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DESENSITIZATION IN THE PROCESS OF HISTAMINE SECRETION INDUCED BY ANTIGEN AND DEXTRAN

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SUMMARY

1. Antigen challenge of sensitized rat peritoneal mast cells in the absence of calcium failed to release histamine. The release when calcium was added subsequently declined rapidly, this desensitization being almost complete in 4 min.

2. Phosphatidyl serine (10 μg/ml.) reduced the rate of desensitization so that decay of the response to added calcium was not complete after 16 min.

3. Exposing normal cells to dextran resulted in a slow rate of desensitization, the response to phosphatidyl serine added with calcium having decayed by only 27% within 10 min.

4. Phosphatidyl serine added with dextran prevented desensitization so that the response to subsequently added calcium did not decay even after an interval of 20 min.

5. Cells activated by dextran and calcium became rapidly desensitized as shown by decay of the response to added phosphatidyl serine which was almost complete by 5 min.

6. Histamine release by the calcium ionophore (A 23187) added to cells at intervals before the addition of calcium did not show significant decay.

7. Desensitization of the cells to antigen did not change their response to the ionophore.

INTRODUCTION

Dale (1913) showed that after a dose of antigen sufficient to produce a maximum effect had induced an anaphylactic reaction in sensitized tissue, subsequent doses of antigen were without effect. The tissue was desensitized to this antigen but retained its ability to produce a response to a different stimulus or to a different antigen when the tissue was sensitized

to more than one antigen. Mongar & Schild (1957*a*, *b*) showed that it was possible to prevent anaphylactic histamine release without inhibiting desensitization, for example by lowering the temperature to 17° C or by the addition of phenol. In guinea-pig chopped lung and human leucocytes, challenge of the sensitized tissue with antigen in the absence of calcium produced no histamine release. Subsequent addition of calcium only partially restored release, indicating that calcium lack partly prevents desensitization (Mongar & Schild, 1958; Lichtenstein & Osler, 1964). We have studied the process of desensitization in rat peritoneal mast cells in relation to the role of calcium in this tissue using both antigen- and dextran-induced histamine release and a specific calcium ionophore, A 23187 (Caswell & Pressman, 1972; Reed & Lardy, 1972). Histamine release by each of these agents is dependent on extracellular calcium (Foreman & Mongar, 1972*a*, *b*; Foreman, Gomperts & Mongar, 1973); in addition, antigen-induced histamine release from sensitized rat peritoneal mast cells is potentiated by phosphatidyl serine, and dextran releases appreciable amounts of histamine from these cells only when phosphatidyl serine is also present (Goth, Adams & Knoohuizen, 1971).

METHODS

Experiments were performed on mixed peritoneal cells of the rat (1–5 % mast cells). The source and preparation of these cells has been described previously (Foreman & Mongar, 1972*a*; Garland & Mongar, 1974). It was assumed that the release of histamine from the mast cells was not accompanied by any appreciable loss of other intracellular material, e.g. lactic dehydrogenase or potassium (Johnson & Moran, 1969). The medium used for incubating the cells was based on Tyrode solution which has the following composition (m-mole/l.): NaCl 137; KCl 2·7; NaH_2PO_4 0·4; $NaHCO_3$ 12·0; $MgCl_2$ 1·0; glucose 5·6. $CaCl_2$ 1·0 or 1·8 m-mole/l. was included in the solution or added later in the experiment as appropriate.

In experiments in which antigen (ovalbumin) was used as the stimulus cells were suspended in 1 ml. calcium-free Tyrode solution at 37° C. Antigen was added to produce a final concentration of 10 μg/ml. and this was followed at intervals of 1–16 min by the addition of calcium to produce a final concentration of 1·0 m-mole/l. Incubation was continued at 37° C for 10 min after the addition of calcium, and the reaction was then stopped by cooling to 0° C. Cells suspended in Tyrode solution containing calcium 1·0 m-mole/l. before the addition of antigen served as controls. When required, phosphatidyl serine was added to produce a final concentration of 10 μg/ml.

In experiments in which dextran was used, the three components necessary for histamine release, namely dextran, phosphatidyl serine and calcium, were added to cells suspended at 37° C in Tyrode solution (with or without calcium) in the following sequences: (*a*) dextran and phosphatidyl serine followed at various time intervals by calcium; (*b*) dextran and calcium followed at various time intervals by phosphatidyl serine; (*c*) dextran alone followed at various time intervals by a mixture of calcium and phosphatidyl serine. In each case, the final concentration of calcium was 1·8 m-mole/l.; of dextran 6 mg/ml., and of phosphatidyl serine 10 μg/ml. After the

addition of the last component incubation was continued for 5 min at 37° C before the reaction was stopped by cooling.

To release histamine with the calcium ionophore, A 23187, cells were suspended in Tyrode solution and incubated for 10 min at 37° C after the addition of ionophore, the reaction then being stopped by cooling (Foreman, Mongar & Gomperts, 1973).

Ovalbumin was supplied by B.D.H. Phosphatidyl serine supplied by Koch-Light Ltd or Lipid Products was mixed with saline in a mechanical blender, after evaporation of the chloroform–methanol solvent in a stream of nitrogen. Dextran (mol. wt. 110,000) was supplied by Fisons as a 6 % (w/v) solution in saline (NaCl 154 m-mole/l.). The ionophore A 23187 (Eli Lilly) was dissolved in ethanol and diluted in saline. The final concentration of ethanol (1·5 m-mole/l.) did not affect the cells's response to antigen. The chemicals used to make Tyrode solution were of Analar quality.

RESULTS

In the absence of antigen or other histamine-releasing agent, the isolated mast cells released approximately 2% of their histamine in 10 min, this being referred to as spontaneous release. The values for histamine release by antigen, dextran and ionophore presented in this paper have been corrected for spontaneous release.

Desensitization to antigen

The histamine release induced by antigen when the cells were suspended in Tyrode solution having a calcium concentration of 1 m-mole/l. served as a control for determining the effect of stimulating the cells in calcium-free solution and then adding calcium at various times afterwards. Fig. 1 shows that there was a rapid decay of the response to calcium following exposure of the cells to antigen, the decay being almost complete in 4 min and having a half-life of about 1 min. The decay could not be explained by the disappearance of antigen since a second dose of antigen failed to elicit a response when the decay had been allowed to proceed. The addition of phosphatidyl serine to the calcium-free medium in which the cells were stimulated delayed the decay of the response to calcium, complete decay taking more than 16 min.

Desensitization in dextran-induced histamine release

The response to calcium added after dextran and phosphatidyl serine was constant with time and there was no decline in histamine release when calcium was added 10 or even 20 min after the dextran and phosphatidyl serine (Fig. 2). For comparison dextran and phosphatidyl serine were added to aliquots of the same cell population suspended in Tyrode solution containing calcium 1·8 m-mole/l. About three times more histamine was released when calcium was added after dextran and phosphatidyl serine than when dextran and phosphatidyl serine were added to cells already in

a calcium-containing medium. This difference was observed even when less than 10 sec separated the addition of dextran and phosphatidyl serine from the subsequent addition of calcium (Fig. 2). Results similar to that in Fig. 2 were obtained in each of three experiments.

Augmentation of histamine release by adding calcium 5 min after the other components was observed at calcium concentrations from 0·3 to 10 m-mole/l. (Fig. 3).

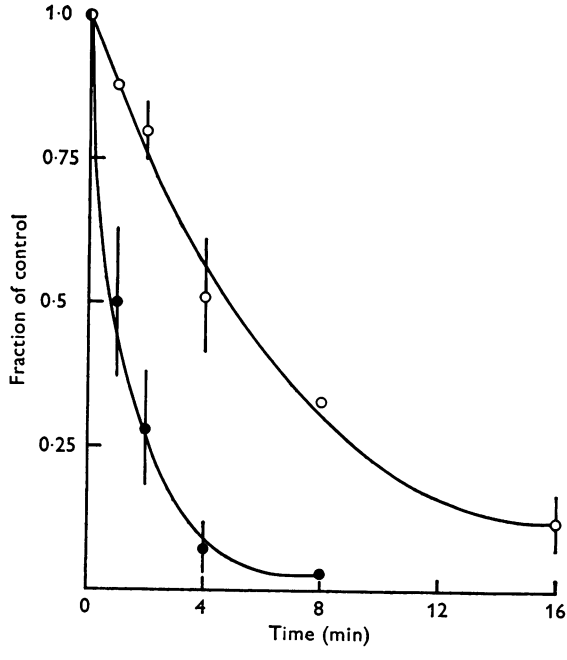

Fig. 1. Time course of the decay in the response to calcium of rat mast cells stimulated by the antigen–antibody reaction. ●—● without phosphatidyl serine; ○—○ phosphatidyl serine, 10 μg/ml. Vertical bars indicate S.E. of the mean which was calculated when three or more values contributed to a point. The control histamine releases were between 18 and 54 % of total in the absence of phosphatidyl serine and 37–64 % in the presence of phosphatidyl serine.

Histamine release induced by dextran and phosphatidyl serine is inhibited when the cells are initially suspended in Tyrode solution containing high calcium concentrations, e.g. 3–10 m-mole/l. (Garland & Mongar, 1974). However, such inhibition was not observed when the calcium was added after the dextran and phosphatidyl serine to cells previously suspended in calcium-free Tyrode solution (Fig. 3).

An alternative reaction in which phosphatidyl serine was added at various times after dextran to cells suspended in complete Tyrode solution

(calcium 1·8 m-mole/l.) provoked histamine release which showed a rapid decay, similar to that observed when calcium was added after antigen stimulation. The decay was almost complete in 5 min and had a half-life of about 1 min (Fig. 4).

When a mixture of phosphatidyl serine and calcium was added at various intervals after the addition of dextran to cells suspended in calcium-free

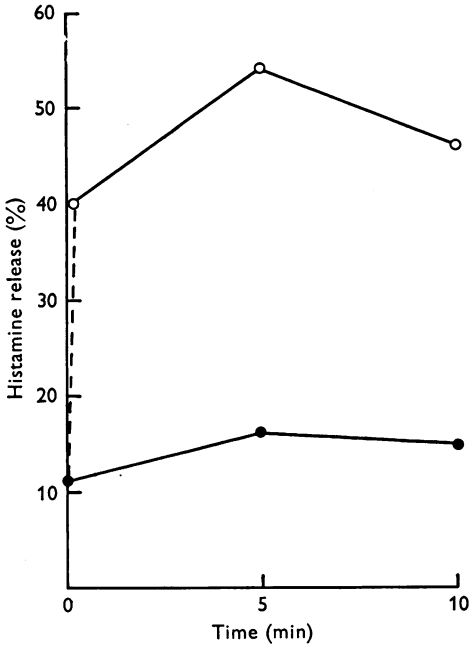

Fig. 2. ○—○ histamine release (%) by the addition of calcium (1·8 m-mole/l.) at various times after dextran (6 mg/ml.) and phosphatidyl serine (10 μg/ml.) had been added to cells in calcium-free Tyrode solution. ●—● histamine release (%) from the same cell population following the addition of dextran (6 mg/ml.) and phosphatidyl serine (10 μg/ml.) to cells suspended in complete Tyrode solution (calcium 1·8 m-mole/l.) for the same times. Results from a single experiment; each point is the mean of duplicates.

Tyrode solution there was a decay of the response, which was much slower than that observed when calcium was added after antigen or when phosphatidyl serine was added after dextran and calcium. After 10 min the response had declined to about two-thirds of the control value (Fig. 5), the control for this reaction being obtained by adding dextran to cells suspended in complete Tyrode solution (calcium 1·8 m-mole/l.) already containing phosphatidyl serine.

The effects of calcium ionophore, A 23187

When calcium was added at various times after the addition of a calcium-specific ionophore, A 23187, to cells suspended in calcium-free Tyrode solution at 37° C, the resulting histamine release was unchanged (Fig. 6). Release observed when calcium was added after A 23187 was compared with that observed when the ionophore was added to cells suspended in Tyrode solution containing calcium.

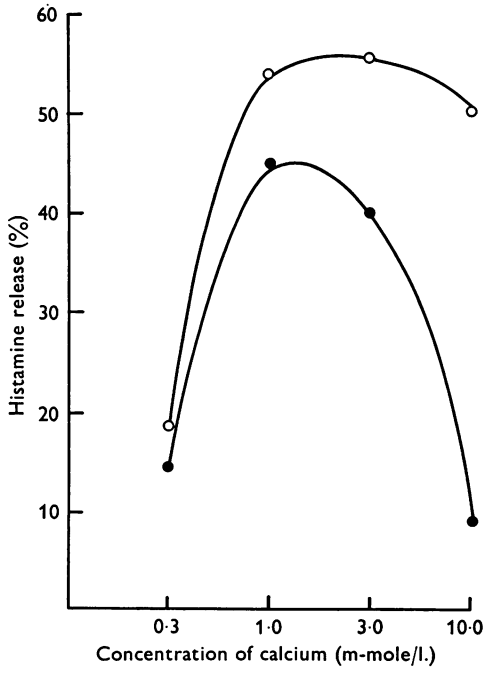

Fig. 3. Histamine release (%) by different calcium concentrations. ●—● dextran (6 mg/ml.) and phosphatidyl serine (10 μg/ml.) added to cells incubated in Tyrode solution containing calcium 0·3–10·0 m-mole/l. ○—○ dextran (6 mg/ml.) and phosphatidyl serine (10 μg/ml) added to the cells suspended in calcium-free Tyrode solution, 5 min before the addition of calcium (0·3 to 10·0 m-mole/l.). Results from a single experiment; each point is the mean of duplicates.

Cells from sensitized rats were challenged with antigen in calcium-free Tyrode solution and 4 min were allowed to elapse before calcium was added (final concentration 1 m-mole/l.). This addition of calcium was immediately followed by a second dose of antigen or A 23187. The histamine release observed under these conditions was compared with that when a single dose

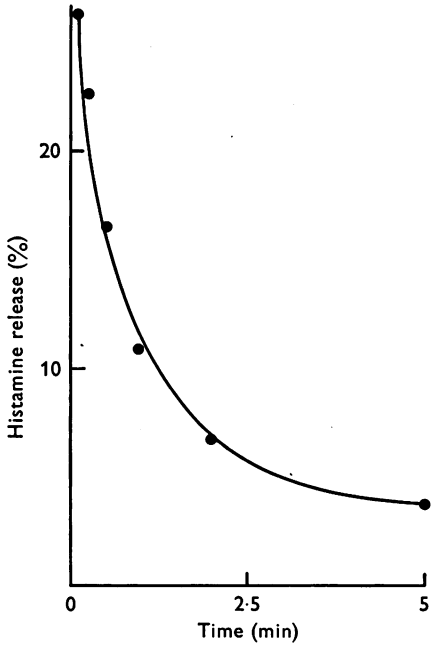

Fig. 4. Histamine release (%) by adding phosphatidyl serine, 10 μg/ml., at various times after the addition of dextran (6 mg/ml.) and calcium, 1·8 m-mole/l. Results from a single experiment; each point is the mean of duplicates.

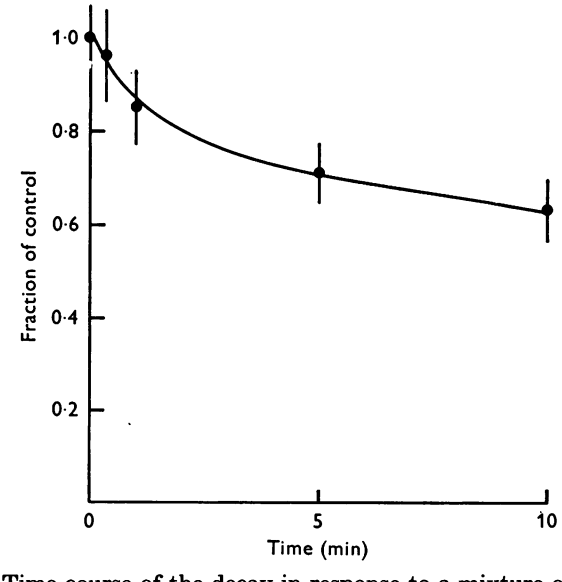

Fig. 5. Time course of the decay in response to a mixture of phosphatidyl serine (10 μg/ml.) and calcium (1·8 m-mole/l.) added at various intervals following dextran (6 mg/ml.) to cells suspended in calcium-free Tyrode solution. Each point is the mean of three experiments; vertical bars indicate S.E. of the mean.

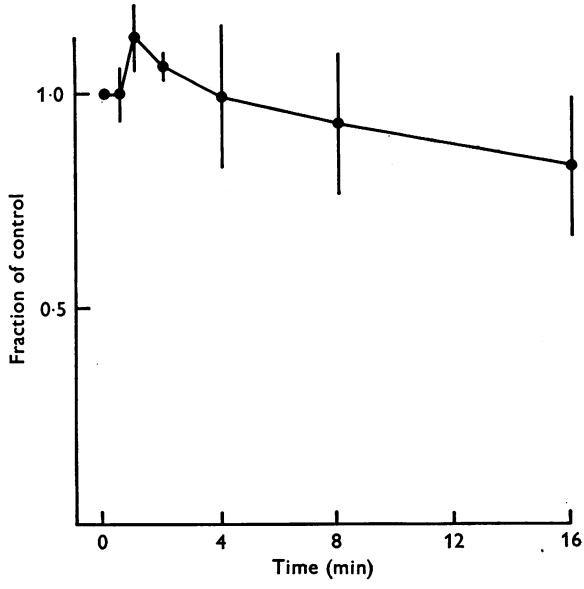

Fig. 6. Effect of adding calcium, 1 m-mole/l., to cells suspended in calcium-free Tyrode solution, at various times after the addition of A 23187, 0·6 μ-mole/l. The release is expressed as a fraction of the control histamine release obtained when A 23187 was added to cells suspended in complete Tyrode solution (calcium, 1 m-mole/l.). Control histamine releases in the four experiments ranged from 44 to 77 %. Vertical bars indicate S.E. of mean.

TABLE 1. The release of histamine (% total) from mast cells in response to antigen or A 23187 (*a*) when the cells were suspended in Tyrode solution containing calcium, 1 m-mole/l., and (*b*) when the calcium concentration had been brought to 1 m-mole/l. 4 min after an antigen stimulus in the absence of calcium

(*a*) Cells suspended in Tyrode solution containing calcium, 1 m-mole/l.		(*b*) Cells suspended with antigen in calcium-free Tyrode solution 4 min before the addition of calcium, 1 m-mole/l.	
Antigen (10 μg/ml.)	A 23187 (0·6 μ-mole/l.)	Antigen (second dose) (10 μg/ml.)	A 23187 (0·6 μ-mole/l.)
22·4	78·9	6·7	80·0
10·6	83·3	0·9	88·0
58·6	99·7	22·0	98·0
14·0	76·4	3·9	74·8
51·6	76·2	6·0	87·6

of antigen or A 23187 was added to cells in complete Tyrode solution (calcium 1·0 m-mole/l.).

Challenge with antigen in the calcium-free medium resulted in desensitization of the cells to antigen as described above. However, desensitization did not affect the response to the ionophore (Table 1).

DISCUSSION

In antigen-stimulated, sensitized rat peritoneal mast cells, desensitization was more rapid than that found in other tissues such as guinea-pig chopped lung, human leucocytes and human skin (Mongar & Schild, 1958; Lichtenstein & Osler, 1964; Yamamoto & Greaves, 1973). The action of phosphatidyl serine on rat peritoneal mast cells appears to be related to the effects of the alkaline earth ions (Mongar & Svec, 1972; Foreman & Mongar, 1973). Thus, it is possible that desensitization to antigen could be the result of a change in membrane 'gates' for calcium. It has been suggested that such 'gates' control the entry of calcium into the mast cell and that the amount of calcium entering the cell is related to the histamine release (Foreman & Mongar, 1973). The antigen–antibody stimulus is supposed to open the gates, and providing calcium is present, histamine release occurs. If calcium is not present when the stimulus is given, no histamine release occurs and the gates close so that subsequent addition of calcium does not bring about histamine release. Phosphatidyl serine may delay desensitization by preventing gate closure, and it is interesting that in the tissues where phosphatidyl serine is inactive (guinea-pig chopped lung and human leucocytes, Mongar & Svec, 1972; E. S. K. Assem & J. L. Mongar, personal communication) desensitization is slow. In rat peritoneal mast cells, phosphatidyl serine potentiates histamine release and slows desensitization.

The calcium ionophore, A 23187, transports calcium into the cell and stimulates histamine release (Foreman, Mongar & Gomperts, 1973). The response to the ionophore is unaffected by prior desensitization of the cells to antigen, which suggests that desensitization is a change in the histamine release reactions occurring at or before calcium entry into the cells. This is further supported by the observation that the response of the cells to calcium added after the ionophore shows no decay.

Desensitization to the dextran stimulus alone occurs slowly so that when calcium and phosphatidyl serine are added 10 min after the dextran the response has fallen to only 33% of the control value. Phosphatidyl serine slows this even further so that no decay of the response is seen after 10 min. Adding calcium with the dextran, and using phosphatidyl serine to trigger the release process, produces a desensitization with a time course similar to that observed for antigen-stimulated cells in the absence of phosphatidyl

serine. In contrast to the antigen-activated release process, the dextran-activated system seems to require calcium for the membrane events associated with the gating of calcium. Both antigen- and dextran-induced histamine release require calcium, but it is possible that in dextran-induced release calcium actually controls the processes which regulate the entry of this ion into the cell. Such an hypothesis could also explain the inhibition of dextran-induced histamine release which occurs at high calcium concentrations if these concentrations inhibit membrane events (gate opening) induced by the stimulus. Similarly, the enhanced histamine release seen when calcium was added after the dextran and phosphatidyl serine could be due to a facilitation of gate opening by calcium deprivation.

We conclude that desensitization may be due to a change in mast cell membrane permeability to calcium. In the case of dextran-induced histamine release, but not antigen-induced release, calcium itself may modify these changes in permeability.

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