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The Mechanism of Anaphylactic Histamine Release from Rabbit Leucocytes

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Summary. Histamine release from rabbit leucocytes is temperature-dependent and requires the presence of calcium but not magnesium ions. It is inhibited by agents which reduce disulphide linkages, and by sulphydryl-blocking agents, but potentiated by certain dicarboxylic acids.

INTRODUCTION

In a previous paper (Greaves and Mongar, 1968b) we demonstrated that leucocyte suspensions, effectively free of platelets, obtained from ovalbumin-sensitized rabbits would release most of their histamine *in vitro* when challenged by the specific antigen under suitable conditions. Evidence was also presented suggesting that the major part of the histamine in this preparation was present in the basophils. The present paper describes experiments undertaken to determine some physical and biochemical aspects of anaphylactic histamine release from rabbit leucocytes. Preliminary results were communicated to the IIIrd International Pharmacological Congress (Greaves and Mongar, 1968a).

METHODS

New Zealand albino female rabbits weighing 2–5 kg were used. The methods of sensitization, isolation of platelet-depleted leucocyte suspensions and histamine release were as described in the preceding paper. The concentration of ovalbumin used to challenge leucocytes *in vitro* was 6×10^{-4} unless otherwise stated.

In some experiments the effect of chemical inhibitors was studied by pre-incubating the cells with the inhibitor for 15 minutes prior to challenge with antigen after which incubation was continued a further 15 minutes. In all inhibition experiments controls were carried out in which the inhibitor was incubated with the cells in the absence of antigen. The presence of certain inhibitors in high concentration tended to interfere with the response of the guinea-pig ileum to histamine at bioassay. In these cases the reaction volume was reduced from 4·0 to 1·5 ml, and during bioassay concentrations of the inhibitors equal to those present in the supernatants were added to the standard histamine solutions.

RESULTS

TEMPERATURE

The effect of temperature on anaphylactic histamine release was studied in two experiments. Variation of temperature from 2 to 47° was achieved by incubating aliquots of a

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suspension in waterbaths thermostatically controlled to within 0·5°, the tubes being covered with Parafilm during incubation. Spontaneous histamine release was measured for each temperature. Fig. 1 shows that release is highly temperature sensitive.

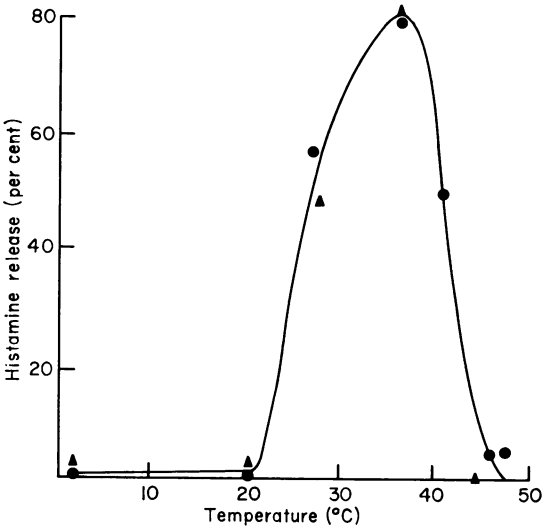

FIG. 1. Anaphylactic histamine release at 2–47°. Leucocytes from two animals studied, the corresponding values being represented by ● or ▲. The total histamine contents of aliquots were 288 and 600 ng, respectively.

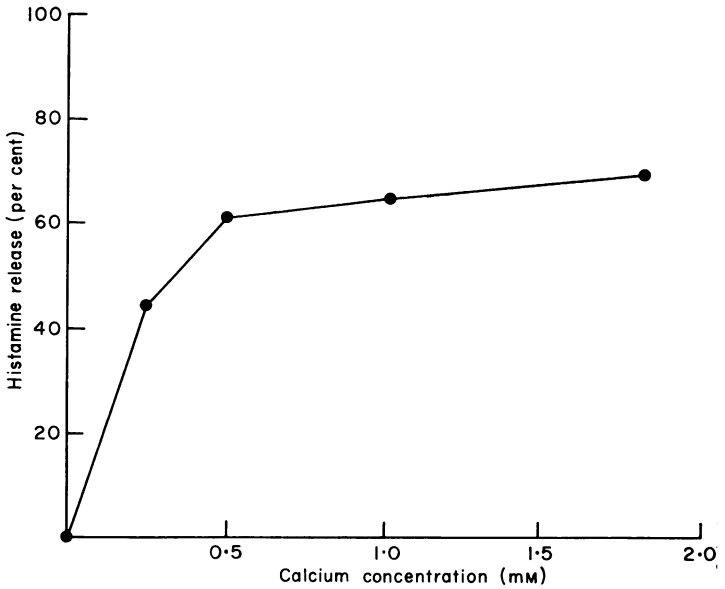

FIG. 2. Relationship between calcium concentration and anaphylactic histamine release. Results represent mean of four experiments.

At 2° and 20° release by antigen was absent or trivial in both experiments. Of the temperatures studied 37° gave maximum release, more than half of this amount being released at 28°. At 41° marked inhibition of histamine release took place. Inhibition was

complete at 44–47°. Heating to 47° had little effect on spontaneous histamine release which was 9 per cent in one experiment and 5 per cent in the other, the corresponding values at 37° being 4 and 3 per cent, respectively.

CALCIUM

Anaphylactic histamine release from rabbit leucocytes can be inhibited by reducing or omitting calcium in the reaction medium. Fig. 2 shows the effect of varying the concentration of calcium on release. Omission of calcium caused complete abolition of release in three experiments and in a fourth there was less than 5 per cent of maximum release. Eight further experiments gave an average inhibition of 97 per cent. Maximum histamine release was attained in the presence of 0·25–0·5 mM calcium. Further increase of calcium concentration to 1·8 mM (the concentration in Tyrode solution) failed to cause appreciable enhancement of histamine release.

MAGNESIUM

The data of Table 1 show that withdrawal of magnesium had no inhibitory effect on anaphylactic histamine release.

TABLE 1

ANAPHYLACTIC HISTAMINE RELEASE IN TYRODE WITH AND WITHOUT MAGNESIUM

Total histamine (ng)	Histamine release by antigen (ng)		Inhibition (%)
	Mg 0·77 mM	Mg nil	
612	412	472	−15
390	190	174	9
159	91	106	−19
212	202	161	22

Leucocyte suspensions from four rabbits studied (spontaneous release in normal Tyrode averaged 5 per cent).

In four experiments release in the presence of 0·77 mM magnesium (the concentration in Tyrode solution) was compared with release in the absence of magnesium. In two experiments release was slightly lowered in the absence of magnesium and in two slightly raised.

Increasing the concentration of magnesium above that in Tyrode failed to mitigate the inhibitory effect of calcium withdrawal on anaphylactic histamine release. In three experiments (Table 2) a rise in the concentration of magnesium from 0·77 to 10 mM completely failed to restore release in the absence of calcium ions.

SULPHYDRYL-BLOCKING AGENTS

The effect of the sulphydryl-blocking agents iodoacetate and the more specific *N*-ethyl maleimide (NEM) and *p*-chloromercuribenzoate (PCMB) on anaphylactic histamine release has been studied. Table 3 shows the effect of 1 mM iodoacetate in three experiments: it caused almost total inhibition of anaphylactic histamine release.

Preliminary control experiments with NEM and PCMB showed that they released histamine from sensitized leucocytes in the absence of antigen. For example, four to eleven times as much histamine was released by 2 mM NEM compared with release in Tyrode alone and 1 mM PCMB released three to five times as much histamine compared with Tyrode. However, by assuming that this direct release by the inhibitor proceeds independently of release by antigen the effect on the anaphylactic reaction could be calculated. In one experiment with 2 mM NEM inhibition of anaphylactic histamine release was 93 per cent. The effect of 1 mM PCMB was similar, a 1 mM solution causing 80 per cent inhibition.

TABLE 2

EFFECT ON HISTAMINE RELEASE OF A HIGH CONCENTRATION OF MAGNESIUM IN THE ABSENCE OF CALCIUM

Total histamine (ng)	Histamine release (ng)			
	Tyrode	Antigen		
	Ca 1·8 mM Mg 0·77 mM	Ca 1·8 mM Mg 0·77 mM	Ca nil Mg 0·77 mM	Ca nil Mg 10 mM
212	15	202	25	25
159	10	129	10	10
122	12	85	17	12

TABLE 3

INHIBITION BY 1 mM IODOACETATE OF ANAPHYLACTIC HISTAMINE RELEASE

Total histamine (ng)	Histamine release (ng)				Inhibition (%)
	Tyrode		Antigen		
	Alone	Iodoacetate	Alone	Iodacetate	
480	50	42	205	55	92
520	30	25	350	44	93
512	25	27	475	28	100

DISULPHIDE REDUCING AGENTS

Two types of disulphide reducing agents were used—sodium sulphite and various thiols (cysteine, thioglycollate and reduced glutathione).

Sodium sulphite was studied at three concentrations: 0·5, 2·0 and 10·0 mM. Results of three experiments with these concentrations are given in Table 4. Considerable inhibition of anaphylactic histamine release (63–81 per cent) was seen with 10 mM sulphite in all three experiments. In one experiment a large inhibition was also seen with the two lower concentrations of sulphite.

In further experiments the effect of two concentrations (10 and 50 mM) of each of the three thiols was investigated. No increased histamine release, compared with spontaneous release in Tyrode alone, took place when leucocytes were incubated with 50 mM thiol in the absence of antigen. Results of histamine release by antigen in the presence of the three

thiols are given in Fig. 3 (means of two experiments). Inhibitions of 28–88 per cent were obtained with 50 mM thiols and 19–46 per cent with 10 mM thiols.

TABLE 4

INHIBITION BY SULPHITE OF ANAPHYLACTIC HISTAMINE RELEASE

Total histamine (ng)	Histamine release (ng)						Inhibition (per cent)		
	Tyrode		Antigen						
				Sulphite (mM)			Sulphite (mM)		
	Alone	Sulphite (10 mM)	Alone	0·5	2	10	0·5	2	10
505	40	40	365	335	297	150	9	21	63
480	50	39	305	113	108	86	71	73	81
520	30	25	350	413	288	133	−20	19	66

TABLE 5

POTENTIATION BY 0·5 mM SUCCINATE AND MALEATE OF ANAPHYLACTIC HISTAMINE RELEASE

Total histamine (ng)	Histamine release by antigen (ng)			Potentiation (per cent)	
	Alone	Succinate	Maleate	Succinate	Maleate
430	209	246	238	18	14
519	172	354	369	106	114
365	195	281	223	44	14

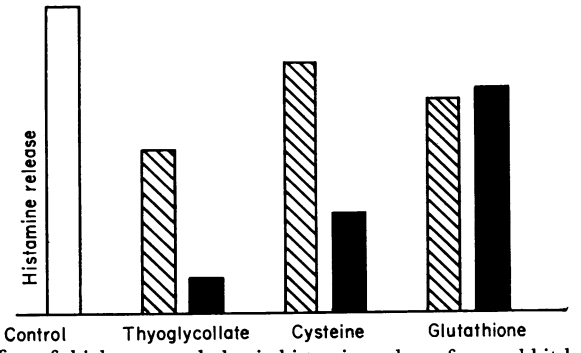

FIG. 3. Inhibitory effect of thiols on anaphylactic histamine release from rabbit leucocytes. Means of two experiments. Solid and cross-hatched columns, Release (as fraction of control) in presence of 10 and 50 mM thiol, respectively. Open columns (control), Maximum release achieved in absence of inhibitor (66 per cent, experiment 1; 70 per cent, experiment 2).

DICARBOXYLIC ACIDS

Succinate and maleate ions both enhance anaphylactic histamine release from rabbit leucocytes. In three experiments aliquots of sensitized leucocyte suspensions were incubated with succinate or maleate in a concentration of 0·5 mM in the presence and absence of a sub-optimal concentration of antigen (62 μg/ml). Neither of these anions

caused release from leucocytes in the absence of antigen, but marked potentiation of anaphylactic histamine release was seen (Table 5) despite the low concentration of anion used.

DISCUSSION

The foregoing results suggest that anaphylactic histamine release from rabbit leucocytes, like that from other systems previously studied (Mongar and Schild, 1957a, b, 1958; Brocklehurst, Humphrey and Perry, 1961; Uvnas and Thon, 1961; Mota and Ishii, 1960; Lichtenstein and Osler, 1964) is mediated by a temperature-sensitive, calcium-dependent mechanism with the characteristics of a sulphydryl enzyme system. But although these systems share many factors in common there are also some well-defined differences.

Release from rabbit leucocytes is sensitive to both heating and cooling, optimum release being achieved at about 37°. At 41° marked inhibition was demonstrated. By contrast no evidence of inhibition at 40·5° was noted by Mongar and Schild (1957b) using chopped guinea-pig lung, although inhibition was demonstrated at 43°. Human leucocytes also show much greater sensitivity to heat, complete inhibition being achieved by raising the temperature to only 40° (Lichtenstein and Osler, 1964).

Inhibition of anaphylaxis due to calcium lack is also a unifying characteristic of *in vitro* anaphylaxis. Although only low concentrations of calcium ions are required to support substantial anaphylactic histamine release from rabbit leucocytes, omission of calcium caused more than 98 per cent inhibition of release in eight out of twelve experiments, and at least 90 per cent inhibition in the remainder. Similar results have been obtained with human leucocytes (Lichtenstein and Osler, 1964). With chopped guinea-pig lung it has been found insufficient to remove calcium from the medium and a calcium chelating agent EDTA had to be added to obtain complete inhibition (Mongar and Schild, 1958). The role, if any, of magnesium ions in anaphylaxis is a minor one. With rabbit leucocytes a thirteen-fold increase in the concentration of magnesium failed to reverse inhibition of release by antigen in the absence of calcium. Similar results have been obtained with the chopped guinea-pig lung (Mongar and Schild, 1958). In the human system maximum release obtainable in the presence of calcium alone can be increased by addition of magnesium (Lichtenstein and Osler, 1964).

Sulphydryl-blocking agents were first used to inhibit *in vitro* anaphylaxis by Mongar and Schild (1955) and Moussatché and Danon (1956). In the present investigation three sulphydryl inhibitors were used: iodoacetate, NEM and PCMB. Although more specific than iodoacetate (which, as well as behaving as an alkylating agent, also reacts with amino groups), NEM and PCMB proved difficult to use owing to their tendency to cause histamine release in the absence of antigen. Nevertheless, results with all three compounds indicated that with rabbit leucocytes, as with chopped guinea-pig lung (Mongar and Schild, 1955, 1957) and rat mast cells (Mota and Ishii, 1960) free sulphydryl groups are essential for anaphylactic histamine release.

The inhibitory effect of disulphide-reducing compounds on release from rabbit leucocytes resembles that occurring with chopped guinea-pig lung but with additional important differences. In the rabbit both thiols and sulphite produced only inhibition within the range of concentrations used. With sensitized guinea-pig lung a dual action of thiols can be demonstrated, potentiation of release at 10 mM and inhibition at 50 mM (Edman, Mongar and Schild, 1964). The potentiating effect of 10 mM thiol is even more marked with isolated

rat peritoneal mast cells and in this system the inhibitory effect of 50 mM thiol has almost disappeared (Perera and Mongar, 1965).

That dicarboxylic acids enhance *in vitro* anaphylaxis was first demonstrated by Moussatché and Danon (1957) using guinea-pig lung and later confirmed by Austen and Brocklehurst (1961). The potentiation of rabbit leucocyte anaphylaxis by low concentrations of maleate or succinate ions extends these findings. The mechanism of this effect awaits elucidation.

Although participation of sulphydryl enzyme-like mechanisms in anaphylactic histamine release from leucocytes is established, the precise stage of the reaction at which the mechanism operates remains speculative. Studies of the relationship between cytological changes in leucocytes and anaphylactic histamine release from them are at present in progress and may provide a useful approach to the solution of this problem.

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