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A possible role for cyclic AMP in the regulation of histamine secretion and the action of cromoglycate

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The secretion of histamine from mast cells and basophil leucocytes is inhibited by the application of substances (adenylate cyclase activators, phosphodiesterase inhibitors and cyclic AMP analogues) which lead to the accumulation of intracellular cyclic AMP [1–7] and it has been shown that the antiallergic drug, cromoglycate inhibits cyclic AMP phosphodiesterase from several tissues [8].

Application of a specific antigen to sensitized mast cells triggers the secretion of histamine in a calcium-dependent manner [9, 10]. The secretion of histamine is also accompanied by an increase in calcium uptake by the mast cells, and cells made selectively permeable to calcium by treatment with the divalent cation carrier substance (ionophore), A 23187, release histamine when subsequently provided with calcium [11]. It has been suggested that the antigen-antibody reaction on the mast cell membrane opens calcium gates in the membrane [12], and the response of the mast cells to calcium following antigen stimulation, decreases rapidly with time; indicating that the gates do not remain open. The response of the cells to calcium has almost completely disappeared 4 min after antigen stimulation [13]. It has therefore been proposed that the control of histamine secretion following the antigen-antibody stimulus is brought about by time-dependent changes in the calcium permeability of the mast cell membrane. It must be pointed out, however, that whereas histamine secretion is complete in 1 min, the decay of the response to calcium following antigen stimulation is not complete before 4 min although it is substantially manifest after 1 min. Supporting the idea that the decay in the response to calcium is related to the control of histamine secretion is the observation that phosphatidyl serine prolongs the release process and slows the decay of the calcium response [13, 14]. In view of these observations, it was of interest to examine the role of cyclic AMP in relation to the action of calcium in rat peritoneal mast cells.

The source of the cells together with the methods of sensitizing them and of inducing and measuring histamine release from them have been described [10, 15]. Dibutyryl cyclic AMP (Sigma) produced a graded inhibition of antigen-evoked histamine release as the concentration was raised in the range from 10 μmoles/l to 10 m-moles/l (Fig. 1).

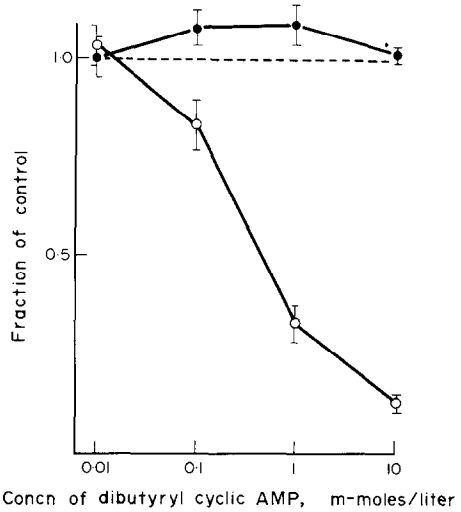

Fig. 1. Concentration–response relationship for the action of dibutyryl cyclic AMP on histamine release induced by antigen, 10 μg/ml (○) and A 23187, 5 μmoles/l (●) from the same cell population. Response is expressed as a fraction of control histamine release from cells suspended in a medium containing calcium, 1 m-mole/l and no dibutyryl cyclic AMP. Control histamine releases were: 22 ± 5 per cent (mean ± S.D.) of total for antigen and 77 ± 4 per cent of total for A 23187. Cells with or without dibutyryl cyclic AMP were preincubated for 30 min at 37° before the addition of the histamine releasing agent. Each point is the mean from four experiments and the vertical bars represent S.E.M.

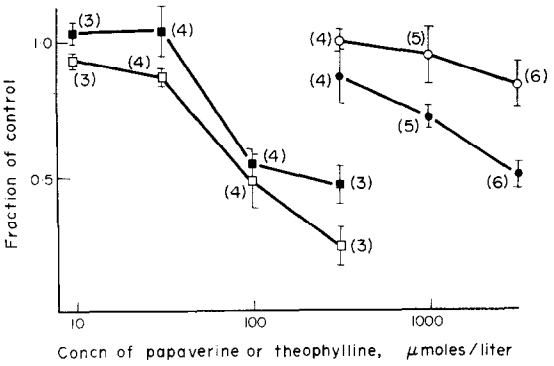

Fig. 2. Concentration–response relationship for the action of theophylline (○) and papaverine (□) on histamine release induced by antigen, 10 μg/ml (filled symbols) and A 23187, 5 μmoles/l (open symbols) from the same cell population. Response is expressed as a fraction of control histamine release from cells suspended in a medium containing calcium, 1 m-mole/l and no papaverine or theophylline. Control histamine releases were: 26 ± 13 per cent of total (mean ± S.D.) for antigen and 66 ± 12 per cent of total for A 23187. Cells with or without papaverine and theophylline were preincubated for 30 min at 37° before the addition of histamine releasing agent. The number of experiments contributing to each point is indicated by the figures in parentheses and the vertical bars represent S.E.M.

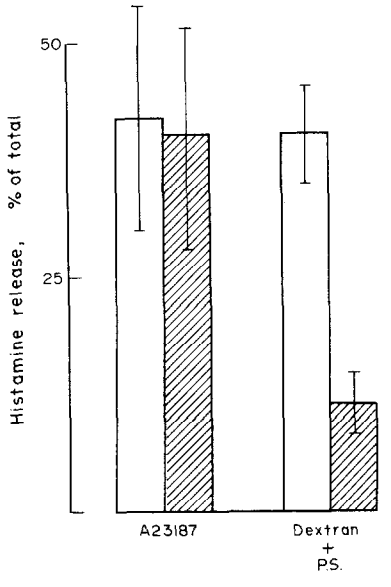

Fig. 3. The effect of cromoglycate, 60 μmoles/l (▨) on histamine release induced by either the ionophore, A 23187, 2·5 μmoles/l or by a mixture of dextran, 6 mg/ml and phosphatidyl serine (PS), 10 μg/ml. Open columns are control histamine releases where no cromoglycate was added. In each case the cromoglycate was mixed with the releasing agent before addition to the cells. Each column is the mean of five experiments and the vertical bars represent S.E.M.

However, these concentrations of dibutyryl cyclic AMP failed to produce any inhibition of histamine release mediated by the calcium ionophore, A 23187. Cyclic AMP and AMP, at a concentration of 1 m-mole/l, failed to produce any inhibition of histamine release evoked by antigen.

The effects of the two phosphodiesterase inhibitors, papaverine (B.P. 1973) and theophylline (B.D.H.) are shown in Fig. 2. Papaverine, in concentrations of 30–3000 μmoles/l inhibited histamine release induced by both antigen and A 23187. Theophylline in the concentration range 0·3–3·0 m-moles/l inhibited antigen-evoked histamine release, but failed to produce a significant inhibition of histamine release mediated by A 23187. The inhibition of antigen-induced histamine release produced by theophylline was not reversed by four-fold increases of the calcium ion concentration in the incubation medium. It should be pointed out that although the effects of dibutyryl cyclic AMP and theophylline on histamine release are measured after 30 min preincubation, further experiments have shown that they are effective after 1 min preincubation: the effect at 1 min being approximately a third of that at 30 min.

Histamine release induced by either antigen or dextran is inhibited by cromoglycate [15, 16], and Fig. 3 shows that a concentration of cromoglycate (60 μmoles/l) which inhibits histamine release induced by dextran does not produce any inhibition of histamine release mediated by the ionophore. In a further 21 experiments, cromoglycate (60 μmoles/l) was without effect on histamine release induced by A 23187 (0·5–2·5 μmoles/l).

Assuming that dibutyryl cyclic AMP, by gaining access to sites within the cell, mimics the action of intracellular cyclic AMP, it follows from our results that raised intracellular levels of cyclic AMP prevent antigen-induced histamine release, but do not affect the release induced by the calcium ionophore, A 23187. Both antigen stimulation and the ionophore appear to trigger histamine secretion by increasing the permeability of the mast cell membrane to calcium [11, 12], though of course the calcium 'channel' is different in each case. The observed effect of dibutyryl cyclic AMP can thus be interpreted in terms of an action of intracellular cyclic AMP on the calcium gate opened by the antigen-antibody reaction.

Similarly, if it is assumed that theophylline raises intracellular levels of cyclic AMP, the observation that it inhibits antigen-evoked histamine release but not ionophore-induced release suggests that cyclic AMP prevents the opening of the calcium gates following antigen stimulation. In our experiments, cromoglycate behaves in a manner similar to dibutyryl cyclic AMP and theophylline, and in view of the report that this drug is a cyclic AMP phosphodiesterase inhibitor [8] it would be interesting to investigate further the proposal that cromoglycate acts by preventing calcium entry into the mast cell, possibly by raising intracellular cyclic AMP.

It is possible that the methylxanthines such as theophylline and caffeine act directly on the calcium gate [17] but if this is so our experiments indicate that theophylline and calcium do not simply compete for the receptor site since the inhibitory effect of theophylline is not overcome by raising the calcium concentration. According to the calcium gate model, the phosphodiesterase inhibitor papaverine would be expected to inhibit antigen-evoked but not ionophore-mediated histamine release. In fact, papaverine was equipotent in inhibiting the release mediated by both agents but we believe that papaverine is exerting one of its many other actions in this situation [18]. Papaverine has been shown to inhibit oxidative phosphorylation [19] and this

would certainly lead to inhibition of histamine release induced by both antigen and A 23187 since both evoke histamine release which is dependent on an intact mechanism for ATP synthesis [11, 20].

Our results are consistent with the hypothesis that intracellular cyclic AMP regulates the calcium gating mechanism which is supposed to control histamine secretion. Measurements of intracellular cyclic AMP and calcium uptake are needed to investigate this further.

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Department of Pharmacology, University College London, London, WC1E 6BT — J. C. FOREMAN, J. L. MONGAR

Department of Experimental Pathology, University College Hospital Medical School, London, WC1E 6JJ — B. D. GOMPERTS

Pharmacology Laboratory, Wellcome Research Laboratories, Langley Court, Beckenham, BR3 3BS, England — L. G. GARLAND

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Trialkyl phosphates and phosphorothiolates—Lack of hydrophobic interaction with acetylcholinesterase

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Several publications from this laboratory [1–3] and Russian laboratories [reviewed in Ref. 4] have indicated that trialkyl phosphates, phosphorothiolates and phosphonothiolates are potent anticholinesterases when at least one alkyl chain is of adequate length. This effect has been attributed to hydrophobic interaction of the inhibitor with the enzyme, resulting in high affinity in the reversible (K_d) step, despite the anticipated low reactivity in the irreversible (k_2) step; the process may be depicted as:

$$(R'O)_2P(O)XR + EOH \overset{K_d}{\rightleftharpoons} (R'O)_2P(O)XR \cdot EOH \xrightarrow{k_2} (R'O)_2P(O)OE + RXH$$

where X = *O* or *S*, EOH represents the enzyme, $K_d (= k_{-1}/k_1)$ is the dissociation constant for the first (binding) step and k_2 is the phosphorylation rate constant.

Recently, I. B. Wilson* raised doubts concerning the kinetic feasibility of the k_2 step in view of the poor leaving-group character of *SR* and *OR* moieties, while Gumbman and Williams [5] have reported the production of potent impurities during the synthesis of triethyl phosphate. Subsequently Gazzard *et al.* [6] found that the synthetic route used by Bracha and O'Brien [1–3], starting with diethyl phosphorochloridate, led to the production of an alkali-labile impurity which accounted for all the anticholinesterase activity in the case of the one compound they studied, namely diethyl *S*-*n*-propyl phosphorothiolate. We did not consider that this single finding, using the *n*-propyl derivative, was sufficient to call into question the hypotheses arising out of the earlier work, unless supported by similar results for other members of the class.

In view of the evidence discussed above, we have now undertaken a re-examination of the activities of these compounds and report here our findings for a series of phos-

* Personal communication.