7.7 at 23 °C; 0.05 M) and filtered through three layers of cheesecloth. The homogenate was washed three times by centrifugation (48000*g*; 10 min; 4 °C) with intermittent resuspensions in fresh buffer. The final pellet was resuspended in sufficient fresh buffer to yield a tissue concentration of 2.5 mg/mL for use in the assay.

One milliliter of the tissue suspension was added in triplicate to tubes containing [^{3}H]QNB (SA = 30.1 Ci/mmol; New England Nuclear, Boston, MA) and various concentrations of the test compound in 1 mL of buffer. Final ligand concentration in the assay was 0.2 nM; atropine (10^{-6} M) was used to determine nonspecific binding. Incubations were conducted for 90 min at room temperature, and the reactions were terminated by vacuum filtration with a modified Brandell cell Harvester and Whatman GF/B glass fiber filters. The filters were washed with 2 × 5 mL of ice-cold buffer and radioactivity determined by liquid scin-

tillation counting. Specific [^{3}H]pirenzepine was determined with bovine striatal membranes. Bovine brains were obtained from a local abattoir and transported to the laboratory packed in ice. The striata were dissected immediately and stored frozen (-80 °C) until the day of assay. For assay, the tissue was thawed, weighed, and homogenized (polytron setting 5.5; 30 s) in 20 volumes (w/v) of assay buffer (HEPES·KOH; 0.05 M; pH 7.7). The tissue was washed as described for the [^{3}H]QNB assay, and the final pellet was resuspended in sufficient buffer to yield a tissue concentration of 5 mg/mL. One milliliter aliquots of the suspension were added in triplicate to tubes containing [^{3}H]pirenzepine and various concentrations of the drugs of interest. Final ligand concentration in the assay was 1 nM, and atropine (10^{-6} M) was used to determine nonspecific binding. Incubations were continued for 60 min at 23 °C, and reaction was terminated by vacuum filtration as described previously; however, filters were presoaked at 23 °C for 45–60 min in a solution (0.04%; v/v) of polyethylimine in assay buffer. Filters were rinsed rapidly with 2 × 5 mL of ice-cold buffer, and radioactivity was determined by liquid scintillation counting.

Functional Assays. Male Sprague–Dawley rats (200–250 g) were sacrificed by decapitation, and the cerebral cortices and hearts were removed for PI and AC assays, respectively.

PI Hydrolysis. Accumulated inositol phosphates (IP) were measured as previously described.[18] Briefly, cross-chopped slices (350 × 350 μm) of rat cerebral cortex prepared on a McIlwain tissue chopper were transferred to 20 volumes of Krebs-bicarbonate buffer and incubated for 60 min at 37 °C with gentle shaking. Thereafter, 50-μL aliquots of packed slices were incubated with 5.0 mM LiCl and 0.32 μM myo-2-[^{3}H]inositol for 30 min. Antagonists were added 20 min prior to carbachol addition, and incubations were continued for 60 min. Reactions were terminated by addition of 940 μL of chloroform/methanol (1:2 v/v). Water-soluble inositol phosphates were extracted by a batch technique with a Dowex anion exchange resin. Labeled IPs were eluted with 1.0 M ammonium formate/0.1 M formic acid.

Adenylate Cyclase (AC). Rat heart membranes were prepared immediately prior to use by modification of a procedure described previously.[18] Crudely minced rat heart tissue was homogenized in 10 mM triethanolamine hydrochloride and 145 mM NaCl (pH 7.4) on ice with a Brinkmann Polytron PT 10/35 (setting 5.5, 20 s). The homogenate was filtered through four layers of cheesecloth and centrifuged (30000*g*) for 20 min at 4 °C. The pellet was resuspended in the original volume of buffer using the polytron and centrifuged, and the process was repeated three times to yield a particulate membrane fraction.

For AC assay, about 120 μg of membrane protein was added to a reaction mixture containing a final concentration of 50 mM triethanolamine hydrochloride (pH 7.4), 5.0 mM $MgSO_4$, 50 μM ATP, 50 μM AMP, 1.0 mM dithiothreitol, 1.0 mM 3-isobutyl-1-methylxanthine (IBMX), 10 mM creatine phosphate, 1.4 mg/mL creatine phosphokinase, 90 mM NaCl, 30 μM GTP, and 1.7 μCi of [^{32}P]ATP in a 100-μL final volume. Incubations were carried out for 15 min at 37 °C. Reactions were terminated with 150 μL of a stock solution containing 100 μL of 2% SDS, 40 mM ATP, 1.4 mM cAMP and 50 μL of [^{3}H]cAMP (200 000 cpm) in Tris·HCl, pH 7.4. [^{32}P]cAMP was isolated by column chromatography according to the procedure of Solomon.[22]

[^{3}H]Myoinositol (16 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). [^{32}P]ATP (800 Ci/mmol), [^{3}H]cAMP (33.5 Ci/mmol), [^{3}H]QNB (30.1 Ci/mmol), and [^{3}H]pirenzepine (76 Ci/mmol) were purchased from New England Nuclear (Boston, MA).

Data Analysis. Inhibition constants (IC_{50}'s) were calculated with the EBDA program. Apparent affinity constants (K_i) were determined according to the method of Cheng and Prusoff.[23] Dissociation constants (K_D) for [^{3}H]pirenzepine (2.90 nM) and [^{3}H]QNB (180 pM) were determined in preliminary experiments by using saturation analysis and LIGAND. K_i values for PI assays were determined from Dixon plot analysis[15] or with the Cheng–Prusoff equation.[23] For AC, pA_2 curves were constructed, and K_i values were determined as described by Tallirida et al.[24]

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Registry No. (*R*)-**1**, 62869-69-6; (*S*)-**1**, 62869-68-5; (*R*)-**2**, 114298-73-6; (*S*)-**2**, 114375-05-2; (*Ra*,*Rb*)-**3**, 114298-75-8; (*Sa*,*Sb*)-**3**, 114298-77-0; (*Sb*,*Ra*)-**3**, 114298-79-2; (*Ra*,*Sb*)-**3**, 114298-81-6; (*R*)-(−)-3-quinuclidinol, 25333-42-0; ethyl benzilate, 52182-15-7; methyl xanthene-9-carboxylate, 39497-06-8; ethyl (*R*)-atrolactate, 29916-14-1; (*S*)-3-quinuclidinol, 34583-34-1; ethyl (*S*)-atrolactate, 2406-23-7; xanthene-9-carboxylate acid, 82-07-5; trifluoroacetic anhydride, 407-25-0.

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Preparation of 7-Oxaaporphine Derivatives and Evaluation of Their Dopaminergic Activity

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A series of 7-oxaaporphine derivatives was prepared. The compounds were evaluated as dopaminergic agents. None of them showed either affinity for dopamine receptors or activity in vivo in the climbing behavior (mice) and turning behavior (6-hydroxydopamine-lesioned rats) tests. The lack of activity is tentatively related to the effect of the oxygen atom on the pK_a of these molecules.

Dopamine agonists are useful in treating diseases such as Parkinsonism, Huntington's chorea, galactorrhea, and hyperprolactinemia.[1] Apomorphine (Ia, Table I) is a prototypical dopaminergic agent, but has undesirable effects such as emesis and also a short duration of action. Its pharmacology has been extensively reviewed,[2] and the in-depth studies by Cannon,[3] Neumeyer,[4] and others have

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