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Influence of hyposensitization on ATP level and CO_2 production of mast cells in anaphylaxis

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SUMMARY

Anaphylaxis in a glucose-free medium containing pyruvate caused a release of histamine and a significant decrease in the ATP level of rat mast cells. The fall was maximal after 10 min and it was found to reverse after 22 min. Glucose completely counteracted the ATP fall without changing the anaphylactic histamine release. Furthermore, the oxidative metabolism of exogenous pyruvate to CO_2 was stimulated in the mast cell.

A high level of protection of mast cells to antigen challenge was obtained following hyposensitization and only a small amount of the intracellular histamine was released in contrast to non-hyposensitized cells. Hyposensitization counteracted the ATP fall by antigen challenge but the increase in oxidative metabolism remained unchanged.

The results indicate that hyposensitization exerts effects in the mast cell consistent with a reduced ATP utilization or with a reduced uncoupling of oxidative phosphorylation. The mechanism of the hyposensitization must be due to inhibition of one or more of the cellular steps leading to histamine release and subsequent morphological changes of the cell or to uncoupling of oxidative phosphorylation.

INTRODUCTION

The mechanism of specific hyposensitization therapy in immediate allergic diseases is still unsettled. Measurable changes occurring as a result of hyposensitization point to the involvement of various mechanisms such as development of blocking antibody (Lichtenstein, Norman & Winterwerder, 1968; Ishizaka *et al.*, 1973), reduction in the specific IgE antibody level after an initial increase before start of the pollen season and changes in the basophil cell (Sadan *et al.*, 1969; Lichtenstein *et al.*, 1973; Stahl Skov, Norn & Weeke, 1976). A change in the target cell resulting in a high protection to antigen challenge was found *in vitro* by anaphylactic hyposensitization of isolated rat mast cells by antigen (Norn & Stahl Skov, 1974).

For a long time anaphylactic histamine release and histamine liberation by compound 48/80 have been regarded as an energy-dependent reaction (Diamant, 1975). Evidence for an increased utilization of ATP in the mast cell by compound 48/80-induced histamine release was given by Peterson & Diamant (1974), and a correlation between mast cell ATP content and histamine release was demonstrated by antigen and compound 48/80 (Johansen & Chakravarty, 1972 and 1975), Peterson (1974a, b).

In this study the influence of hyposensitization on ATP level and oxidative metabolism of mast cells in anaphylaxis was investigated.

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MATERIALS AND METHODS

Sensitized rats. Adult female Wistar rats, weight 200–230 g, were sensitized to horse serum, which was complement inactivated by heating to 56°C for 30 min. 1·0 ml of the serum was given by subcutaneous injection mixed with 1·0 ml (10^{10} bacilli) pertussis vaccine as adjuvant. The rats were used 3–6 weeks after sensitization.

Antigen. Horse serum, inactivated as described above.

Cell suspension. Rat mast cells from the peritoneal and pleural cavities were isolated by centrifugation in a Ficoll density gradient according to the method of Thon & Uvnäs (1966). The mast cells, collected from the Ficoll, were washed three times with a salt solution, buffered to pH 7·0 with 10% Sørensen phosphate buffer and containing 0·131 M NaCl, 2·4 mM KCl, 1·0 mM $CaCl_2$, 4·7 mM Na_2HPO_4, 2·0 mM KH_2PO_4, and 1 mg/ml human serum albumin. If not otherwise stated, 1 mM pyruvate (labelled or cold) was used as metabolic substrate.

The mast cells were finally suspended in the above buffered salt solution in a concentration of 100,000–500,000 cells/ml.

Hyposensitization and challenge. The mast cell suspension was kept at 37°C and equilibrated for 15–25 min. The cells were then hyposensitized to antigen by incubation for 1 hr with increasing concentrations of horse serum up to 0·023% by means of an infusion technique which permits a slow and continuous increase of the antigen concentration (Fig. 1). Challenge with antigen was performed by the addition of horse serum with a final concentration of 0·6%.

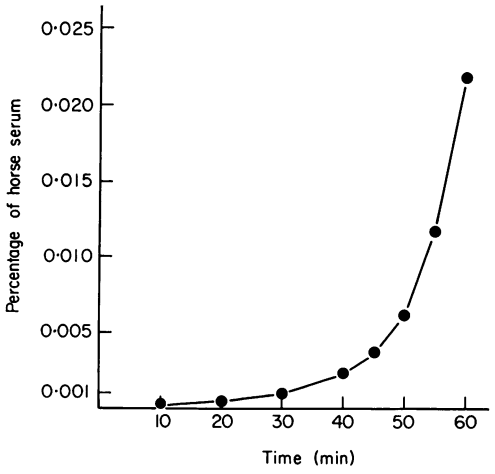

FIG. I

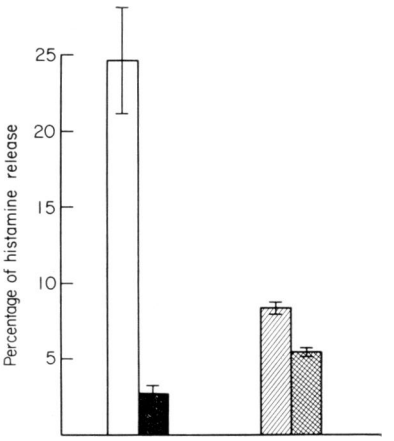

FIG. 2

FIG. 1. Hyposensitization of mast cells by infusion of antigen at 37°C. The antigen (horse serum) concentration in the cell suspension is plotted against the infusion time.

FIG. 2. Histamine release from hyposensitized and non-hyposensitized mast cells. Open column, anaphylaxis; solid column, spontaneous histamine release; hatched column, hyposensitized cells challenged; cross-hatched column, histamine release caused by hyposensitization. Mean ± s.e. mean of six experiments.

Histamine release. Anaphylactic histamine release was determined 15 min after the challenge with antigen in cell suspensions not hyposensitized but infused with buffered salt solution. Concomitantly, the release of histamine was determined in hyposensitized cells, challenged or not challenged with antigen, and in cells neither hyposensitized nor challenged (spontaneous histamine release). The release of histamine was determined in the supernatant and expressed as a percentage of the total histamine content of the sample. The spectrofluorometric method of Shore, Burkhalter & Cohn (1959) was used omitting the extraction procedure according to Bergendorff & Uvnäs (1972). The fluorescence was measured in a Farrand Ratio Fluorometer (Farrand Optical Company Ltd, U.S.A.).

ATP content. The ATP level in mast cells was analysed by the firefly extract bioluminescence method according to Diamant *et al.* (1974).

CO_2 production. $^{14}CO_2$ production from mast cells was measured in a closed tube system with a side-arm containing a filter moistened with NaOH for absorption of $^{14}CO_2$ (Diamant *et al.*, 1974).

RESULTS

Histamine release

Fig. 2 shows the release of histamine in mast cell suspensions from sensitized rats. By anaphylaxis 22% of the total histamine content of the sample was released. When hyposensitized cells were challenged with

antigen, the histamine release was only 3%. The values are corrected for spontaneous histamine release and histamine liberation caused by hyposensitization, respectively. Identical values were obtained in cell suspensions when glucose (5 mM) was substituted for pyruvate as metabolic substrate.

ATP level

The fall in ATP level of mast cells by anaphylaxis in a glucose-free medium and the counteraction of hyposensitization are shown in Fig. 3 (a single experiment) and Table 1 (a survey). Before challenge with antigen some decrease was found in the baseline of mast cell ATP level in hyposensitized as well as in

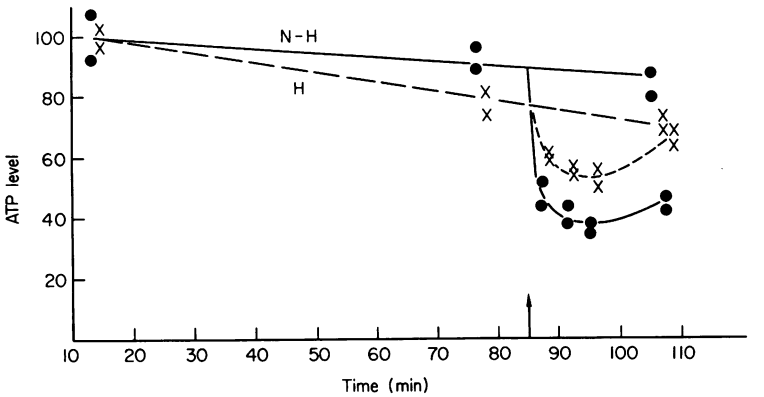

FIG. 3

FIG. 3. Decrease of mast cell ATP level by anaphylaxis and counteraction by hyposensitization. All values are expressed as a percentage of the initial ATP level (ordinate value). The cells were challenged with antigen at the time indicated by the arrow. Hyposensitized (H) and non-hyposensitized (N-H) cells were studied. Duplicate determinations from one experiment are presented.

TABLE 1. Decrease of mast cell ATP level by anaphylaxis and its counteraction by hyposensitization

	Percentage decrease after challenge with antigen			
	2 min	6 min	10 min	22 min
Non-hyposensitized (N-H)	31·8 ±3·9	47·3 ±6·2	52·7 ±6·5*	36·5 ±6·9*
Hyposensitized (H)	13·2 ±3·8	18·3 ±5·1	24·0 ±3·2†	7·7 ±3·8†
Ratio (H: N-H)	0·42	0·39	0·46	0·21

The fall from baseline is given in percentage of the initial ATP level.
Mean of six experiments ± s.d. between H and N-H at all times ($P<0·01$ by paired differences).
* s.d. ($P<0·05$ by paired differences).
† s.d. ($P<0·05$ by paired differences).

non-hyposensitized cells. The average falls from six experiments amounted to 16 and 21%, respectively. The difference is not significant ($P>0·1$ by paired differences), and a similar fall was obtained with cells from non-sensitized rats as well as when glucose was substituted for pyruvate. By anaphylaxis the ATP level was reduced by 32% after 2 min. The fall was maximal after 10 min, i.e. 53%, and it was found to reverse after 22 min. Within the period observed the fall from the baseline was significant ($P<0·01$ by paired differences). No fall from the baseline was obtained by challenge of non-sensitized rats with antigen (horse serum) or when glucose (5 mM) was substituted for pyruvate.

The fall in ATP level observed after antigen challenge was counteracted by previous hyposensitization of the cells to antigen. The fall was reduced by 60–80% and the reduction was significant at all times ($P<0·01$ by paired differences).

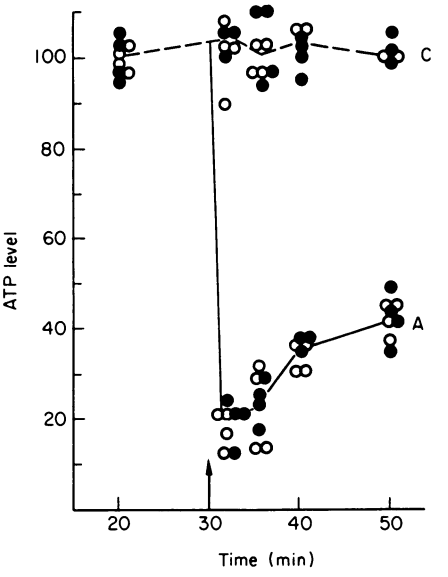

FIG. 4

FIG. 4. No influence of histamine on ATP level in mast cells exposed to anaphylaxis (A) and in cells not challenged with antigen (C). (●) Histamine, 9 μg base/ml cell suspension added at zero time; (○) no histamine added. Challenge time indicated by the arrow. Individual values from two experiments are given.

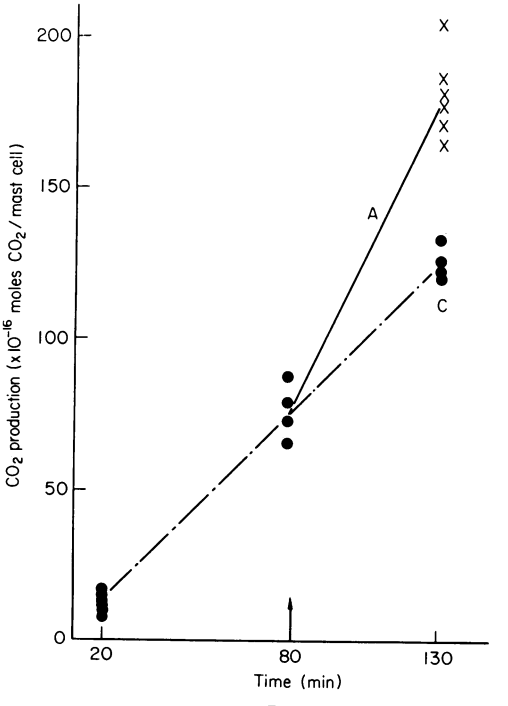

FIG. 5

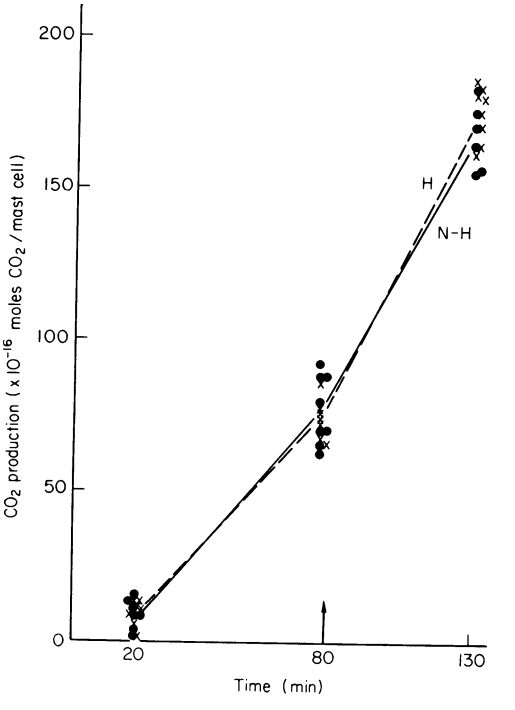

FIG. 6

FIG. 5. CO_2 production from mast cells exposed to anaphylaxis (A) and from cells not challenged with antigen (C). Challenge time indicated by the arrow. Individual values are given.

FIG. 6. CO_2 production from hyposensitized (H) and non-hyposensitized (N-H) mast cells challenged with antigen. Challenge time indicated by the arrow. Individual values are given.

Fig. 4 shows that the addition of histamine does not influence the mast cell ATP level. Thus, no significant difference was obtained in cell suspensions with or without addition of histamine. This was the case by anaphylaxis as well as in controls, i.e. cells not challenged with antigen.

CO_2 production

The CO_2 production from mast cells from sensitized rats is shown in Fig. 5. In cells not challenged with antigen the production of CO_2 increased linearly with time from 20–130 min and amounted to 10^{-16} moles CO_2/mast cell/min. Anaphylaxis increased the CO_2 production by 100 or 56% (Figs 5 and 6). Exactly the same increase was obtained when hyposensitized cells were challenged with antigen (Fig. 6).

DISCUSSION

When non-hyposensitized cells were challenged with antigen in a glucose-free medium containing pyruvate, anaphylaxis caused a significant fall in the mast cell ATP level. The fall was maximal after 10 min and it was found to reverse after 22 min (Table 1, Fig. 3). Glucose completely counteracted the fall in ATP level without changing the anaphylactic histamine release. Furthermore, as judged from the CO_2 production, the oxidative metabolism of exogenous pyruvate was stimulated in the mast cell (Fig. 5).

These effects on ATP level and CO_2 production confirm our earlier findings (Diamant *et al.*, 1974) which were explained by an effect on energy production consistent with an uncoupling of oxidative phosphorylation. This assumption was based mainly on the findings that 2,4-dinitrophenol mimics the effects of antigen on the ATP level as well as on the CO_2 production and glucose counteracts the fall in ATP level. Further, it was found that the histamine liberation by compound 48/80 in a glucose-free medium was not accompanied by a change in the mast cell ATP content (Diamant, 1967; Peterson & Diamant, 1974). Compound 48/80 was not expected to differ from antigen, on the contrary, one would expect the same utilization of ATP since both histamine release processes are considered to involve energy and since the morphological changes in the cell seem to be the same (Bloom & Chakravarty, 1970; Anderson, Slorach & Uvnäs, 1973). As it has been shown that histamine liberation by compound 48/80 was accompanied by an increased utilization of ATP which was supposed to be balanced by an increased ATP production (Peterson & Diamant, 1974; Diamant, 1975), the fall in ATP level by the antigen–antibody reaction was considered to be the consequence of an uncoupling effect on oxidative phosphorylation. The uncoupling would be expected to be a side reaction or be secondary to the histamine release.

Later, however, Johansen & Chakravarty (1975) were able to demonstrate a decrease in ATP level both by compound 48/80 and also by antigen. Therefore, an alternative hypothesis for the changes in ATP level and CO_2 production during anaphylaxis seems to be possible: anaphylaxis increased the utilization of ATP in mast cells. By exceeding the capacity for ATP production derived from oxidative metabolism the level of ATP decreases in a glucose-free medium. Exogenous glucose prevents the fall in ATP content by supplying ATP by glycolysis, the glycolysis being stimulated by the high ADP/ATP ratio (Lehninger, 1971).

The fall in ATP level induced by anaphylaxis was already significant during the phase of histamine release (Diamant *et al.*, 1974) and it continued to decrease after the release of histamine (Table 1, Fig. 3). Therefore, a direct utilization of ATP in the release process as well as in the repairing processes after termination of the histamine release seems likely. The fall in mast cell ATP level during anaphylaxis was not influenced by histamine, since no change was obtained by extracellular histamine in a concentration corresponding to that present in the cell suspension. Neither did histamine change the basal ATP level (Fig. 4).

Anaphylactic hyposensitization of isolated rat mast cells *in vitro* was demonstrated by Norn & Stahl Skov (1974) by pre-incubation of the cells with antigen in suboptimal concentrations. In the present study a more effective method for hyposensitization has been developed by means of an infusion technique which permits a slow and continuous increase of the antigen concentration causing a very low release of histamine during hyposensitization and a high level of protection of the cells against antigen

challenge (Fig. 2). When these cells were challenged with antigen, only a small amount of the intracellular histamine content was released in contrast to non-hyposensitized cells. The reduced release of histamine induced by hyposensitization cannot be explained by a depression of the cell function based on a reduced energy level, since the ATP level in hyposensitized cells is identical with that of non-hyposensitized cells. However, some decrease of the baseline ATP level was obtained before challenge with antigen (Fig. 3), but the decrease did not differ from that of non-hyposensitized cells or from hyposensitized cells of non-sensitized rats, and it was not counteracted by glucose (i.e. no starvation of the cells). It might, therefore, be explained by some mechanical damage of the cells resulting in a leakage of ATP which is then hydrolysed by the mast cell ecto-ATPase, the existence of which was demonstrated by Diamant (1969).

By challenge of hyposensitized cells with antigen in a glucose-free medium the fall in ATP level was significantly lower than that of non-hyposensitized cells (Fig. 3 and Table 1). Hyposensitization thus counteracts the ATP fall by antigen challenge. The increase in oxidative metabolism induced by anaphylaxis was not changed by previous hyposensitization of the cell (Fig. 6). The findings of an unchanged stimulation of oxidative metabolism and of a reduced fall in ATP level associated with hyposensitization fit with the picture of a mild anaphylaxis in which challenge was performed with a small antigen concentration instead of the usual shock dose (Diamant *et al.*, 1974).

The results indicate that hyposensitization exerts effects in the mast cell consistent with a reduced uncoupling of oxidative phosphorylation or with a reduced ATP utilization in the repairing processes after termination of histamine release. A possible counteracting effect on ATP utilization within the histamine releasing process itself will demand investigation at an early stage of the active release process, but unfortunately, this presents great technical difficulties.

The mechanism of hyposensitization must be due to inhibition of one or more of the cellular steps leading to histamine release and subsequent morphological changes of the cell or to uncoupling of oxidative phosphorylation. A reduced antigen-antibody reaction might be the result of a progressive binding of cell-fixed antibody with antigen during hyposensitization. Other possible steps for inhibition are in the sequence of events involved in the intracellular signal.

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