*Short communications*

# Effect of cromoglycate on anaphylactic histamine release from rat peritoneal mast cells

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Cromoglycate (1–30 $\mu$M) produced a concentration-dependent inhibition of anaphylactic histamine release from actively sensitized rat peritoneal mast cells but at lower concentrations (0·01–0·1 $\mu$M) occasionally produced a concentration-dependent enhancement of histamine release.

Disodium cromoglycate has gained widespread acceptance in the prophylactic treatment of allergic asthma and *in vitro* it inhibits anaphylactic histamine release from several preparations including human and monkey lung (Assem & Mongar, 1970) and rat peritoneal cells (Assem & Richter, 1971). It has also been shown to inhibit *in vivo* anaphylactic histamine release from rat peritoneal cells (Morse, Austen & Bloch, 1969).

This paper describes the quantitative relationship between cromoglycate concentration and anaphylactic histamine release from rat peritoneal cells *in vitro*.

**Methods.**—Female rats of the Canterbury Ash Wistar strain were sensitized by a modification of the method described by Mota (1964). Each rat received an intramuscular injection of 2·5 mg/100 g ovalbumin (Koch-Light, 5 times crystallized) in saline (154 mM NaCl) and at the same time 0·5 ml/100 g i.p. *Bordetella pertussis* ($8\times10^{10}$ organisms/ml, Burroughs Wellcome & Co.). Twenty-one days later each was anaesthetized with diethyl ether, decapitated and 10 ml cold saline (154 mM NaCl) containing heparin 100 $\mu$g/ml (Evans Medical) was injected into the peritoneal cavity. After the peritoneum had been massaged for approximately one minute the fluid was withdrawn through a mid-line abdominal incision. The peritoneal washings from at least five rats were pooled and kept on ice until used. The washings were centrifuged at 250 *g* for 6 min, the supernatant was discarded and the cell pellet resuspended in cold Tyrode solution (3 ml per rat) of the following composition (mM): NaCl 137, $NaHCO_3$ 12, $NaH_2PO_4$ 0·3, KCl 2·7, $MgCl_2$ 1·0, $CaCl_2$ 1·8, dextrose 5·6.

The cell suspension (0·5 ml in duplicate) was pre-incubated at 37° C for 2 min and 0·5 ml antigen (ovalbumin 2 $\mu$g/ml) was added alone or with cromoglycate. Incubation was continued at 37° C for one minute, almost maximal release of histamine having been found in this time. The reaction was then stopped by adding 4 ml ice cold Tyrode and each sample was centrifuged at 250 *g* for 6 minutes. The supernatant was decanted, the pellets resuspended in 5 ml Tyrode and boiled for 10 min to release residual histamine.

Released and residual histamine were assayed on the atropinized guinea-pig ileum and the released histamine was expressed as per cent total and corrected for spontaneous release in Tyrode alone (normally about 2%). Cromoglycate (0·1–1,000 $\mu$M) was found not to influence spontaneous release or to interfere with the histamine assay. Disodium cromoglycate was equilibrated with atmospheric moisture when it was converted to the pentahydrate with a molecular weight of 602. Concentrations are expressed in terms of the acid.

**Results.**—Cromoglycate at concentrations above 1 $\mu$M inhibited anaphylactic histamine release. Above this concentration inhibition was linearly related to log concentration, 50% inhibition being achieved at 7 $\mu$M (Fig. 1a) and a maximum inhibition of approximately 80% was obtained at 30 $\mu$M. Analysis by $\chi^2$ test showed that the slopes from individual experiments were not homogeneous ($P<0·001$).

The inhibitory phase was occasionally preceded at lower concentrations of cromoglycate by a phase of augmented histamine release. Figure 1(b) illustrates an extreme example where augmentation increased with concentration to a maximum of 87% at 0·1 $\mu$M. Enhancement of histamine release by low concentrations of cromoglycate was seen in 3 out of 6 experiments (114, 128 and 187% of control), whilst in the remaining 3 experiments these low concentrations had no significant effect.

**Discussion.**—Anaphylactic histamine release from rat peritoneal mast cells *in vitro* can be used for the quantitative investiga-

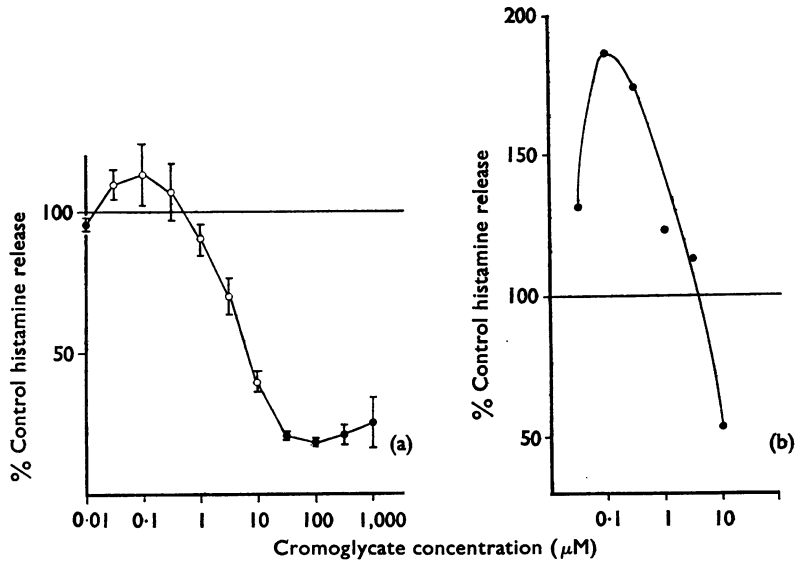


FIG. 1. (a) Effect of cromoglycate on anaphylactic histamine release. Open circles are the means (±S.E.) from 6 duplicate experiments, closed circles are the means from 2 duplicate experiments. (b) Results from a single experiment (each point being the mean of duplicates) showing an extreme example of augmentation of anaphylactic histamine release.

tion of antiallergic drugs like cromoglycate. The concentration-effect curve regularly had an adequate slope, threshold to maximum inhibition occurring within the range of 1–30 μM. These results are of particular interest since earlier *in vitro* studies, in which primate chopped lung and rat peritoneal cells were used, failed to show a consistent increase in inhibition with increasing concentrations of cromoglycate (Assem & Mongar, 1970 ; Assem & Richter, 1971).

The enhancement of histamine release observed in some experiments with low concentrations of cromoglycate (<1 μM) cannot be attributed to histamine release by the drug alone but may be due to stimulation of the process activated by antigen combining with cell fixed antibody. This stimulation followed at higher concentrations by inhibition has also been reported for the effect of thioglycollate on anaphylactic histamine release from rat peritoneal cells (Perera & Mongar, 1965).

Moussatché & Danon (1957) reported that succinate, a dicarboxylic acid, enhanced anaphylactic histamine release from guinea-pig chopped lung. This was not attributable to it being a Krebs cycle intermediate because fumarate had no effect whilst maleate, which is not part of the cycle, did enhance histamine release (Austen & Brocklehurst, 1961). Cromoglycate is also a dicarboxylic acid and might stimulate anaphylactic histamine release by the same mechanism as succinate and maleate.

Cromoglycate has also been shown to augment another anaphylactic response. Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard (1970), reported that if passive cutaneous anaphylaxis reactions in rats were elicited by large antigen doses, low doses of cromoglycate increased whilst higher amounts inhibited the reaction. It is tempting also to relate these findings to a clinical report of cromoglycate possibly worsening the condition of an asthmatic patient (Lobel, Machtey & Eldror, 1972).

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## REFERENCES

ASSEM, E. S. K. & MONGAR, J. L. (1970). Inhibition of allergic reactions in man and other species by cromoglycate. *Int. Arch. Allergy*, **38**, 68–77.

ASSEM, E. S. K. & RICHTER, A. W. (1971). Comparison of *in vivo* and *in vitro* inhibition of the anaphylactic mechanism by β-adrenergic stimulants and disodium cromoglycate. *Immunology*, **21**, 729–739.

AUSTEN, K. F. & BROCKLEHURST, W. E. (1961). Anaphylaxis in chopped gunea pig lung. II Enhancement of the anaphylactic release of histamine and slow reacting substance by certain dibasic aliphatic acids and inhibition by monobasic fatty acids. *J. Exp. Med.*, **113**, 541–557.

COX, J. S. G., BEACH, J. E., BLAIR, A. M. J. N., CLARKE, A. J., KING, J., LEE, T. B., LOVEDAY, D. E. E., MOSS, G. F., ORR, T. S. C., RITCHIE, J. T. & SHEARD, P. (1970). Disodium Cromoglycate (Intal). *Adv. Drug Res.*, **5**, 115–196.

LOBEL, H., MACHTEY, I. & ELDROR, M. Y. (1972). Pulmonary infiltrates with eosinophilia in an asthmatic patient treated with disodium cromoglycate. *The Lancet*, ii, 1032.

MORSE, H. C., AUSTEN, K. F. & BLOCH, K. J. (1969). Biologic properties of rat antibodies. III. Histamine release mediated by two classes of antibodies. *J. Immunol.*, **102**, 327–337.

MOTA, I. (1964). The mechanism of anaphylaxis. I. Production and biological properties of "mast cell sensitizing" antibody. *Immunology*, **7**, 681–699.

MOUSSATCHÉ, H. & DANON, A. P. (1957). On the action of succinate and malonate upon the histamine released during the anaphylactic reaction *in vitro*. *Naturwissenschaften*, **44**, 330.

PERERA, B. A. V. & MONGAR, J. L. (1965). Effect of anoxia, glucose and thioglycollate on anaphylactic and compound 48–80 induced histamine release in isolated rat mast cells. *Immunology*, **8**, 519–525.

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