On the ineffectiveness of indomethacin against rheumatoid swelling

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Previously, it was suggested that indomethacin-like drugs fail to arrest rheumatoid swelling because of their inability to prevent the inflammatory changes initiated by circulating sensitized lymphocytes (Jasani, 1975). The effectiveness of indomethacin against lymphocyte-initiated swelling and the accumulation of fibrin has been tested using the skin graft model (Bach & Jasani, 1976) and compared with that of two cortisol-like steroids: fluocinolone acetonide and chlorflumethasone.

To secure the development of circulating sensitized lymphocytes in the test animal, six homografts were transplanted on to each hind limb of the recipient rabbit and the drug was applied topically to one set only (Jasani, Parsons, Roberts & Tweed, 1974). The placebo-treated contralateral set provided the antigenic stimulus leading to the emergence of circulating sensitized lymphocytes (Jasani, 1976).

The results showed that indomethacin (0.02%) was virtually ineffective against the swelling in homografts; a failure which cannot be due to lack of absorption of topically applied indomethacin, which is known to be as active topically as when administered orally (Lewis, 1976). In contrast, the two steroids were definitely effective against both the inflammatory accompaniments. Compared with indomethacin which reduced the swelling only slightly ($96.6 \pm 0.9\%$) and tended to increase fibrin accumulation ($110.6 \pm 4.7\%$), fluocinolone acetonide (0.0025%) reduced the swelling and fibrin content to $66.2 \pm 6.3\%$ and $53.6 \pm 6.0\%$ respectively of control, whereas chlorflumethasone (0.00025%) reduced them to $62.2 \pm 5.1\%$ and $69.2 \pm 1.9\%$ (mean $\pm$ s.e. mean, $n = 4$).

As the accumulation of fibrin occurs in the rheumatoid joint in a manner similar to that in homografts (Bullock, Jasani & Roberts, 1976), the observations suggest that new anti-rheumatic agents should also exhibit steroid-like properties in their ability to depress lymphocyte-initiated inflammation but should be devoid of undesirable effects.

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Site of action of the antiallergic drugs cromoglycate and doxantrazole

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The degree of antigen-induced histamine secretion from sensitized mast cells is correlated with 45-calcium uptake (Foreman, Hallett & Mongar, 1975). Inhibition of oxidative and glycolytic metabolism prevents histamine secretion but not ^{45}Ca uptake, whereas dibutyryl cyclic AMP inhibits both ^{45}Ca uptake and secretion. Histamine secretion induced by the calcium ionophore, A23187 is not inhibited by dibutyryl cyclic AMP or by antiallergic drugs (Foreman, Mongar, Gomperts & Garland, 1975) indicating that these inhibitors exert their effect on antigen-induced secretion at the level of calcium entry into the mast cell.

The methods of measuring histamine secretion from, and ^{45}Ca uptake by sensitized rat peritoneal mast cells have already been described (Foreman, Mongar & Gomperts, 1973).

Cromoglycate and doxantrazole produce a dose-related inhibition of antigen-stimulated ^{45}Ca uptake by the mast cells. The concentration ranges for inhibition of ^{45}Ca uptake are similar to those for inhibition of histamine secretion (Garland & Mongar, 1976). Inhibition of ^{45}Ca uptake by dibutyryl cyclic AMP is

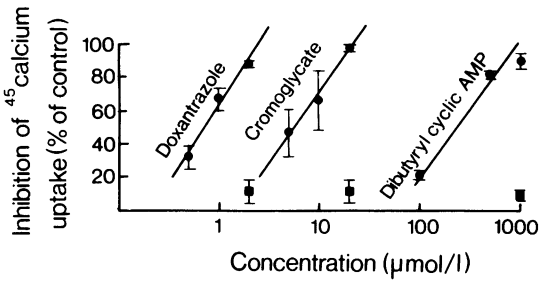

Figure 1 Inhibition by doxantrazole, cromoglycate and dibutyryl cyclic AMP of ^{45}Ca uptake induced by antigen (●) or A23187 (■) from sensitized rat peritoneal mast cells. Inhibition is expressed as a percentage of ^{45}Ca uptake in the absence of inhibitor which was 462 ± 158 ct/min for antigen and 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.

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

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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