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MECHANISM OF HISTAMINE RELEASE FROM RAT MAST CELLS INDUCED BY THE IONOPHORE A23187: EFFECTS OF CALCIUM AND TEMPERATURE

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1 The mechanism of histamine release from a pure population of rat mast cells induced by the lipid soluble antibiotic, A23187, has been studied and compared with data for anaphylactic histamine release reported in the literature.

2 Histamine release induced by A23187 in the presence of calcium 10^{-3} mol/l was completed in 10 minutes. By preincubation of the mast cells with A23187 for 10 min in the absence of calcium the histamine release induced by calcium, 10^{-3} mol/l or 5×10^{-3} mol/l, was completed in 90 s and 45 s, respectively.

3 A23187-induced histamine release was maximal with calcium 10^{-3} mol/l when the cells were incubated at 33 to 39°C for 10 minutes.

4 The cellular mechanism, which was stimulated by A23187 and calcium for the release of histamine, was irreversibly inactivated by incubation at 45°C.

5 An inhibition of energy metabolism was excluded as the cause of the heat inactivation.

6 The dependence of A23187-induced histamine release on calcium and temperature, the time course of histamine release and the heat inactivation are consistent with the view that the same mechanism is involved in A23187-induced and anaphylactic histamine release.

Introduction

A new carboxylic acid, lipid-soluble antibiotic, A23187, specifically forms complexes with calcium and magnesium and transports these ions across a variety of membranes (Reed & Lardy, 1972). In the presence of calcium, A23187 acts as a histamine releasing agent on isolated mast cells (Foreman, Mongar & Gomperts, 1973), and extrusion of secretory granules has been observed from rat mast cells exposed to A23187 in the presence of calcium (Cochrane & Douglas, 1974). Furthermore, injection of calcium into individual mast cells induced similar morphological changes (Kanno, Cochrane & Douglas, 1973).

The present study was performed in order to compare the mechanism of A23187-induced histamine release with that of anaphylactic histamine release. The effect of calcium concentration on the time course, the effect of temperature and the effect of heat inactivation on A23187-induced histamine release have been studied and compared with data for anaphylactic histamine release reported in the literature.

Methods

Isolation of rat mast cells

Male Sprague-Dawley rats (360–555 g) were used for the experiments. Mast cells were isolated by differential centrifugation in concentrated human serum albumin by a slight modification of the method described by Chakravarty (1965) and Chakravarty & Zeuthen (1965). Rats were killed by bleeding from the carotid arteries under light ether anaesthesia. Mixed peritoneal cells were collected by injection of Krebs-Ringer solution containing 50 μg/ml heparin into the abdominal cavity through a small incision. After differential centrifugation the mast cell fraction was washed twice to remove the excess albumin and suspended in Krebs-Ringer solution containing human serum albumin, 1 mg/ml, final pH 7.0 to 7.1.

In experiments in which mixed peritoneal cells were used, the cell suspensions were washed twice in Krebs-Ringer solution containing human serum albumin and suspended in the same solution.

Incubation procedures

Mast cells pooled from 1 to 6 rats were divided into groups of samples which in each experiment contained the same cell density in a final volume of 0.5 ml both for determination of the adenosine triphosphate (ATP) content of the mast cells and for the histamine release experiments. The cell density for different experiments varied from 10^5 cells to 1.35×10^5 cells per ml, except for the experiments illustrated in Figure 2 (see legend to Figure 2). The cell suspensions were prewarmed in a 37°C bath for 10 to 20 min and the incubation was continued with A23187 for 1 to 60 minutes. In some experiments histamine release was initiated by incubation with calcium after preincubation with A23187 (see legend to Figure 4 and results). Samples without the releaser and other appropriate controls were included. The results of each experiment presented in the figures represent the mean value from 2 to 3 samples. For details of the ATP experiments, see legends to figures.

Determination of histamine release and the ATP content of the mast cells

The histamine release experiments were performed as described previously (Johansen & Chakravarty, 1975). Histamine was determined by a modification of the fluorimetric method (Shore, Burkhalter & Cohn, 1959). Ionophore A23187, ethanol, glucose, pyruvate or succinate did not interfere with the determination of histamine. The release of histamine was calculated as a percentage of the total histamine content of the mast cells. For the determination of ATP, the reaction after incubation of the samples was stopped by chilled perchloric acid. After neutralization of the supernatant the ATP content was determined by the bioluminescence technique using luciferin-luciferase from firefly lanterns as described earlier (Johansen & Chakravarty, 1975).

Human serum albumin was supplied by AB KABI (Stockholm, Sweden), succinate (disodium salt), pyruvate (sodium salt) by Sigma Chemical Company (St. Louis, U.S.A.) and glucose by BDH Chemicals Ltd. All other chemicals were of analytical grade. Ionophore A23187 was kindly supplied by Eli Lilly & Co. (Indiana, U.S.A.). A23187 was dissolved in ethanol 96% v/v and diluted in Krebs-Ringer solution for the experiments. The final concentration of ethanol during incubation never exceeded 0.3% v/v.

Control experiments showed no change in A23187-induced histamine release when the final concentration of ethanol was increased to 0.5% v/v. Ethanol 2% v/v did not change the ATP content of the mast cells (Johansen & Chakravarty, 1975).

Krebs-Ringer solution had the following composition (mmol/l): NaCl 141.9, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.0, Na_2HPO_4 2.5, KH_2PO_4 0.6. When $CaCl_2$ was 2.5 mmol/l, NaCl was reduced to 139.8 mmol/litre. In calcium-free Krebs-Ringer solution, NaCl was increased to 143.3 mmol/litre.

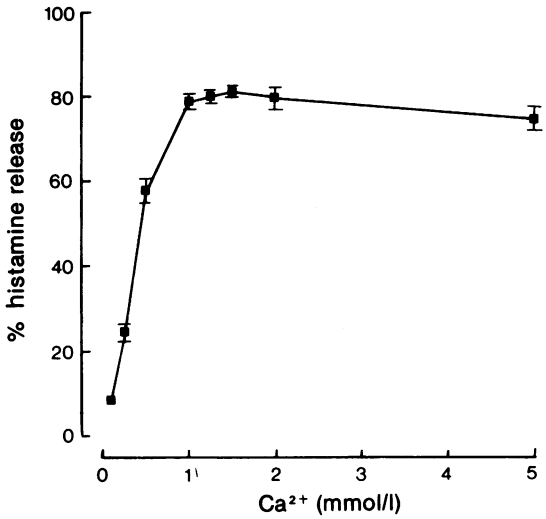

Figure 1 Histamine release induced by A23187 (10^{-5} mol/l) in the presence of increasing concentrations of calcium. Spontaneous histamine release (mean 2.5%) deducted. Cell density 1 to 1.2×10^5 cells per ml; average purity: 99.7%. Mean values from 4 experiments; vertical lines show s.e. means.

Results

Concentration dependence

Histamine release from mast cells induced by A23187 (10^{-5} mol/l) increased rapidly when the concentration of calcium in the suspending medium was increased from 10^{-4} mol/l and the release was maximal with calcium 10^{-3} mol/l (Figure 1).

The concentration of ionophore A23187 which induced maximal histamine release from a pure population of mast cells was 10^{-6} mol/l with a calcium concentration of 2.54×10^{-3} mol/l (Figure 2a). When the calcium concentration was reduced to 10^{-3} mol/l, maximal histamine release was caused by 5×10^{-6} mol/l ionophore A23187. However, 90% of the maximal release was induced by 2×10^{-6} mol/l A23187 (Figure 2b). A further shift to the right was observed in the presence of calcium 10^{-3} mol/l when the cell density was increased 18 times by using mixed peritoneal cells (Figure 2c).

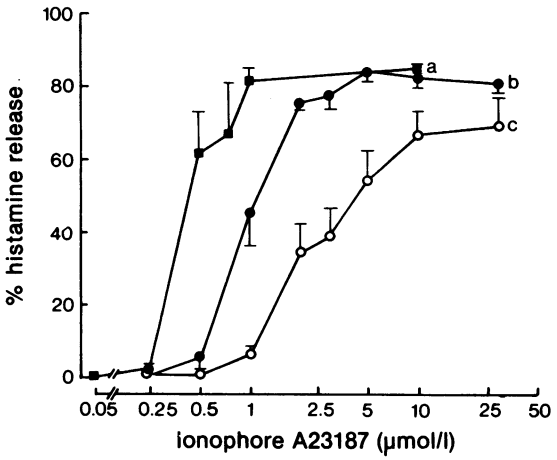

Figure 2 Histamine release induced by increasing concentrations of A23187 in the presence of calcium 2.5×10^{-3} mol/l, curve a(■), or 10^{-3} mol/l, curves b(●) and c(○). Curves (a) and (b) show the effect of A23187 on a pure population of mast cells (average purity: 97.2% and 98.6%, respectively. Cell density: 1 to 2×10^{5} cells per ml). Curve (c) shows the effect of A23187 on a mixed population of peritoneal cells (cell density: 2.2 to 3.7×10^{6} cells per ml; mast cells: 11.4%). Spontaneous histamine release in the absence of A23187 (mean 3.4%) deducted. Mean from 4 (a), 7 (b) and 6 (c) experiments; vertical lines show s.e. means.

Time course of histamine release

The release of histamine by incubation of the mast cells with A23187 was a slow process. After 5 min incubation, 90% of the total release of histamine had occurred and the release was completed after 10 min incubation with A23187 (Figure 3). Diffusion of A23187 into the mast cells may be the rate limiting process in A23187-induced histamine release. In the experiments illustrated in Figure 4, mast cells were therefore preincubated with A23187 for 10 min in the absence of calcium before histamine release was initiated by adding calcium to the medium. The time course of histamine release after incubation with calcium was dependent on the concentration of calcium. In the presence of calcium 5×10^{-3} mol/l histamine release was completed after 45 s, while with calcium 10^{-3} mol/l, 90% of the maximal release occurred after 90 seconds. This was followed by a slow histamine release up to 5 min, when the release was identical to the release induced by calcium 5×10^{-3} mol/litre.

Temperature dependence

In these experiments the dependence of A23187-induced histamine release on temperature

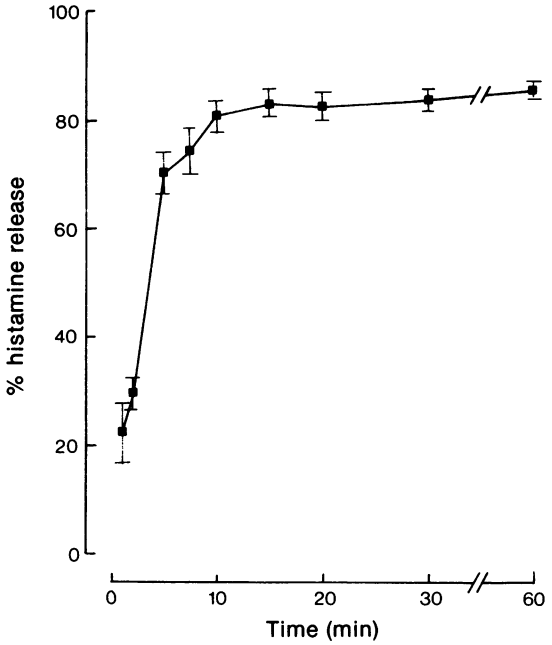

Figure 3 Time course of histamine release induced by A23187 (10^{-5} mol/l) in the presence of calcium (10^{-3} mol/l). Abscissa scale: time of incubation with A23187. Spontaneous histamine release (mean 3.9%) deducted. Average purity of mast cells: 97.8%. Mean values mean from 4 experiments; vertical lines show s.e. means.

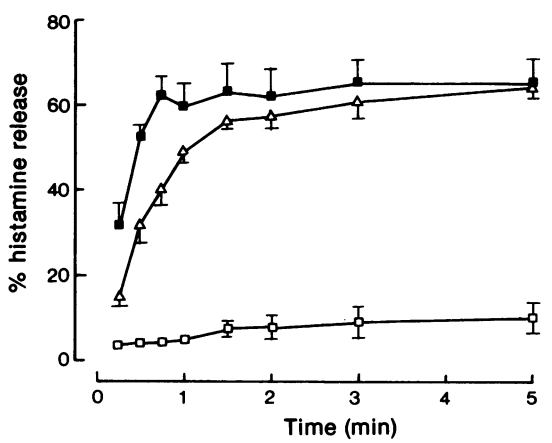

Figure 4 Time course of histamine release induced by calcium. The mast cells were preincubated with A23187 for 10 min before calcium was added. Abscissa scale: time of incubation with calcium 2×10^{-4} mol/l (□), 10^{-3} mol/l (△) or 5×10^{-3} mol/l (■). Spontaneous histamine release (mean 2.9%) deducted. Average purity: 97.8%. Mean values from 4 (□, ■) and 8 (△) experiments; vertical lines show s.e. means.

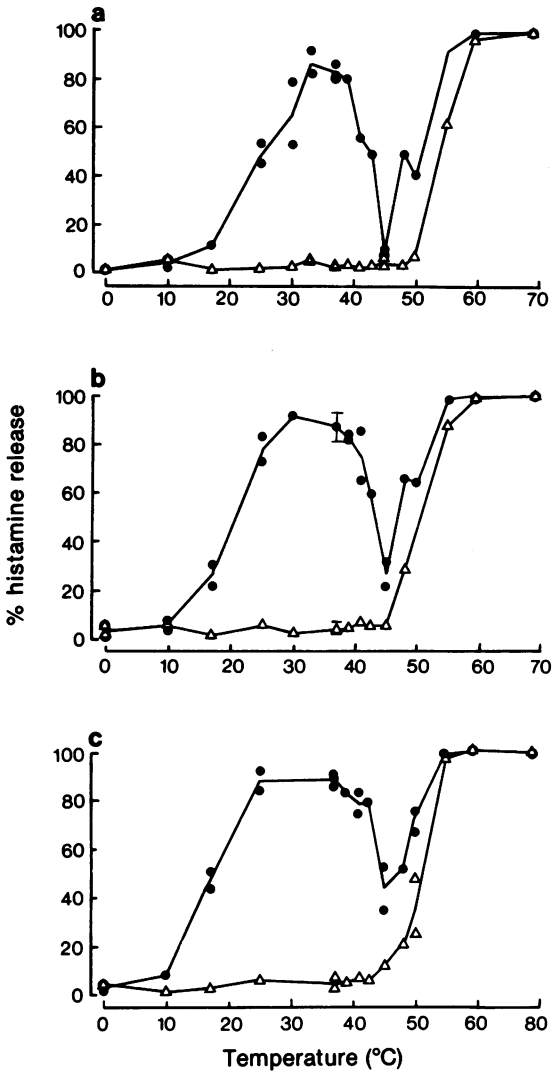

Figure 5 Dependence of A23187-induced histamine release on temperature. Abscissa scale: incubation temperature (°C). Mast cells were equilibrated for 10 min at the temperatures indicated before incubation with A23187 for 10 min (a), 30 min (b) or 60 min (c). Individual results from 9 experiments. A23187-induced histamine release (●); spontaneous histamine release (△) not deducted. Histamine release at 37°C in Figure 5b is mean and range from 9 experiments. Average purity: 98.1%.

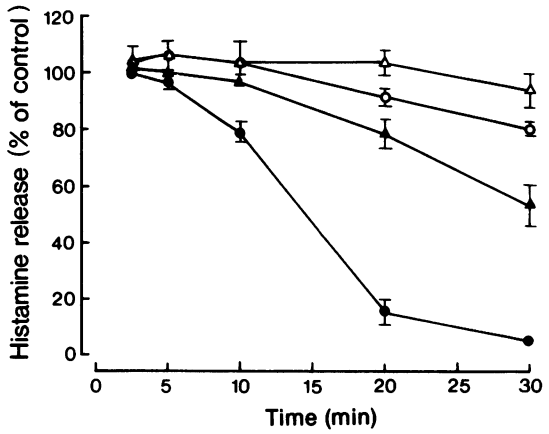

Figure 6 Inhibition of A23187-induced histamine release at 37°C by pretreatment at 42.5 to 44.9°C. Abscissa scale: time of pretreatment at 42.5°C (△), 43.1°C (○), 43.7°C (▲) or 44.9°C (●). Ordinate scale: histamine release as % of control without pretreatment; 100 on the ordinate scale: 83.1% ± 3.4% (mean ± s.e. mean). Spontaneous histamine release with or without pretreatment (mean: 4.4% and 4.0%, respectively) deducted. Average purity: 96.5%. Mean values from 4 (△, ○, ▲) and 3 (●) experiments; vertical lines show s.e. mean.

was studied. Maximal histamine release from mast cells, incubated with ionophore A23187 for 10 min, was observed between 33° to 39°C (Figure 5a). Below and above that temperature range histamine release was inhibited and at 10°C and 45°C the release process was completely blocked. Spontaneous histamine release was 1.3 to 6.8% when the mast cells were incubated at temperatures below 50°C. With incubation above 50°C the spontaneous release increased rapidly. Essentially the same pattern of A23187-induced and spontaneous histamine release was obtained when the incubation time was increased and the spontaneous release due to the cytotoxic effect of higher temperature was observed at around 45°C when the incubation time was increased to 30 to 60 min (Figure 5b,c).

In order to study heat inactivation of A23187-induced histamine release, mast cells were incubated twice in succession as follows. After incubation at 42.5°C to 44.9°C for 2.5 to 30 min, the mast cells were cooled in an ice-chilled water bath and then incubated again with A23187 at 37°C for 20 minutes. Samples without A23187 and samples with or without A23187 incubated only at 37°C were included. No change in histamine release occurred after preincubation at 42.5°C (Figure 6). A significant reduction in histamine release was observed after preincubation for 20 min at 43.1°C ($P < 0.025$, t test for paired data). The release was inhibited after preincubation at 44.9°C for 10 min and almost completely blocked after 30 minutes.

Effect of incubation at 45°C on mast cell ATP content

A gradual and identical decrease in mast cell ATP

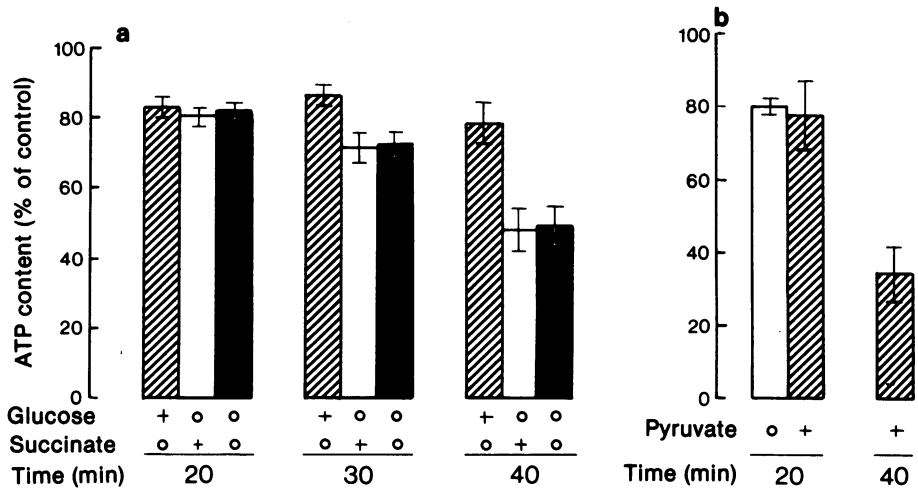

Figure 7 Changes in the ATP content of mast cells after incubation at 45°C in the presence and absence of glucose 5×10^{-3} mol/l and succinate 5×10^{-3} mol/l (a) or pyruvate 10^{-3} mol/l (b). Abscissa scale: time of incubation. Ordinate scale: ATP content as % of control; 100% on the ordinate scale represents the ATP content of mast cells incubated for 20 to 40 min at 37°C without substrate (mean ± s.e. mean: 1.58 ± 0.02 pmol/10^3 cells, $n = 13$). Average purity: 98.1%. Mean values from 3 to 4 experiments for (a) and 3 experiments for (b). Vertical lines show s.e. means.

content was observed after incubation for 20 to 40 min at 45°C in the presence or absence of pyruvate which is metabolized by oxidative metabolism in the rat mast cell (Diamant Norn, Felding, Olsen, Ziebell & Nissen, 1974) or succinate which stimulates respiration in rat mast cells by 50% (Chakravarty & Zeuthen, 1965) (Figure 7a,b). After 20, 30, and 40 min incubation the ATP content was 80%, 70%, and 45% respectively of the normal value (Figure 7a). By contrast in the presence of glucose which stimulates glycolysis in rat mast cells (Chakravarty, 1965; Diamant & Peterson, 1971) the ATP content was reduced 20% after incubation for 20 min at 45°C and no further reduction was observed after 30 min and 40 min (Figure 7a).

Dissociation between the ATP content of the mast cell and the ability of the cell to release histamine

In these experiments mast cells were incubated at 37°C for 30 min to restore the ATP content following a preincubation period of 30 min at 45°C. In the presence or absence of pyruvate the ATP content was reduced 70% after the second incubation at 37°C. By contrast, in the presence of glucose only 25% reduction in ATP was observed (Figure 8). During the first incubation (45°C) the ATP content was reduced 12% in the presence of glucose and 40% in the presence or absence of pyruvate. However, the difference between the ATP content after the first and second incubation in the presence of glucose was not statistically significant ($P > 0.05$). Histamine release induced by incubation with A23187 after the second incubation at 37°C remained completely blocked in the presence or absence of substrates.

Discussion

Anaphylactic histamine release from guinea-pig lung and disruption of rat mast cell due to antigen is dependent on the incubation temperature (Mongar & Schild, 1957; Chakravarty, 1960; Högberg & Uvnäs, 1960). Histamine release from guinea-pig lung induced by the antigen-antibody reaction is dependent on calcium (Mongar & Schild, 1958; Chakravarty, 1960) and the anaphylactic histamine release from rat mast cells is maximal at a calcium concentration of 1 to 2×10^{-3} mol/l (Foreman & Mongar, 1972). A23187-induced histamine release shows essentially the same dependence on both incubation temperature and calcium concentration of the incubation medium.

Foreman *et al.* (1973) reported that the cells extracted A23187 from the aqueous medium. The release of histamine by the different concentrations of A23187 may therefore be expected to decrease when the density of the cell suspension (either pure mast cells or mast cells and other peritoneal cells) increases as demonstrated in Figure 2.

The secretory response induced by A23187 was slow compared to anaphylactic histamine release which was complete after 30–40 s (Johansen & Chak-

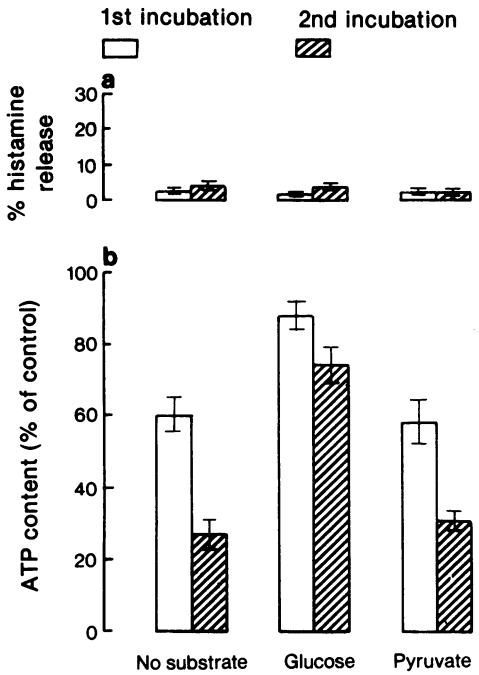

Figure 8 Effect of incubation at 37°C on mast cell ATP content and A23187-induced histamine release following incubation at 45°C. Ordinate scale: % histamine release (a) and ATP content as % of control value (b); 100 on the ordinate scale represents ATP content in mast cells incubated in the absence of substrate (mean ± s.e. mean: 1.42 ± 0.15 pmol/10^3 cells). The cells were incubated twice in the presence or absence of glucose (5×10^{-3} mol/l) or pyruvate (10^{-3} mol/l). The first incubation at 45°C lasted 30 min; after cooling in ice-chilled water bath the cells were incubated for 30 min at 37°C. The ATP content after the first and second incubation is shown in (b). Histamine release induced by incubation with A23187 for 20 min after the first and second incubation is shown in (a). The release of histamine without preincubation at 45°C in the presence of glucose, pyruvate or without substrate was: 91.2%, 85.9% or 84.4%, respectively. Spontaneous histamine release from the incubations after 1st (mean: 4.0%) and 2nd (mean: 3.0%) incubation or without incubation at 45°C (mean: 3.2%) deducted. Average purity: 97.8%. Mean ± s.e. mean from 3 experiments.

ravarty, 1975). However, when the cells were preincubated with A23187 without calcium and histamine release induced thereafter by calcium, the time course of the release was similar to anaphylactic histamine release.

Varying degrees of inactivation of A23187-induced histamine release at 37°C were produced by heating the cells to 43.1°C to 44.9°C. An increase in the temperature of 0.6°C produced a significant reduction in histamine release which demonstrates a high temperature sensitivity of the histamine release mechanism. Essentially the same sensitivity for heat inactivation was demonstrated for anaphylactic histamine release from guinea-pig lung and rat mast cells (Mongar & Schild, 1957; Högberg & Uvnäs, 1960). Histamine release induced by antigen-antibody reaction and compound 48/80 is dependent on the ATP content of the mast cell (Johansen & Chakravarty, 1972; 1975). During incubation at 45°C a reduction in mast cell ATP content was observed. Glucose partly counteracted this reduction in ATP, in contrast to pyruvate or succinate which did not influence the level of ATP during incubation at 45°C. When incubation at 45°C was followed by an incubation at 37°C, no restoration of ATP occurred. In the presence or absence of pyruvate, ATP content was further reduced during the second incubation, but in the presence of glucose no significant change in ATP was observed. This difference may be explained by an inhibition of the aerobic ATP synthesis almost without change in glycolysis by incubation at 45°C. An inadequacy of substrate for oxidative metabolism could not explain the results. CO_2 production from glucose is not inhibited during incubation of mast cells at 45°C (Svendstrup & Chakravarty, 1977). The progressive decrease in ATP during incubation at 45°C and 37°C in the absence of a substrate or in the presence of pyruvate or succinate may therefore be caused by a reduction of the aerobic phosphorylation capacity without change in the capacity to metabolize glucose. Histamine release at 37°C was irreversibly blocked by preincubation at 45°C even in the presence of glucose when mast cell ATP content was more than 75% of the normal level and therefore present in sufficient amount for almost normal histamine release to occur with other selective histamine liberators (Johansen & Chakravarty, 1972; 1975).

Histamine release from rat mast cells induced by A23187 is dependent on extracellular calcium and temperature, and has a time course similar to that of anaphylactic histamine release. Moreover, the sensitivity of A23187-induced histamine release to heat inactivation was essentially the same as that demonstrated previously for anaphylactic histamine release from guinea-pig and rat. Disturbances in the energy metabolism could not explain the inhibition of A23187 induced histamine release of incubation at 45°C.

These observations are consistent with the view that the same mechanism is involved in A23187-induced and anaphylactic histamine release. The observed effects of ionophore A23187 on histamine release may possibly be explained on the assumption that an increase in the concentration of

calcium in mast cell cytosol is the trigger of the release mechanism.

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