Br. J. Pharmac. (1973), **48**, 527–537.

The action of lanthanum and manganese on anaphylactic histamine secretion

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Summary

1. In the concentration range 1 nM to 1 μM, lanthanum inhibited the calcium-dependent component of anaphylactic histamine release, but was without effect on the component which was independent of calcium.

2. The inhibition of anaphylactic histamine release by lanthanum can be reversed by increasing the calcium ion concentration. The pA_2 for the lanthanum-receptor interaction was found to be 7·6.

3. Lanthanum also inhibited the activation of anaphylactic histamine release by strontium ions.

4. The inhibition of anaphylactic histamine release by lanthanum was reversed by eluting the lanthanum from the cells.

5. In the concentration range 1 to 300 nM, lanthanum had no effect on the histamine release induced by compound 48/80 in a calcium-free medium, but the effect of calcium on the histamine release by compound 48/80 was antagonized by lanthanum in this range of concentrations.

6. At concentrations of 10 μM and greater, lanthanum induced a release of histamine in the absence of antigen.

7. In the concentration range 0·5 to 4·0 mM, manganese inhibited the calcium-dependent component of anaphylactic histamine release, but was without effect on the component which is independent of calcium. The inhibition of anaphylactic histamine release by manganese could be reversed by increasing the calcium ion concentration.

Introduction

Lanthanum has a crystal ionic radius of 0·115 nm which is similar to the values for calcium and strontium (0·099 and 0·113 nm) (Pauling, 1960), and it has been suggested by Lettvin, Pickard, McCulloch & Pitts (1964) that lanthanum would be expected to bind at calcium sites and mimic the action of calcium, though because of its higher valency, lanthanum would be expected to have a greater affinity than calcium. However, in most biological systems, lanthanum, while showing a high affinity for calcium sites, acts as a calcium antagonist rather than an agonist (Williams, 1970).

At the neuromuscular junction, Heuser & Miledi (1971) have shown that lanthanum inhibits the calcium-dependent, evoked secretion of acetylcholine, but the ion also increases the frequency of the miniature endplate potentials which represent the spontaneous release of transmitter and thus the ion appears to have a dual action on this tissue. A similar dual action of lanthanum has also been described at the adrenal medulla (Borowitz, 1972) where secretion of catecholamines

is a calcium-dependent process (Douglas & Rubin, 1961) and it has recently been shown that lanthanum causes a release of oxytocin and neurohypophysin from the hypophysis, whilst it inhibits the evoked release of vasopressin (Russell & Thorn, 1972; Matthews, Legros, Grau, Nordmann & Dreifuss, 1973). Lanthanum reduces the movement of calcium across artificial lipid membranes (van Breeman, 1969), and Weiss & Goodman (1969) have shown that it reduces resting tone and inhibits potassium- and acetylcholine-induced contractions of the smooth muscle of guinea-pig ileum. These effects of lanthanum were related to the replacement of calcium by lanthanum at superficial membrane sites, a decrease in the mobility of calcium at the membrane sites and an inhibition of calcium uptake by the muscle.

The antigen-stimulated secretion of histamine from mast cells is a calcium-dependent process (Mongar & Schild, 1958) but there is also a small component of the stimulated release of histamine which does not require calcium (Foreman & Mongar, 1972a). Lanthanum has been used, in this study, to investigate the action of calcium in the process of histamine release. A preliminary account of some of our results, which show that lanthanum possesses a dual action on histamine release from isolated mast cells has already been published (Foreman & Mongar, 1972b).

Methods

Male and female rats were used for these experiments: they were from a closed, random bred colony of Lister Hooded rats and weighed between 150 and 300 g.

The rats were sensitized to egg albumin 15 to 30 days prior to the experiment by a modification of Mota's method (1964) which, together with the method used for isolating the mast cells from the peritoneal cavity, has been described in an earlier paper (Foreman & Mongar, 1972a). The final volume of the cell suspension was 0·5 ml, and 0·01 ml of antigen or compound 48/80 was added immediately before a 10 min incubation at 37° C. The final concentration of antigen was 20 μg/ml and that of compound 48/80 was 0·4 μg/ml unless otherwise stated. After incubation, the reaction was stopped by the addition of 2·0 ml of ice-cold Tyrode solution which was free from alkaline earth ions. The cell suspension was then centrifuged at 1,000 *g* for 5 min and the supernatant removed for histamine assay. The pellets were resuspended in Tyrode solution and heated to 100° C for 10 min to release the remaining histamine, which was also assayed.

Histamine assays were performed on the isolated ileum of guinea-pig, the calcium concentration of the histamine solution being adjusted to ensure an adequate concentration for contraction of the smooth muscle. At the dilutions encountered, lanthanum and manganese ions did not affect the histamine assays.

The medium used to incubate the cells had the following composition (mM): NaCl, 137; KCl, 2·7; glucose, 5·6; HEPES buffer, 20. The pH of this medium was adjusted to 7·0 with hydrochloric acid before the addition of the appropriate amount of lanthanum chloride. The pH for the experiments with manganous chloride was 7·5. At the pH used, no precipitation of manganese was observed, and lanthanum did not appear to precipitate at concentrations up to 0·1 mM, but at a concentration of 1 mM, although there was no immediate precipitation, a gelatinous deposit formed when the solution was left standing overnight at 4° C. All solutions were prepared on the day of the experiment. Tyrode solution, used to dilute the histamine for the assays, had the following composition (mM): NaCl 137, KCl 2·7, NaH_2PO_4 0·4, $NaHCO_3$ 12·0, glucose 5·6, $CaCl_2$ 1·8, $MgCl_2$ 1·0.

Materials

Lanthanum chloride was a B.D.H. laboratory reagent, but all other inorganic chemicals were of Analar quality. HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acids) was supplied as a 1 M buffer solution by Burroughs Wellcome, who also supplied compound 48/80. The antigen, egg albumin, was a general purpose chemical supplied by B.D.H.

Results

In this paper, the histamine release in the absence of antigen is referred to as ' spontaneous release ' and the release in the presence of antigen or compound 48/80 is referred to as ' total release '. Subtraction of spontaneous histamine release from total release provides a value for the antigen-stimulated or ' anaphylactic histamine release '. The correction is small since spontaneous release is only about 2% unless strontium is present at a concentration of 10 mM, when it increases to about 10% (Foreman, 1973).

Effect of lanthanum on anaphylactic histamine release

The anaphylactic release of histamine increases as the calcium concentration is raised from 0·1 to 1·0 mM, but a small release of histamine occurs in the absence of calcium, and this is not reduced by the addition of EDTA (ethylene diamine tetra-acetic acid, disodium salt) (Foreman & Mongar, 1972a). Lanthanum, in low concentrations, inhibits the calcium-dependent component of anaphylactic histamine release, but it was without effect on the component of release which occurs in the absence of calcium (Figure 1). A mean concentration-effect curve for the inhibitory action of lanthanum is shown in Fig. 2, where the effect is expressed as a percentage inhibition of the anaphylactic histamine release in a medium containing calcium, 1·8 mM, which is the amount normally present in Tyrode solution.

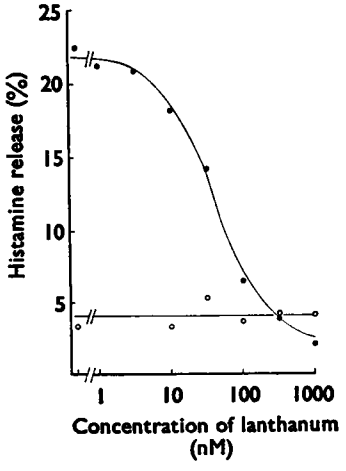

FIG. 1. The action of lanthanum on anaphylactic histamine release from rat mast cells. ●——●, in the presence of calcium, 1·8 mM; ○——○ in a calcium-free medium. Results from a single experiment.

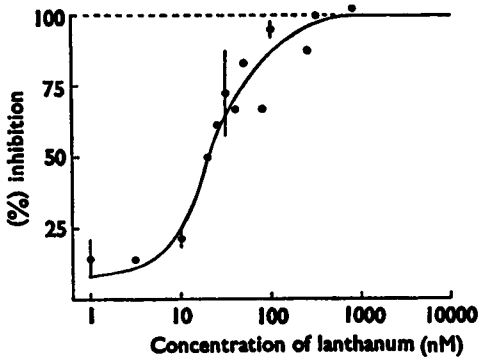

FIG. 2. Mean concentration-effect curve for the inhibition by lanthanum of the calcium-dependent anaphylictic histamine release. The concentration of calcium was 1·8 mM. The standard error (vertical bars) was calculated when three or more values contributed to the mean.

The interaction of calcium and lanthanum in anaphylactic histamine release

The inhibition of anaphylactic histamine release produced by lanthanum can be reversed by increasing the calcium ion concentration. Assuming that the lanthanum and calcium combine with the same receptor site on the cell, in a reversible manner:

$$Ca + R \rightleftharpoons CaR$$

$$La + R \rightleftharpoons LaR$$

and that only the CaR complex is capable of activating histamine release, then, as Schild (1949) has shown, application of the Law of Mass Action to the equilibrium conditions yields the following relationship:

$$x - 1 = K_{La}\,[La^{3+}],$$

where x, the dose-ratio, is the ratio of the concentrations of calcium required to produce the same effect in the presence and absence of lanthanum; $[La^{3+}]$ is the concentration of free lanthanum in solution and K_{La} is the affinity constant for the lanthanum-receptor complex.

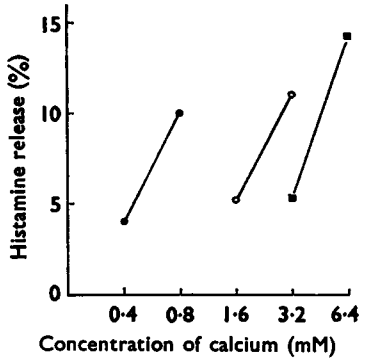

FIG. 3. The action of lanthanum on the concentration-effect curve for calcium in anaphylactic histamine release. ●——●, no lanthanum; ○——○, lanthanum, 50 nM; ■——■, lanthanum, 100 nM. Results from a single experiment.

A plot of log (x−1) against log [La^{3+}] should yield a straight line with a slope of 1 and an intercept on the abscissa corresponding to $-\log K_{La}$.

The theory has been tested for the calcium-lanthanum interaction in anaphylactic histamine release in experiments such as the one shown in Figure 3. When the concentration of lanthanum was increased it produced a graded, parallel shift of the concentration-effect curve for calcium to the right, as predicted by the competitive theory for interaction. When the concentration-effect curves were not exactly parallel, a mean value for x was determined over the response range tested.

A plot of log (x−1) against lanthanum concentration on a log co-ordinate was constructed from eight experiments and is shown in Figure 4. The slope of the line is 1·1 and the intercept on the abscissa gives a value of 27 nM for the dissociation constant of the lanthanum-receptor complex. The results are in agreement with a competitive model for the interaction of calcium and lanthanum, but it should be pointed out that equilibrium conditions have been assumed. The minimum period between the addition of calcium and lanthanum to the cells and the challenge with antigen was five minutes. Also, if it is assumed that only a small fraction of receptors need be occupied by calcium to give a maximum response, then it follows that a non-competitive antagonist at low concentrations will produce a parallel shift of the concentration-effect curve to the right, and the plot of log (x−1) against log of the antagonist concentration for small values of x, will produce a straight line with a slope of 1.

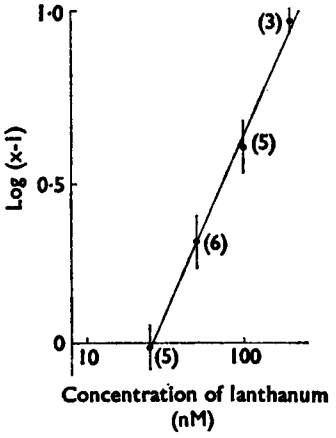

FIG. 4. Plot of log (x−1) against lanthanum ion concentration on a logarithmic scale, for calcium-lanthanum antagonism. The line was fitted by eye and the vertical bars represent standard error. The figures in parentheses indicate the number of experiments contributing to each point.

Effect of lanthanum on the activation of anaphylactic histamine release by strontium ions

Strontium has been shown to substitute for calcium in the anaphylactic release of histamine (Foreman & Mongar, 1972a), and so it was of interest to determine the effect of lanthanum on the response to strontium. Figure 5 shows that the anaphylactic release of histamine in the presence of 10 mM strontium was inhibited by lanthanum. The concentration of strontium was that which has been shown to produce a maximum effect.

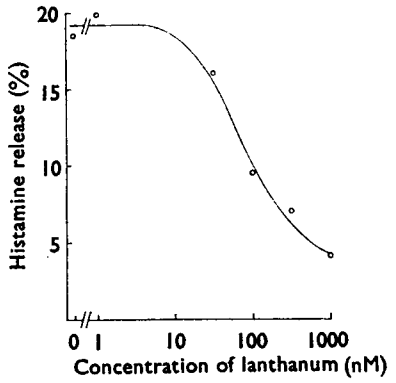

FIG. 5. Concentration-effect curve for the action of lanthanum on anaphylactic histamine release in the presence of strontium 10 mM. Results from a single experiment.

Reversal of the effect of lanthanum by elution

The mast cells were preincubated for 10 min at 37° C in the presence of calcium 1 mM and various concentrations of lanthanum. The cells were then spun down and washed one to three times by resuspension and centrifugation in a medium containing calcium 1 mM. Control cells, not pre-incubated with lanthanum were treated in a similar manner. Finally, the cells were challenged with antigen for 10 min at 37° C in the presence of calcium 1 mM. Figure 6 shows an experiment in which the anaphylactic release after treatment with lanthanum, 100 nM and washing, is the same as the anaphylactic release of histamine from cells which were not treated with lanthanum. When higher concentrations of lanthanum (1 μM) were used it was found in some experiments that only a partial reversal of the inhibition of anaphylactic histamine release could be achieved by washing the cells in calcium-free medium. In other experiments a complete reversal of the effect of lanthanum 1 μM was observed.

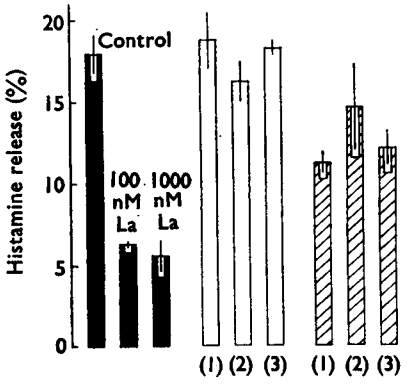

FIG. 6. Elution of lanthanum from isolated mast cells. The closed columns show the effect of lanthanum on the control anaphylactic release of histamine in the presence of calcium 1 mM. The open columns show the responses after elution of a concentration of 100 nM lanthanum, and the hatched columns after the elution of lanthanum 1 μM. The figures in parentheses indicate the number of washes (volume of wash=1 ml). Each column is the mean of duplicate samples and the vertical bars represent the range.

Effect of lanthanum on histamine release by compound 48/80

The histamine releasing agent, compound 48/80, has been shown to be capable of producing its effects in the absence of calcium (Uvnäs & Thon, 1961). However, addition of calcium at concentrations up to 1 mM, potentiates submaximal release of histamine by compound 48/80 (Diamant & Krüger, 1967). A comparison of the effect of calcium on histamine release by the antigen-antibody reaction and compound 48/80 is shown in Table 1. It is clear that at all concentrations of 48/80, the release by this agent is less dependent on free calcium in solution than the anaphylactic release of histamine. A 'calcium-free medium', however, is a medium to which no calcium is added and it is possible that the apparent lower sensitivity to calcium of histamine release by compound 48/80 compared with anaphylactic histamine release, is due to the dependence of the release by 48/80 on calcium bound to the cell surface, and not free in solution. A calcium antagonist such as lanthanum should indicate whether the large component of histamine

TABLE 1. *The release of histamine by compound* 48/80 *and the antigen-antibody reaction in the presence and absence of calcium*

Experiment	Concentration of releaser (μg/ml)	Histamine release (%) No Ca^{2+}	Histamine release (%) *Ca^{2+}	Increase in release by Ca^{2+}
(i) Release by compound 48/80				
1	0·3	10·6	16·8	6·2
2	0·4	34·5	69·2	34·7
3	0·4	36·9	59·8	22·9
4	0·4	8·5	17·8	9·3
5	0·5	61·6	84·2	22·6
6	1·0	60·0	68·0	8·0
7	1·0	73·0	96·0	23·0
(ii) Release by supramaximal concentration of antigen				
a	20	14·1	39·8	25·7
b	20	5·4	25·0	19·6
c	20	6·7	13·3	6·6
d	20	11·6	27·7	16·1
e	20	3·9	35·9	32·0
f	20	5·4	62·5	57·1
g	20	4·0	11·0	7·0

The mean increase of release produced by calcium has been expressed as a percentage of the mean histamine release in the presence of calcium to give the following figures:
Antigen—76%, Compound 48/80—32%

*The concentration of calcium (1mM) was sufficient to produce a maximum effect.

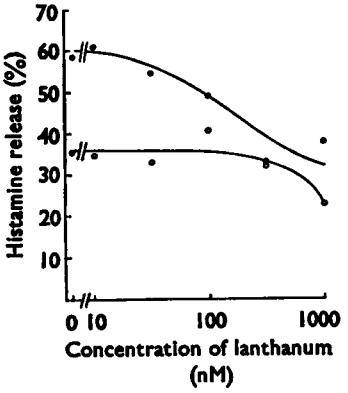

FIG. 7. Concentration-effect curve for lanthanum on histamine release induced by compound 48/80. ●——●, histamine release in the presence of calcium, 1 mM; ○——○, histamine release in a calcium-free medium. Results from a single experiment.

release by compound 48/80, in the absence of calcium is, in fact, independent of calcium. Experiments such as the one shown in Fig. 7 demonstrated that lanthanum, whilst inhibiting the potentiation by calcium of compound 48/80-induced histamine release, failed to inhibit the release in the absence of calcium, except at a concentration of 1 μM. At this concentration, it is possible that the lanthanum may be entering the cells and acting at other calcium binding sites, since this is the concentration of lanthanum whose effect could only be partially reversed by elution.

Lanthanum and spontaneous histamine release

In the range of lanthanum concentrations which inhibit the anaphylactic release of histamine, the ion is without effect on the spontaneous release of histamine, and this is in agreement with the observation that spontaneous histamine release is independent of the extracellular calcium concentration (Foreman, 1973). However, at lanthanum concentrations greater than 10 μM, the ion causes a release of histamine, and this effect is shown in Figure 8.

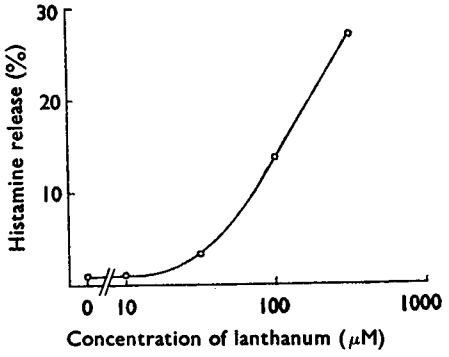

FIG. 8. Concentration-effect curve for lanthanum on spontaneous histamine release. Results from a single experiment.

It is not possible to investigate the relationship between concentration and effect above a concentration of about 1 mM because the lanthanum precipitates. The possibility that lanthanum lysed the mast cells was investigated indirectly by determining the effect of lanthanum on red blood cell haemolysis. Compared with the detergent Triton X 100, which produced 100% haemolysis at a concentration of 1 mg/ml, lanthanum at a concentration of 1 mM produced only 1% haemolysis. At this concentration, however, lanthanum did cause aggregation of the red cells and produced a similar effect on mast cells.

Effect of manganese on anaphylactic histamine release

Manganese produces a concentration-related inhibition of anaphylactic histamine release in the presence of calcium ions, as shown in Fig. 9, but the small fraction of the anaphylactic release of histamine which is not dependent on calcium is not inhibited by manganese.

The inhibitory action of manganese on anaphylactic histamine release can be reversed by increasing the calcium ion concentration, and manganese 1 mM produces a dose-ratio of about 4 (Figure 10).

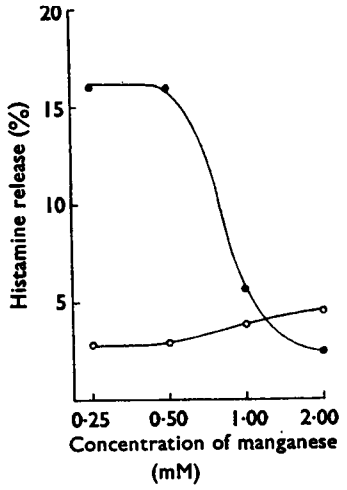

FIG. 9. The action of manganese on anaphylactic histamine release. ●——●, histamine release in the presence of calcium, 1·8 mM; ○——○, histamine release in a calcium-free medium. Results from a single experiment.

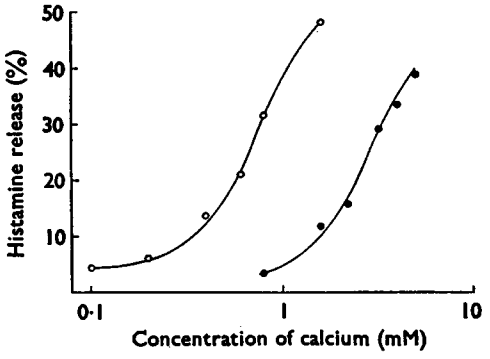

FIG. 10. The action of manganese on the concentration-effect curve for calcium in the anaphylactic release of histamine. ○——○, no manganese; ●——●, manganese, 1 mM. Results from a single experiment.

Discussion

Anaphylactic histamine release requires the presence of calcium ions (Mongar & Schild, 1958; Foreman & Mongar, 1972a), and Dalhquist & Diamant (1972) have reported that histamine release induced by ATP is accompanied by an uptake of calcium into the mast cell. However, it is not yet established whether calcium uptake follows the process of histamine release or whether histamine release is dependent on calcium uptake. The observed effects of the alkaline earth ions and their interaction with phosphatidyl serine (Foreman & Mongar, 1973) can be explained in terms of a model involving divalent cation gates in the mast cell membrane which, during the passage of calcium and strontium, trigger histamine secretion, but evidence from the direct measurement of calcium movement across the mast cell membrane is still lacking. In smooth muscle it has been shown that lanthanum inhibits the movement of calcium across the cell membrane (van Breemen, 1969; Weiss & Goodman, 1969), and it is conceivable that the ion has

a similar action in the mast cell, though it is, of course, possible that lanthanum competes for calcium sites involved in histamine secretion other than those involved with the entry of the ion into the mast cell.

The pA_2 scale of Schild (1947) has been used in two ways. Firstly, when dealing with a particular class of receptors, the pA_2 values obtained for various competitive antagonists provide a method for classifying the antagonists, and secondly, a single antagonist can be used to identify different sets of receptors (Arunlakshana & Schild, 1959). Lanthanum appears to be a competitive antagonist for calcium in the mast cell, and the elution experiments indicate that the binding of the ion to the cells is reversible. The inability to elute completely the effect of the ion at higher concentrations is unexplained, but it may be the result of entry of the ion into the cells, where other calcium binding sites may exist. It is interesting to compare the pA_2 for calcium-lanthanum antagonism with that for calcium-magnesium antagonism in the rat mast cell. The pA_2 value in the former case was found to be 7·6 whilst in the latter case (Foreman & Mongar, 1972a) it has been shown to be 2·03, indicating that the affinity of the calcium receptor in the mast cell is almost 10^6 times greater for the lanthanum ion than for the magnesium ion. Assuming manganese to be a competitive antagonist of calcium in the mast cell, it can be calculated that the pA_2 for the MnR complex is about 3·3 (Figure 10). The action of strontium is also antagonized by lanthanum, which confirms the conclusion, from studies with magnesium, that calcium and strontium are acting at the same site.

The use of lanthanum as a high affinity calcium antagonist, has provided further evidence that both anaphylactic histamine release and compound 48/80-induced histamine release have components which are independent of extracellular calcium concentration. However, it appears that histamine release induced by compound 48/80 has a larger calcium-independent component than antigen-induced release. It is interesting that Rothschild (1970) has also described two components of histamine release induced by compound 48/80, from rat lung, in terms of energy requirement: one is not blocked by metabolic inhibitors, whilst the other component is blocked by these inhibitors and is, therefore, said to be energy-dependent. It seems possible that the calcium-dependent and energy-dependent components of compound 48/80-induced histamine release may be the result of the same process and this is distinct from another mechanism for histamine release which is both independent of calcium and metabolic energy. It should be pointed out that there is no direct evidence to relate calcium dependence with energy dependence, but both of these properties are general features of secretory processes. The anaphylactic reaction is largely dependent on calcium and cellular energy (Mongar & Schild, 1962; Chakravarty, 1967; Perera & Mongar, 1965) and can be considered to be the physiological process for the secretion of histamine from mast cells, which is similar to the processes for secretion of acetylcholine from motor nerves and adrenaline from the adrenal medulla (Douglas, 1968). It appears that part of the action of compound 48/80 results in histamine release which is independent of the processes associated with secretion.

J. C. F. is supported by a grant from the Medical Research Council.

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(*Received January* 26, 1973)