Volume 268, number 1, 103–106 FEBS 08645 July 1990

Estimation of adenosine triphosphate utilization of rat mast cells during and after anaphylactic histamine secretion

Torben Johansen

Institute of Medical Biology, Department of Pharmacology, The Medical School, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

Received 14 May 1990

Determination of the cellular content of adenosine triphosphate (ATP) and the rate of ATP-synthesis were used to estimate the cellular utilization of ATP in relation to anaphylactic histamine secretion. There was an increased rate of oxidative ATP-synthesis and a decreased cellular ATP content during the time period of histamine secretion and immediately after its completion. During secretion the additional ATP-utilization above the basal level of ATP-synthesis was 0.51 pmol/10^3 cells. 2.5 min after cell activation, the rate of additional ATP-utilization was 0.30 pmol/10^3 cells/min, and the persistent ATP-decrease observed after 30 and 120 min may be due to a decreased rate of oxidative ATP-synthesis.

Mast cell; Adenosine triphosphate; Anaphylaxis; Histamine secretion; Exocytosis

1. INTRODUCTION

Anaphylactic histamine secretion of rat peritoneal mast cells is an energy-dependent mechanism (for review, see [1,2]). During the secretory process the cellular content of ATP decreased [3,4] and aerobic glucose oxidation was stimulated [5]. This may indicate an increased utilization of cellular ATP during the time period of histamine secretion, and it seems necessary that ATP is synthesized as the anaphylactic histamine secretion takes place [6]. After completion of the secretion a further decrease of the cellular ATP content was found 2.5 min after activation of the secretory mechanism. There was no recovery of the decreased level of ATP after prolonged incubation of the cells up to 2 h [3,4].

The aim of this study was to estimate the amount of ATP used for anaphylactic histamine secretion and to examine if the longterm decrease of the ATP content of the mast cells occurring after completion of the secretion was associated with changes in the cellular metabolism of ATP.

2. MATERIALS AND METHODS

2.1. *Sensitization of rats. Isolation of mast cells*

Male wistar rats, 290–450 g, were used for the experiments. The rats were sensitized to egg albumin, and the peritoneal mast cells were isolated as described earlier [3]. Mast cells: 98.3% of the cell population (range 96.5–99.8%, $n = 22$).

Correspondence address: T. Johansen, Institute of Medical Biology, Department of Pharmacology, The Medical School, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

2.2. *Incubation procedure*

Mast cell suspensions from 2–5 rats were pooled and divided into aliquots with the same density in a final volume of 0.5 ml. These were used for the determination of the ATP content of the cells and for the histamine secretion experiments. The cell density was mean 9.8×10^4 cells/ml (range $5.2–23.0 \times 10^4$ cells/ml).

The cell suspensions in Krebs-Ringer solution with phosphatidyl-L-serine, 50 μg/ml, were equilibrated in a 37°C bath for 10 min. Then 2-deoxyglucose (2-DG), 5 mM, was added and antigen (egg albumin 0.2 mg/ml) was added 20–30 min later. The cells were incubated with antigen for 15 s, 30 s, 2.5 min, 30 min and 120 min. A combination of antimycin A (1 μM) and oligomycin (1 μg/ml) was then added to the samples used for determination of the cellular ATP content. Samples with the inhibitors but without antigen were run in parallel. This was also the case concerning samples with 2-DG alone, with or without antigen, as well as with samples without inhibitors and without antigen.

For the histamine secretion experiments the cells were temperature-equilibrated and preincubated with 2-DG as described above. Then antigen was added and 15 s later antimycin A and oligomycin were added to the cell suspension. The incubation continued for 60 s giving 75 s total exposure of the cells to antigen (related to the ATP determinations in Figs. 1 and 2: 15 s). The samples for determination of histamine secretion related to the remaining ATP measurements were preincubated with 2-DG as above and incubated with antigen for 5 min (related to the ATP determinations in Figs. 1 and 2: 2.5 min), 30 min and 120 min but without the addition of antimycin A and oligomycin. The experiments were performed as described previously [4]. Samples without antigen for determination of the spontaneous histamine secretion were included, which was not influenced by the metabolic inhibitors. Control samples containing non-sensitized mast cells were incubated as above for determination of the cellular ATP content and for secretion experiments.

2.3. *Determination of histamine secretion and the cellular ATP content*

Histamine was determined by the fluorometric method [7], omitting the extraction procedure. Egg albumin, antimycin A, oligomycin and 2-DG did not interfere with the determination of histamine. The secretion of histamine was calculated as a percentage of the total

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

histamine content of the mast cells. For the determination of ATP the reaction after incubation of the samples was stopped with ice-chilled perchloric acid (333 mM). After neutralization of the supernatant the ATP content was determined by the bioluminescence technique using luciferin- luciferase from firefly tails as described earlier [4]. Internal standards were used for correction of the inhibition of the reaction by potassium perchlorate. None of the reagents interfere with the ATP determination.

2.4. *Materials*

Human serum albumin was supplied by AB Kabi (Stockholm, Sweden), egg albumin by Mallingckrodt Chemical Works (St. Louis, USA), antimycin A, oligomycin, 2-deoxy-D-glucose, phosphatidyl-L-serine by Sigma Chemical Co. (St. Louis, U.S.A.). All other chemicals were of analytical grade. The Krebs-Ringer solution contained (in mM) NaCl 139.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, Na_2HPO_4 2.5, KH_2PO_4 0.6, pH 7.0–7.1

3. RESULTS

Addition of antimycin A and oligomycin to the 2-DG-treated cells caused a rapid depletion of the mast cell ATP content (Fig. 1). Apparently, the decay-curve demonstrated a linear relation between the time of incubation of the cells with the inhibitors and the log value of the cellular ATP content. The correlation coefficient was 0.9888 and 0.0149 (mean and SD, $n = 19$, range: 0.9229–0.9995, $P < 0.05$ or lower). The time period for 50% decrease of the ATP content ($t_{1/2}$) was 45 s (mean value) in the absence of antigen (Table I). In

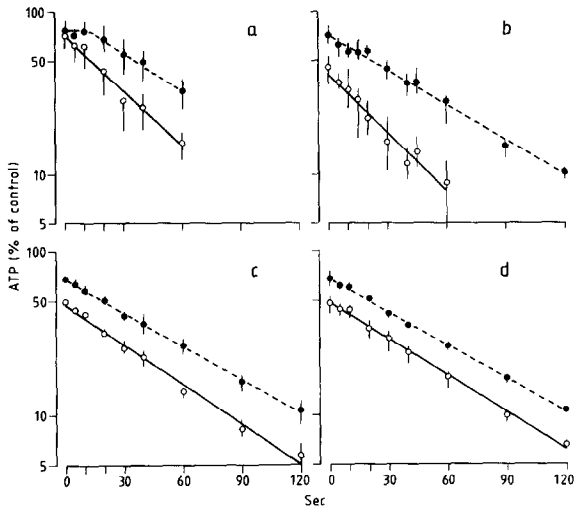

Fig. 1. Rate of ATP depletion. The cells were preincubated with 2-DG and incubated with (○) or without (●) antigen for 15 s (a), 2.5 min (b), 30 min (c) and 120 min (d). Then the respiratory inhibitors were added for 5 to 60–120 s (abscissa scale). Ordinate scale: the cellular content of ATP (log scale). 100 on the ordinate represent the control value from mast cells incubated without inhibitors and without antigen: 1.43 and 0.04 pmol/10^3 cells (mean and SE, $n = 19$). Histamine secretion: 40.2% (a), 46.3% (b), 36.1% (c), and 35.4% (d) (mean values, $n = 5$ (15 s) and 3). Spontaneous histamine secretion deducted (8.1% and 0.9%, mean and SE, $n = 19$). There was a tendency to an increase in the spontaneous histamine secretion after 30 min and 120 min incubation. Mean value from 5 (15 s), 3–8 (2.5 min) and 3 experiments. Vertical lines show range.

Table I

Rate of cellular ATP depletion during and after histamine secretion

Incubation	ATP depletion, $t_{1/2}$ (s)	
	− Ag	+ Ag
15 s	46	26
2.5 min	45	25
30 min	44	37
120 min	44	40

Mean values

presence of antigen there was an initial decrease of $t_{1/2}$ followed by a gradual increase towards the control value.

2-DG caused a decrease of the cellular ATP content to 72% (mean value) of the value found in untreated cells (Fig. 2). This level was very stable up to 120 min incubation (the longest period of observation). Upon the addition of antigen to the 2-DG-treated cells the ATP content decreased by (in pmol/10^3 cells) 0.07 ($P < 0.01$), 0.14 ($P < 0.001$), and 0.38 after incubation for 15 s, 30 s, and 2.5 min, respectively, and then there was no change in the ATP level up to 120 min incubation of the cells with antigen (Fig. 2, *t*-test for paired data). It may be observed that during the time interval from 30 sec to 2.5 min incubation of the cells with antigen, the ATP level was decreased by 0.24 pmol/10^3 cells, i.e. 0.12 pmol/10^3 cells/min.

The ATP content of non-sensitized rat mast cells was changed by the metabolic inhibitors as described above for the sensitized cells. However, addition of antigen the suspensions of non-sensitized cells had no effect on the ATP content of the cells and no histamine secretion occurred above the value of the spontaneous release of histamine (data not shown).

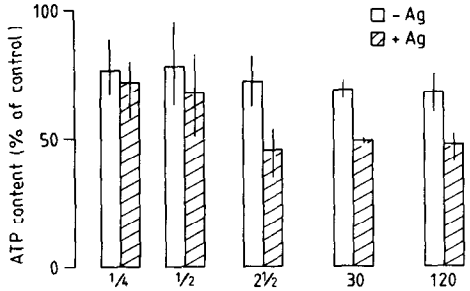

Fig. 2. ATP content of the mast cells. After preincubation with 2-DG the cells were incubated for 15 s to 120 min with (□) or without antigen (▨). Abscissa scale: time of incubation (min). Ordinate scale: ATP content of the cells. 100 on the ordinate represent control value from mast cells incubated without 2-DG and without antigen: 1.43 and 0.04 pmol/10^3 cells (mean and SE, $n = 20$). Histamine secretion: see legend to Fig. 1. Mean value from 5 (15 s), 9 (30 s), 8 (2.5 min) and 3 experiments. Vertical lines show range.

4. DISCUSSION

Preincubation of the cells with 2-DG made them dependent on the oxidative phosphorylation for the synthesis of ATP [8]. High concentrations of respiratory inhibitors [8] were used to inhibit the oxidative ATP-synthesis. It is likely, therefore, that almost no ATP was produced by mast cells incubated with both glycolytic and respiratory inhibitors. The ATP-decay curves obtained by these inhibitors are consistent with a pseudo-first order reaction because of the linear relation between time and the log value of the cellular ATP content (Fig. 1). The rate of the oxidative ATP-synthesis in the 2-DG-treated cells was, therefore, calculated by use of the following equations:

$$\ln C_t = \ln C_o - k \times t \quad (1)$$

$$k = \ln 2 \times (t_{1/2})^{-1} \times s^{-1} \quad (2)$$

C_o is the cellular ATP content at zero time, i.e. the steady state level of the 2-DG-treated cells, and C_t is the ATP content t seconds later. Ideally a value of t approximating zero should be used in the calculation. In this study $t = 1$ second was chosen for the calculations.

The rate of oxidative ATP-synthesis was the same after short and long time of incubation of the cells with 2-DG (Table II), the mean value was 0.96 pmol/10^3 cells/min. After exposure of the cells to antigen for 15 s and 2.5 min the rate of ATP-synthesis was significantly increased by 58% and 20%, respectively. Anaphylactic histamine secretion is completed in 40 s [4]. Estimation of the amount of ATP-utilization due to the secretory process was performed by adding the ATP-decrease observed after 30 sec incubation of the cells with antigen (Fig. 2) and the amount of ATP synthesized in 40 s (corrected for the basal rate of ATP-synthesis) (Table III). Taken together this value is 0.51 pmol/103 cells/40 s, and the value for the basal level of ATP-utilization is the same as the basal ATP-synthesis, 0.64 pmol/10^3 cells/40 s. Thus, the cellular ATP-utilization during antigen-antibody induced histamine secretion is increased by 80%. The amount of ATP-utilization due to the secretion induced by A23187 and compound 48/80 was 0.14 and 0.15 pmol/10^3 cells, respectively [9,10]. Peterson and Diamant [11] reported a decrease in mast cell ATP content of 0.52 mmol/kg dry weight/25 s after initiation of the secretory process by compound 48/80, and this was equivalent to 0.26 pmol ATP/10^3 cells [12]. This value is similar to although somewhat higher than the value reported by Johansen [10]. This may be due to the fact that the cellular ATP content was determined after the secretory process was completed, and it has been shown that the ATP content of the mast cells decreases not only during compound 48/80-induced secretion but also for some time after completion of the histamine secretion [9]. Estimation of the ATP-utilization 2.5 min after addition of antigen for the cell activation show an additional ATP-utilization of 0.30 pmol/10^3 cells/60 s above the basal value. The large increase in cellular utilization of ATP during the secretion of histamine (80%) is changed to a value of 33% after completion of the secretion. Chakravarty [13] reported a 30% increase in the oxygen uptake of rat mast cells after anaphylactic histamine secretion. This value was observed 5 min after cell activation and it lasted for 15–20 min.

The persistent decrease of the cellular ATP content after completion of the secretion confirm previous observations with mast cells incubated both without and with metabolic inhibitors [3,4]. The estimated decrease in the rate of oxidation ATP-synthesis in the mast cells after 30 and 120 min incubation with antigen (Table II) may explain the persistent ATP-decrease.

It has been proposed that following anaphylactic histamine secretion there is an uncoupling of the oxidative phosphorylation in rat mast cells [14]. However, this is not a likely explanation of the persistent ATP-decrease in the present experiments. The ATP content of the mast cells was very stable after 2.5–120 min incubation of the cells with antigen. In order to maintain this steady state level of cellular ATP synthesis of ATP is required to replace the basal level of cellular ATP-utilization.

Since the cells were incubated in a substrate-free medium and because of the increased rate of ATP-synthesis during and immediately after the secretion,

Table II

Rate of cellular ATP-synthesis during and after anaphylactic histamine secretion

Incubation (min)	ATP-synthesis (pmol/10^3 cells/min)		Ratio
	− Ag (a)	+ Ag (b)	(b/a) × 100%
0.25	0.96	1.52*	158%
2.5	0.92	1.10*	120%
30	0.98	0.86	88%
120	0.98	0.76*	78%

Mean values, 5 (0.25 min), 8 (2.5 min) and 3 experiments
*$P < 0.025$ by t-test for two groups of data

Table III

Calculation of the amount of additional ATP-utilization of the mast cells due to the anaphylactic histamine secretion

Incubation (min)	ATP-synthesis (pmol/10^3 cells)		Decrease of ATP (pmol/10^3 cells)	Total amount of ATP used for secretion (pmol/10^3 cells)
	− Ag	+ Ag		
for 40 s:				
0.25	0.64	1.01	0.14	0.37 + 0.14 = 0.51
for 60 s:				
2.5	0.92	1.10	0.12	0.18 + 0.12 = 0.30

there may have been an insufficient supply of endogenous substrate for the oxidative ATP-synthesis, and this could explain the persistent ATP-decrease at 30 min and 120 min. However, lack of substrate did not explain the previous observations of persistent ATP-decreases in similar experiments performed in presence of exogenous glucose and with or without the use of respiratory inhibitors [3,4].

In conclusion, there was a large additional utilization of ATP above the basal level of cellular ATP-utilization during anaphylactic histamine secretion from actively sensitized rat mast cells. This was not related to the reaction between antigen and the cell-bound antibody (4). Rather it may be related to energy-requiring reactions leading to exocytosis, since a decreased secretory response was observed if the cellular ATP-synthesis was inhibited by the addition of metabolic inhibitors during the release process but after activation of the cells by the antigen-antibody reaction [6].

Acknowledgements: Thanks are due to Mrs Annette Kragh Rasmussen and Mrs Susanne Nielsen for their excellent technical assistance. This work was supported by The Danish Medical Research Council (512-15082), Klestrup's Foundation, Novo's Foundation, F.L. Smidth's Foundation and Lily B. Lund's Foundation.

REFERENCES

[1] Chakravarty, N. (1977) in: Handbook of Experimental Pharmacology (Rocha e Silva, M. ed) vol. XVIII, pp. 93–108, Springer-Verlag, Berlin.
[2] Johansen, T. (1987) Pharmacol. Toxicol. 61 Suppl. II, 1–20.
[3] Johansen, T. (1979) Eur. J. Pharmacol. 58, 107–115.
[4] Johansen, T. and Chakravarty, N. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 288, 243–260.
[5] Svendstrup, F. and Chakravarty, N. (1977) Exp Cell Res. 106, 223–231.
[6] Johansen, T. (1981) Eur. J. Pharmacol. 72, 281–286.
[7] Shore, P.A., Burkhalter, A. and Cohn, V.H. (1959) J. Pharmacol. Exp. Ther., 127, 182–186.
[8] Johansen, T. (1979) Br. J. Pharmacol. 65, 103–109.
[9] Johansen, T. (1980) Life Sci. 26, 61–69.
[10] Johansen, T. (1983) Acta Pharmacol. Toxicol. 53, 245–249.
[11] Peterson, C. and Diamant, B. (1974) Acta Pharmacol. Toxicol. 34, 337–346.
[12] Diamant, B. and Lowry, O.H. (1966) J. Histochem. Cytochem. 14, 519–524.
[13] Chakravarty, N. (1968) Exp. Cell Res. 49, 160–168.
[14] Diamant, B., Norn, S., Felding, S., Olsen, N., Ziebell, A. and Nissen, J. (1974) Int. Arch. Allergy Appl. Immunol., 47, 894–908.