Muscarinic receptors: ionic perturbation of the binding properties

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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.

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Muscarinic acetylcholine receptors and cyclic GMP in rat brain

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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