The kinetics of action of acetylcholine antagonists on guinea-pig ileum

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Several workers, e.g. Paton (1961), Paton & Rang (1965), have used the observed rates of onset and offset of antagonism to calculate the antagonist-receptor association and dissociation rate constants, k_1 and k_2. This involves the assumption that access of drug to the receptors is not rate-limiting. We have performed three types of experiment with the very slow antagonist benziloyl tropine methyl iodide (BTrMe) and the results indicate that some form of access limitation is involved.

Pieces of guinea-pig ileum were suspended in Tyrode solution, at 37° C, through which air was bubbled. The contractions produced by carbachol were recorded isotonically. The experiments were analysed on the assumption that dissociation was rate-limiting and the results compared with the predictions of this model.

1. Onset of and recovery from various concentrations of antagonist were followed by adjusting the concentration of carbachol, as described by Paton & Rang (1965), to keep the responses within a narrow range. The antagonist occupancy at any time was calculated from the observed dose ratio. Although occupancy changed exponentially during both onset and offset as predicted by the dissociation-limited model, apparent values of k_2, calculated from the offset rate constant, varied with the concentration of BTrMe—an increase in concentration from 10×10^{-10} M to 40×10^{-10} M, increased the apparent value of k_2 from 0.52×10^{-4}/s to 2.38×10^{-4}/s—whereas the dissociation-limited model predicts that the calculated value of k_2 should be constant. This model also predicts that the ratio of k_1, calculated from the onset rate constant, to k_2 should be equal to the affinity constant, calculated from the equilibrium dose ratio. This was found to be so only at low concentrations of antagonist.

2. The decrease in occupancy of BTrMe produced when a concentration of the 'fast' antagonist, *n*-pentyl triethylammonium iodide, was superimposed was followed as before. Although occupancy decreased exponentially, apparent values of k_2, calculated from the rate constant, increased as the concentration of fast antagonist was increased to a limiting value of 8.33×10^{-4}/s. According to the dissociation-limited model the calculated value of k_2 should be constant.

3. Experiments were also performed with these two antagonists in which, as the pentyl triethylammonium was added, the concentration of BTrMe was increased so that its occupancy at equilibrium with the pentyl triethylammonium was the same as that produced by the lower concentration alone. Contrary to the predictions of the dissociation-limited model, a transitional stage was observed.

These observations are not consistent with the dissociation-limited model and so some form of access limitation must be involved. Also antagonist-receptor rate constants cannot be calculated from kinetic measurements made under these conditions.

This conclusion is supported by the results of similar experiments with lachesine.

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Aldosterone, moulting and the number of sodium channels in frog skin

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In amphibian skin aldosterone causes an increase in the transepithelial transport of sodium (Crabbé, 1964) and in addition induces a moult (Nielsen, 1969). During the moult the stratum corneum separates from the stratum granulosum, and the slough is shed. The newly moulted skin has different characteristics from normal skin in that there is an increase in sodium transport (Nielsen, 1969), and the blocking action of amiloride on transport is inhibited (Nielsen & Tomlinson, 1970). This abstract reports attempts to discover

the mechanisms of these changes.

Aldosterone was injected into the lymph sacs of frogs (Rana temporaria, 70 μg per frog) which were then placed in individual tanks containing running deionized water, overnight. Afterwards the ventral skin was dissected free and the stratum corneum peeled off. Animals which had already shed the stratum corneum were rejected. The skins were mounted horizontally in perspex cells for voltage clamping at zero potential and for labelling experiments. [^{14}C]-amiloride was used to label sodium channels in the mucosal surface of the skins, as described previously (Cuthbert, 1973).

It was confirmed that newly moulted skins were less sensitive to the blocking actions amiloride, although the sensitivity increased to normal ($Km = 2 \times 10^{-7}$ M, for skins bathed in solutions containing 111 mEq/l Na^+) within 4 hours. Omission of calcium from the mucosal bathing solution increased the insensitivity to amiloride in newly moulted skins, and delayed the recovery of sensitivity. Since treatment of normal skins with EGTA-Ringer reduces or abolishes the effect of amiloride (Cuthbert & Wong, 1972) it is considered that interaction of calcium with newly exposed (or induced) sodium channels is, at least, part of the mechanism involved before the blocking action of amiloride can be fully developed.

The number of sodium channels, measured in the presence of 1.1 mEq/l Na^+, in newly moulted skins was $712 \pm 59/\mu m^2$ (four determinations), a significant increase ($P < 0.05$) on the number for normal skins ($201 \pm 14/\mu m^2$, 15 determinations). Treatment of normal skins with EGTA in the absence of calcium and in the presence of 1.1 mEq/l Na^+ did not reduce the amount of amiloride bound. For example, in one group of normal skins exposed to ^{14}C-amiloride (10^{-8} M) the amount bound was 0.1 ± 0.01 pmole/9.6 cm^2 (four determinations) and the mean inhibition of transport was $22.9 \pm 1.4\%$. In the absence of calcium the amount bound was 0.15 ± 0.01 pmole/9.6 cm^2 (seven determinations) while transport was inhibited by only 4%. In four skins which moulted spontaneously the amount of amiloride bound was 0.44 ± 0.03 pmole/9.6 cm^2, in the absence of calcium.

The observations suggest that the increase in sodium transport following moulting is due to an increase in the density of sodium channels in the mucosal surface. Whether this results from uncovering occluded channels, or by induction of new channels by aldosterone is not yet clear.

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Voltage-clamp experiments on the potential-dependent behaviour of membrane ion channels operated by the muscarinic receptor of smooth muscle

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Stimulation of the muscarinic receptor increases the conductance of the cell membrane of smooth muscle (Bolton, 1972). The present experiments were done to discover if the additional conductance which appears when the muscarinic receptor is activated, varies as the membrane potential is charged.

Experiments were done on strips of smooth muscle either from guinea-pig uterus (after previous treatment with 200 μg oestradiol benzoate) or from guinea-pig ileum. The strips were 5 mm x 50-100 μm x 100-300 μm in size and were introduced into a double sucrose-gap apparatus (Rougier, Vassort & Stämpfli, 1968). In this, potential is recorded across one sucrose-gap (width 0.6-0.8 mm) while current can be passed across the other. The portion of active tissue ('node') between the sucrose gaps was 100-200 μm wide. Nodal potential was clamped using a conventional voltage-clamp circuit.

After clamping at the resting membrane potential, rectangular potentials of different sizes were imposed either in a hyperpolarizing or in a depolarizing direction. The currents required to clamp the potential at values positive to the resting membrane potential were changing only slowly