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EFFECT OF DISODIUM CROMOGLYCATE AND CYCLIC AMP-ACTIVE DRUGS ON CYTOTOXIC HISTAMINE RELEASE FROM RAT MAST CELLS

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Abstract—Disodium cromoglycate and compounds which elevated levels of cyclic AMP in the mast cell variously inhibited cytotoxic histamine release induced by the surface active agents melittin, Tween 20 and Triton X-100. These results are inconsistent with the postulated effects of the drugs on receptor mediated calcium channels and alternative explanations of their action are considered.

Histamine release from the mast cell may be produced by a range of pharmacological, immunological and mechanical stimuli [1]. The inducing agents involved may be broadly classified into two groups. One, non-selective releasers, act by disruption of the mast cell membrane [2, 3]. These agents are cytotoxic and liberate all of the intracellular contents including histamine. Two 'selective releasers' liberate histamine by the sequential exocytosis of secretory granules and without the loss of characteristic cytoplasmic markers such as the enzyme lactate dehydrogenase [2, 3]. The response requires an intact cell metabolism and is blocked by inhibitors of glycolysis and oxidative phosphorylation and by extremes of temperature. Operationally, these parameters may be used to differentiate experimentally between the two mechanisms of release [2, 3].

As in other secretory systems, the selective liberation of histamine is triggered by an increased level of ionized calcium in the cell cytosol [4]. The ligand–receptor interaction on the cell surface is believed to increase the permeability of the membrane to external calcium ions (that is, to open calcium gates in the membrane) or to mobilize internal reservoirs of the cation [4, 5]. It has been suggested that a variety of anti-allergic compounds and drugs, which elevate intracellular levels of cyclic AMP, may inhibit histamine release by acting directly on the calcium gating mechanism to prevent movement of the cation from the extracellular environment into the cytosol [5–9]. Recent experiments in our laboratories have, however, cast serious doubt on this model [10–16] and to examine the hypothesis further, we here wish to report the effects of such drugs on cytotoxic histamine release, induced by a number of surface active agents.

MATERIALS AND METHODS

Mixed peritoneal cells were recovered by direct lavage of male and female Sprague–Dawley rats (200–400 g) and histamine release was determined as previously reported [17]. As a positive control, some animals were sensitized to the nematode *Nippostrongylus brasiliensis* and cells subsequently challenged with secretory allergen (10–20 worm equivalents/ml) as formerly described [16]. In further experiments, mast cells were purified to greater than 95% homogeneity by gradient centrifugation over Percoll [18] and release of lactate dehydrogenase was measured according to established procedures [2, 3].

To investigate the metabolic requirements for histamine release, cells were preincubated (20 min) in the absence of glucose but in the presence of 2-deoxyglucose or antimycin A. To examine the effects of inhibitors, cells were pretreated (10–20 min) with theophylline or prostaglandin E_1 (PGE_1), or for longer periods (30 min) with N^6-$O^{2'}$-dibutyryl adenosine-3′,5′-cyclic monophosphate (dibutyryl cyclic AMP, Bu_2 cAMP), or 8-bromoadenosine-3′,5′-cyclic monophosphate (8-bromo cyclic AMP, Br-cAMP). Disodium cromoglycate was added to the cells simultaneously with the inducer. In each case, histamine release was assessed 10 min after the addition of the liberator and expressed as a percentage of the total content of the amine or as the percentage inhibition of the control value.

Disodium cromoglycate was a gift from Mr. P. Sheard (Fisons Pharmaceuticals, Loughborough, U.K.) and melittin from bee venom was generously provided by Dr. C. E. Dempsey (Department of Chemistry, University College London). Octyl phenoxy polyethoxyethanol (Triton X-100), polyoxyethylene sorbitan monolaurate (Tween 20) and all other reagents were purchased from the Sigma (London) Chemical Co. (Poole, U.K.).

RESULTS

Melittin, Tween 20 and Triton X-100 produced a graded release of histamine from rat peritoneal mast cells (Fig. 1). The dose–response curve for melittin was bell-shaped, possibly reflecting the tendency of the peptide to form aggregates at higher concentrations [19]. Since we, and others [10–15], have shown that the effect of a given inhibitor is markedly dependent on the strength of the releasing stimulus, concentrations of the liberators (melittin, 1 μg/ml; Tween 20, 0.33 μl/ml and Triton X-100, 0.06 μl/ml)

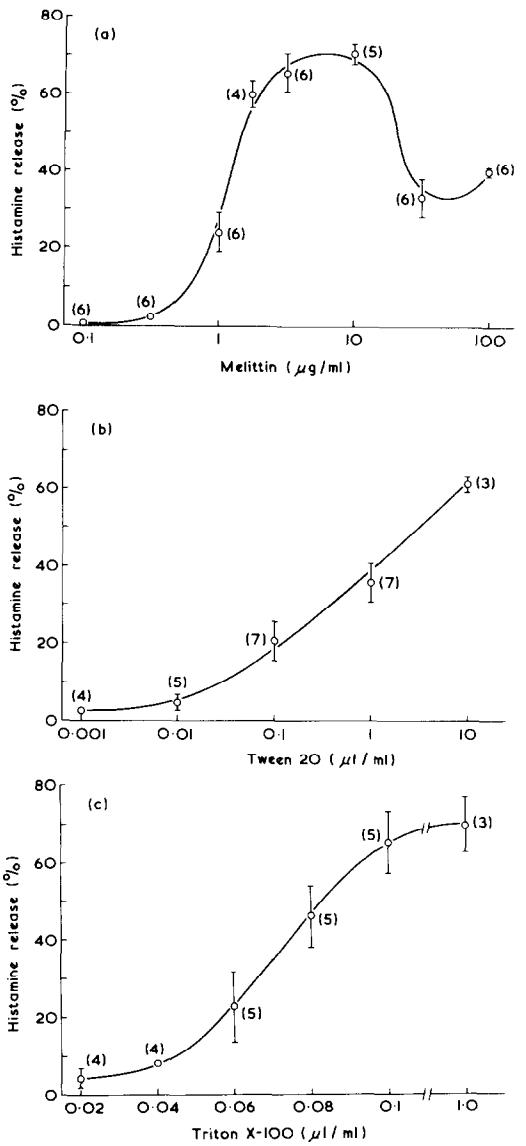

Fig. 1. Dose–response curves for the release of histamine from rat peritoneal mast cells treated with (a) melittin, (b) Tween 20 and (c) Triton X-100. The points are the means from the number of experiments shown in parentheses and vertical bars (shown where larger than the symbol) denote S.E.

which gave releases of histamine (*ca.* 30%) comparable to those normally observed with the anaphylactic reaction were used in all subsequent experiments. Under these conditions, the cells gave parallel releases of lactate dehydrogenase. In three experiments, the liberation of histamine and corresponding releases of the enzyme were respectively: melittin, 27.2 ± 1.3 and 31.4 ± 3.0; Tween 20, 29.0 ± 3.2 and 35.5 ± 7.3; and Triton X-100, 25.7 ± 3.0 and 30.4 ± 3.0.

The responses to all three surfactants were unaffected by glucose deprivation or the presence of metabolic inhibitors (Table 1). Histamine release was unaffected or even potentiated by elevated temperatures (45°) but was reduced or abolished by submaximal (0°) temperatures (Table 1). In sharp contrast, anaphylactic histamine release was blocked by inhibitors of glycolysis (2-deoxyglucose) and oxidative phosphorylation (antimycin A) and by both extremes of temperature (Table 1). The effect of Triton X-100 was independent of the concentration of external calcium ions (0–20 mM) but the responses to melittin and Tween 20 were abrogated by high concentrations of the cation. Thus, in three experiments the release of histamine in the absence of added calcium and at a cation concentration of 20 mM were respectively: melittin, 26.3 ± 2.7 and 0.5 ± 0.5; Tween 20, 30.1 ± 5.8 and 7.4 ± 2.7; and Triton X-100, 22.3 ± 8.8 and 31.4 ± 5.1.

Histamine release induced by melittin was inhibited in dose-dependent fashion by cromoglycate (1–1000 μM, Fig. 2). In contrast, the drug was virtually ineffective against Tween 20 and Triton X-100, producing ≤ 30% inhibition over the concentration range tested (data not shown). Similar results were obtained with PGE_1 (5–100 μM, Table 2). Theophylline (1–20 mM) blocked the release evoked by all three surfactants but was again most active against melittin (Table 2). Analogues of cyclic AMP (0.1–10 mM) comparably inhibited the effect of the three agents, with Bu_2cAMP being more active than Br-cAMP (Table 3).

DISCUSSION

The cytotoxic nature of histamine release induced by melittin, Tween 20 and Triton X-100 is well established [1–3, 20, 21] and was confirmed in the present work by the accompanying liberation of lactate dehydrogenase and the lack of inhibition by metabolic poisons and elevated temperatures. As a positive control, the latter conditions were shown effectively to block anaphylactic histamine secretion. In contrast, submaximal temperatures abrogated the effect of both antigen and the detergents. Similar results have been reported for the surfactant *n*-decylamine by other workers [22]. Inhibition under these conditions clearly cannot then be taken as a criterion for selective histamine release and probably reflects a reduction in fluidity of the membrane, thus minimizing access and activity of the detergent. High concentrations of calcium also inhibited the effect of melittin and Tween 20 and, as has long been suggested, may have a similar stabilizing effect on the cell membrane.

Cromoglycate, cyclic AMP analogues and compounds which are known to elevate intracellular levels of cyclic AMP in the mast cell [23, 24], variously inhibited cytotoxic histamine release. In general, the effect of melittin was more readily blocked than that of Tween 20 or Triton X-100, possibly indicating some difference in the detailed mechanism of the disruptive effect produced by these agents, and the drugs were active at concentrations very similar to those previously found by us to prevent IgE-mediated secretion [13, 15, 16]. These results extend those of Kaliner and Austen [15] who showed that Bu_2cAMP, PGE_1 and aminophylline inhibited complement or water induced lysis of mast cells and of Marshall [26] who reported a protective effect of

Table 1. Effect of metabolic inhibitors and temperature on histamine release induced by surfactants and antigen

Conditions	Histamine release (%) induced by: Melittin	Tween 20	Triton X-100	Antigen
Medium				
Complete	27.7 ± 2.5	31.7 ± 1.1	30.9 ± 2.4	19.8 ± 2.0
Glucose free	23.9 ± 2.8	32.3 ± 2.3	31.1 ± 1.1	29.0 ± 2.2
2-Deoxyglucose (5 mM)	27.0 ± 2.2	31.3 ± 3.0	30.5 ± 0.8	2.4 ± 0.7
Antimycin A (1 μM)	27.6 ± 4.1	30.6 ± 3.3	29.9 ± 2.6	2.5 ± 0.6
Temperature				
0	0	7.4 ± 1.1	1.8 ± 1.6	3.1 ± 0.8
25	10.2 ± 3.3	22.8 ± 4.4	8.3 ± 0.5	7.8 ± 2.2
37	43.7 ± 7.3	20.5 ± 3.1	21.9 ± 1.5	29.0 ± 1.1
45	31.7 ± 4.3	31.2 ± 5.5	52.1 ± 3.1	3.2 ± 0.8

Cells were preincubated (20 min, 37°) in the media shown and then challenged with surfactants or allergen at the concentrations given in the text. Histamine release was assessed after a further 10 min. Values are means ± S.E. for 3–4 experiments.

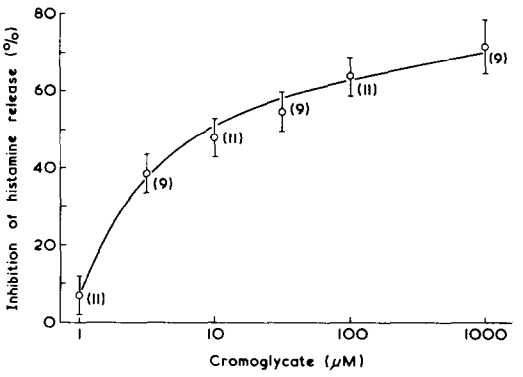

Fig. 2. Inhibition by cromoglycate of histamine release from rat peritoneal mast cells treated with melittin (1 μg/ml). Control release in the absence of the drug was 26.9 ± 3.2. The points are the means from the number of experiments shown in parentheses and vertical bars denote S.E.

cromoglycate on mast cell damage induced by Tween 20.

These data should then be considered in terms of the proposed mechanism of action of the named compounds. Cromoglycate and cyclic AMP-active drugs have been claimed selectively to inhibit the receptor mediated activation of calcium gates in the cell membrane [5–9]. They are, therefore, suggested to prevent influx of the cation from the external environment and consequently to block exocytosis. However, cytotoxic histamine release circumvents the ligand–receptor interaction, the subsequent biochemical events which lead to secretion, and involves a direct disruptive effect on the membrane. The activity of the test drugs under these conditions clearly cannot be explained in terms of any specific effect on the calcium gating mechanism. Previous studies in our laboratories [10–16], which have demonstrated inhibition of histamine release in the absence of extracellular calcium and prevention of

Table 2. Effect of prostaglandin E_1 (PGE_1) and theophylline on histamine release induced by surfactants

Drug	Inhibition (%) of histamine released induced by: Melittin	Tween 20	Triton X-100
(a) PGE_1 (μM)			
5	37.0 ± 9.8	14.5 ± 6.1	2.2
10	50.0 ± 9.4	15.5 ± 1.8	−21.8
50	60.5 ± 6.9	15.8 ± 5.4	−2.3
100	70.1 ± 1.7	28.5 ± 8.3	−4.4
(b) Theophylline (mM)			
0.625	17.0 ± 6.8	2.3 ± 1.5	11.9 ± 4.9
1.25	33.9 ± 7.5	13.0 ± 7.6	7.8 ± 3.3
2.5	43.8 ± 8.6	29.6 ± 9.5	16.0 ± 5.9
5	57.5 ± 6.1	39.8 ± 5.8	19.9 ± 7.9
10	77.0 ± 8.6	45.7 ± 10.2	36.4 ± 4.8
20	98.7 ± 1.3	53.2 ± 7.4	50.3 ± 6.4

Cells were preincubated (10–20 min, 37°) with the test drugs and then challenged with surfactants at the concentrations given in the text. Histamine release was assessed after a further 10 min.

Control releases in the absence of the drugs were: melittin (a) 21.6 ± 4.4 (n = 3), (b) 19.6 ± 3.8 (n = 3); Tween 20 (a) 34.2 ± 1.9 (n = 3), (b) 22.8 ± 1.4 (n = 5); and Triton X-100 (a) 38.6 (n = 2), (b) 38.2 ± 2.4 (n = 6).

All values are means ± S.E. (where given) for the number (n) of experiments noted.

Table 3. Effect of cyclic AMP analogues on histamine release induced by surfactants

Analogue	Inhibition (%) of histamine release induced by: Melittin	Tween 20	Triton X-100
(a) Bu_2cAMP (mM)			
0.1	29.5 ± 4.5	34.4 ± 4.3	24.4 ± 5.7
1	53.6 ± 4.2	63.5 ± 10.1	48.6 ± 6.4
10	82.0 ± 9.0	83.7 ± 5.4	75.9 ± 6.0
(b) Br-cAMP (mM)			
0.1	8.0 ± 2.9	0	0
1	15.6 ± 3.3	17.7 ± 2.9	10.0 ± 7.1
10	41.0 ± 5.1	29.4 ± 4.6	42.4 ± 9.0

Cells were preincubated (30 min, 37°) with the test compounds and then challenged with surfactants at the concentrations given in the text. Histamine release was assessed after a further 10 min.

Control releases in the absence of the drugs were: melittin (a) 44.7 ± 3.2 (n = 3), (b) 40.7 ± 2.5 (n = 4); Tween 20 (a) 31.0 ± 2.3 (n = 6), (b) 30.7 ± 2.6 (n = 3); and Triton X-100 (a) 23.1 ± 4.7 (n = 6), (b) 38.1 ± 2.6 (n = 4).

All values are means ± S.E. for the number (n) of experiments noted.

secretion induced by the calcium ionophore A23187, are similarly not in accord with this model. The present results suggest instead that cromoglycate and cyclic AMP may exert a general stabilizing effect on the mast cell membrane. Such an effect could protect the cell against the cytolytic effect of detergents in a manner similar to reduced temperatures or high concentrations of calcium. An increased stability or reduced fluidity of the membrane would also have manifold effects on the selective release of histamine induced by various agents, possibly leading to an inhibition of the fusional processes involved in exocytosis, the lateral diffusion of ionophores, the mobilization of membrane-bound calcium and the opening of calcium channels. The latter would then be part of a general phenomenon rather than reflecting a specific, direct effect of the test drugs. This model is then in accord with all of the available experimental data but it must be emphasized that the biochemical basis for the proposed stabilizing effect remains completely obscure and requires further detailed investigation.

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REFERENCES

1. W. Kazimierczak and B. Diamant, *Prog. Allergy* **24**, 295 (1978).
2. A. R. Johnson and N. C. Moran, *Fedn Proc Fedn Am. Socs exp. Biol.* **28**, 1716 (1969).
3. A. R. Johnson and N. C. Moran, *Am. J. Physiol.* **216**, 453 (1969).
4. M. Berridge, *Adv. cyclic Nucleotide Res.* **6**, 1 (1975).
5. J. C. Foreman, L. G. Garland and J. L. Mongar, in *Calcium in Biological Systems* (Ed. C. J. Duncan), p. 193. Cambridge University Press, Cambridge (1976).
6. L. G. Garland and J. L. Mongar, *Int. Archs Allergy appl. Immun.* **50**, 27 (1976).
7. J. C. Foreman, J. L. Mongar, B. D. Gomperts and L. G. Garland, *Biochem. Pharmac.* **24**, 538 (1975).
8. F. Eckstein and J. C. Foreman, *FEBS Letts* **91**, 182 (1978).
9. J. C. Foreman, M. B. Hallett and J. L. Mongar, *Br. J. Pharmac.* **59**, 473P (1977).
10. M. Ennis and F. L. Pearce, *Int. Archs Allergy appl. Immun.* **60**, 461 (1979).
11. M. Ennis, G. Atkinson and F. L. Pearce, *Agents Acts* **10**, 222 (1980).
12. M. Ennis, F. L. Pearce and P. M. Weston, *Br. J. Pharmac.* **70**, 329 (1980).
13. M. Ennis, A. Truneh, J. R. White and F. L. Pearce, *Nature, Lond.* **289**, 186 (1981).
14. F. L. Pearce and A. Truneh, *Agents Acts* **11**, 44 (1981).
15. A. Truneh and F. L. Pearce, *Int. Archs Allergy appl. Immun.* **66**, 76 (1981).
16. J. R. White and F. L. Pearce, *Immunology* (in press).
17. G. Atkinson, M. Ennis and F. L. Pearce, *Br. J. Pharmac.* **65**, 395 (1979).
18. L. Enerbäck and I. Svensson, *J. Immun. Meths* **39**, 135 (1980).
19. J. Gauldie, J. M. Hanson, F. D. Rumjanek, R. A. Shipolini and C. A. Vernon, *Eur. J. Biochem.* **61**, 369 (1976).
20. B. Jasani and D. R. Stanworth, *Int. Archs Allergy appl. Immun.* **45**, 74 (1973).
21. B. Jasani, G. Kreil, B. F. Mackler and D. R. Stanworth, *Biochem. J.* **181**, 623 (1979).
22. N. C. Moran, B. Uvnäs and B. Westerholm, *Acta physiol. scand.* **56**, 26 (1962).
23. T. J. Sullivan, K. L. Parker, W. Stenson and C. W. Parker, *J. Immun.* **114**, 1473 (1975).
24. B. D. Fredholm, I. Guschin, K. Elwin, G. Schwab and B. Uvnäs, *Biochem. Pharmac.* **25**, 1583 (1976).
25. M. Kaliner and K. F. Austen, *Science* **183**, 659 (1971).
26. R. Marshall, *Thorax* **27**, 38 (1972).