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Effects of muscarinic receptor agonists and antagonists on α_2-adrenoceptors in rat brain

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The specific binding of [^{3}H]clonidine to α_2-adrenoceptors on neural membranes isolated from six brain areas was determined with rats treated for various periods of time with the muscarinic agonists, oxotremorine or pilocarpine, or with the muscarinic antagonists atropine, atropine methyl nitrate, scopolamine and scopolamine methyl bromide. Administration of pilocarpine, 10 mg/kg, twice daily i.p. for 1 and 14 days increased markedly the number of α_2-adrenoceptors on neural membranes from all six brain areas. In contrast, oxotremorine, 0.3 mg/kg, twice daily i.p., for 7 days decreased the number of α_2-adrenoceptors on membranes from all brain areas except the brainstem and caudate nucleus. Both atropine and scopolamine increased the density of α_2-adrenoceptors in specific brain areas. Neither atropine methyl nitrate nor scopolamine methyl bromide had an appreciable effect upon the specific binding of [^{3}H]clonidine to neural membranes from most brain areas.

α_2-Adrenoceptors; Muscarinic receptor agonists; Muscarinic receptor antagonists; [^{3}H]Clonidine binding; Brain (rat)

1. Introduction

Recently, long-term treatment of rats with various cholinesterase inhibitors was found to increase markedly the density of α_2-adrenoceptors in various areas of the brain as determined by measuring the specific binding of [^{3}H]clonidine to neutral membranes (Hollingsworth, 1988). The presynaptic α_2-adrenoceptor plays an important role in the regulation of noradrenaline release from neurons (Langer, 1981; Starke, 1981). Very few studies have been conducted, however, to assess the effects of either short- or long-term cholinergic stimulation or blockade on monoaminergic nerve function, and the possibility that cholinergic agonists and antagonists might modify α_2-adrenoceptors in the rat brain has not been evaluated previously.

Oxotremorine, the active metabolite of tremorine, and pilocarpine are cholinergic agonists or partial agonists which have actions within the central nervous system that are blocked by centrally acting muscarinic receptor antagonists (Cho et al., 1962; Cho and Jenden, 1964; Janssen and Niemegeers, 1967). The purpose of the present study was to determine whether short- or long-term administration of pilocarpine and oxotremorine might influence neuronal function by modifying the number and/or affinity of α_2-adrenoceptors in the rat brain in a manner similar to that seen after chronic treatment with cholinesterase inhibitors. In addition, the effects of short- and long-term administration of the centrally acting muscarinic antagonists, atropine and scopolamine, and of their peripherally acting quaternary derivatives, atropine methyl nitrate and scopolamine methyl bromide, were evaluated.

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2. Materials and methods

Groups of 6 male, Sprague-Dawley rats, 170-230 g, were injected intraperitoneally with saline or with various muscarinic agonists and antagonists for 1, 7 or 14 days. The duration of treatment with any specific drug was determined by the ability of the animals to tolerate the drug. Twelve hours after the last injection, the rats were sacrificed by decapitation, their brains removed rapidly and placed in ice-cold Krebs physiological buffer solution and subsequently dissected according to a modification of the method described by Glowinski and Iversen (1966). The Krebs buffer consisted of (mM): NaCl 118; KCl 4.8; $CaCl_2$ 1.3; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25; disodium EDTA 0.03; glucose 10; ascorbic acid 0.06. The following regions were studied: hippocampus, amygdala, hypothalamus, parietal-occipital cortex, caudate nucleus, and an area of the brainstem which contained the locus coeruleus.

2.1. Measurement of specific binding of [^{3}H]clonidine to neural membranes in rat brain homogenates

The specific binding of [^{3}H]clonidine to α_2-adrenoceptors in homogenates was measured as follows. Tissues pooled from the six rats were homogenized in 5 ml of ice-cold Tris-sucrose buffer (Tris-HCl, 5 mM; sucrose, 250 mM; $MgCl_2$, 1 mM; pH 7.4). The homogenates were centrifuged at $1\,100 \times g$ for 10 min, and the supernatants were saved. The supernatants were next centrifuged at $40\,000 \times g$ for 10 min, and the pellet saved. The pellet was washed twice with 2 ml of fresh incubation buffer (Tris-HCl, 40 mM; $MgCl_2$, 10 mM; pH 7.5) and recentrifuged at $40\,000 \times g$ for 10 min. The final pellet was resuspended in an appropriate volume of Tris-incubation buffer. In equilibrium studies total [^{3}H]clonidine binding was measured in 1 ml aliquots of the fresh membranes which were incubated in duplicate for 30 min at 25°C with 0.5 to 64 nM [^{3}H]clonidine. Non-specific binding was determined by adding unlabelled clonidine, 10 μM, as well as the radioligand to a second pair of incubates. This concentration of cold clonidine displaced the labelled ligand to the same degree as maximally effective concentrations of either idazoxan or yohimbine, α_2-adrenoceptor antagonists. Specific binding was defined as the difference between total and non-specific binding. In drug competition studies, the neural membranes were incubated with [^{3}H]clonidine at a concentration of 4 nM in the presence of concentrations of the various muscarinic agonists and antagonists up to and including 100 μM. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10 ml aliquots of Tris-incubation buffer (25°C). After termination of the incubation, the glass fiber filters were air dried and then placed in glass scintillation vials and counted for radioactivity as described by Smith et al. (1972). Protein determinations were performed by the method of Lowry et al. (1951) in which bovine serum albumin was used as the protein standard. A computerized program (McPherson, 1983) which utilizes Scatchard (Scatchard, 1949) and Hill (Hill, 1910) transformations was used to develop preliminary estimates of the apparent dissociation constants (K_D) and the maximum number of binding sites (B_{max}) for the various radioligands. The values obtained from this program were used as preliminary estimates which are required for the non-linear regression analysis program devised by Munson and Rodbard (1980). This program, LIGAND, was used to develop the final values for K_D and B_{max}.

2.4. Drugs used

The following drugs were used: atropine methyl nitrate (Sigma), atropine sulfate (Sigma), clonidine hydrochloride (Boehringer-Ingelheim), [^{3}H]clonidine hydrochloride (New England Nuclear, specific activity 30-41.5 mCi/mmol; radiochemical purity 98%), oxotremorine sesquifumarate (Aldrich), pilocarpine hydrochloride (Sigma), scopolamine hydrobromide (Sigma), and scopolamine methyl bromide (Upjohn). All drugs were dissolved in glass-distilled water, and all doses of drugs are expressed in terms of their salts.

2.5. Analysis of results

For each drug which was studied, values obtained with groups of rats treated for 1 day and

for either 7 or 14 days were compared with an equivalent number of values obtained with the saline-treated control group which was studied concurrently with each drug-treated group. Mean values ± the S.E. are given for each group. Statistical comparisons of groups were by analysis of variance, and P values were generated by the Neuman-Keuls multiple comparison test.

3. Results

3.1. Binding of [^{3}H]clonidine to neural membranes

As reported previously (Smith et al., 1981; Hollingsworth, 1988), the regional distribution of specific binding of [^{3}H]clonidine to neural membranes was uneven (tables 1-6). For rats treated with saline alone, the highest degree of binding occurred with membranes from the amygdala, hypothalamus and parietal cortex. An intermediate degree of binding occurred with membranes from the hippocampus and brainstem, and the lowest binding was found with membranes from the caudate nucleus. Non-specific binding ranged from 5% in the amygdala and parietal cortex to 14% for the caudate nucleus. For individual brain areas, there were no statistically significant differences among the values obtained from the various saline-treated control groups. None of the drug treatments altered the degree of non-specific binding to membranes from any brain area. When added in vitro in concentrations up to 100 μM none of the muscarinic agonists or antagonists used in this study modified the specific binding of [^{3}H]clonidine to neural membranes.

3.2. Effects of muscarinic agonists

Treatment of rats with pilocarpine hydrochloride, 10 mg/kg i.p., twice daily for 14 days increased significantly the binding of [^{3}H]clonidine to neural membranes isolated from all areas of the rat brain studied (table 1). The largest increases occurred with membranes isolated from the amygdala (+122%, $P < 0.02$) and brainstem (+128%, $P < 0.001$). Intermediate increases occurred with membranes from the hypothalamus (80%, $P < 0.0005$), the parietal cortex (+77%, $P < 0.005$) and the hippocampus (+53%, $P < 0.05$). The smallest increase occurred with membranes from the caudate nucleus (+37%, $P < 0.05$).

TABLE 1

Effects of acute and chronic treatment with pilocarpine hydrochloride on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		14 days	
	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)
Amygdala	145.1	2.67	269.5 [a]	3.36	322.3 [b]	3.39
	±10.7	±1.11	±22.0	±0.59	±56.8	±1.45
Hypothalamus	122.1	3.98	254.0 [f]	5.09	220.1 [f]	4.73
	±8.4	±1.61	±16.7	±1.12	±16.4	±1.20
Parietal cortex	109.8	2.06	170.2 [e]	2.80	193.8 [d]	4.04
	±4.4	±0.85	±5.4	±0.34	±18.4	±1.60
Hippocampus	86.1	3.06	132.9 [a]	3.68	132.1 [a]	5.79
	±1.9	±0.62	±6.7	±0.24	±16.6	±1.49
Brainstem	72.1	1.40	155.0 [e]	3.02	164.7 [e]	6.02
	±7.8	±0.14	±14.2	±0.44	±11.5	±1.38
Caudate nucleus	47.7	1.25	69.1 [a]	2.70	65.5 [a]	4.67
	±2.3	±0.27	±2.0	±0.30	±9.0	±1.50

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.005$, [e] $P < 0.001$, [f] $P < 0.0005$. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with pilocarpine hydrochloride, 10 mg/kg i.p., twice daily for either 1 or 14 days. Each point represents the mean ± S.E. of 3-4 determinations.

TABLE 2

Effects of acute and chronic treatment with oxotremorine sesquifumarate on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		7 days	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
Amygdala	146.8	1.18	151.4	2.11	69.3 [c]	0.89
	±5.6	±0.24	±12.6	±0.77	±7.1	±0.07
Hypothalamus	136.4	2.37	111.2	2.00	68.1 [c]	2.04
	±11.1	±0.44	±9.8	±0.02	±10.1	±0.29
Parietal cortex	127.2	1.76	129.6	2.18	78.7 [b]	1.51
	±6.5	±0.39	±15.1	±0.63	±3.1	±0.50
Hippocampus	85.9	2.16	69.6	1.79	40.6 [a]	1.23
	±15.4	±0.34	±3.5	±0.08	±3.1	±0.21
Brainstem	63.9	2.02	69.8	1.24	48.3	2.50
	±10.9	±0.34	±1.6	±0.06	±4.1	±1.08
Caudate nucleus	43.6	1.61	57.7	2.33	31.8	1.95
	±1.6	±0.36	±7.9	±0.30	±3.0	±0.32

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with oxotremorine sesquifumarate, 0.3 mg/kg i.p., twice daily for either 1 or 7 days. Each point represents the mean ± S.E. of 3 determinations.

Short-term treatment of rats with pilocarpine hydrochloride, 10 mg/kg i.p., twice daily for 1 day also increased significantly the binding of [^{3}H]clonidine to neural membranes isolated from all areas of the rat brain (table 1). The largest increases occurred with membranes isolated from the brainstem (+115%, $P < 0.001$), hypothalamus (+108, $P < 0.0005$) and amygdala (+86%, $P < 0.05$). Intermediate increases occurred with membranes from the hippocampus (54%, $P < 0.05$) and the parietal cortex (+55%, $P < 0.002$). The smallest increase occurred with membranes from the caudate nucleus (+45%, $P < 0.01$). Neither short- nor long-term treatment of treatment of rats with

TABLE 3

Effects of acute and chronic treatment with scopolamine hydrobromide on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		14 days	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
Amygdala	149.1	1.73	165.3	1.71	230.5 [b]	1.46
	±15.5	±0.21	±22.3	±0.44	±8.0	±0.06
Hypothalamus	114.7	1.64	152.5	3.07	173.4	3.90
	±10.9	±0.28	±22.1	±0.43	±24.3	±0.96
Parietal cortex	104.8	2.15	139.3 [a]	1.78	152.4 [a]	1.96
	±12.9	±0.53	±8.5	±0.11	±10.5	±0.18
Hippocampus	84.4	2.27	82.6	2.23	114.7 [a]	3.01
	±12.1	±0.64	±5.8	±0.20	±13.7	±0.58
Brainstem	72.1	1.40	88.8	1.66	96.7	2.10
	±7.8	±0.15	±7.6	±0.11	±11.1	±0.40
Caudate nucleus	57.6	2.08	49.9	1.73	64.0	2.17
	±3.5	±0.24	±4.0	±0.05	±9.3	±0.39

[a] $P < 0.05$, [b] $P < 0.01$. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with scopolamine hydrobromide, 3 mg/kg i.p., twice daily for either 1 or 14 days. Each point represents the mean ± S.E. of 4 determinations.

TABLE 4

Effects of acute and chronic treatment with atropine sulfate on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		14 days	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
Amygdala	147.6	2.08	165.5	1.12	180.6 [a]	1.20
	±19.7	±0.37	±20.0	±0.16	±10.1	±0.08
Hypothalamus	133.2	3.45	127.4	2.53	122.5	2.64
	±9.9	±0.71	±17.6	±0.40	±5.3	±0.34
Parietal cortex	119.7	3.08	139.8	1.56	139.5 [a]	1.43
	±9.9	±0.43	±6.8	±0.27	±20.1	±0.08
Hippocampus	91.0	3.21	75.8	1.70	87.9	2.02
	±3.4	±0.32	±9.7	±0.23	±8.9	±0.23
Brainstem	67.0	1.81	84.5	1.54	83.1	1.68
	±5.9	±0.23	±10.5	±0.12	±7.3	±0.18
Caudate nucleus	57.1	2.46	64.6	1.82	60.7	1.63
	±4.8	±0.32	±20.6	±0.20	±8.5	±0.14

[a] $P < 0.01$. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with atropine sulfate, 3 mg/kg i.p., twice daily for either 1 or 14 days. Each point represents the mean ± S.E. of 3-6 determinations.

pilocarpine caused a change in the K_D for the binding of [^{3}H]clonidine to neural membranes from any brain area.

Treatment of rats with oxotremorine sesquifumarate, 3 mg/kg i.p., twice daily for 7 days had an effect upon α_2-adrenoceptor density which was the opposite of that seen with pilocarpine. Chronic treatment with oxotremorine significantly decreased the specific binding of [^{3}H]clonidine to membranes from the amygdala (table 2, −53%, $P < 0.01$), hypothalamus (−50%, $P < 0.001$), parietal cortex (−38%, $P < 0.001$), and hippocampus (−53%, $P < 0.05$). Treatment with oxotremorine was not continued after 7 days be-

TABLE 5

Effects of acute and chronic treatment with scopolamine methyl bromide on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		14 days	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
Amygdala	155.1	1.85	193.5	4.16	151.7	1.30
	±10.2	±0.31	±19.3	±2.62	±6.1	±0.04
Hypothalamus	134.3	4.39	166.6	2.97	96.6	2.01
	±10.8	±0.76	±22.3	±0.12	±7.8	±0.31
Parietal cortex	123.8	3.28	130.1	1.52	131.7	1.58
	±6.9	±0.87	±7.1	±0.21	±6.1	±0.10
Hippocampus	88.5	3.25	84.4	2.21	80.0	2.06
	±4.7	±0.28	±9.2	±0.17	±7.6	±0.46
Brainstem	74.6	1.94	86.2	2.19	60.2	1.15
	±12.1	±0.80	±12.1	±0.27	±2.8	±0.07
Caudate nucleus	51.8	2.28	52.9	1.60	47.6	1.76
	±4.5	±0.34	±3.0	±0.05	±5.8	±0.17

Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with scopolamine methyl bromide, 3 mg/kg i.p., twice daily for either 1 or 14 days. Each point represents the mean ± S.E. of 3-4 determinations.

cause the animals were unable to tolerate the drug for longer periods of time. Treatment of rats for 1 day with oxotremorine, 3 mg/kg i.p., every 12 h, did not alter significantly the specific binding of [^{3}H]clonidine to neural membranes from any of the brain areas which were studied. Neither short- nor long-term treatment with oxotremorine produced a significant change in the K_D for the binding of [^{3}H]clonidine to membranes from any brain area.

3.3. *Effects of muscarinic antagonists*

Both scopolamine and atropine had qualitatively similar effects upon the specific binding of [^{3}H]clonidine to membranes isolated from various brain areas. Treatment of rats with scopolamine hydrobromide, 3 mg/kg i.p., twice daily for 14 days, increased significantly the specific binding of [^{3}H]clonidine to membranes from the amygdala (+55%, $P < 0.05$), parietal cortex (+45%, $P < 0.05$) and hippocampus (+36%, $P < 0.05$, table 3). Treatment for 1 day significantly altered binding only with membranes isolated from the parietal cortex (+33%, $P < 0.05$).

Administration of atropine sulfate, 3 mg/kg i.p., twice daily for 14 days, significantly increased the specific binding of [^{3}H]clonidine to membranes isolated from the rat parietal cortex (table 4, +17%, $P < 0.5$).

In contrast to scopolamine, scopolamine methyl bromide, 3 mg/kg i.p., twice daily for 14 days did not alter binding to membranes from any area of the rat brain (table 5). Administration of atropine methyl nitrate, 3 mg/kg i.p., twice daily for 14 days, decreased the specific binding of [^{3}H]clonidine but only to membranes isolated from the hypothalamus (−28%, $P < 0.05$, table 6). Treatment with either antagonist for 1 day was associated with no significant changes in binding. None of the muscarinic antagonists, when administered for either 1 or 14 days, altered the K_D for clonidine binding to neural membranes from any brain area.

4. Discussion

Previously, the chronic administration of cholinesterase inhibitors, neostigmine, triorthocresyl phosphate (TOCP), diisopropylfluorophosphate (DFP) and paraoxon, was found to increase markedly the density of α_2-adrenoceptors in various areas of the rat brain (Hollingsworth, 1988).

TABLE 6

Effects of acute and chronic treatment with atropine methyl nitrate on the specific binding of [^{3}H]clonidine to neural membranes isolated from various areas of the rat brain.

Brain area	Controls		1 day		14 days	
	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D
Amygdala	146.5	1.87	124.9	1.31	165.1	1.32
	±7.5	±0.38	±11.7	±0.13	±19.7	±0.18
Hypothalamus	132.5	3.12	105.7	2.34	95.2 [a]	2.27
	±6.2	±0.70	±8.5	±0.20	±11.7	±0.17
Parietal cortex	107.2	3.07	94.7	1.77	118.6	2.00
	±10.2	±0.63	±7.6	±0.21	±13.3	±0.27
Hippocampus	80.3	2.04	75.8	1.74	84.0	2.23
	±6.4	±0.37	±9.9	±0.20	±9.7	±0.12
Brainstem	64.7	1.78	76.5	1.80	69.4	1.41
	±5.5	±0.20	±8.7	±0.27	±8.1	±0.19
Caudate nucleus	50.6	2.08	41.7	1.92	34.8	1.79
	±2.5	±0.24	±4.7	±0.51	±6.0	±0.22

[a] $P < 0.05$. Binding parameters were estimated by use of the non-linear curve-fitting program, LIGAND (Munson and Rodbard, 1980). Animals were treated with atropine methyl nitrate, 3 mg/kg i.p., twice daily for either 1 or 14 days. Each point represents the mean ± S.E. of 5-7 determinations.

In the present study, similar increases in the density of α_2-adrenoceptors in specific areas of the rat brain were found after both acute (1 day) and chronic (14 days) treatment of rats with pilocarpine, a muscarinic agonist with pronounced cholinergic activity both within and outside of the central nervous system. The magnitude of the increases in receptor density and the distribution of the receptors in the brain which were affected were very similar to those seen after long-term administration of cholinesterase inhibitors. The observations with pilocarpine would suggest that the changes in α_2-adrenoceptor density in the rat brain after long-term administration of cholinesterase inhibitors were due to stimulation of muscarinic cholinergic receptors.

In contrast, the administration of oxotremorine for 7 days resulted in marked decreases in α_2-adrenoceptor density in all brain areas studied except the brainstem. Oxotremorine presumably produces its characteristic tremor by a mechanism which involves an action of catecholamines upon β-adrenoceptors in the rat brain (Hallberg and Almgren, 1987). It is possible that the changes in α_2-adrenoceptor number seen in the present study are secondary to increases in the synaptic content of catecholamines within the brain. Oxotremorine has been shown to deplete brain noradrenaline stores in rats (Rogers and Slater, 1971), increase brain noradrenaline turnover in rats (Corrodi et al., 1967), and increase brain dopamine turnover in rats (Nose and Takemoto, 1974) and cats (Laverty and Sharman, 1965). Also, oxotremorine increases markedly plasma catecholamine content when administered to rats (Weinstock et al., 1978, 1980). Long-term administration of other drugs, such as the tricyclic antidepressants, which are thought to increase synaptic catecholamine content in the brain, also decreased the specific binding of [^{3}H]clonidine to α_2-adrenoceptors in the same brain areas as those affected by oxotremorine (Smith et al., 1981; Smith and Hollingsworth, 1983; 1984). Thus, the changes in the density of α_2-adrenoceptors in rat brain after long-term oxotremorine administration might reflect sustained increases in synaptic catecholamine content.

Differences in efficacy in stimulation of muscarinic receptors or in selectivity for various types of muscarinic receptors might explain the differing effects of the two muscarinic agonists, oxotremorine and pilocarpine, upon the density of α_2-adrenoceptors in various areas of the rat brain. Pilocarpine has relatively low efficacy upon presynaptic muscarinic receptors on noradrenergic neurons in the rabbit iris (Bognar et al., 1988), similar to that reported for gastrointestinal smooth muscle (Furchgott and Bursztyn, 1967). Both oxotremorine and pilocarpine are partial agonists upon muscarinic receptors which stimulate phosphoinositol turnover in rat cerebral cortical membranes (Fisher et al., 1983; Freedman et al., 1988). However, Evans et al. (1985) reported that oxotremorine was considerably more efficacious than pilocarpine in stimulating both phosphoinositol turnover and unidirectional efflux of $^{45}Ca^{2+}$ in human astrocytoma cells. In embryonic chick heart cells, oxotremorine is a full agonist upon muscarinic receptors which inhibit catecholamine-stimulated cyclic-AMP formation but devoid of agonistic activity on those muscarinic receptors which stimulate phosphoinositol turnover (Brown and Brown, 1984). Radioligand binding experiments with rat cerebral cortical membranes designed to evaluate efficacy of muscarinic agonists demonstrated a good correlation for pilocarpine but none for oxotremorine between binding and stimulation of phosphoinositol turnover (Freedman et al., 1988). These results were interpreted to indicate that the receptor selectivity of oxotremorine differed from that of pilocarpine. Thus, although differences in agonist efficacy might explain the difference between oxotremorine and pilocarpine observed in the present study, differences in receptor selectivity might also be a factor.

Considerable evidence exits that there are more than one type of muscarinic receptor in the nervous system (Hammer et al., 1980; Watson et al., 1983; Luthin and Wolfe, 1984; Evans et al., 1985). In the isolated guinea-pig ileal preparation pilocarpine appears to stimulate a population of muscarinic receptors which differs from that stimulated by oxotremorine. Pilocarpine has been found to enhance the release of acetylcholine from neurons in the guinea-pig myenteric plexus

whereas oxotremorine inhibited acetylcholine release from this preparation (Kilbinger, 1984; Kilbinger and Wagner, 1975). Kilbinger (1984) has speculated that there are two types of presynaptic neuronal muscarinic receptors, one which is stimulated by pilocarpine to enhance neurotransmitter release and the other which is stimulated by oxotremorine to inhibit neurotransmitter release. It is possible that similar differences in selectivity for muscarinic receptors in the central nervous system could be responsible for the differences between the changes in α_2-adrenoceptor density caused by pilocarpine and those caused by oxotremorine.

In general, administration for 14 days of the centrally active muscarinic antagonists, atropine sulfate and scopolamine hydrobromide, produced changes in α_2-adrenoceptor density similar to those seen with administration of pilocarpine for 1 or 14 days, and opposite to those seen with administration of oxotremorine for 7 days. The increases in receptor density seen with atropine sulfate and scopolamine hydrobromide appear to be mediated through an action within the central nervous system since neither of their quaternary derivatives, which do not readily penetrate the central nervous system, produced similar results when administered chronically. The results of the present study suggest that atropine and scopolamine might block the actions of endogenous acetylcholine at muscarinic receptors preferentially stimulated by oxotremorine rather than those preferentially stimulated by pilocarpine.

The possibility that these muscarinic antagonists interact directly with α_2-adrenoceptors must be considered. A number of studies have shown that certain muscarinic antagonists possess significant affinity for α_1-adrenoceptors (Cantor et al., 1983; Varma and Yue, 1986). However, the present study confirms the findings of Varma and Yue (1986) that neither scopolamine nor atropine have significant affinity for α_2-adrenoceptors in rat brain.

The present study shows that muscarinic receptor stimulation and blockade result in appreciable changes in the density of α_2-adrenoceptors in various areas of the rat brain. Furthermore, it suggests that muscarinic receptor subtypes preferentially stimulated by either pilocarpine or by oxotremorine or differences in relative efficacy of muscarinic agonists might be factors related to these changes in receptor number. Whether the changes in α_2-adrenoceptor density represent changes in presynaptic or postsynaptic α_2-adrenoceptors and the functional significance of such changes remain to be determined.

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