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THE EFFECT OF VARYING CALCIUM CONCENTRATION ON THE KINETIC CONSTANTS OF HYOSCINE AND MEPYRAMINE ANTAGONISM

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The kinetic constants for the interaction of hyoscine and mepyramine with the receptors of the longitudinal muscle of the guinea-pig ileum have so far been measured on intact ileum, and in the presence of a bathing solution of approximately normal composition (Paton, 1961). The use of the longitudinal muscle in isolation presents certain advantages for analytic work, particularly when the ionic composition of the bathing fluid is varied, since it avoids the risk that the mucous membrane or circular muscle could function as an ionic reservoir. In the present paper, earlier experiments with hyoscine and mepyramine on the whole gut are repeated for the longitudinal strip, as a preliminary to testing how far with an antagonist the drug-receptor interaction depends on the calcium concentration.

METHODS

The kinetic constants for onset (k_1), and offset (k_2), of antagonistic action, as well as the equilibrium constant (k_e), were determined on the isolated longitudinal muscle of the guinea-pig ileum, dissected and mounted as described in the preceding paper (Paton & Rothschild, 1965). The sensitivity of the preparations in the normal, high and low calcium media was tested by determining the dose/response curve over a range of 1.0 to 10 or 20 ng of acetylcholine per ml., and a dose, usually of 5.0 ng/ml., was selected as the standard. Rate of onset of antagonism was determined by treating the muscle with a given dose (x) of antagonist, and measuring its decreasing sensitivity to the stimulant by finding the dose of the latter required to match the standard response. A series of dose ratios (DR) could thus be obtained and the corresponding values determined of receptor occupancy, $p = (DR-1)/DR$. Equilibration of the preparation with the dose of antagonist given was considered to have been reached when responsiveness to the same dose of stimulant no longer decreased in a consistent manner after eight or ten trials. If p_e is receptor occupancy at equilibrium, the difference (p_e-p) represents the extent to which occupancy falls short of its maximum value at any given moment.

Since $p = \frac{x}{x+k_2/k_1}[1-e^{-(k_1+x_3)t}]$, the time constant of onset of antagonism, (k_1x+k_2), is obtained from the slope of the line relating log (p_e-p) to time. From this, together with k_2, k_1 was calculated.

k_2, the rate constant for the dissociation of the drug from its receptor sites, was obtained in a similar manner by determining the decreasing sequence of dose ratios representing loss of antagonism following wash-out. According to the equation, $p = \frac{x}{x+k_2/k_1}e^{-k_2t}$, a plot of log p against time will yield a line of slope k_2.

k_e, the equilibrium constant of the drug-receptor reaction, was obtained from the equation, $k_e = x/(DR-1)$.

The doses of drugs quoted are in terms of their salts, acetylcholine bromide, hyoscine bromide and mepyramine maleate.

RESULTS

The kinetic constants for the action of hyoscine

The results of nine experiments on the kinetic constants for hyoscine, tested at 2.2, 4.4 and 8.8×10^{-10} g/ml., are shown in Table 1. The calculated value for k_e, obtained from

TABLE 1

KINETIC CONSTANTS OF THE ACTION OF HYOSCINE ON THE ISOLATED LONGITUDINAL MUSCLE OF THE GUINEA-PIG ILEUM

	k_e (g/ml.$\times 10^{-10}$)	k_1 ($s^{-1}g^{-1}ml.\times 10^6$)	k_2 ($s^{-1}\times 10^{-4}$)	k_2/k_1 (g/ml.$\times 10^{-10}$)
	0·59	2·60	2·00	0·76
	0·88	3·00	1·45	0·48
	0·63	3·14	2·03	0·95
	0·88	1·53	1·52	1·00
	0·88	1·27	1·92	1·51
	0·98	2·14	3·06	1·41
	1·47	2·80	2·50	0·89
	1·76	2·60	1·66	0·64
	1·06	1·38	1·66	1·20
Mean$\pm$s.e.	1·01$\pm$0·13	2·27$\pm$0·22	1·98$\pm$0·19	0·98$\pm$0·11

the ratio k_2/k_1, is also shown; it did not differ significantly from the value of the equilibrium constant obtained by direct measurement. It is worth noting that the average kinetic constants for hyoscine, determined on the isolated longitudinal muscle preparation, differed by at most 30% from equivalent values obtained using the guinea-pig intact ileum preparation (Paton, 1961).

Effect of calcium deficiency on the kinetic constants of hyoscine

Fig. 1 shows the changes in the values of the kinetic constants of hyoscine brought about by changing the concentration of calcium in the perfusion fluid. Alteration of this factor over a tenfold range failed to affect the value of the equilibrium constant, so that the extent of receptor occupation, provided equilibration with the antagonist had been reached, was independent of the concentration of calcium in the medium.

In contrast, the rate constants both of onset and offset of the antagonism were found to be significantly reduced ($P<0.05$) when the concentration of calcium was decreased to one-fifth of its normal value. This change caused both constants to drop to approximately 50% of their values in the normal medium. An intermediate drop of approximately 25% was observed when the calcium concentration had been decreased to 0.4 of its normal value, but this effect lacked statistical significance.

A limited number of experiments were also performed to test the effect of calcium deficiency on the kinetic constants of the antihistaminic action of mepyramine. Only effects on k_e, the equilibrium constant, and on k_2, the rate constant of the offset of antagonism, were determined. Fig. 2 shows that the latter was markedly decreased in the low calcium media, whilst, as with hyoscine, the equilibrium constant remained the same over a fivefold range of alteration of the calcium concentration.

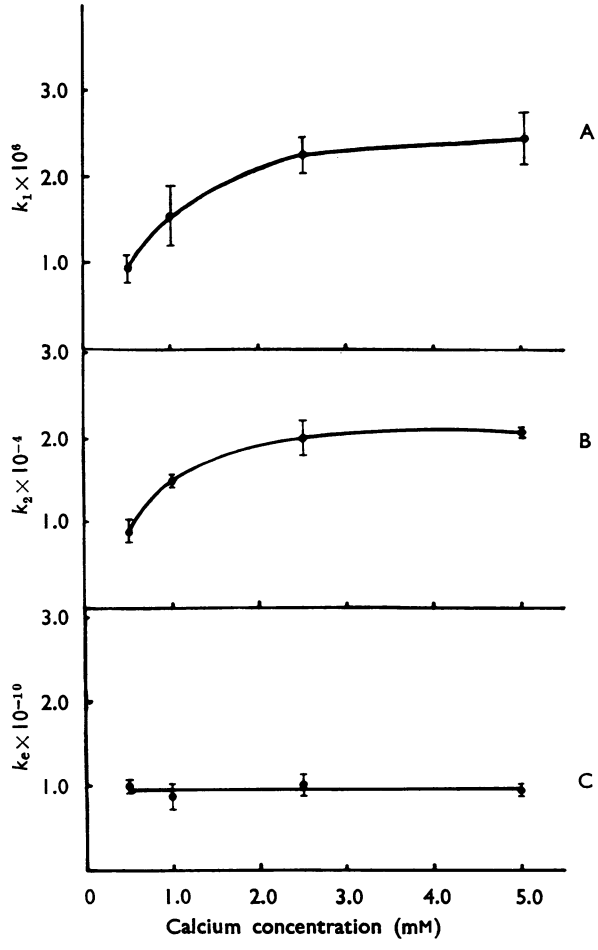

Fig. 1. Effect of calcium on the rate constants of onset and offset and on the equilibrium constant for the action of hyoscine bromide on the isolated longitudinal muscle of the guinea-pig ileum. A, Onset rate constant (k_1); B, offset rate constant (k_2); C, equilibrium constant (k_e). Points represent mean values and standard errors and were obtained from two, eight, four and three experiments performed with muscles from different animals at respectively 5.0, 2.5, 1.0 and 0.5 mM-calcium. Ordinates: A, $k_1 \times 10^6$; B, $k_2 \times 10^{-4}$; C, $k_e \times 10^{-10}$. Abscissa: calcium concentration, mM.

DISCUSSION

The results of these experiments can be briefly stated: that calcium does not influence the equilibrium state of the drug-receptor reaction, but modifies equally the forward and reverse reaction rates. Change of calcium concentration differs in this respect from change of temperature; if the tissue is cooled, the dissociation rate constant declines faster than the association rate constant, so that the potency of the antagonist increases (Paton, 1964). Our knowledge of the state of the drug molecule at the receptor and of the receptor itself is too scanty to justify much speculation about the mechanism underlying this calcium effect. It is as though calcium ion catalyses the drug-receptor reaction, reducing the energy

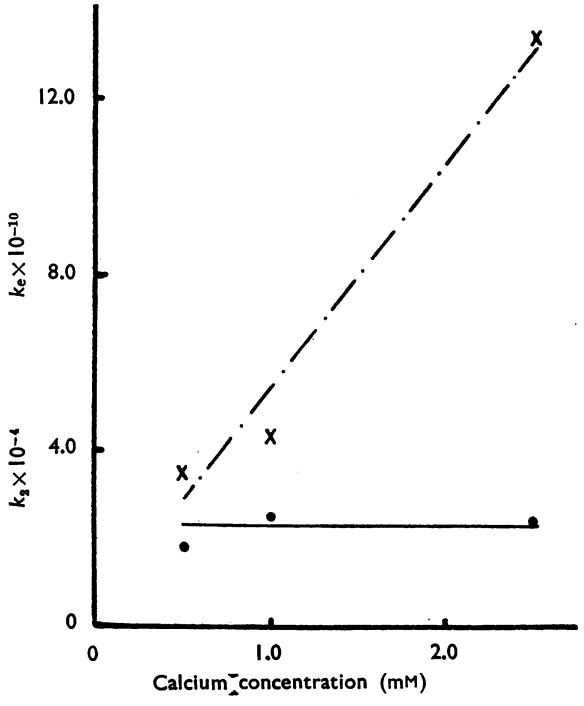

Fig. 2. Effect of calcium on the rate constant of offset and on the equilibrium constant for the action of mepyramine against histamine. ×——×, Offset rate constant; ●——●, equilibrium constant. Points represent mean values and standard errors and were obtained from two, two and one experiments performed with muscle from different animals at respectively 2.5, 1.0 and 0.5 mM-calcium. Ordinate: $k_2 \times 10^{-4}$; $k_e \times 10^{-10}$. Abscissa: calcium concentration, mM.

required to form an activated drug molecule-receptor complex. Further, the calcium effect appears to be brought about by some saturable reaction, with an equilibrium constant of approximately 1 mM. One envisages, therefore, a calcium-binding site associated with the receptor area, and facilitating receptor reactivity.

SUMMARY

1. The kinetic rate constants for the antagonism of hyoscine against acetylcholine have been determined on strips of longitudinal muscle from the guinea-pig ileum exposed to various calcium concentrations.

2. Calcium deficiency reduces both forward and backward rate constants but not the equilibrium constant. Similar findings were obtained with mepyramine. The results are interpreted in terms of a calcium-binding site associated with the receptor area which facilitates receptor reactivity.

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