Br. J. Pharmac. (1977), **61**, 237–242

THE RELATIONSHIP BETWEEN ENERGY METABOLISM AND THE ACTION OF INHIBITORS OF HISTAMINE RELEASE

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1 Dextran-induced release of histamine from rat mast cells was inhibited equally in complete and glucose-free Tyrode solution by doxantrazole (0.03–3 μmol/l), theophylline (0.1–3 mmol/l) and dicumarol (0.01–10 μmol/litre).

2 Doxantrazole (3 μmol/l), theophylline (3 mmol/l) and dicumarol (10 μmol/l) did not reduce the adenosine 5′-triphosphate (ATP) content of mast cells in glucose-free medium. Higher concentrations of dicumarol (56–100 μmol/l) markedly reduced the cellular ATP content. This reduction was reversed by glucose.

3 Papaverine was a more potent inhibitor of histamine release from mast cells incubated in glucose-free solution than in complete Tyrode solution (dose-ratio = 20). Like antimycin A (1 μmol/l), papaverine (3 μmol/l) caused a depletion of mast cell ATP that was greater in the absence (85%) than in the presence (25%) of extracellular glucose.

4 These results suggest that dicumarol, like doxantrazole and theophylline, inhibits histamine release without affecting mast cell energy metabolism. In contrast, papaverine probably inhibits release by depleting ATP that is required for exocytosis.

5 Inhibition of histamine release by dibutyryl cyclic adenosine 3,5′-monophosphate (1–3 mmol/l) was significantly greater when cells were incubated in complete rather than in glucose-free medium.

Introduction

In addition to being dependent upon extracellular calcium (Foreman & Mongar, 1972a) the anaphylactic release of histamine from rat peritoneal mast cells has a requirement for metabolic energy, being supported by adenosine 5′-triphosphate (ATP) derived from either glycolytic or oxidative pathways. This conclusion is based on experiments with metabolic inhibitors and measurement of the cellular content of ATP (Peterson, 1974; Diamant, Norn, Fielding, Olsen, Ziebell & Nissen, 1974; Johansen & Chakravarty, 1975). Experiments using the divalent cation ionophore, A23187, which facilitates the passage of calcium across cell membranes, suggest that ATP is required for exocytosis at a stage following calcium entry into the cell (Foreman, Mongar & Gomperts, 1973).

The dextran-induced release of histamine from rat mast cells *in vitro* shares many properties with the anaphylactic reaction including calcium-dependence (Foreman & Mongar, 1972b), enhancement by phosphatidyl serine (Goth, Adams & Knoohuizen, 1971), desensitization and cross-desensitization with antigen (Foreman & Garland, 1974; Foreman, Garland & Mongar, 1976) and inhibition by antiallergic agents such as cromoglycate (Garland & Mongar, 1974). Furthermore, the reaction is diminished by metabolic inhibitors such as 2,4-dinitrophenol (Beraldo, Dias da Silva & Lemos Fernandes, 1962).

In recently described differential release experiments, several compounds were compared as inhibitors of histamine release induced by either dextran or the ionophore (A23187) (Garland & Mongar, 1976). Two compounds, papaverine and dicumarol, had effects that were not consistent with their generally accepted properties. Thus, papaverine which is known as a phosphodiesterase inhibitor, appeared to have the effect of a metabolic inhibitor while dicumarol, which is known to uncouple oxidative phosphorylation in some systems (Mahler & Cordes, 1967) had a profile of effect resembling cromoglycate rather than 2,4-dinitrophenol. Therefore, the present

experiments were undertaken to examine the effects of these inhibitors of histamine release on energy metabolism in rat mast cells.

Methods

Histamine release was induced by dextran from mixed peritoneal cells of male Wistar rats (1–5% mast cells) prepared *in vitro* as described previously (Garland & Mongar, 1974). A 0.5 ml volume of a mixture of dextran and phosphatidyl serine (PS) was added to 0.5 ml cells preincubated for 30 min at 37°C in Tyrode solution with or without glucose (5.6 mmol/l); the final concentrations were dextran 6 mg/ml, PS 30 μg/ml. Where appropriate, drugs were included with dextran and PS in the releasing mixture. Histamine release was stopped after 5 min incubation at 37°C by the addition of 4 ml ice-cold calcium-free Tyrode and each sample was centrifuged at 250 *g* for 6 minutes. The supernatant was decanted, the pellet resuspended in 5 ml Tyrode and boiled for 10 min to release residual histamine. Released and residual histamine was assayed fluorimetrically by the method of Shore, Burkhalter & Cohn (1959) but omitting the extraction step as described by Loeffler, Lovenberg & Sjoerdsma (1971). The control histamine release from cells preincubated in complete medium was $35.7 \pm 1.3\%$ (mean ± s.e. mean of 45 observations) compared with $49.6 \pm 1.6\%$ from samples of the same cell suspensions incubated in glucose-free medium. This difference is consistent with the inhibition by glucose of dextran anaphylactoid reactions in rats described by Beraldo *et al.* (1962) and Poyser & West (1968). Inhibition of dextran-induced release from cells incubated in either glucose-free or complete medium was calculated by reference to the appropriate control release corrected for the spontaneous histamine release which was the same in each medium ($6.7 \pm 0.4\%$).

Measurement of ATP was made using cells isolated from recently killed male Sprague-Dawley rats (320–500 g) and fractionated by differential centrifugation through human serum albumin (Chakravarty & Zeuthen, 1965; Chakravarty, 1965). 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 7.0–7.1). The purity of the mast cells was $96.4 \pm 0.8\%$ (mean ± s.e. mean of 12 experiments). Mast cells pooled from 1–5 rats were divided into aliquots containing 40,000–95,000 cells in a final volume of 0.5 ml. The cell suspensions were prewarmed in a 37°C water bath for 10 min and the incubation continued thereafter with different drugs for between 10 to 30 minutes. Samples without drugs and other appropriate controls were included. After incubation, the reaction was stopped by quickly adding double the volume of chilled perchloric acid (PCA) giving a final PCA concentration of 330 mmol/litre. Extraction and determination of the mast cell ATP content was performed as described previously with a specially constructed photometer to measure the peak initial flash from the firefly lantern luciferin-luciferase reaction (Johansen & Chakravarty, 1975). Each experiment was performed in either duplicate or triplicate. The ATP content of mast cells after incubation with drugs is given as a percentage of the control value which was 1.22 ± 0.04 pmol per 10^3 cells (mean ± s.e. mean of 12 experiments). There was no significant difference between the ATP levels in control cells incubated with or without glucose.

Dextran (mol. wt. 110,000) was supplied by Fisons as a 6% (w/v) solution in saline (NaCl 154 mmol/l). Phosphatidyl serine supplied by Koch-Light Ltd. was mixed with saline in a mechanical blender after evaporation of the chloroform-methanol solvent in a stream of nitrogen. Desiccated firefly tails (lanterns), ATP, antimycin A, dicumarol and N^6-O_2'-dibutyryl cyclic adenosine 3′,5′-monophosphate, sodium salt (dibutyryl cyclic AMP) were purchased from the Sigma Chemical Company. Theophylline and doxantrazole were supplied by the Wellcome Research Laboratories. Antimycin A was dissolved in ethanol and then diluted with Krebs-Ringer solution containing albumin 1 mg/ml. The final concentration of ethanol was 0.02% v/v. As shown previously ethanol even in a concentration of 2% v/v did not influence the ATP content of the mast cells (Johansen & Chakravarty, 1975).

The incubation media had the following compositions (mmol/l): Tyrode solution – NaCl 137, KCl 2.7; $MgCl_2$ 1.0, $CaCl_2$ 1.8, $NaHCO_3$ 12 and NaH_2PO_4 0.3; Krebs-Ringer solution – NaCl 141.9, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.0, Na_2HPO_4 2.5 and KH_2PO_4 0.6. Where appropriate, glucose was added to achieve a final concentration of 5.6 mmol/litre. All chemicals were of Analar quality.

Results

The dextran-induced release of histamine from rat peritoneal mast cells was inhibited by the antiallergic agent, doxantrazole, at concentrations of between 0.03–3 μmol/l added to cells concomitantly with the releasing stimulus (Figure 1a). This dose-related inhibition was identical in cells from the same original population but suspended in glucose-free rather than complete Tyrode solution. Inhibition of histamine release by theophylline, 0.1–3 mmol/l (Figure 1b) and dicumarol, 0.01–10 μmol/l (Figure 1c) was also unaffected by the glucose content of the preincubation medium. Figure 2a shows that dibutyryl cyclic AMP was slightly more potent when added to cells preincubated in glucose-containing medium. In

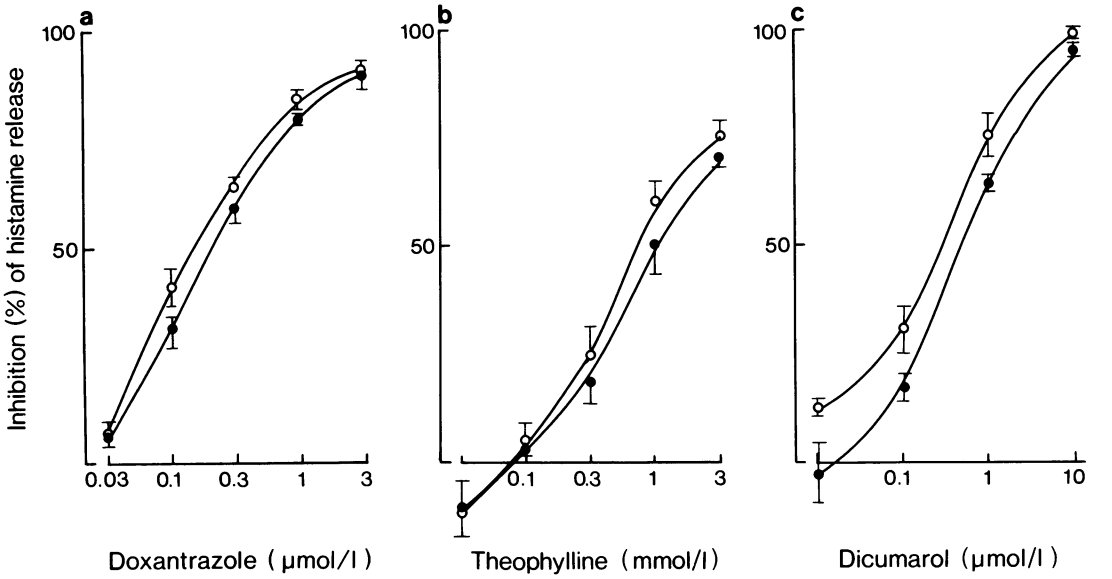

Figure 1 Inhibition (%) of histamine release from rat peritoneal cells incubated at 37°C for 30 min in either glucose-free (●) or complete (○) Tyrode solutions before the addition of a solution containing dextran, phosphatidyl serine and where appropriate various concentrations of inhibitor which was in (a) doxantrazole, (b) theophylline and (c) dicumarol. Each point is the mean of three separate experiments. Vertical lines show s.e. mean. Inhibition in the presence and absence of glucose was not significantly different at the 5% level when compared by Student's *t* test.

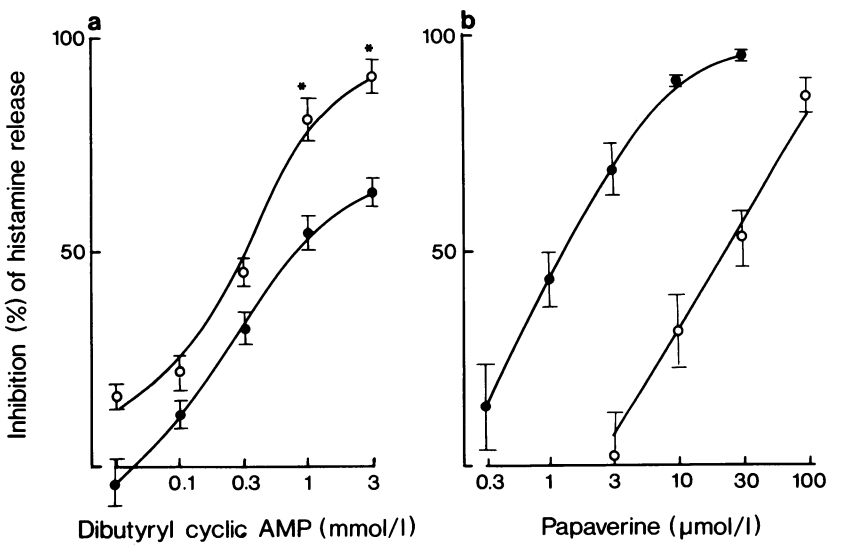

Figure 2 Inhibition (%) of histamine release from rat peritoneal cells incubated at 37°C for 30 min in either glucose-free (●) or complete (○) Tyrode solution before the addition of a solution containing dextran, phosphatidyl serine and where appropriate various concentrations of inhibitor which was in (a) dibutyryl cyclic AMP and in (b) papaverine. Each point is the mean of three separate experiments, vertical lines show s.e. mean. * $P < 0.005$ (by *t* test).

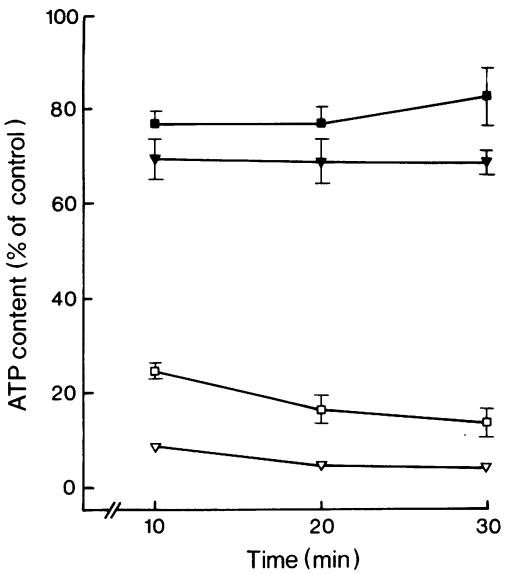

Figure 3 The ATP content of mast cells incubated for 10 to 30 min at 37°C in either complete (■,▼) or glucose-free (□, ▽) Krebs-Ringer soluton containing either papaverine 3 μmol/l (squares) or antimycin A 1 μmol/l (triangles). Each point is the mean of three separate experiments; vertical lines show s.d.

contrast to each of the foregoing inhibitors, papaverine was 20 times more active when added with the histamine-releasing stimulus to cells preincubated in glucose-free Tyrode solution than it was when added to cells in complete Tyrode (Figure 2b).

In order to examine further the effect of these compounds on mast cell energy metabolism, direct measurements were made of the cellular ATP content. As shown in Table 1, incubation of purified mast cells in glucose-free medium for 10–30 min at 37°C with doxantrazole (3 μmol/l), theophylline (3 mmol/l) or dibutyryl cyclic AMP (3 mmol/l) did not alter their ATP content. Furthermore, dicumarol did not reduce the cellular ATP content until concentrations of 56 or 100 μmol/l were used (Table 2). The fall in ATP content produced by dicumarol (100 μmol/l) was less in cells incubated in Krebs-Ringer medium containing glucose (Table 2).

When mast cells were incubated with papaverine (3 μmol/l) in the absence of glucose their ATP content fell by 75% below the control value after 10 min and became further reduced when incubation was continued for up to 30 min (Figure 3). However, in the presence of glucose, the reduction of ATP levels by papaverine was only about 25%. This effect of papaverine was compared with that of antimycin A. As shown in Figure 3, antimycin A (1 μmol/l) reduced the ATP content almost completely, this reduction also being largely counteracted when the mast cells were incubated in glucose-containing medium.

Discussion

Histamine release from rat mast cells incubated in glucose-free medium is more susceptible to inhibitors of oxidative metabolism, such as antimycin A, than release from cells in glucose-containing medium (Peterson, 1974; Diamant *et al.*, 1974). This is, presumably, because in the absence of glucose, mast cells are unable to derive sufficient ATP from glycolysis to support histamine release. Figures 1a and 1b show that both doxantrazole and theophylline inhibited histamine release from cells incubated in glucose-free medium to the same extent as from cells in complete medium. Furthermore, neither compound reduced the ATP content of mast cells (Table 1). Therefore, it is unlikely that these two compounds inhibit histamine release through an effect on energy metabolism, a conclusion that is consistent with previous observations (Garland & Mongar, 1976).

When incubated with rat mast cells, dicumarol reduced their ATP content apparently through inhibition of oxidative metabolism since the effect was reversed by glucose (Table 2). However, the concentration required to reduce the ATP level was higher

Table 1 The ATP content, expressed as a percentage of control, of mast cells incubated at 37°C in glucose-free medium for various periods with dibutyryl cyclic 3′,5′-adenosine monophosphate (3 mmol/l), theophylline (3 mmol/l), or doxantrazole (3 μmol/l).

	ATP content		
Incubation (min)	*Dibutyryl cyclic AMP*	*Theophylline*	*Doxantrazole*
10	99.9 ± 3.7	101.2 ± 0.6	101.8 ± 1.7
20	97.9 ± 2.5	102.4 ± 0.5	102.4 ± 4.4
30	99.2 ± 3.6	106.2 ± 5.6	104.3 ± 2.4

Values are mean ± s.e. mean of 3 separate experiments.

Table 2 The ATP content, expressed as a percentage of control of mast cells incubated at 37°C in either glucose-free or complete (*) medium for 30 min with various concentrations of dicumarol.

Dicumarol (μmol/l)	*ATP content*
10	93.9 ± 3.8 (9)
17.8	94.6 ± 0.2 (3)
31.6	92.8 ± 3.2 (3)
56.2	6.7 ± 1.9 (3)
100	3.9 ± 0.2 (3)
100 (*)	55.6 ± 2.1 (3)

Values are mean ± s.e. mean. Figures in parentheses indicate number of experiments.

than that which, in separate experiments, completely inhibited the dextran-induced release of histamine. Most notably, there was no reduction of ATP content by dicumarol after 30 min incubation at a concentration of 32 μmol/l, while a concentration of 10 μmol/l added concomitantly with the dextran and PS completely inhibited histamine release. Consistent with these findings, dicumarol inhibited equally histamine release from cells preincubated in either glucose-free or glucose-containing Tyrode (Figure 1c). These two pieces of evidence support the conclusion from earlier experiments (Garland & Mongar, 1976) that dicumarol prevents histamine release by a mechanism separate from its effect on energy metabolism.

Papaverine was a more potent inhibitor of histamine release from cells preincubated in glucose-free Tyrode than from cells in complete Tyrode (Figure 2b). Furthermore, direct measurements of the mast cell ATP content show a similarity of action between papaverine (3 μmol/l) and antimycin A (1 μmol/litre). Both compounds reduced the cellular ATP content, the reduction being greater in cells incubated in glucose-free medium. These findings support the hypothesis that papaverine inhibits dextran-induced histamine release from rat mast cells through an effect on oxidative metabolism (Garland & Mongar, 1976) even when the inhibitor is added at the same times as the releaser. In agreement with these observations, Fredholm, Guschin, Elwin, Schwab & Uvnäs (1976) found that inhibition of Compound 48/80-induced histamine release by a high concentration of papaverine (100 μmol/l) was accompanied by depletion of mast cell ATP content.

Inhibition of release by dibutyryl cyclic AMP was significantly greater when cells were preincubated in complete rather than in glucose-free Tyrode solution. This suggests that dibutyryl cyclic AMP requires glucose for its action, possibly associated with its transport into the cell. This hypothesis is consistent with the gradual development of inhibition observed when cells are incubated with dibutyryl cyclic AMP before the addition of the histamine-releasing agent (Stechschulte & Austen, 1973; Garland, 1975). However, further experiments are required to clarify this suggestion.

The technical assistance of Miss Susanne Kristensen, Mrs Annette Rasmussen, Mr J.J. Adcock and Mr H.C. Cox is gratefully acknowledged.

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(Received February 1, 1977.
Revised April 6, 1977.)