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# THE EFFECT OF ALKALINE EARTH CATIONS ON THE RELEASE OF HISTAMINE FROM RAT PERITONEAL MAST CELLS TREATED WITH COMPOUND 48/80 AND PEPTIDE 401

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**1** Extracellular calcium ions have a dual effect on the release of histamine from rat peritoneal mast cells treated with compound 48/80 and peptide 401. The release is either potentiated or inhibited according to the relative concentrations of ion and inducer.

**2** Strontium similarly potentiates the release produced by optimal concentrations of inducer but higher concentrations are required than in the case of calcium. Strontium is markedly less inhibitory than calcium.

**3** Mast cells may be depleted of intracellular calcium by incubation for short periods with the chelating agent, ethylenediamine tetraacetic acid (EDTA). They thereby become unresponsive to compound 48/80 and peptide 401 unless calcium is reintroduced into the incubation medium. Strontium and barium, but not magnesium, will substitute for calcium in this system. Barium additionally produces a marked release of histamine even in the absence of inducer. Pretreatment with the ionophore A23187 similarly inhibits the subsequent response to peptide 401 in divalent cation-free medium. This inhibition is reversed on the reintroduction of calcium.

**4** Compound 48/80 and peptide 401 release histamine from mast cells incubated in isotonic sucrose in the complete absence of added metal ions. However, the corrected release under these conditions is potentiated by both mono and divalent cations.

**5** On the basis of these results, the possible mechanism of action of the basic releasing agents and their usefulness as models for studying histamine secretion is discussed.

## Introduction

The physiological stimulus for the release of histamine from mast cells is provided by the combination of antigen with specific antibody fixed to the cell surface. This combination is believed to increase transiently the permeability of the membrane to calcium ions (Foreman, Garland & Mongar, 1976) and, as in many other systems in which the movement of calcium couples the stimulus to the secretory response (Douglas 1968), influx of the ion initiates the release process. Strontium (and to a lesser extent barium) will act as a partial substitute for calcium and it appears that both cations may enter the activated cell through the same channels (Foreman & Mongar, 1972a; Foreman, Hallett & Mongar, 1977a).

Histamine may also be selectively released by a variety of agents which simulate the immunologically induced mechanism. Many of these agents, for example adenosine 5'-triphosphate (ATP), dextran, the ionophore A23187 and concanavalin A, resemble the anaphylactic reaction in that they are calcium-dependent and stimulate calcium uptake by treated cells (Dahlquist 1974; Foreman, Hallett & Mongar, 1977b). Two inducers, compound 48/80 and the much less widely studied peptide 401, the mast cell degranulating (MCD) peptide from bee venom (Breithaupt & Habermann, 1968), are however of particular interest in that they promote the release of histamine in the absence of extracellular calcium (Uvnäs & Thon, 1961; Assem & Atkinson, 1973). The mechanism of action of peptide 401 has previously not been investigated in detail but compound 48/80 is apparently able to mobilize intracellular stores of calcium and cells deprived of this reserve by treatment with chelating agents or the calcium ionophore A23187 become unresponsive (Douglas & Ueda, 1973; Cochrane & Douglas, 1974; Diamant & Patkar, 1975).

In this paper we describe the results of experiments in which we have examined further the effects of calcium and other ions on the release of histamine induced by compound 48/80 and peptide 401.

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## Methods

Male and female Lister hooded rats, of body weight 150 to 250 g and from a closed random-bred colony, were used. Individual animals were anaesthetized with ether, decapitated and Tyrode solution (5 ml) containing heparin (5 units/ml) was injected into the peritoneal cavity. The Tyrode solution had the composition (mM): NaCl 137, $NaHCO_3$ 12, glucose 5.6, KCl 2.7, $NaH_2PO_4$ 0.4, $MgCl_2$ 1 and $CaCl_2$ 1.8. Where necessary the pH was adjusted to 7.4 before use. The abdomen was massaged (1 to 2 min) and the peritoneal cavity opened by midline incision. The fluid was removed with a siliconized pipette and the cells recovered by centrifugation (room temperature, 2 min, 150 *g*). Polystyrene or siliconized glass centrifuge tubes were used throughout.

In simple release experiments, cells were washed and suspended in Tyrode solution from which calcium and magnesium had been omitted. Where necessary, cells from several rats were combined to provide sufficient material for a given experiment. One rat typically provided about 12 samples, each containing approx. 5% mast cells and 0.2 to 1 μg histamine. Aliquots (0.5 ml) were then added to centrifuge tubes containing an equal volume of Tyrode solution appropriately modified according to the experiment. This solution contained either alkaline earth ions to the stated final concentrations or, in all experiments in which these ions were omitted, ethylenediamine tetraacetic acid (EDTA) to a final concentration of $10^{-4}$ M. The suspension was allowed to equilibrate (37°C, 5 min) in a metabolic shaker with gentle mechanical agitation and then a solution of the releasing agent (10 μl) was added. Secretion was allowed to proceed for a further 10 min and the reaction was terminated by the addition of ice-cold Tyrode solution (2 ml) from which calcium and magnesium had been omitted. Cells and supernatants were recovered by centrifugation (4°C, 2 min, 150 *g*). The cell pellets were resuspended in Tyrode solution (3 ml) and allowed to stand in a boiling water bath (10 min) to release residual histamine. Individual supernatants were treated similarly. Histamine was then estimated by the spectrofluorimetric assay of Shore, Burkhalter & Cohn (1959) but omitting the extraction steps (Loefler, Lovenberg & Sjoerdsma, 1971). Histamine release is given as the amount of amine found in the supernatant expressed as a percentage of the total found in cells and supernatant and is corrected, unless otherwise stated, for the spontaneous release (normally approx. 5%) in the absence of inducer.

In some experiments, N-2-hydroxyethyl piperazine-N'-2-ethane sulphonic acid (HEPES, 10 mM) was used as a buffer in place of the bicarbonate. Cells were harvested as before and aliquots (100 μl) added to the appropriate medium (final volume 2 ml). Cells were incubated (37°C, 10 min) and a solution of releasing agent (100 μl) added to the desired final concentration. Samples were recovered as above, acidified with an equal volume of perchloric acid (0.8 N) and histamine determined by the automated fluorimetric assay (Auto-Technicon, industrial method 164-73E).

In experiments involving depletion of intracellular calcium, cells were incubated for the length of time noted in Tyrode solution free of divalent cations and containing EDTA ($10^{-4}$ M). They were then recovered by centrifugation (room temperature, 2 min, 150 *g*) and suspended in the appropriate incubation medium (1 ml). A solution of releasing agent (10 μl) was then added as required and secretion was estimated as above. Alternatively cells were preincubated (5 min, 37°C) in divalent cation-free medium containing the calcium ionophore A23187 ($3 \times 10^{-7}$ M) before being resuspended and challenged in the noted media.

In other experiments, peritoneal cells were obtained as described but rapidly washed and suspended in sucrose (0.25 M) buffered with tris-(hydroxymethyl)-methylamine-HCl (1 mM, pH 7.4). Aliquots (0.5 ml) were then incubated in an equal volume of sucrose containing EDTA or appropriate ions and releaser was added as required. The cells were incubated (37°C, 10 min) and cooled in ice-water (5 min) before addition of ice-cold Tyrode solution (without divalent cations, 2 ml) to displace histamine from discharged granules. Release of histamine was then measured as before.

Compound 48/80 was a gift from Dr A. N. Payne of the Wellcome Research Laboratories, Beckenham and peptide 401, prepared from bee venom essentially according to the method of Gauldie, Hanson, Rumjanek, Shipolini & Vernon (1976), was generously provided by Dr A. J. Garman of the Chemistry Department, University College London. The calcium ionophore A23187 was donated by the Lilly Research Centre Limited, Windlesham.

## Results

### *Effect of extracellular calcium*

Dose-response curves for the release of histamine in the presence and absence of calcium are shown for compound 48/80 and peptide 401 in Figure 1. Calcium (1.8 mM) potentiated secretion at high concentrations of releasing agent but was inhibitory at lower concentrations. These effects are discussed further below.

### *Requirement for intracellular calcium*

Mast cells incubated for up to 45 min in the presence of calcium (1.8 mM) showed a slight, progressive de-

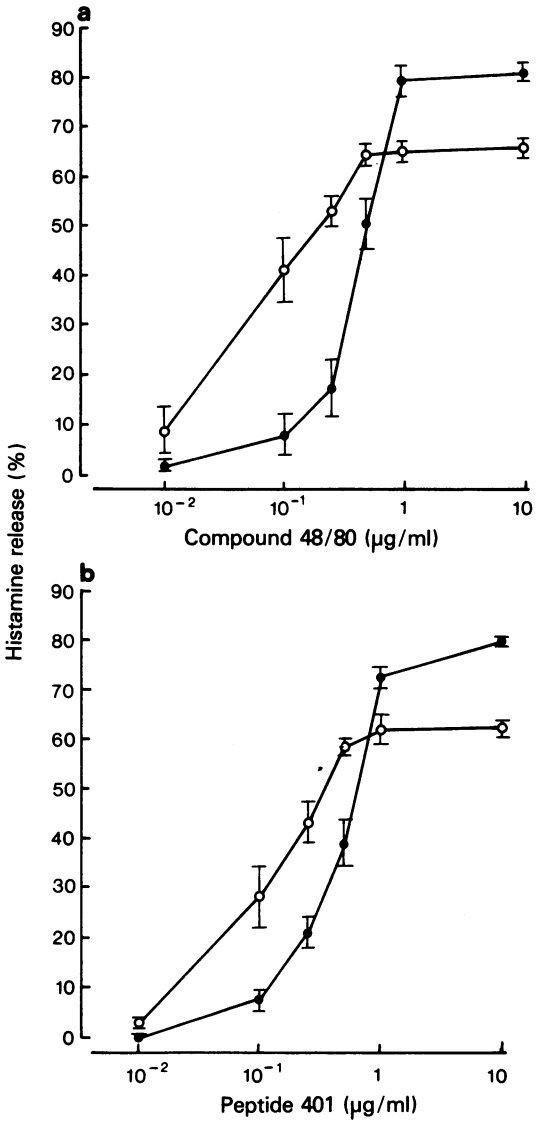


**Figure 1** Dose-response curves for release of histamine by compound 48/80 (a) and peptide 401 (b) from rat peritoneal mast cells in the presence (●) and absence (○) of calcium (1.8 mM). The points are the means from 8 (48/80) or 4 (401) separate experiments and the vertical bars show s.e. mean.

crease in responsiveness to subsequent challenge with compound 48/80 (Figure 2). In sharp contrast, cells incubated in EDTA ($10^{-4}$ M) became very markedly desensitized over the same period. However, these cells were fully or partially responsive if resuspended in a medium containing calcium before challenge. Identical results were obtained with peptide 401 and pretreatment of cells with the calcium ionophore also

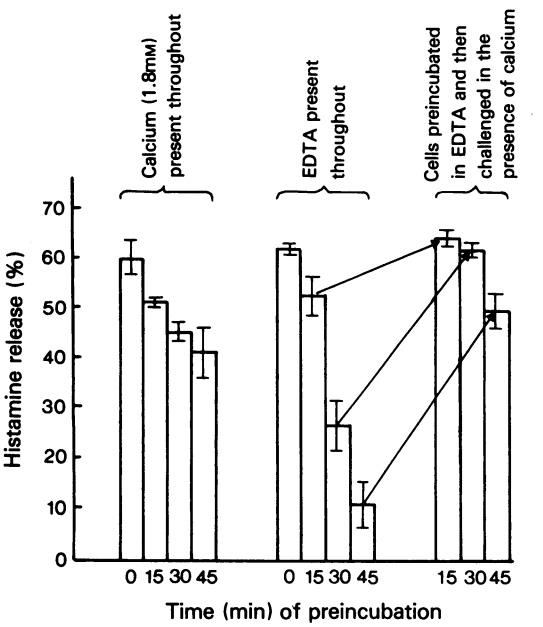


**Figure 2** Calcium-requirement for the histamine release from rat peritoneal mast cells produced by optimal amounts of compound 48/80. Cells were preincubated in the presence of calcium (1.8 mM) or EDTA ($10^{-4}$ M) before challenge. Samples of the latter were also transferred (→) to a calcium-containing medium before challenge. Columns represent the means from 5 separate experiments and the vertical bars show s.e. mean.

inhibited the subsequent response to this agent (Figure 3). This inhibition was reversed when calcium was present at the time of challenge. The addition of calcium after pretreatment with ionophore did not induce histamine release in the absence of inducer.

*Effect of strontium, barium and magnesium*

The effect of increasing concentrations ($10^{-4}$ to $10^{-2}$ M) of strontium or calcium on the release induced by compound 48/80 and peptide 401 are shown in Figure 4. Optimal amounts of both ions potentiated the release but higher concentrations were required in the case of strontium. However, this ion did not produce the marked inhibition observed with calcium.

The effects of barium and magnesium (1 and 10 mM) are shown, together with the results for cells incubated in cation-free media, in Table 1. Barium (10 mM) produced a significant elevation of the spontaneous release and a corresponding increase in the uncorrected induced releases. Magnesium had an inhibitory effect on the induced release, although the effect was statistically significant only in the case of compound 48/80.

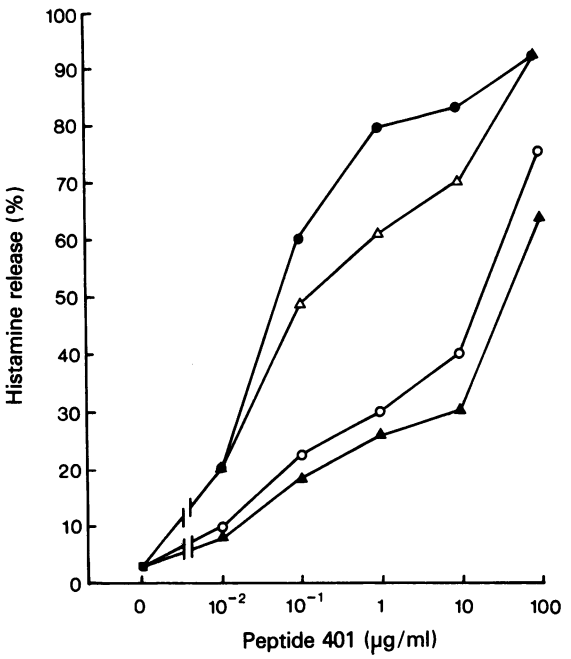


**Figure 3** Calcium-requirement for the histamine release produced by optimal amounts of peptide 401. Rat peritoneal mast cells-were preincubated in calcium-free medium with the ionophore A23187 ($3 \times 10^{-7}$ M) and transferred to media without calcium (▲) or the ion at concentrations of $10^{-5}$ M (○), $10^{-4}$ M (△) or $10^{-3}$ M (●) before challenge. Points are the means from 3 experiments.

The ability of strontium, barium or magnesium to substitute for calcium in cells deprived of divalent cations by incubation with EDTA is shown in Figure 5. Treated cells released some histamine on subsequent reintroduction of barium (10 mM) even in the absence of inducer but both compound 48/80 and peptide 401 produced a marked release provided calcium, strontium or barium, but not magnesium were present.

*Effect of metal ions in isotonic sucrose*

The effect of various metal ions on the spontaneous and induced release of histamine from cells incubated in buffered isotonic sucrose is shown in Table 2.

In these experiments, it was necessary to add sodium ions at the end of the incubation to displace histamine from the released granules (Thon & Uvnäs, 1967). Preliminary tests showed that, provided the solutions and suspensions were cooled in ice before mixing, addition of the inducing agent along with the saline did not lead to any release of amine. The results obtained therefore reflect only induced exocytosis during the incubation period and not subsequent degranulation produced by the addition of sodium ions.

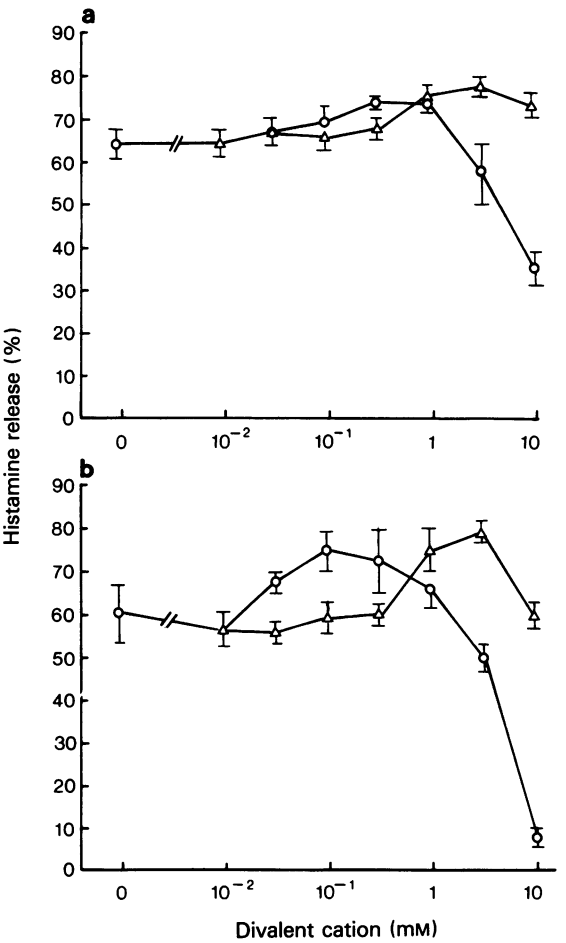


**Figure 4** Effect of increasing concentrations of calcium (○) and strontium (△) on the histamine release from rat peritoneal mast cells produced by optimal amounts of compound 48/80 (a) and peptide 401 (b). The points are the means from 4 separate experiments and the vertical bars show s.e. mean.

The spontaneous release in the absence of any metal cations is very high (approx. 30%) but is significantly suppressed by calcium (1 mM), strontium (1 and 10 mM), magnesium (1 and 10 mM), barium (1 mM) and sodium (1 and 10 mM) but not by potassium (1 or 10 mM). Compound 48/80 produces a significant corrected release (27.9 ± 3.2) of histamine in the complete absence of added metal ions. This value is increased by all the metals tested but most notably by calcium, strontium and barium. In contrast, there is less variation between the uncorrected releases. Essentially similar results were obtained in a more limited study with peptide 401.

## Discussion

In the present study, compound 48/80 and peptide 401 were found to behave in a very similar fashion. Extracellular calcium had a two fold effect on the secretion of histamine evoked by both agents and either inhibited or enhanced the release according to the relative concentrations of ion and inducer. The potentiating effect of moderate amounts of calcium at optimal and supraoptimal concentrations of compound 48/80 has been previously reported (Uvnäs & Thon, 1961; Diamant & Kruger, 1967) but the antagonistic effect at lower concentrations of inducer has not been recorded. However, inhibitory effects of excess calcium have been observed for cells stimulated by antigen, dextran and ATP (Foreman & Mongar, 1972a, b; Dahlquist, Diamant & Krüger, 1974). The mechanism of this inhibition is not known but it may reflect a general stabilizing effect of calcium on the cell membrane or it might result from a competition between calcium and the releasing agent for common receptor binding sites.

The inability of compound 48/80 to evoke the release of histamine or to stimulate exocytosis following treatment of rat mast cells with chelating agents has been examined by Douglas and his coworkers (Douglas & Ueda, 1973; Cochrane & Douglas, 1974). However, these workers routinely incubated the cells for very long periods (2 to 2 h) before challenge whereas in the present study, cells exposed to EDTA for periods in excess of 30 to 45 min had a much decreased responsiveness to the subsequent reintroduction of calcium. Diamant & Patkar (1975) found a similar detrimental effect of long exposure to EDTA and showed that cells could be deprived of calcium and made insensitive to compound 48/80 by pretreatment with the ionophore A23187. Similar results were obtained in the present work with peptide 401.

The effect of other alkaline earth cations was also examined. Strontium was found to be markedly less inhibitory than calcium but resembled this ion in potentiating the release produced by optimal concentrations of compound 48/80 and peptide 401. However, higher concentrations of strontium were required to produce this effect. At high concentrations of barium (10 mM) both the spontaneous and uncorrected releases were significantly increased. In contrast, magnesium was generally inhibitory to the induced release. These experiments, which may ulti-

**Table 1** Effect of barium and magnesium on the histamine release produced by compound 48/80 and peptide 401

| *Ion* (mM) | | *Histamine release (%)* *Spontaneous* | *Uncorrected* | *Corrected* |
|---|---|---|---|---|
| | | *Compound 48/80* | | |
| None | | 4.5 ± 0.5 | 67.1 ± 4.2 | 62.7 ± 4.0 |
| Barium | 1 | 4.5 ± 0.6 | 70.7 ± 5.9 | 66.2 ± 5.4 |
| | 10 | 38.6 ± 9.1* | 83.1 ± 1.1* | 44.6 ± 9.6 |
| Magnesium | 1 | 5.1 ± 0.7 | 65.3 ± 5.2 | 60.3 ± 4.7 |
| | 10 | 6.8 ± 1.7 | 31.0 ± 4.7* | 24.2 ± 3.7* |
| | | *Peptide 401* | | |
| None | | 6.2 ± 1.1 | 67.3 ± 2.3 | 61.2 ± 2.5 |
| Barium | 1 | 7.0 ± 0.8 | 73.6 ± 2.2 | 66.5 ± 2.8 |
| | 10 | 31.2 ± 8.0* | 85.0 ± 0.8* | 53.9 ± 7.8 |
| Magnesium | 1 | 8.5 ± 1.7 | 60.0 ± 5.0 | 51.6 ± 5.0 |
| | 10 | 6.6 ± 1.2 | 48.1 ± 8.4 | 41.5 ± 9.6 |

Values are given as means ± s.e. mean for 4 (48/80) or 5 (401) experiments. Concentrations of releasing agents were 1 μg/ml.
* Denotes values which are significantly ($P \leq 0.05$) different from the appropriate release (spontaneous, uncorrected or corrected) in the absence of added ions. All other values are not significantly different.

**Table 2** Effect of metal ions on the histamine release produced by compound 48/80 and peptide 401 in isotonic sucrose

| *Ion* (mM) | | *Histamine release (%)* *Spontaneous* | *Uncorrected* | *Corrected* |
|---|---|---|---|---|
| | | *Compound 48/80* | | |
| None | | 35.6 ± 6.3 | 63.5 ± 4.8 | 27.9 ± 3.2 |
| Ca | 1 | 8.0 ± 1.7* | 73.7 ± 3.8 | 65.7 ± 3.8* |
| Sr | 1 | 8.9 ± 2.1* | 78.0 ± 2.5* | 69.1 ± 2.7* |
| | 10 | 11.2 ± 2.5* | 80.4 ± 1.7* | 69.1 ± 2.8* |
| Mg | 1 | 8.4 ± 1.5* | 66.6 ± 4.9 | 58.2 ± 4.6* |
| | 10 | 8.7 ± 1.6* | 66.6 ± 4.9 | 57.9 ± 5.1* |
| Ba | 1 | 13.6 ± 2.8* | 79.1 ± 1.8* | 65.4 ± 2.8* |
| | 10 | 20.3 ± 4.7 | 81.6 ± 1.5* | 61.3 ± 4.8* |
| Na | 1 | 19.5 ± 1.7* | 73.7 ± 3.7 | 54.2 ± 3.6* |
| | 10 | 14.5 ± 2.3* | 67.7 ± 4.9 | 53.1 ± 5.7* |
| K | 1 | 20.5 ± 1.9 | 73.4 ± 2.8 | 53.0 ± 2.7* |
| | 10 | 23.9 ± 2.6 | 70.3 ± 3.4 | 46.4 ± 3.9* |
| | | *Peptide 401* | | |
| None | | 27.7 ± 3.1 | 72.7 ± 3.2 | 45.0 ± 3.3 |
| Ca | 1 | 10.3 ± 1.7* | 80.0 ± 3.2 | 69.7 ± 4.0* |
| Sr | 1 | 16.7 ± 3.3* | 80.9 ± 1.2 | 64.2 ± 2.7* |

Values are given as means ± s.e. means for 7 experiments in the case of compound 48/80 (except Na (1 mM) and K (1 and 10 mM) which were based on 6 experiments, and Na (10 mM) which was based on 5 experiments) and 4 experiments in the case of peptide 401. Concentrations of releasing agents were 1 μg/ml.
* Denotes values which are significantly ($P \leq 0.05$) different from the appropriate release (spontaneous, uncorrected or corrected) in the absence of added ions. All other values are not significantly different.

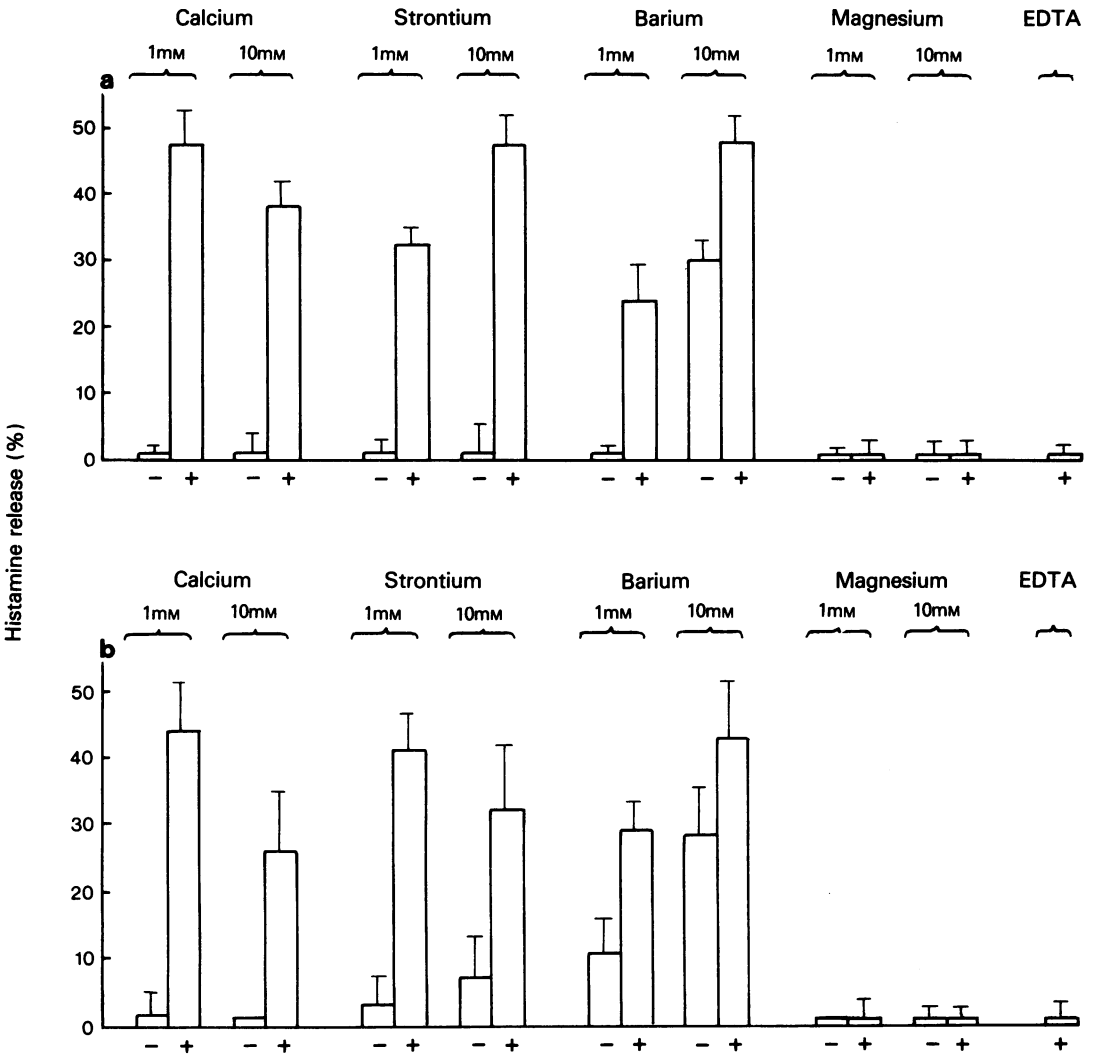


**Figure 5** Effects of alkaline earth ions on the histamine release produced by compound 48/80 (a) and peptide 401 (b) from rat peritoneal mast cells pretreated with EDTA. Cells were incubated in the presence of the chelating agent ($10^{-4}$ M, 35 min) and then transferred to media containing the ions shown. Tyrode (−) or optimal amounts of releasing agent (+) were then added. All values are corrected for the spontaneous release occurring during the preincubation in EDTA. Columns represent the means from 4 experiments and vertical lines (shown where larger than the symbol) are s.e. mean.

mately be of value in elucidating the role of divalent cations in histamine secretion, do not however demonstrate that strontium and barium ions are themselves able to initiate exocytosis since it is possible that an influx of these ions leads to a displacement of bound calcium from sites within the cell. This displacement could then trigger the release mechanism. This possibility was examined by depleting the cells of divalent cations by treatment with EDTA before the challenge step. Such cells still release histamine provided that calcium, strontium or barium but not magnesium were added along with the inducer. It then follows that all three cations are individually able to promote histamine release or that these ions (but not magnesium) displace bound calcium which is inaccessible to the action of both EDTA and the basic releasers.

Cells incubated in isotonic sucrose responded most favourably to compound 48/80 and peptide 401 in the presence of calcium, strontium or barium but, in

keeping with the results of Thon & Uvnäs (1967) and Yamasaki & Sugiyama (1972), there was still a significant, corrected release of histamine in the absence of any added metal cations. The spontaneous release of histamine was very high under these conditions but was much reduced by low concentrations of divalent cations. Similar results have been previously reported by Cochrane & Douglas (1976).

The present and previous studies suggest a dual mechanism of action for compound 48/80 and peptide 401. These agents can clearly utilize intracellular calcium to initiate the release process but can also stimulate cells depleted of this store, provided that calcium is present in the extracellular medium. Under the latter conditions, the inducers may evoke exocytosis by promoting calcium uptake, presumably by activating specific calcium-gates (Foreman *et al.*, 1976) in the membrane, in a way similar to antigen and other agents. The mechanism by which the basic releasers mobilize intracellular calcium is not clear. Cochrane & Douglas (1976) have suggested that calcium may be bound to the inner plasma membrane in a manner which requires or is facilitated by the binding of sodium to the outer surface of the cell. Cationic agents like compound 48/80 and peptide 401 might then displace sodium from these sites with an accompanying liberation of intracellular calcium and release of histamine.

Finally, the basic releasers may provide information about the mechanisms which control histamine secretion. Adenosine 3',5'-cyclic monophosphoric acid (cyclic AMP) is an important modulator of the process and increased intracellular levels of the nucleotide inhibit release. Mongar and his coworkers (Foreman *et al.*, 1976; Garland & Mongar, 1976; Foreman, Hallett & Mongar, 1977c) believe that this agent acts directly on the calcium-gating mechanism, so blocking influx of the ion and preventing exocytosis. However, we have recently shown (unpublished observations) that cyclic AMP and related antiallergic compounds block the release induced by compound 48/80 and peptide 401 in the absence of extracellular calcium and hence under conditions where the gating mechanism is not operative. These results suggest that such inhibitors may have a more general role in the regulation of intracellular concentrations of calcium whether derived from internal or external sources.

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