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THE ROLE OF THE ALKALINE EARTH IONS IN ANAPHYLACTIC HISTAMINE SECRETION

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SUMMARY

1. Anaphylactic histamine release from rat peritoneal mast cells is dependent on the presence of calcium ions. Graded increase of histamine release occurs as the calcium ion concentration is raised from 0·1 to 1·0 m-mole/l.

2. Magnesium antagonizes the effect of calcium. The dissociation constant of the Mg-receptor complex was found to be 9·4 m-mole/l.

3. Strontium will replace calcium ions in the activation of anaphylactic histamine release. Graded increase of histamine release occurs when the strontium ion concentration is raised from 1·0 to 10·0 m-mole/l.

4. Magnesium also antagonizes the effect of strontium. The dissociation constant of the magnesium-receptor complex was found to be 11·0 m-mole/l.

5. The log concentration–effect curve for strontium has a greater maximum and steeper slope than the curve for calcium.

6. Measurements of the interaction of calcium and strontium ions are in agreement with the hypothesis that strontium possesses a higher efficacy but a lower affinity than calcium, for the 'calcium receptor' in mast cells.

7. Barium will replace calcium in the activation of anaphylactic histamine release.

INTRODUCTION

Calcium ions are required for the anaphylactic release of histamine from chopped guinea-pig lung (Mongar & Schild, 1958), human leucocytes (Lichtenstein & Osler, 1964) and rabbit basophil leucocytes (Greaves & Mongar, 1968). Mongar (1970) found that strontium will replace calcium in chopped guinea-pig lung, and that magnesium and barium antagonize this effect of calcium. Strontium and barium replace calcium in the activation of histamine release induced by compound 48/80 in the perfused cat paw (Strandberg, 1971).

There have been several studies of the antagonism between calcium and magnesium at the neuromuscular junction (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Bloich, Glagoleva, Liberman & Nenashev, 1968), and strontium is able to substitute for calcium in the activation of transmitter release at the neuromuscular junction (Miledi, 1966; Dodge, Miledi & Rahmamimoff, 1969). Douglas & Rubin (1964) have reported that strontium will substitute for calcium in the activation of catecholamine release from adrenal chromaffin tissue. Strontium will also replace calcium in the process leading to the secretion of neurohypophysial hormones (Buchs, Dreifuss, Grau & Nordmann, 1972).

The alkaline earth ions are, therefore, useful tools for examining the mechanism of secretory processes, and the experiments described in this paper form part of a study of the actions of these ions in histamine release from isolated mast cells.

A preliminary communication of part of this work has already been published (Foreman & Mongar, 1972).

METHODS

A closed, random bred colony of Lister Hooded rats, weighing between 150 and 350 g, were used for these experiments. Male and female rats were used according to availability.

The method of sensitizing the rats is a modification of a method described by Mota (1964). The rats were sensitized to egg albumin (B.D.H.) by the intramuscular injection into each hind limb of 0·25 ml. of a suspension containing egg albumin 5 mg/ml. in Freund's incomplete adjuvant (Difco). An I.P. injection of 0·5 ml. of Pertussis vaccine B.P. (Burroughs Wellcome) was administered to each rat at the same time as the albumin injection.

Fifteen to 30 days after injection, the rats were lightly anaesthetized with diethyl ether, and decapitated. 4 ml. saline (NaCl, 154 m-mole/l.) containing heparin 100 μg/ml. (Evans Medical), was injected into the peritoneal cavity of each rat. The peritoneum was massaged for approximately one minute before the cavity was opened by a mid line incision, and the fluid withdrawn. The peritoneal washings from several rats were pooled to provide sufficient cells for an experiment: one rat providing about six samples.

The cell suspension obtained was divided into samples of equal volume, each containing between 0·2 and 1·0 μg histamine and these were centrifuged at 50 g for 5 min. The supernatants were discarded and the pellets resuspended in a medium containing the appropriate concentration of alkaline earth ions. The final volume of the cell suspension was usually 0·5 ml. The antigen was added to produce a final concentration of between 20 and 100 μg/ml. in the incubating medium, and the samples were incubated at 37° C for 10 min. At the end of the incubation period, the reaction was stopped with 2·0 ml. ice-cold Tyrode solution free from calcium and magnesium ions. The samples were centrifuged at 1000 g for 5 min and the supernatants assayed for histamine. The pellets were resuspended in Tyrode solution and heated in a water bath at 100° C for 10 min to release residual histamine.

Assay of released and residual histamine was performed biologically on the isolated guinea-pig ileum (Boura, Mongar & Schild, 1954). The calcium concentration of the

samples was adjusted where necessary to ensure an adequate concentration of calcium for the contraction of the ileum. Barium ions were precipitated before assay by adding a small excess of sodium sulphate, and at the dilutions used, the other alkaline earth ions did not affect the assay.

The media used for incubating the cells was based on Tyrode solution, which has the following composition: NaCl 137·0 m-mole/l.; KCl 2·7 m-mole/l.; NaH_2PO_4 0·4 m-mole/l.; $NaHCO_3$ 12·0 m-mole/l.; glucose 5·6 m-mole/l.; $CaCl_2$ 1·8 m-mole/l.; $MgCl_2$ 1·0 m-mole/l. The required amounts of alkaline earth ions were added to Tyrode solution from which the calcium and magnesium had been omitted. At high concentrations of alkaline earth ions, which were likely to be precipated, the bicarbonate in the medium was replaced by 10–30 m-mole/l. HEPES (4-(2 hydroxy ethyl)-1-piperazine ethane sulphonic acids) (Burroughs Wellcome or Biocult). The chemicals used to make Tyrode solution and the chlorides of the alkaline earth ions were of Analar quality.

RESULTS

In the absence of antigen, the isolated mast cells released 1·8 ± 0·1 % (mean ± S.E.) of their histamine, this release being referred to as spontaneous release. In the results presented in this paper, unless otherwise stated, the spontaneous release has been subtracted from the total release in the presence of antigen, to give a value for the anaphylactic histamine release.

The effect of calcium

Removal of calcium from the incubating medium does not reduce the anaphylactic histamine release to zero. In six experiments, the spontaneous release was 1·5 ± 0·1 % (mean ± S.E.) while the total release in calcium-free Tyrode solution was 7·7 ± 1·2 % (mean ± S.E.). On average, therefore, addition of antigen to cells in calcium-free medium resulted in 6·2 % release above the spontaneous level. Addition of EDTA 0·1 m-mole/l. to the calcium-free medium did not reduce the anaphylactic histamine release.

Increasing the concentration of calcium in the incubating medium causes a corresponding increase in the anaphylactic histamine release. The concentration–effect curve is shown in Fig. 1, the maximum effect being achieved at a concentration of 1–2 m-mole/l. At calcium concentrations above 2 m-mole/l. histamine release was depressed in some experiments. In terms of percentage histamine release, the maximum effect varied, depending on the degree of sensitization of the cells to egg albumin, and so Fig. 2 represents the mean concentration–effect curve, where effect is expressed as a percentage of the maximum histamine release obtainable with calcium.

In experiments where the spontaneous release was determined at different calcium ion concentrations, no concentration related effect was observed over a range of calcium ion concentrations from 0·1 to 1·8 m-mole/l.

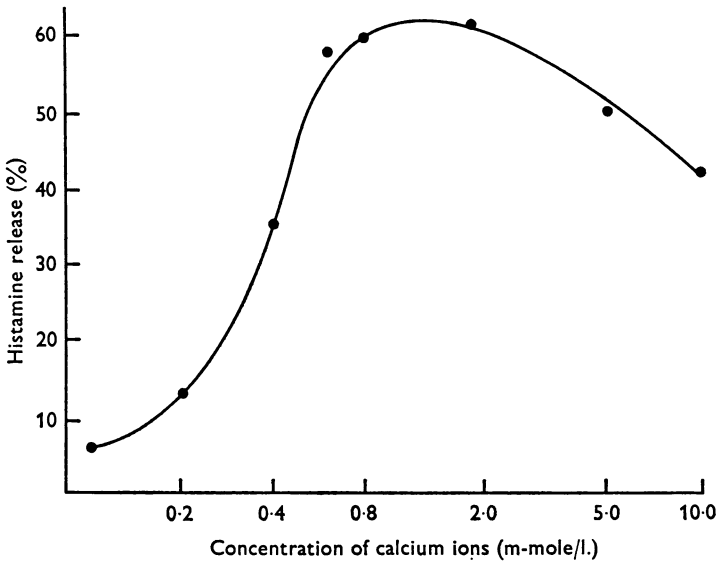

Fig. 1. Concentration–response curve for calcium in the anaphylactic release of histamine. Results from a single experiment.

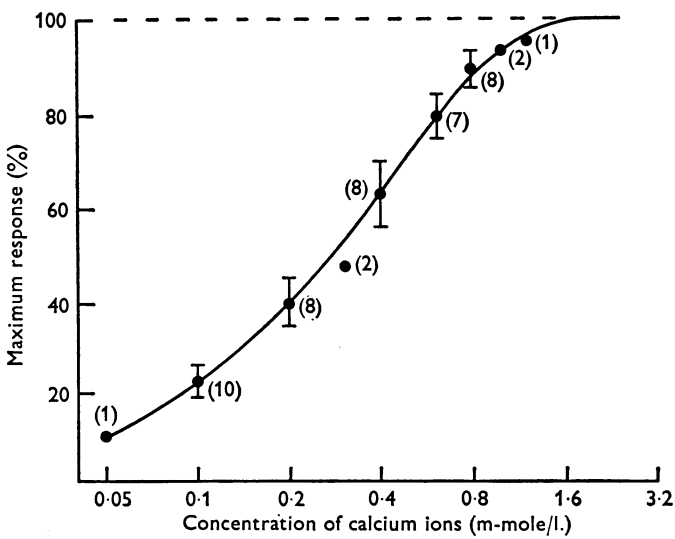

Fig. 2. Mean concentration–effect curve for calcium in the anaphylactic release of histamine. Figures in parentheses indicate number of experiments contributing to each point. Vertical bars refer to S.E. of mean.

Antagonism of the effect of calcium by magnesium

In media containing a constant concentration of calcium, increasing the concentration of magnesium reduces the anaphylactic histamine release. The inhibition caused by magnesium can be reversed by increasing the concentration of calcium.

Assuming that calcium and magnesium interact reversibly with a receptor site in the cells

$$Ca + R \rightleftharpoons CaR,$$

$$Mg + R \rightleftharpoons MgR,$$

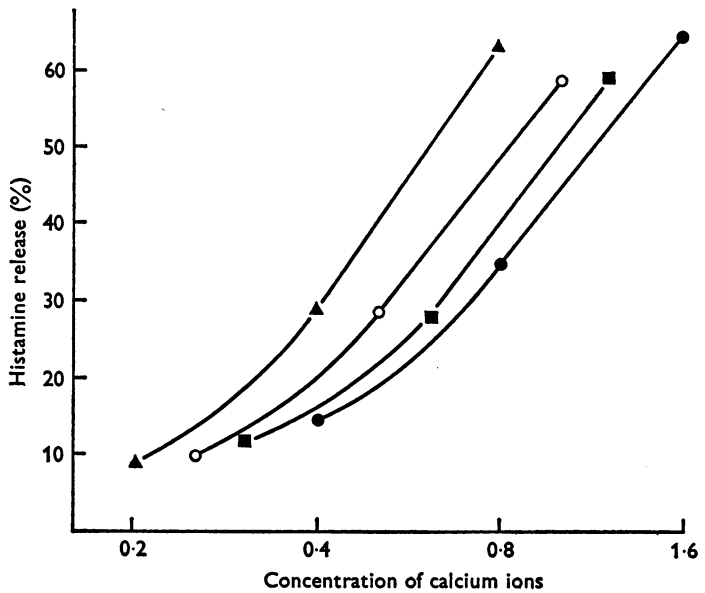

Fig. 3. Action of magnesium on concentration–effect curve for calcium. ▲—▲ no Mg^{2+}; ○—○ Mg^{2+} 3 m-mole/l.; ■—■ Mg^{2+} 6 m-mole/l.; ●—● Mg^{2+} 9 m-mole/l. Results from a single experiment.

and that only CaR is capable of activating histamine release, it can be shown (Schild, 1949) that at equilibrium, the following relationship should hold:

$$x - 1 = K_{Mg}[Mg]$$

where the affinity constant of the magnesium-receptor complex is K_{Mg}, and x is the ratio of calcium concentrations in the presence and absence of magnesium, required to produce the same effect and [Mg] is the concentration of magnesium in solution. Thus a plot of $\log(x-1)$ against log of magnesium concentration should produce a straight line with a gradient of 1 and an intercept on the abscissa corresponding to $-\log K_{Mg}$.

Addition of magnesium ions to a calcium containing medium shifts the log concentration–effect curve to the right in a graded and parallel manner, as concentration of magnesium ions is increased.

Fig. 4 illustrates a plot of log $(x-1)$ against log [Mg], constructed from a series of seven experiments of the kind shown in Fig. 3. The slope of the line is 0·9, and the value of the dissociation constant obtained from the intercept on the abscissa is 9·4 m-mole/l.

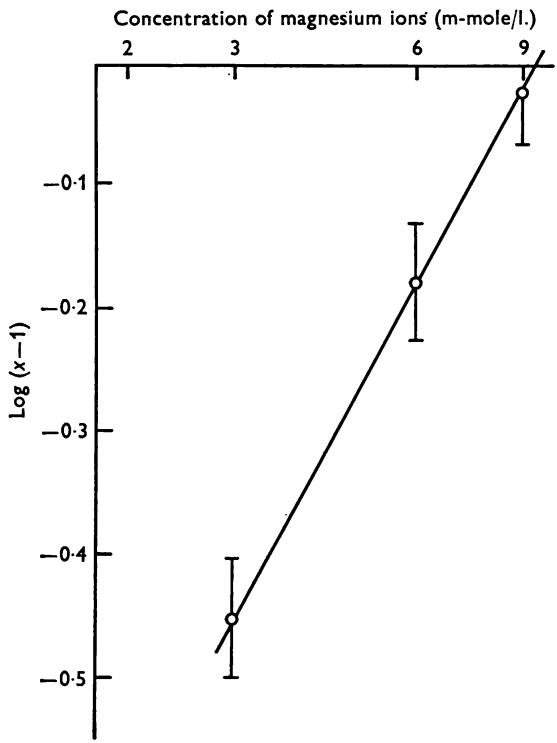

Fig. 4. Plot of log $(x-1)$ against magnesium concentration on a log scale, for calcium-magnesium antagonism. The line was fitted by eye to the points, which represent the means from seven experiments ± S.E.

In experiments where the concentration–effect curves were not exactly parallel a mean value of x was calculated for the range of the curves determined in the experiment. The results are compatible with the concept that magnesium and calcium compete for the same receptor, but in experiments of this kind it should also be mentioned that if it assumed that only a small fraction of receptors is activated, on the theory tested, parallel log concentration–effect curves would be obtained for small magnesium concentrations even if the antagonism were non-competitive. Also, for small

values of x, the relationship between log $(x-1)$ and log [Mg] would be linear, with a slope of unity, if the antagonism were non-competitive.

Equilibrium conditions for the measurement of the calcium-magnesium antagonism have been assumed. The minimum time between the addition of magnesium and calcium ions to the cells at 0° C and the challenge with antigen at 37° C was 5 min.

Effect of strontium ions

Anaphylactic release of histamine from the mast cells occurred on substituting strontium for calcium (Fig. 5).

Increase of strontium ion concentration, in contrast to calcium, potentiates the spontaneous release of histamine though it remains at a low level

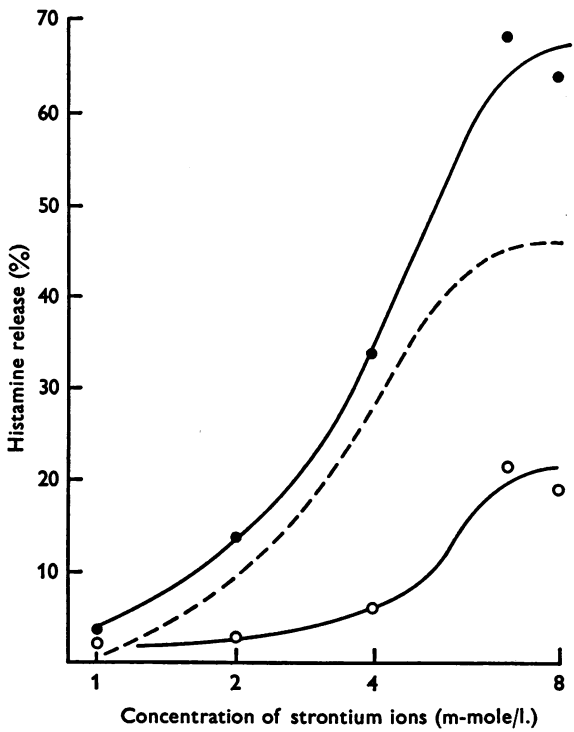

Fig. 5. Concentration–effect curve for strontium ions. ○—○ spontaneous release; ●—● total release in presence of antigen. Broken line is derived by subtracting spontaneous from total release and represents anaphylactic release. Results from a single experiment.

compared with the total release in the presence of antigen. Fig. 6 shows the mean concentration–effect curve for strontium, the maximum effect occurring at a concentration of about 10 m-mole/l. The range of strontium concentrations producing graded increase in histamine release is of the

order of ten times greater than the corresponding range of calcium concentrations.

Antagonism of the effect of strontium by magnesium

A graded, parallel shift to the right of the strontium concentration–effect curve was obtained when increasing concentrations of magnesium were added to the strontium containing incubating medium (Fig. 7).

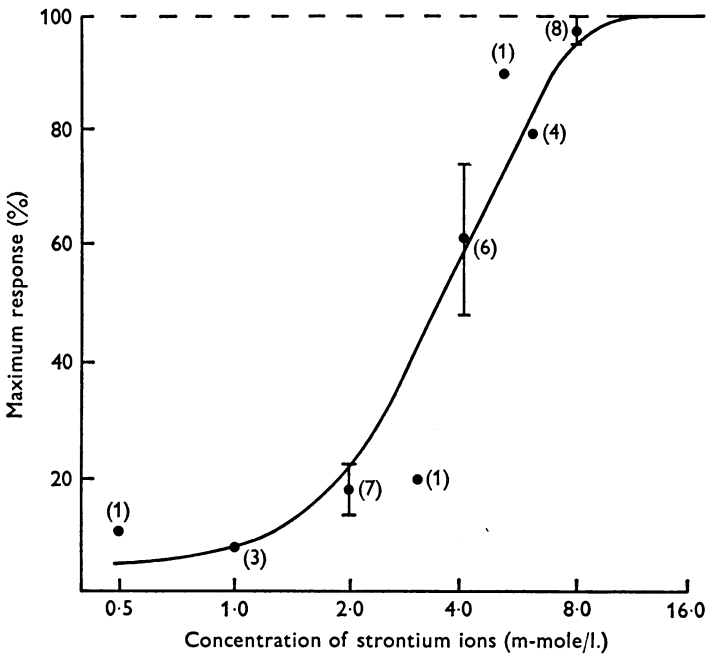

Fig. 6. Mean concentration–effect curve for strontium in the anaphylactic release of histamine. Figures in parentheses indicate the number of experiments contributing to each point. Vertical bars refer to S.E. of mean.

From a series of eight experiments, a plot of log $(x-1)$ against log [Mg] was constructed, and the slope of the line was found to be 1·17. The value for the dissociation constant for the magnesium-receptor complex was found to be 11·0 m-mole/l. (Fig. 8).

In all experiments, controls were included to estimate the spontaneous release in the presence of the various mixtures of strontium and magnesium. The results showed that magnesium depressed the concentration–related effect of strontium on spontaneous histamine release.

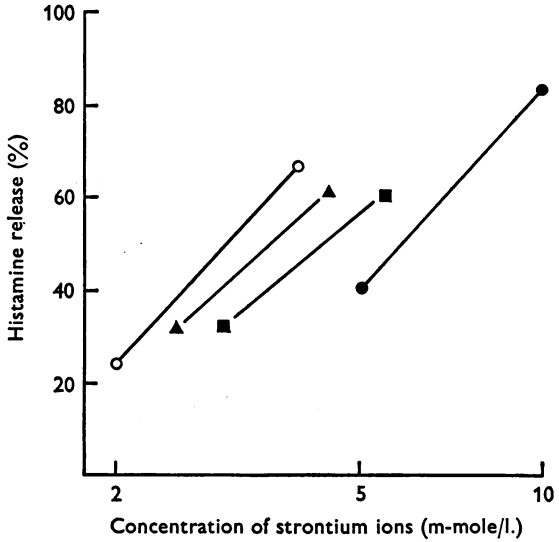

Fig. 7. Action of magnesium on the concentration–effect curve for strontium. ○—○ no Mg^{2+}; ▲—▲ Mg^{2+} 2 m-mole/l.; ■—■ Mg^{2+} 4·5 m-mole/l.; ●—● Mg^{2+} 12 m-mole/l. Results from a single experiment.

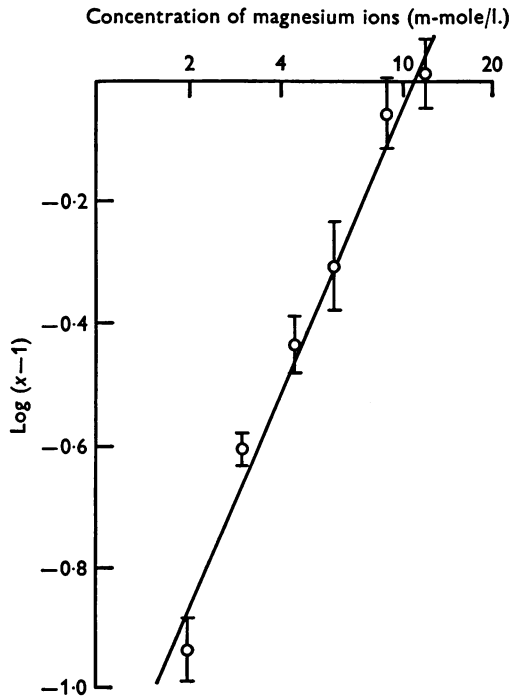

Fig. 8. Plot of $\log(x-1)$ against concentration of magnesium on a log scale for strontium–magnesium antagonism. The line was fitted by eye to the points, which represent the means from eight experiments ± S.E.

Comparison of the effect of calcium and strontium

In the initial experiments with strontium, it was observed that higher maximum releases of histamine were being obtained than in the experiments with calcium. Large differences in the degree of sensitization between batches of rats could have explained this observation, and so concentration–effect relationships for calcium and strontium were determined on the same pooled cell population. A marked difference between the concentration–effect curves for the two ions was observed in all experiments, and a typical result is shown in Fig. 9.

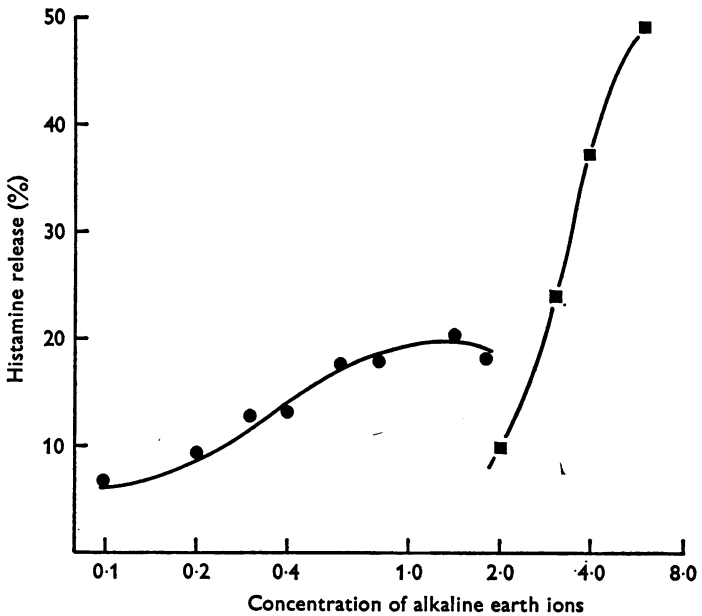

Fig. 9. Comparison of the concentration–effect curves for calcium and strontium in a single experiment on the same cell population. ●—● calcium; ■—■ strontium.

Strontium consistently produced a greater maximum release than calcium, and the curve for strontium is steeper than that for calcium. In a series of five experiments in which maxima were determined for calcium and strontium the ratio of the maxima (Sr/Ca) had a mean value of $2{\cdot}2 \pm 0{\cdot}2$ (mean $\pm$ S.E.).

Another approach to the difference between the two ions is illustrated by the type of experiment shown in Fig. 10. Antigen concentration–effect curves were constructed by incubating aliquots of cells from a pooled sample with a medium containing either calcium, 1 m-mole/l., or strontium,

10 m-mole/l.; both concentrations being sufficient to produce a maximum response.

Cells suspended in the medium containing strontium consistently released more histamine than those suspended in a medium containing calcium, at all antigen concentrations.

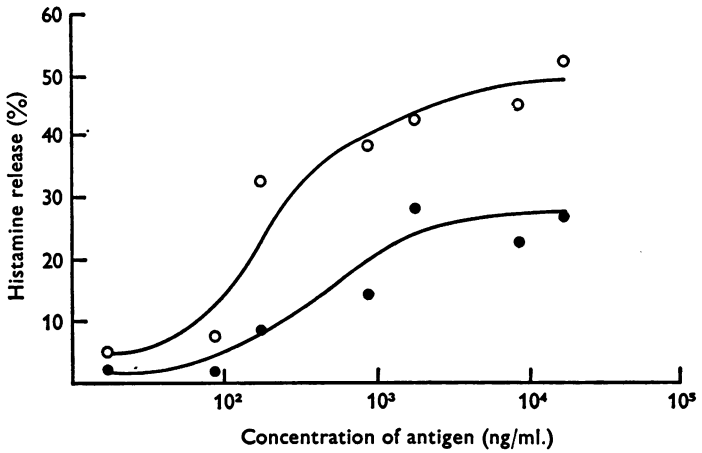

Fig. 10. Antigen concentration–effect curves: ●—● in a medium containing calcium 1 m-mole/l.; ○—○ in a medium containing strontium 10 m-mole/l. Results from a single experiment.

Interaction between calcium and strontium

The close similarity of the dissociation constants for the magnesium-receptor complex obtained when calcium or strontium are used as agonists suggests that the two ions are acting at the same site. Further support for this hypothesis is gained from experiments involving the interaction of calcium and strontium. The reaction of the two ions with the receptor can be expressed as follows

$$Ca + R \rightleftharpoons CaR,$$

$$Sr + R \rightleftharpoons SrR.$$

Application of the Law of Mass Action, and the concept of affinity and efficacy, introduced by Stephenson (1956), to these equilibria yields the following relationships:

$$S_{Ca} = e_{Ca} \frac{K_{Ca}[Ca]}{1 + K_{Ca}[Ca] + K_{Sr}[Sr]}, \quad (1)$$

$$S_{Sr} = e_{Sr} \frac{K_{Sr}[Sr]}{1 + K_{Ca}[Ca] + K_{Sr}[Sr]} \quad (2)$$

where S is the stimulus to the tissue and is a function of the efficacy of the ion (e) and the fraction of receptors occupied. K_{Ca} and K_{Sr} are the affinity constants for the ion-receptor complexes.

Assuming the response to be directly proportional to the stimulus, the response in the presence of calcium and strontium can be calculated by combining eqns. (1) and (2)

$$\text{Response} = f(S_{Ca} + S_{Sr}). \tag{3}$$

Estimates of affinity constants for calcium and strontium can be obtained from the reciprocals of the concentrations of the ions required to

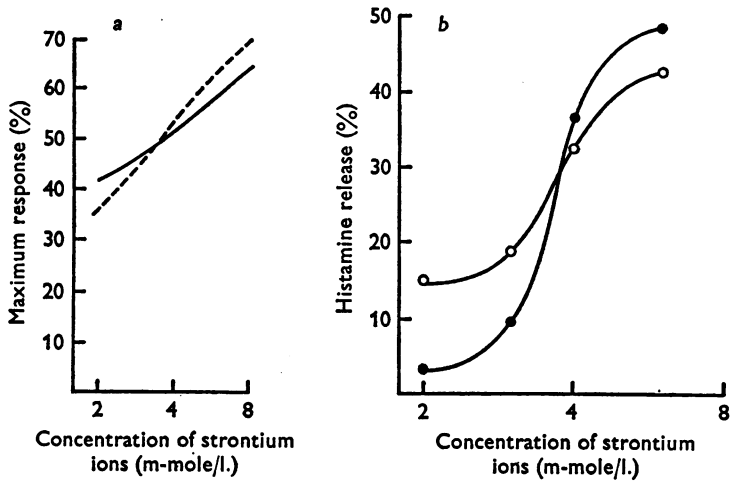

Fig. 11. Interaction of calcium and strontium. *a*, concentration–effect curves for strontium in the absence of calcium (interrupted line) and in the presence of calcium 0·3 m-mole/l. (continuous line) constructed from theory – see text. *b*, relationship between concentration and histamine release for strontium alone (●—●) and for strontium in the presence of calcium 0·3 m-mole/l. (○—○). Results from a single experiment.

produce 50 % of the maximum response. Again, it is assumed that response is directly proportional to the fraction of receptors occupied. The values obtained are $3{\cdot}3 \times 10^3$ and $0{\cdot}3 \times 10^3$ l./mole (Figs. 2 and 6) for calcium and strontium respectively.

The ratio of maximum effects produced by the two ions should be the same as the ratio of their efficacies provided the response is directly proportional to the fraction of receptors occupied. Hence, from the value for the ratio of maximum quoted above, strontium has been assigned an efficacy twice as great as that for calcium.

Fig. 11*a* shows the concentration–effect curve for strontium constructed from eqn. (3) and the effect of calcium 0·3 m-mole/l. on this curve. Fig. 11*b*

shows the actual experimental results from a determination of the effects of calcium, 0·3 m-mole/l. on the concentration–effect curve to strontium. The concentration of strontium at which calcium, 0·3 m-mole/l. does not change the response was found to be 3·6 m-mole/l. which is in agreement with the theoretically predicted value of 3·5 m-mole/l. A series of similar experiments with various concentrations of calcium all show agreement with the prediction from eqn. (1).

The observed interactions of calcium and strontium with the receptor are in agreement with the hypothesis that calcium possesses a higher affinity for the receptor than strontium but has a smaller efficacy.

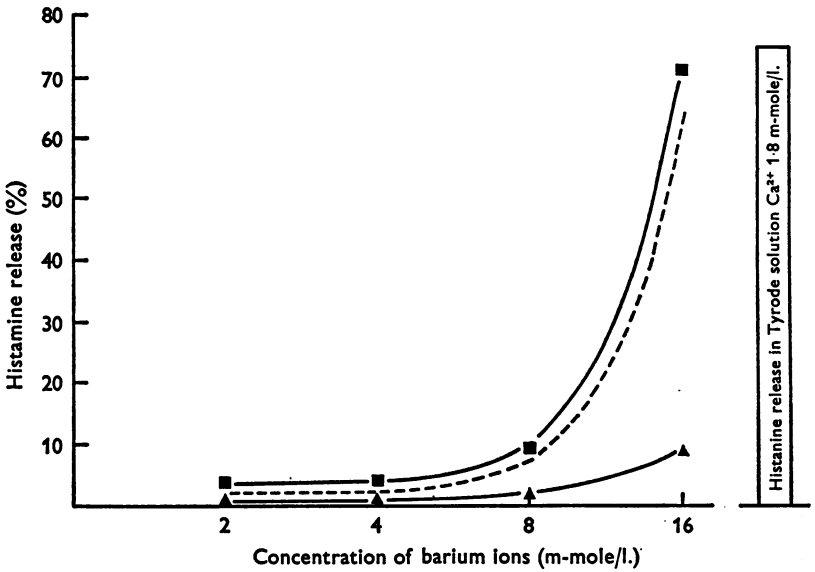

Fig. 12. Concentration–effect curve for barium ions. ▲—▲ spontaneous release; ■—■ total release in the presence of antigen. Broken line is derived by subtracting spontaneous from total release and represents anaphylactic release. Results from a single experiment.

The effect of barium

Barium is capable of replacing calcium in the activation of anaphylactic histamine release from rat peritoneal mast cells which contrasts with the antagonistic interaction of these two ions in chopped lung (Mongar, 1970). The concentration–effect curve shown in Fig. 12 indicates that barium is required in higher concentrations (16 m-mole/l.) than strontium to produce an effect comparable with the effect observed at maximal calcium concentration. At the concentrations required for activation of anaphylactic histamine release barium also potentiates spontaneous release.

DISCUSSION

The requirement of calcium for the anaphylactic reaction in rat peritoneal mast cells confirms the reports of the calcium dependence of the anaphylactic reaction in other tissues (Mongar & Schild, 1958; Lichtenstein & Osler, 1964; Greaves & Mongar, 1968). The observation that in calcium-free media containing EDTA some degree of anaphylactic histamine release occurs is unexplained. It may be that the EDTA was incapable of chelating all calcium ions present, or that part of the release process is independent of calcium, as is the case with the histamine release induced by compound 48/80 (J. C. Foreman & J. L. Mongar, unpublished observations). The inhibition of histamine release observed in some experiments

TABLE 1. Values of pA_2 for the antagonist action of magnesium ions

Test preparation	Agonist	pA_2	Reference
Neuromuscular junction	Ca^{2+}	2·40	Jenkinson (1957)
Neuromuscular junction	Ca^{2+}	2·53	Dodge & Rahamimoff (1967)
Rat mast cells	Ca^{2+}	2·03	This paper
Rat mast cells	Sr^{+}	1·97	This paper
Guinea-pig mast cells (chopped lung)	Ca^{2+}	2·52	Mongar (1970)
Snail heart	Ca^{2+}	1·94	Burton & Loudon (1972)

with high calcium concentrations is also seen in rabbit basophil leucocytes (Greaves & Mongar, 1968) but not in guinea-pig chopped lung preparations. Heuser, Katz & Miledi (1971) have reported an inhibitory effect of high calcium concentrations at the motor nerve terminal, which apparently represents a disturbance of the secretory mechanism, and in particular the vesicle distribution.

The role of calcium in secretory process has been discussed by Douglas (1968) and the experiments presented in this paper indicate a parallel between histamine release and other secretory processes. Schild (1947) has proposed the use of pA_2 as a convenient method for expressing the activity of an antagonist in terms of dissociation constant of the antagonist-receptor complex in competitive antagonism, and Arunlakshana & Schild (1959) have demonstrated the use of pA_2 as a method for classifying receptors. Table 1 provides pA_2 values calculated from published work on calcium-magnesium antagonism. The similarity of pA_2 values obtained in mast cells using either calcium or strontium as agonist suggests that these ions are acting on the same site, but the general agreement between pA_2 values obtained in various systems suggests the existence of a 'calcium receptor' which is common to widely differing tissues. The role of calcium in the

stimulus-secretion coupling at the neuromuscular junction is well documented (del Castillo & Stark, 1952; Dodge & Rahamimoff, 1967; Rahamimoff, 1970) and the observation that the calcium receptor in mast cells is similar to that in motor nerve terminals may mean that the role of calcium in activating histamine release is similar to that in the activation of transmitter release.

The replacement of calcium by strontium and barium has been described in a number of tissues, strontium being found less effective than calcium in chopped lung (Mongar, 1970) at the neuromuscular junction (Miledi, 1966) and in adrenal chromaffin in tissue (Douglas & Rubin, 1964). In contrast, the action of strontium in the mast cell is more effective than that of calcium if histamine release is used as an index. The greater efficacy of strontium with respect to calcium places calcium in the role of a partial agonist. Strontium, however, apparently has a lower affinity for the receptor than calcium and this finding is in keeping with observations in other tissues where strontium was used in higher concentrations than calcium to produce a similar effect.

The demonstration by Chakravarty, Gustafson & Phil (1967) that histamine is stored in discrete granules within the mast cell, and that in the anaphylactic reaction these granules are exuded from the cell is another parallel between histamine release and other secretory processes. The precise site of action of calcium in this secretory mechanism is unknown though from the work of Uvnas (1971) it does not appear to be at the level of histamine release from the granule. Calcium probably acts at the stage between the stimulus to the cell provided by the antigen-antibody reaction and the process of degranulation. The findings of Mongar & Svec (1972) that phospholipids potentiate histamine release in anaphylaxis is particularly interesting in the light of the binding of calcium to phospholipids (Hauser & Dawson, 1967). The phospholipids which bind calcium are of the acidic type, and this is also the type which potentiates histamine release.

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