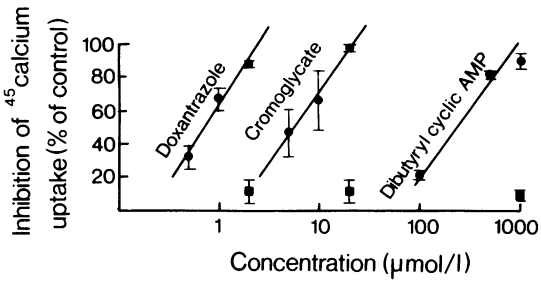

Figure 1 Inhibition by doxantrazole, cromoglycate and dibutyryl cyclic AMP of ^{45}Ca uptake induced by antigen (●) or A23187 (■) from sensitized rat peritoneal mast cells. Inhibition is expressed as a percentage of ^{45}Ca uptake in the absence of inhibitor which was 462 ± 158 ct/min for antigen and 2637 ± 459 ct/min for A23187. ^{45}Ca uptake in the absence of stimulus was 76 ± 14.6 ct/min. Uptakes refer to counts associated with approx. 5×10^5 cells and means are given together with s.e. mean for three experiments. The histamine secretions in the absence of inhibitor were $26 \pm 2.8\%$ for antigen and $55\% \pm 3.1$ for A23187. Concentration of antigen was supramaximal and that of A23187 was 3.0 μmol/l.

compared with that produced by the antiallergic drugs in Figure 1. Relative to cromoglycate, doxantrazole is eight times more effective in inhibiting ^{45}Ca uptake, whereas dibutyryl cyclic AMP is only 0.025 times as active. The activities for the inhibition of histamine secretion, cromoglycate: doxantrazole: dibutyryl cyclic AMP: are 1:20:0.02. None of the three agents inhibits ^{45}Ca uptake (Figure 1) or histamine secretion induced by the calcium ionophore, A23187.

Experiments with the ionophore A23187 have suggested that calcium entry into the mast cell is a sufficient stimulus to secretion (Foreman *et al.*, 1973). The antigen-antibody reaction appears to allow entry of calcium into the cell by increasing membrane permeability. The results presented here show that cromoglycate and doxantrazole inhibit the antigen-induced ^{45}Ca uptake and may exert their antisecretory effects by this mechanism. It is unlikely that these agents act at a point after calcium entry into the cell since they do not inhibit ionophore-induced secretion. The antiallergic agents are also inhibitors of phosphodiesterase (Roy & Warren, 1974; Tateson & Trist, 1976) and may prevent ^{45}Ca uptake indirectly by raising intracellular levels of cyclic AMP, since dibutyryl cyclic AMP itself prevents calcium uptake.

M.B.H. is an MRC scholar.

References

FOREMAN, J.C., HALLETT, M.B. & MONGAR, J.L. (1975). 45-calcium uptake in rat peritoneal mast cells. *Br. J. Pharmac.*, **55**, 283–284P.

FOREMAN, J.C., MONGAR, J.L. & GOMPERTS, B.D. (1973). Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process. *Nature, Lond.*, **245**, 249–251.

FOREMAN, J.C., MONGAR, J.L., GOMPERTS, B.D. & GARLAND, L.G. (1975). A possible role for cyclic AMP in the regulation of histamine secretion and the action of cromoglycate. *Biochem. Pharmac.*, **24**, 538–540.

GARLAND, L.G. & MONGAR, J.L. (1976). Differential histamine release by dextran and the ionophore A23187: the actions of inhibitors. *Int. Archs. Allergy appl. Immun.*, **50**, 27–42.

ROY, A.C. & WARREN, B.T. (1974). Inhibition of cAMP phosphodiesterase by disodium cromoglycate. *Biochem. Pharmac.*, **23**, 917–920.

TATESON, J.E. & TRIST, D.G. (1976). Inhibition of adenosine 3′5′-cyclic monophosphate phosphodiesterase by potential antiallergic compounds. *Life Sci.*, **18**, 153–162.

Dependence of histamine release from rat mast cells induced by the ionophore A23187 on endogenous adenosine triphosphate

T. JOHANSEN, (introduced by G.P. LEWIS)

Department of Pharmacology, Odense University, Denmark

Pure populations of rat peritoneal mast cells were used to study the ATP content of the mast cell in relation to histamine release induced by the ionophore A23187. When the ATP content was reduced to varying levels by preincubation with antimycin A and A23187, a good correlation was found between the ATP levels of the mast cells and the amounts of histamine released by incubation with calcium. The time course of A23187-induced histamine release and the effect of A23187 on the ATP content of the mast cells were studied under aerobic and anaerobic conditions. Histamine release was completed within 10 min from cells incubated under aerobic conditions, and there was a reduction in the ATP content of the cells

incubated in the presence or absence of glucose in close time relation to the histamine release. In the anaerobic experiments antimycin A was used to block the oxidative phosphorylation. After preincubation with antimycin A, glucose and A23187, calcium was added to the cell suspension. There was a marked reduction in the ATP content during the same period, when histamine release occurred. In other anaerobic experiments oligomycin was added to the cells during the preincubation period together with antimycin A, glucose and A23187. By incubation with calcium the ATP content of the mast cells was reduced during the histamine release. The observations are consistent with the view that energy requiring processes are involved in A23187 induced histamine release from mast cells, although part of ATP reduction in the aerobic experiments may be due to an uncoupling effect of Ca^{++} on the oxidative phosphorylation.

The effect of histamine antagonists on antigen-induced contractions of sensitized human bronchus *in vitro*

LYN S. DUNLOP & A.P. SMITH (introduced by PRISCILLA J. PIPER)

Chest Unit, King's College Hospital Medical School, London SE5 8RX

The nature and mechanisms of the participation of histamine in bronchial asthma are unclear despite its long history. The object of this work was to identify the relative importance of histamine in allergic bronchospasm in a passively sensitized human bronchial muscle preparation.

Human bronchus, 2–3 mm in diameter, dissected free of macroscopically normal tissue removed for carcinoma, was passively sensitized in undiluted serum from patients with house dust mite allergy. Sensitized bronchi were cut into spiral strips and suspended in 10 ml organ baths under a load of 250 mg, perfused with Krebs at 37°C and gassed with 95% O_2 and 5% CO_2. Changes in muscle length were recorded electronically with an isotonic transducer and recorded on moving paper. When the muscle tone was steady responses to a maximum dose of acetylcholine chloride (200 μg/ml for 3 min) and to a series of doses of histamine acid phosphate (5, 20, 80 μg/ml for 5 min) were obtained for each preparation. This was repeated after half the tissues had been treated with the inhibitor being tested, the remainder being parallel controls. Finally, all the tissues were challenged with freeze-dried house dust mite antigen (D. Pteronyssius 10 μg/ml). These responses were expressed as a percentage of the maximum acetylcholine response (MAR) recorded prior to challenge to enable comparison between tissues. In two series of experiments the effect of the H_1 receptor antagonist mepyramine (25 μg/ml), and the H_2 receptor antagonist metiamide (10 μg/ml) were studied on the dose response curve to histamine and response to antigen challenge. The effect of metiamide on a submaximal challenge (0.1 μg/ml D. Pteronyssius antigen) was also studied.

Dose related bronchial muscle contraction occurred in response to histamine but in the presence of mepyramine dose related relaxation occurred which was subsequently abolished by metiamide.

Antigen challenge caused contraction of the muscle in all cases. Mepyramine significantly reduced this response from 117.3 ± 6.2 in controls to $89.2 \pm 8.3\%$ MAR ($P < 0.005$, $n = 53$). Metiamide alone increased the response to histamine when compared with parallel controls, but it has no significant effect on the maximum antigen challenge response which was 124.0 ± 10.0 in controls and $114.6 \pm 10.3\%$ MAR in metiamide treated tissues ($P > 0.05$, $n = 21$). It did, however, cause a significant increase in the submaximal antigen challenge response from 81.2 ± 7.1 in controls to $116.4 \pm 21.1\%$ MAR ($P < 0.02$, $n = 42$). The challenge response in the presence of the two antagonists together, $109.3 \pm 5.1\%$ MAR, was not significantly different from the parallel control experiments, $107.9 \pm 6.2\%$ MAR ($P > 0.05$, $n = 38$).

The results of the experiments indicate that both H_1 and H_2 receptors occur in human bronchus and that both participate in allergic bronchospasm, H_1 receptors stimulating and H_2 receptors inhibiting muscle tone. Their effects, however, appear to cancel each other out, which suggests that if histamine plays any role in asthmatic bronchospasm, it is more likely to be via an indirect (DeKock, Nadel, Zwi, Colebatch & Olsen, 1966) rather than a direct action.

Reference

DeKOCK, M.A., NADEL, J.A., ZWI, S., COLEBATCH, H.J.H. & OLSEN, C.R. (1966). *J. appl. Physiol.*, **21**, 185–192.