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Muscarinic receptors discriminated by pirenzepine are involved in the regulation of neurotransmitter release in rat nucleus accumbens

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1 The effect of pirenzepine, a selective muscarinic antagonist, was tested on the oxotremorine facilitation of the K^+-evoked release of [^{14}C]-dopamine from tissue slices of rat nucleus accumbens.

2 The effect of pirenzepine was compared with that of scopolamine and other antagonists which show no heterogeneity in their action on muscarinic receptors in order to determine whether a selective action at a single receptor subtype, M_1 or M_2, could be distinguished.

3 Pirenzepine and scopolamine both antagonized the oxotremorine-induced ($EC_{50} = 3 \times 10^{-7}$ M) facilitation of [^{14}C]-dopamine release with pA_2 values of 7.5 and 8.9 respectively. This result indicated that the high affinity pirenzepine receptor (M_1) was involved in this response. Low concentrations of 3-quinuclidinyl benzilate (3×10^{-10} M), N-methylscopolamine (3×10^{-9} M) and methyl atropine (10^{-8} M) also abolished this facilitatory effect of oxotremorine.

Introduction

Cholinoceptor agonists modulate the release of noradrenaline through muscarinic receptors in peripheral structures such as the heart (Fuder, 1982), and the release of dopamine in the central nervous system, the nucleus accumbens and corpus striatum (Giorguieff *et al.*, 1977; de Belleroche & Gardiner, 1982). In addition, muscarinic receptors are involved in the presynaptic control of acetylcholine release from peripheral autonomic nerves of myenteric plexus, parasympathetic neurones of heart and iris (Gustafsson *et al.*, 1980; Alberts *et al.*, 1982; Kilbinger, 1984) as well as central cholinergic neurones of cerebral cortex, hippocampus, corpus striatum and nucleus accumbens (Szerb & Somogyi, 1973; Molenaar & Polak, 1980; de Belleroche & Gardiner, 1982). The question arises as to whether these receptors modulating transmitter release can be characterized by their affinity for agonists or selective antagonists. Analysis of agonist binding indicates that three populations of binding site are present in varying amounts in different tissues which have affinity constants that differ by approximately three orders of magnitude (Birdsall *et al.*, 1980). The functional significance of the three affinity binding sites is not fully understood nor is it known whether they mediate separate physiological actions. Pirenzepine discriminates between muscarinic receptors present on sympathetic ganglia and those in ileum (Brown *et al.*, 1980), with high affinity antagonism of muscarinic-induced depolarization in sympathetic ganglia ($pA_2 = 8.3$) but low affinity antagonism ($pA_2 = 6.5–7.0$) of cholinoceptor-mediated contraction and slowing of rat heart (Barlow *et al.*, 1981). High affinity sites for pirenzepine (M_1-receptor subtype) predominate in sympathetic ganglia and low affinity sites (M_2-receptors) are found on noradrenergic nerves in rabbit and rat heart and on cholinergic neurones in guinea-pig ileum (Hammer & Giachetti, 1984). The M_1-receptor subtype is also concentrated in discrete brain regions such as cerebral cortex, corpus striatum and hippocampus (Watson *et al.*, 1983) and as with the low affinity agonist binding site in cerebral cortex, agonist binding is regulated by guanine nucleotides (Birdsall & Hulme, 1983; Birdsall *et al.*, 1984).

In this study, the antagonistic effects of pirenzepine on the oxotremorine-induced facilitation of the K^+-evoked release of dopamine in nucleus accumbens have been compared with those of scopolamine and other antagonists.

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Methods

Male CFY rats (250 g body weight) were stunned, killed by cervical dislocation and brain removed. Two coronal cuts were made at right angles to the axis of the brain, the first 1 mm rostral to the optic chiasma and the second at 1.5 mm rostral to the first cut. The nucleus accumbens was dissected out from this section a rat brain atlas (König & Klippel, 1963) being used for reference.

Tissue slices of nucleus accumbens were cut in a plane parallel to the coronal section (0.35 mm thickness approximately) and immediately immersed in Krebs bicarbonate medium of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4 7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1, pargyline 1.0, ascorbate 2.3, pH 7.4 gassed with 95% O_2:5% CO_2. The initial incubation of tissue slices was carried out in the presence of 4 μM [7-^{14}C]-dopamine (57 mCi mmol^{-1}) at 37°C for 30 min. After preincubation in medium containing isotope the slices were transferred to a perspex block containing 8 tissue chambers and superfused with isotope-free Krebs bicarbonate medium maintained at 37°C, at a rate of 1 cm^3 min^{-1} for a further 30 min to reach a steady baseline of release. Tissue slices were then transferred after the washout period to Krebs bicarbonate medium (1 ml) under control or test conditions, incubated for 10 min at 37°C and transferred to fresh medium containing 34 mM K^+ in the presence or absence of drugs, as indicated, for a further 5 min incubation period. At the end of incubation, tissue slices were removed and aliquots of media taken for analysis of ^{14}C by liquid scintillation counting. Aquasol 2 (New England Nuclear Chemicals) was used as the scintillant. The ^{14}C content of the tissue slices was also determined after they had been dissolved in Soluene 350 (Packard Instrument Company, Inc.). Efflux of transmitter is expressed as the fractional release min^{-1}, i.e. the total amount released in each incubation period as a percentage of the total remaining in the tissue at the beginning of the time period divided by the time of incubation in min. Basal efflux rates have not been subtracted from the rates in the presence of K^+. The assay procedure has been described previously in detail (de Belleroche & Neal, 1982; de Belleroche & Gardiner, 1982; 1983).

Concentration-response curves were plotted of the effect of oxotremorine on the K^+-evoked release of [^{14}C]-dopamine (% control response in the presence of K^+) in the presence and absence of scopolamine and pirenzepine. The concentration of half maximal response was calculated by linear regression analysis where necessary. The shift in EC_{50} or IC_{50} as log (dose-ratio − 1) was plotted against the negative log of the antagonist concentration according to Arunlakshana & Schild (1959) and the pA_2 values were calculated by linear regression analysis.

Pirenzepine, N-methylscopolamine bromide (NMS), methylatropine bromide, decamethonium

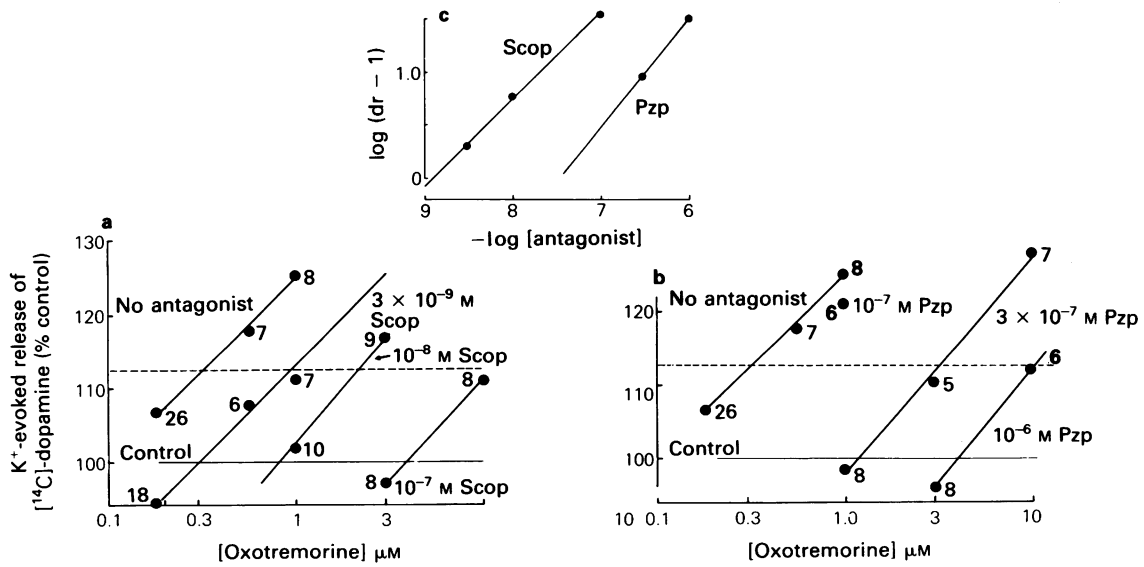

Figure 1 Concentration-response curves for the effect of oxotremorine (log scale) on the 34 mM K^+-evoked release of [^{14}C]-dopamine from tissue slices of rat nucleus accumbens (a) in the presence of scopolamine (Scop) and (b) in the presence of pirenzepine (Pzp). Values are means for the number of experiments indicated. The dotted line indicates the half maximal effect of oxotremorine. (c) The inset above is a Schild plot of the shift in EC_{50} as log (dose-ratio − 1) against log of antagonist concentration.

diiodide and 3-quinuclidinylbenzilate (QNB) were gifts from Dr N.J.M. Birdsall. QNB was dissolved in HCl and all other drugs were dissolved in incubation medium.

Results

Effect of oxotremorine on the release of [^{14}C]-dopamine from tissue slices of nucleus accumbens

Oxotremorine (10^{-7}–10^{-5} M) facilitated the K^+-evoked release of [^{14}C]-dopamine from tissue slices of nucleus accumbens (Figure 1). The maximal effect produced by oxotremorine is an increase of 25% ($EC_{50} = 3 \times 10^{-7}$ M) as previously observed (de Belleroche & Gardiner, 1982). This facilitatory effect was antagonized by both scopolamine (Figure 1a) and pirenzepine (Figure 1b). The pA_2 values for scopolamine and pirenzepine, determined using a Schild plot, were 8.9 and 7.5 respectively (Figure 1c).

Table 1 Effect of cholinoceptor antagonists on the oxotremorine-induced facilitation of [^{14}C]-dopamine release from rat nucleus accumbens

	K^+-evoked release of [^{14}C]-dopamine (% control)
Oxotremorine (5.6×10^{-7} M)	124.2 ± 5.7 (23)
Oxotremorine (5.6×10^{-7} M) + QNB (3×10^{-10} M)	105.3 ± 8.6 (9)
Oxotremorine (5.6×10^{-7} M) + NMS (3×10^{-9} M)	103.8 ± 9.0 (8)
Oxotremorine (5.6×10^{-7} M) + methylatropine (10^{-8} M)	87.3 ± 11.4 (6)
Oxotremorine (5.6×10^{-7} M) + decamethonium (10^{-6} M)	102.7 ± 9.8 (7)

Tissue slices of rat nucleus accumbens were incubated as described in the legend to Figure 1. The K^+-evoked release of [^{14}C]-dopamine in the presence of drugs is expressed as a percentage of that obtained in the absence of drugs for each animal. Values are means ± s.e.means for the number of experiments in parentheses. NMS = N-methylscopolamine and QNB = quinuclidinyl benzilate.

The resting release of [^{14}C]-dopamine was unaffected by the drug concentrations used in this study.

The antagonistic effect of scopolamine was also shown by other muscarinic antagonists. Thus, low concentrations of QNB (3×10^{-10} M), NMS (3×10^{-9} M) and methyl atropine (10^{-8} M) abolished the facilitatory effect of oxotremorine on the K^+-evoked release of [^{14}C]-dopamine (Table 1).

Discussion

In this study, scopolamine was shown to have a potent effect in antagonizing the action of oxotremorine on [^{14}C]-dopamine release. The pA_2 value is very similar to that obtained for the antagonism of the action of oxotremorine on smooth muscle contraction and acetylcholine release in guinea-pig myenteric plexus (Halim *et al.*, 1982; Kilbinger, 1984), and atrial tension and noradrenaline release from sympathetic nerves in heart (Fuder, 1982). From these findings, it is likely that a homogeneous population of muscarinic receptors mediate these actions of scopolamine. The potent effect of pirenzepine in antagonizing the action of oxotremorine on [^{14}C]-dopamine release indicates that the high affinity pirenzepine binding site is involved in this effect. The pA_2 value of 7.5 obtained in the nucleus accumbens is greater than that found for pirenzepine in ileum and heart (6.5–7.0) where the low affinity binding site predominates. However, in sympathetic ganglia a slightly higher pA_2 value is obtained (8.3).

These results indicating that high affinity pirenzepine binding sites are involved in the regulation of [^{14}C]-dopamine release are consistent with this being the predominant receptor subtype found in brain regions such as cerebral cortex and corpus striatum (Birdsall *et al.*, 1984). Furthermore, this receptor subtype may play a significant functional role since it is reduced in rat cerebral cortex following lesion of the ascending cholinergic projection by kainate injection into substantia innominata (de Belleroche *et al.*, 1985).

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