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THE ROLE OF CELL-FIXED CALCIUM IN HISTAMINE RELEASE BY COMPOUND 48/80

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1 Histamine release by compound 48/80 was substantially reduced in a time-dependent manner (maximum at 30 min) by pre-incubating mast cells in calcium-free medium at 37°C but not at 2°C. This effect was optimal at pH 7.0 to 7.5.

2 The re-introduction of calcium (0.1 to 3 mmol/l) restored histamine release to the control value; this effect was independent of temperature.

3 Strontium (1 to 30 mmol/l) partially reversed the effect of calcium deprivation but the same concentrations of barium and magnesium depressed histamine release even further. Magnesium (3 to 15 mmol/l) antagonized the effect of calcium replacement.

4 Results suggest that the level of cell-fixed calcium involved in compound 48/80-induced histamine release may be controlled by the combinaton of rapid passive influx and slow active efflux.

Introduction

In common with many other secretory processes, the release of histamine from mast cells is believed to be mediated by elevation of the intracellular calcium concentration (Foreman, Garland & Mongar, 1976) and it is generally accepted that calcium influx follows a transient change in membrane permeability (Foreman, Mongar & Gomperts, 1973). However, whilst anaphylactic histamine release is largely dependent on extracellular calcium (Mongar & Schild, 1958; Foreman & Mongar, 1973), release induced by compound 48/80 occurs in the absence of added calcium (Uvnäs & Thon, 1961; Kruger, 1976). This is an important distinguishing feature between the two secretory reactions, which otherwise share many properties (Perera & Mongar, 1965; Johnson & Moran, 1969). The prior incubation of rat mast cells with either disodium edetate (EDTA) (Douglas & Ueda, 1973; Kagayama & Douglas, 1974; Cochrane & Douglas, 1974) or the ionophore A23187 under calcium-free conditions (Diamant & Patkar, 1975) prevents the response to compound 48/80, recovery occurring on the re-introduction of calcium. These results have been interpreted as evidence for the utilization of cell-fixed calcium by compound 48/80 to stimulate secretion. However, it is not clear that such treatments do not have other effects possibly deleterious to cell integrity. Therefore, in the present study the effect of simply pre-incubating rat peritoneal and pleural mast cells in calcium-free media on compound 48/80-induced histamine release has been investigated.

Methods

Peritoneal and pleural washings, containing approximately 4% mast cells, from at least 4 male Wistar rats were centrifuged (150 *g*, 6 min) and the pellets resuspended (10 ml per rat) in ice-cold, calcium-free HEPES Tyrode solution (pH 7.4) having the following composition (mmol/l): NaCl 137.0; KCl 2.7, $MgCl_2$ 1.0, HEPES (N-2 hydroxyethyl-piperazine-N'-2-ethane sulphonic acid) 12.0 and D(+)-glucose 5.6. Aliquots (1.5 ml) of this cell suspension were incubated in polypropylene centrifuge tubes at 37°C for 0 to 30 min. The cells were then washed twice by centrifugation at 4°C (150 *g*, 6 min) and resuspension in ice-cold calcium-free Tyrode (0.5 ml per tube) and equilibrated at 37°C for 2 min before addition of 0.5 ml of prewarmed Tyrode solution containing compound 48/80 (final concentration 0.5 μg/ml). Five min later the histamine release reaction was stopped by adding 4 ml of ice-cold calcium-free Tyrode solution and each sample was centrifuged. The supernatants were decanted and the pellets resuspended in 5 ml of Tyrode solution and boiled for 10 min to release residual histamine. Released and residual histamine were assayed fluorimetrically by the method of Shore, Burkhalter & Cohn (1959) but omitting the extraction procedure since histamine was the only substance present in the mast cell samples which fluoresced in the presence of *o*-phthaldialdehyde (Bergendorff & Uvnäs, 1972). Histamine release values (as a percent-

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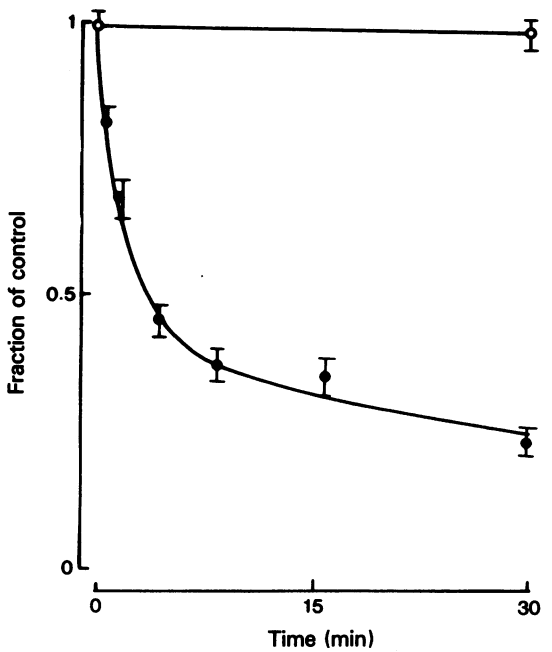

Figure 1 Compound 48/80-induced histamine secretion from mast cells pre-incubated for varying times up to 30 min in calcium-free Tyrode solution at either 37°C (●) or 2°C (○). Points represent the mean from three experiments, each in duplicate; vertical lines show s.e. mean.

age of total histamine content) were corrected for spontaneous release of 4 to 10% (which remained unchanged during each of the described experiments) and expressed as fractions of the appropriate control release value (which ranged from 37 to 47% of total histamine).

Compound 48/80 and *o*-phthaldialdehyde were obtained from Wellcome Reagents Ltd and Sigma respectively; all other reagents were of Analar quality.

Results

When mast cell suspensions were incubated in calcium-free Tyrode solution at 37°C before addition of compound 48/80 the subsequent release of histamine was reduced to about 30% of that from aliquots of the same cell sample incubated under identical conditions but in calcium-containing medium (1.0 mmol/l). This reduction was dependent upon the length of the pre-incubation period, being maximal after 30 min. In contrast, when cells were pre-incubated at 2°C in calcium-free media, the release of histamine was not reduced (Figure 1). In two duplicate experiments where the pH of the incubation medium was varied

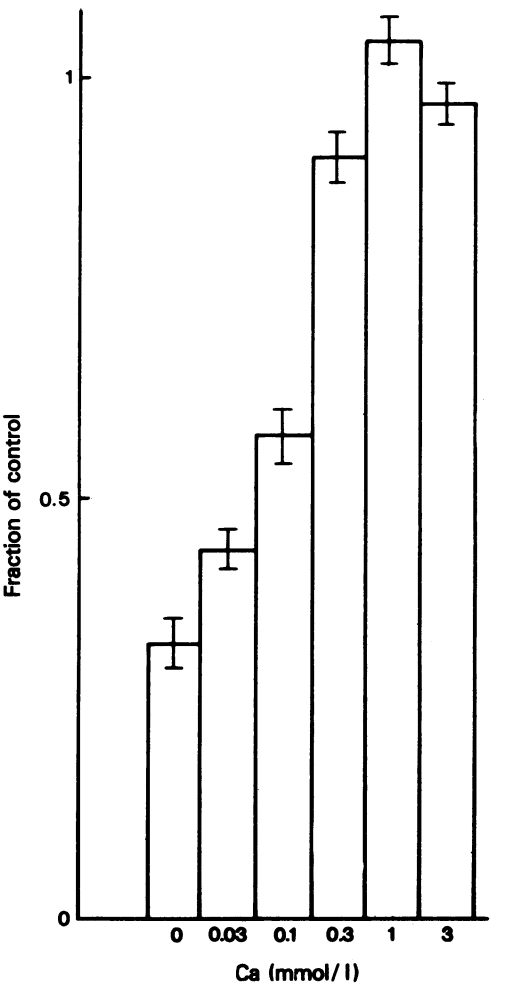

Figure 2 Histamine secretion from mast cells pre-incubated in calcium-free Tyrode solution for 30 min at 37°C, washed, and pretreated with increasing concentrations of calcium at 2°C before being washed again and exposed to compound 48/80. Each column represents the mean from at least six duplicate experiments; vertical lines show s.e. mean.

by increments of 0.5 of a pH unit in the range pH 4.5 to 8.5, the reduction in histamine release was directly related to increasing alkalinity, becoming optimal at pH 7 to 7.5. Analysis of total histamine contents revealed no significant difference between cell samples pre-incubated either at 2°C or at 37°C. Thus the reduced response to compound 48/80 cannot be attributed to the loss of cell histamine.

The role of cell-fixed calcium in compound 48/80-induced histamine release was investigated in the following way. Cells that had been previously incubated in calcium-free medium at 37°C for 30 min

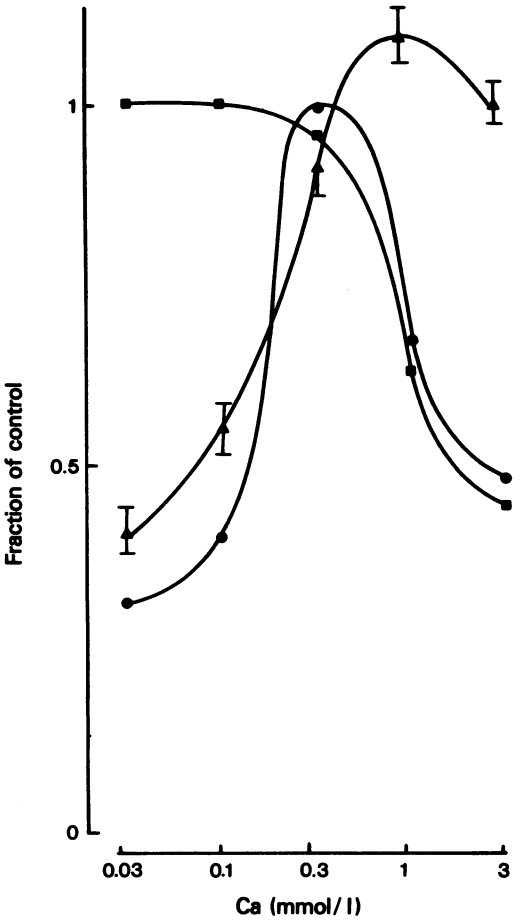

Figure 3 Histamine secretion from mast cells deprived of calcium for 30 min at either 37°C (●) or 2°C (■) before simultaneous addition of compound 48/80 and increasing concentrations of calcium; (▲) indicate response of cells that were washed between addition of calcium and compound 48/80. Results from a duplicate experiment are compared with data from Figure 2 (▲).

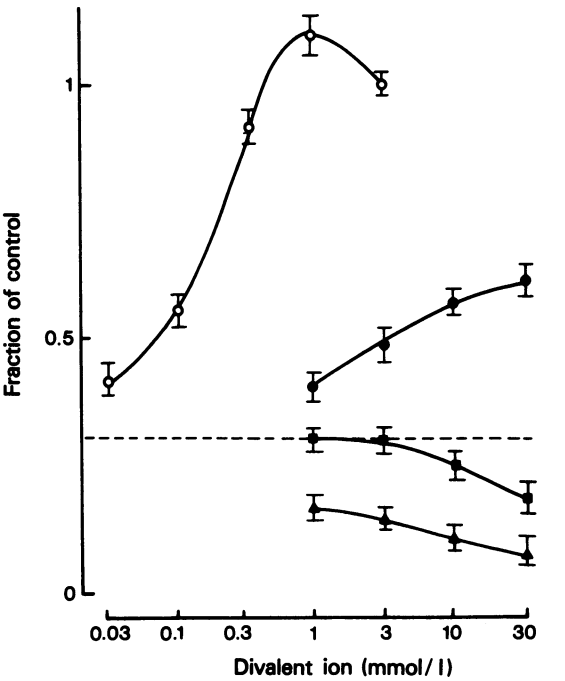

Figure 4 Log concentration effect curves for strontium (●), magnesium (■) and barium (▲) on compound 48/80-induced histamine release from calcium-deprived mast cells. The curve for calcium (○) is included for comparison. Points represent the means from at least six experiments, each in duplicate; vertical lines show s.e. mean. The broken line represents the level of histamine release from calcium-deprived cells to which no cation was added (0.32 ± 0.02 that of control).

were washed twice (see Methods), resuspended in ice-cold Tyrode solution containing calcium at 0.03 to 3.0 mmol/l and then immediately washed twice more with ice-cold, calcium-free Tyrode solution (to remove extracellular calcium) before incubating with compound 48/80 at 37°C. Figure 2 shows that such pretreatment restored histamine release to the control value (cells treated comparably but kept throughout at 2°C) and that this effect was related to the concentration of calcium added, becoming maximal at 1 to 3 mmol/l. The simultaneous addition of calcium (0.3 mmol/l) with compound 48/80 completely restored histamine release to the control value, but concentrations above this were inhibitory. Similarly, histamine release from control cells was inhibited by calcium concentrations above 0.3 mmol/l added with compound 48/80. Figure 3 compares the results from these three types of calcium addition experiments.

The effect of calcium deprivation was partially reversed by pretreating cells with strontium before adding compound 48/80, histamine release recovering to a maximum of 62 ± 3.5% of the control value, with a strontium concentration of 30 mmol/l. In contrast, pretreatment with either magnesium or barium in the same concentration range (1 to 30 mmol/l) reduced histamine release even further (Figure 4). The addition of magnesium (3 to 15 mmol/l) together with calcium (0.1 to 3 mmol/l) at this pretreatment stage resulted in a graded shift of the log concentration-effect curve for calcium-induced recovery of secretory competence (Figure 5); the maximum calcium effect was progressively depressed by increasing the concentration of magnesium.

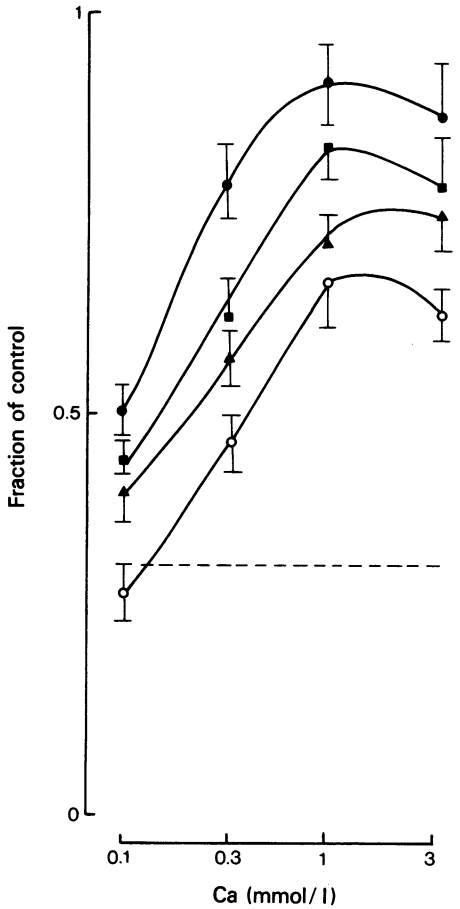

Figure 5 The effect of increasing concentrations of magnesium on the log concentration-effect curve for calcium in restoring histamine secretion from calcium-deprived cells: Mg, 1 mmol/l (●), 3 mmol/l (■), 10 mmol/l (▲), 15 mmol/l (○). Each point represents the mean from the duplicates of three experiments; vertical lines show s.e. mean. The broken line represents the level of histamine release from calcium-deprived cells to which no cations were added (0.33 ± 0.03 that of control).

Discussion

Previous observations (Douglas & Ueda, 1973; Kagayama & Douglas, 1974; Cochrane & Douglas, 1974; Diamant & Patkar, 1975) have suggested that cell-fixed calcium is important for the release of histamine induced by compound 48/80. The present results support this hypothesis, whilst having the advantage of being obtained without the use of agents such as EDTA or the ionophore A23187 which in previous studies may have had effects apart from depleting cellular calcium. Figure 1 clearly shows that histamine release by compound 48/80 was substantially reduced by simply pre-incubating cells in calcium-free Tyrode solution at 37°C for 30 min. Furthermore, this reduction was immediately reversed by addition of extracellular calcium and it is particularly interesting that the concentrations of calcium required to have this effect were identical to those previously found to augment anaphylactic histamine release (Foreman & Mongar, 1972). This suggests that antigen- and compound 48/80-induced histamine release share a common requirement for calcium. Compound 48/80, but not antigen, is able to meet this requirement by drawing on cellular calcium reserves which the present experiments suggest are depleted by calcium deprivation.

The temperature- and pH-dependence of the calcium deprivation effect described above suggests the involvement of enzymatic activity. In contrast, the recovery of secretory competence conferred by calcium was not dependent on either the temperature or the time at which it was added. Thus, it may be envisaged that levels of calcium stored within the mast cell are controlled by a combination of rapid passive influx and a slow active efflux, possibly by a calcium-dependent ATPase as has already been suggested to be responsible for the maintenance of a low cytosolic calcium concentration (Fewtrell & Gomperts, 1977).

In contrast to calcium, magnesium inhibits secretory responses (Rubin, 1970; Baker, 1972; Blaustein, 1974; Douglas, 1974) and in particular competitively antagonizes the augmenting effect of calcium on anaphylactic histamine release (Foreman & Mongar, 1972). Magnesium also antagonized the restorative effect of calcium in the present experiments. This antagonism seemed not to be competitive, the maximum effect of calcium being depressed by each concentration of magnesium. However, magnesium may have had a dual effect in these experiments as pretreatment with magnesium alone augmented the effect of calcium deprivation.

Foreman & Mongar (1972) have shown that strontium will replace calcium ions in the anaphylactic release of histamine from rat mast cells. It is interesting, therefore, that the addition of strontium partially restored compound 48/80-induced histamine release from calcium-deprived cells. The observation that higher concentrations of strontium than calcium were required to produce an equivalent effect agrees with their findings. However, the efficacy of strontium is higher than calcium in the anaphylactic reaction, but appeared to be lower in the release reaction induced by compound 48/80 (Figure 4). An alternative explanation may be that strontium was removed more readily from the cells than calcium by washing, which in these experiments always preceded the addition of compound 48/80.

Barium also substitutes for calcium in the anaphylactic reaction but only at concentrations that potentiate spontaneous histamine release (Foreman & Mongar, 1972). However, barium did not restore the release of histamine induced by compound 48/80 from calcium-deprived cells. Indeed, concentrations of 1 to 30 mmol/l further reduced the release. This may be attributable to possible toxicity of high concentrations of barium or displacement by this ion of residual calcium from already depleted stores.

A.N.P. is a Wellcome student.

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