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.

References

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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