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ANTIGEN-INDUCED HISTAMINE RELEASE FROM SENSITIZED TISSUE AND THE MEASUREMENT OF CALCIUM ION FLUXES

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Summary Antigen stimulated release of histamine from sensitized guinea-pig lung was inhibited by the exclusion of calcium ions from the incubation fluid. Subsequent addition of calcium ions induced release, but the magnitude of this release decreased with time. When the releasing potential had declined to zero, addition of an alternative antigen together with calcium ions induced further release. If the primary challenge was inhibited by the presence of an antiallergic agent, challenge by a second antigen was similarly inhibited, in contrast to the effect when there was no primary antigen challenge. Antigen challenge induced a flux of ^{45}Ca into cells, these fluxes were inhibited by compounds which inhibited histamine release. Inhibition of release did not correlate with inhibition of calcium flux with some agents, suggesting that the measured flux is the sum of at least two fluxes, one secondary to release. These results are explained in a scheme for antigen-induced histamine release.

INTRODUCTION

It has been known for many years that chopped lung from sensitized guinea-pigs will release histamine when challenged with specific antigen. This release requires the presence of external calcium ions and can be inhibited by β-stimulants and other compounds which increase cellular cyclic-AMP levels (1). Non-cytolytic histamine release can be induced by calcium ionophore A23187, which is believed to transport calcium ions into the cell. Antigen challenge to sensitized mast cells similarly induces a movement of calcium ions into the cell, it is believed that this movement results from a 'gating' phenomenon (2). The flux is inhibited by antiallergic compound disodium cromoglycate (3). The experiments reported below investigate the relationships between external calcium ion concentration, antigen-induced histamine release and calcium ion fluxes. Inhibition of these phenomena by various agents which affect mast cell function are studied.

EXPERIMENTAL

Sensitization of animals and histamine release from guinea-pig and rat tissues was determined using methods previously described (4)(5). ^{45}Ca fluxes were measured using a modification of the method of Foreman et al.(3), histamine release was determined in these experiments by sampling the supernatant above the silicone oil layer. Histamine was measured using an automated fluorometric assay. Dual sensitization experiments were performed

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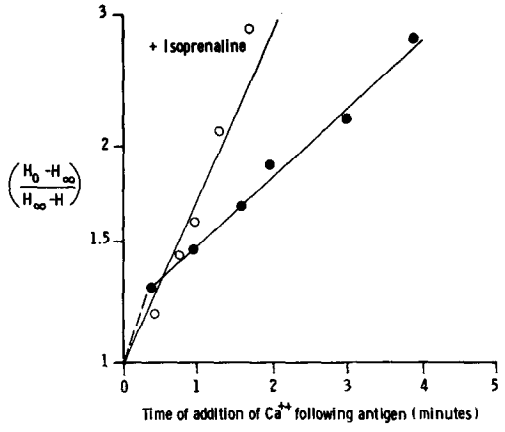

Figure 1. The decay of histamine releasing potential of antigen challenged guinea pig lung and the effect of a β-stimulant

Following antigen challenge to ovalbumin sensitized tissue (in the absence of calcium ions), Ca^{2+} is added at varying delay times. H represents histamine release at time t, H_∞ the residual release at 30 minutes, H_0 the release when calcium is added with antigen. The dotted line represents a schematic small rapid phse with $t_{\frac{1}{2}} < \frac{1}{2}$ minute.

under conditions where the initial release was inhibited i.e. in the absence of calcium ions or in the presence of the antiallergic agent 'Bufrolin'. The samples were challenged 20 minutes later under conditions where histamine release would be expected i.e. in the presence of calcium ions or when the effect of 'Bufrolin' has declined. All experiments were performed in quadruplicate and repeated twice, results shown are from typical experiments, together with statistical evaluation using Student's t test.

RESULTS

When sensitized guinea-pig lung tissue was challenged with specific antigen in the absence of external calcium ions there was little histamine release. If calcium ions were added to the incubation medium subsequent to antigen, the amount of histamine released declined as the time between antigen challenge and calcium addition increased. When isoprenaline, a β-stimulant, was added to the tissue with antigen, the rate of decay markedly increased (Fig.1). This result suggests that the proposed calcium 'gate' was induced to close. This closure excluded calcium ions from the cell and inhibited further histamine release.

Table I shows the results of experiments with dual sensitized tissue. When the first challenge was in the absence of calcium ions and the second in their presence, further histamine release was detected. This result indicates that the second challenge, in the presence of external calcium ions, was able to trigger the release mechanism again, even though the initial antigen-

Table I. The effect of calcium ions and Bufrolin on antigen induced histamine release from dual sensitised guinea-pig lung in vitro.

	Addition at times shown		% Specific Histamine Release ± SEM (4)	
	0 mins	20 mins		
a	Ag1	-	3 ± 2	P < .001
b	Ag1, Ca^{++}		19.2 ± 2.5	
c	-	Ag2, Ca^{++}	10.9 ± 1.2	NS
d	Ag1, Ag2, Ca^{++}		19 ± 2	
e	Ag1, Ca^{++}	Ag2	19.3 ± 1.8	P < .002
f	Ag1,	Ag2, Ca^{++}	11 ± 1	
g	Ag1, Bufrolin, Ca^{++}		9.8 ± .8	P < .005
h	Bufrolin, Ca^{++}	Ag2	10.9 ± 2	
i	Ag1, Bufrolin, Ca^{++}	Ag2	15.8 ± 1.8	

c vs. h NS; c vs. f NS; d vs. e NS; b vs. g P< .005.

Ag1, Ovalbumin; Ag2 Bovine γ Globulin, Ca^{++} concentration 1.5mM, Bufrolin 2.5 10^{-6}M (1μg/ml)

induced histamine releasing potential had decayed, there being no calcium ions present (see Figure 1). This result means that the cells were not desensitized by the first challenge. However, when the first challenge was in the presence of calcium ions, the second antigen did not elicit further release. When the primary challenge was in the presence of 'Bufrolin' the histamine released by the second challenge was inhibited to the same extent as that induced by the first challenge. If, however, the first antigen was not added with the 'Bufrolin', there was no inhibition of the release induced by the second antigen, this phenomenon results from the strict time dependence of addition of antiallergic agents (4)(6). This result shows that the 'Bufrolin' has a long lasting effect on the challenged cell, as opposed to its short lived effect on a non-challenged cell. The results shown in Table I are for Ovalbumin as primary challenge. If γ globulin was used as primary challenge the conclusions are identical.

A movement of ^{45}Ca into mast cells has been shown to be induced by antigen challenge to isolated rat mast cells. This movement can be inhibited by the

Table II. The effect of inhibitors on antigen-induced histamine release from, and calcium flux into, rat mast cells.

Addition	$^{45}Ca^{++}$ flux cpm ± SEM(4)	% inhibition of Histamine Release
Saline	5136 ± 187	-
Antigen	12776 ± 822 a	-
Antigen, Bufrolin	6148 ± 478 b, c	61
Antigen, Dantrolene	6355 ± 313 b, c	75
Antigen, Colchicine	8115 ± 850 b, c	23
Ruthenium Red	3423 ± 183	-
Antigen, Ruthenium Red	5332 ± 223	-5
La^{3+}	4076 ± 185	-
Antigen, La^{3+}	4482 ± 201 d	82

Antigen, Ovalbumin; Bufrolin 2.5μM; Lanthanum chloride 0.5mM; Ruthenium Red 5 x 10^{-5}M; Colchicine 0.5mM; Dantrolene 40μM.

a $P < .0001$ relative to saline control

b $P < .001$ relative to antigen

c $P < .05$ relative to saline

d Not significantly different to control

antiallergic agent disodium cromoglycate (3). A time course of the flux of ^{45}Ca shows that the bulk of the ion flux occurs after the release of histamine is complete (7). This result infers that the flux does not entirely result from the release mechanism but is a consequence of it. The data in Table II comes from experiments where ^{45}Ca flux was measured and compared with histamine release. 'Bufrolin' inhibited the ion flux and histamine release but the results with lanthanum ions and ruthenium red showed that it was possible to inhibit histamine release and calcium ion flux differentially, indicating a separation of effects. Dantrolene sodium is a skeletal muscle relaxant which inhibits excitation-contraction coupling by inhibiting calcium release from the sarcoplasmic reticulum (8). This compound inhibits antigen

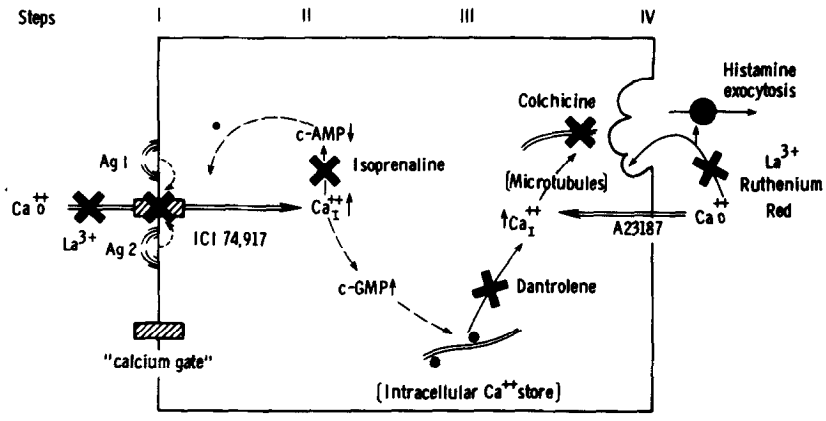

Figure 2. A Scheme for antigen - and ionophore - induced release of histamine from mast cells.

Points of inhibition ✖ and stimulation ⇒

induced release of histamine from mast cells together with the calcium flux. Colchicine, a microtubule disaggregation agent, inhibited histamine release by a small amount but the calcium flux by 60%. Neither of these two compounds would be expected to affect the initial stages of the histamine release mechanism.

DISCUSSION

The results presented above can be explained by the hypothetical scheme shown in figure 2. This represents a series of steps resulting from the combination of antigen with antibody and the extrusion of histamine. Step I is the antigen-antibody combination which leads to calcium movement into the cell, i.e. a 'calcium gate' is opened. The inward downhill calcium flux elicits a change in cellular cyclic nucleotide levels, viz. Cyclic-AMP falls and Cyclic-GMP rises (Step II). It has been proposed that changes in Cyclic-AMP can affect the rate of closure of the calcium gate, i.e. if cyclic-AMP does not fall following antigen stimulation, the gate closes rapidly and there is a reduction in histamine release (2). This is believed to be the mechanism of action of β-stimulants which increase cellular C-AMP (see Fig.1). Further increases in cytoplasmic calcium concentration would then be expected to reverse the changes in cyclic nucleotide levels by activation of phospho-diesterase and return the cells to their basal state, followed by closure of the calcium gate. Excess calcium ions would be removed by membrane bound calcium dependent ATPase (9).

It is proposed (Step III) that the consequence of an increase in cellular cyclic-GMP level is the release of further calcium ions from an intracellular

store - possibly mitochondria or reticulum. This is in parallel with Rasmussen's proposal that cyclic-GMP can induce a flux of calcium ions from sarcoplasmic reticulum (10). The flux of intracellular calcium triggers changes in the microtubules associated with the extrusion of histamine from the cell (Step IV). Exocytosis increases the surface area of the cellular membrane and external calcium ions bind to the newly formed sites, this will occur during and after the histamine has been released.

Figure 2 also shows the point of action of several compounds reported above. We propose that antiallergic compounds inhibit histamine release by binding to the newly formed calcium gate and inactivating it, this explains the long duration of action seen in table II with the dual sensitized tissue, i.e. a second antigen cannot activate a gate 'frozen' in a non active state. Lanthanum ions compete with calcium ions for their sites whilst not being transported into the cell. Isoprenaline inhibits by increasing cellular cyclic-AMP levels and inducing closure of the calcium gate. Inhibition of antigen-stimulated release by dantrolene sodium can be explained if it inhibits the movement of calcium ions out of the intracellular store, this is its mode of action in muscle. Dantrolene does not inhibit ionophore-induced histamine release (5); this is to be expected if ionophore bypasses the steps up to the activation of the microtubular system. Colchicine and vinblastine inhibit ionophore release as demanded by this model (5).

The ^{45}Ca flux data above can be explained if the net flux measured is the sum of two components. These are firstly a small trigger calcium movement induced by antigen and secondly a non-specific binding of ^{45}Ca to the newly exposed membrane. The first, and smaller, of the calcium ion fluxes was inhibited by antiallergic compounds which interferred with the calcium gate. Lanthanum ions also inhibit this phase of the flux, presumably by displacing calcium from the gate. The second, and major component was inhibited not only by ruthenium red and lanthanum ions, which compete for calcium binding sites directly, but also by agents which prevent the initial triggering and consequent histamine release, i.e. by preventing the formation of the secondary sites. Colchicine and dantrolene sodium, since they also reduce histamine release, inhibit the secondary sites forming and hence the binding of calcium ions.

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