Muscarinic receptors: ionic perturbation of the binding properties

N.J.M. BIRDSALL, A.S.V. BURGEN, E.C. HULME & J.W. WELLS

Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA

Both in brain synaptosome preparations and in intact smooth muscle, the binding pattern of antagonists to the muscarinic receptor differs sharply from that of agonists. Whereas the former pattern is described adequately by a simple mass action isotherm, the latter exhibits a functional heterogeneity characterized by flat binding curves exhibiting minimum Hill slopes as low as 0.3. To a first approximation, this pattern can be analysed on the basis of a model (Birdsall & Hulme, 1976) in which two classes of non-interconverting binding sites exist within the receptor population. In both cases, the binding of agonists and antagonists is competitive and mutually exclusive (Birdsall, Burgen, Hiley & Hulme, 1976; Hulme, Burgen & Birdsall, 1976). In the present investigation, it has been possible to perturb the affinity of both agonists and antagonists for brain synaptosome preparations by altering the ionic composition of the medium.

The cerebral cortex from male Wistar rats was used to prepare a P_2 pellet containing approximately 1.4 nmol of muscarinic receptor per gram of protein. The pellet was resuspended in appropriate media and binding studies were carried out at 2°C using equilibrium dialysis, a centrifugation assay, or rapid filtration through glass fibre filters. Antagonist binding was measured directly with N-[^{3}H]-methyl-atropine (2.2 Ci/mmol), N-[^{3}H]-methyl-scopolamine (3.3 Ci/mmol), or N-[^{3}H]-propyl-benzilylcholine (40 Ci/mmol).

The binding of antagonists to the receptor is sensitive to the ionic strength. For (–)-N-[^{3}H]-methyl-scopolamine, the value of pK_d decreases from 10.88 in isotonic sucrose-sodium phosphate (10 mM, pH 7) to 9.94 in Krebs–Henseleit solution and to approximately 9.6 in isotonic sucrose-sodium (10 mM, pH 7) containing 1M NaCl. The effects of NaCl, KCl, and $CaCl_2$ are quantitatively similar when compared as a function of ionic strength. In contrast, the trivalent cation La^{3+} causes a greater reduction in pK_d at low ionic strength than do either the monovalent or the divalent ions. With antagonists, neither the shape nor the maximum amplitude of the binding curve is perturbed.

When measured in Krebs–Henseleit solution by the competitive displacement of N-[^{3}H]-propyl-benzilylcholine, the binding curve for the agonist carbachol can be resolved into a high ($pK_d = 7.1$) and a low ($pK_d = 4.5$) affinity component as reported previously (Birdsall *et al.*, 1976; Hulme *et al.*, 1976). As with antagonists, the agonist binding curve is shifted to lower ligand concentrations at low ionic strength. In addition, however, there is a concomitant steepening of the curve. This is caused by the change in the apparent pK_d at the low affinity site ($\Delta pK_d = 1.2$) exceeding that at the high affinity site ($\Delta pK_d = 0.6$). The relative proportion of the two sites appears to remain unchanged. The failure to observe changes in this ratio argues against a facile interconvertibility between the two receptor populations.

References

BIRDSALL, N.J.M., BURGEN, A.S.V., HILEY, C.R. & HULME, E.C. (1976). Binding of agonists and antagonists to muscarinic receptors. *J. Supramol. Struct.*, **4**, 367–371.

BIRDSALL, N.J.M. & HULME, E.C. (1976). Biochemical studies on the muscarinic acetylcholine receptors. *J. Neurochem.*, **27**, 7–16.

HULME, E.C., BURGEN, A.S.V. & BIRDSALL, N.J.M. (1976). Interactions of agonists and antagonists with the muscarinic receptor. *INSERM*, **50**, 49–70.

Muscarinic acetylcholine receptors and cyclic GMP in rat brain

M.R. HANLEY & L.L. IVERSEN

MRC Neurochemical Pharmacology Unit, Department of Pharmacology, University of Cambridge

Slices of rat corpus striatum (250 μm × 250 μm) were incubated as described previously (Minneman & Iversen, 1976), and cyclic (c) GMP was measured in boiled tissue extracts by a radioimmunoassay procedure (Radiochemical Centre, Amersham). In parallel experiments the binding of [^{3}H]-quinuclidinyl benzilate (QNB) (1.4 nM) was measured, using the same slice preparation incubated for 30 min at 20°C. Specific binding of [^{3}H]-QNB was defined as that portion which could be displaced by 1 μM atropine (70–80% of total binding) (Yamamura & Snyder, 1974).

The agonists oxotremorine, arecoline and carbachol caused increases in tissue cGMP with similar time courses and maximum effects, amounting to an approximate doubling over resting cGMP levels (0.7 pmol/mg protein). The cGMP increase reached a maximum after 2 min and declined rapidly thereafter. The cGMP response to oxotremorine (100 μM) was not blocked by α-bungarotoxin or by hexamethonium

Table 1 Comparison of drug effects on cGMP response and [^{3}H]-QNB binding

Agonist	*EC_{50}–μM for cGMP increase*	*Ki for inhibition ^{3}H-QNB binding—μM*
Oxotremorine	5.6	6.4
Arecoline	22.0	61.0
Carbachol	120.0	115.0
Antagonist	*IC_{50}–nM against cGMP (100 μM oxotremorine)*	*Ki for ^{3}H-QNB binding–nM*
Scopolamine	5.6	5.8
QNB	23.0	5.0
Atropine	54.0	7.7
Clozapine	600.0	350.0
Thioridazine	1300.0	940.0
Chlorpromazine	9300.0	7700.0

(10 μM), but was completely prevented by atropine (1 μM) or QNB (0.1 μM). The muscarinic response was abolished if calcium ions were omitted from the incubation medium, and was additive to the increase in cGMP elicited by sodium azide.

The agonists oxotremorine, arecoline and carbachol yielded EC_{50} values for increasing GMP which agreed well with the corresponding values for these compounds in displacing [^{3}H]-QNB binding (Table 1). The cGMP response thus appears to correspond directly to muscarinic receptor occupancy.

The muscarinic antagonists QNB, scopolamine and atropine and the neuroleptic drugs thioridazine, clozapine and chlorpromazine inhibited the cyclic GMP response to 100 μM oxotremorine. The potencies of the various antagonists in the cGMP and [^{3}H]-QNB tests showed a less close agreement than seen for agonists, and calculated apparent Ka values suggested that the antagonists were more potent in blocking the cGMP response than in displacing [^{3}H]-QNB.

References

MINNEMAN, K.P. & IVERSEN, L.L. (1976). Enkephalin and opiate narcotics increase cyclic GMP accumulation in slices of rat neostriatum. *Nature, (Lond.).*, **262**, 313–314.

YAMAMURA, H.I. & SNYDER, S.H. (1974). Muscarinic cholinergic binding in rat brain. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1725–1729.

Hyperpolarization of myenteric neurones by enkephalin

R.A. NORTH

Neurophysiology Laboratory, Department of Pharmacology, Loyola University Medical Center, Maywood, Illinois 60153, U.S.A.

In the guinea-pig myenteric plexus, morphine and the enkephalins inhibit the neuronal firing which is induced by a glass suction electrode (Dingledine & Goldstein, 1976; North & Williams, 1976). The inhibitory action of morphine in the myenteric plexus has been attributed to a membrane hyperpolarization (Dingledine & Goldstein, 1976; North & Tonini, 1976). The aim of the present experiments was to determine the effects of the enkephalins upon the membrane properties of myenteric neurones.

Intracellular recordings were made from myenteric ganglia which were removed from the ilea of adult guinea-pigs. The isolated ganglia were immobilized and perfused as previously described (Nishi & North, 1973). Cells were impaled under visual control (Nomarski optics, × 500) with glass micro-electrodes which contained 2M potassium chloride. Drugs were applied by changing the perfusing solution to one which differed only in its content of the drug(s). The majority of Type 1 cells were hyperpolarized by met-enkephalin (100–300 nM). The hyperpolarization began as soon as the met-enkephalin reached the tissue and continued throughout the period of exposure (up to 5 min) and rapidly reversed when the tissue was washed with drug-free Krebs solution. The hyperpolarization ranged in amplitude from 3 to 40 mV, the larger effects being more often seen in cells with low resting membrane potentials. In most cells the hyperpolarization was associated with a fall in membrane resistance—this was evident as a reduction in the amplitude of the membrane potential