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INCREASED Na^+/K^+-PUMP ACTIVITY AND ADENOSINE TRIPHOSPHATE UTILIZATION AFTER COMPOUND 48/80-INDUCED HISTAMINE SECRETION FROM RAT MAST CELLS

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Abstract—The Na^+/K^+-pump activity and the utilization of adenosine triphosphate (ATP) were studied in rat peritoneal mast cells after histamine secretion induced by compound 48/80. We measured the ouabain-sensitive K^+-uptake by a radioactive technique ($^{86}Rb^+$). The ATP content and the glycolytic ATP-production were measured by the bioluminescence technique (firefly lantern) and by measurement of the lactate production under anaerobic conditions (antimycin A, oligomycin), respectively. There was an increased requirement for ATP after the secretory response associated with an increased activity of the Na^+/K^+-pump. The anaerobic, but not the aerobic, pathway for ATP-synthesis was able to respond to the increased ATP-requirement. The ATP-requirement of the Na^+/K^+-pump was only partly satisfied when ATP was supplied from either the glycolytic or the oxidative pathway. This may indicate that the availability of ATP was the limiting factor for the activity of the Na^+/K^+-pump following histamine secretion under these conditions. It is concluded that the large increase in Na^+/K^+-pump activity after a secretory response is a likely explanation for the long lasting ATP-decrease in mast cells that follows histamine secretion.

Key words: Na^+/K^+ ATPase; adenosine triphosphate; mast cells; secretion

Histamine secretion induced by compound 48/80 is considered to be an energy-dependent mechanism (for review, see [1, 2]). During and after the secretory process, the cellular ATP content is decreased and this may indicate an increased utilization of ATP in relation to the secretory response. Recovery of the decreased ATP level was not observed within 2 hr of incubation [3]. Recently, we have observed a large increase in the activity of the Na^+/K^+-pump immediately after histamine secretion induced by compound 48/80 and the antigen-antibody reaction. The Na^+/K^+-pump activity gradually decreased and attained the level of unstimulated cells in 2 hr [4].

The aim of this investigation was to estimate the amount of ATP-utilization in relation to the secretory process and to examine if the persistent decrease of the cellular ATP was associated with the change in the pump activity.

METHODS AND MATERIALS

Isolation of rat peritoneal mast cells. Male Sprague–Dawley rats, 240–510 g, were used for the experiments. The rats were killed by decapitation under light ether anaesthesia or after asphyxiation in CO_2. Pure populations of mast cells were isolated from mixed peritoneal cell suspensions by density gradient centrifugation [5, 6]. The number of cells was measured by an automatic cell counter. The purity of the suspensions was determined by inspection in a light microscope of stained (toluidine blue) smears (counting 400 cells). The fraction of mast cells was 96.8 ± 0.004% (mean ± SEM, N = 39).

Incubation procedure. Mast cell suspensions from one to seven rats were pooled and divided into aliquots with the same cell density in a final volume of 0.5 mL. These were used for the determination of the ATP content of the cells (58.2–146.3 × 10^3 cells/mL), for the histamine secretion experiments (55.5–238.0 × 10^3 cells/mL), for the production of lactate (147.2–535.9 × 10^3 cells/mL) and for measurements of the activity of the Na^+/K^+-pump (the ouabain-sensitive K^+-uptake) (230.0–474.0 × 10^3 cells/mL). The procedures for pre-incubation and incubation of the cells were performed at 25° in order to decrease the rate of histamine secretion. After temperature equilibration, the cells were preincubated with 5 mM 2-DG† for 20–25 min (Figs 1–4). The cells in Fig. 1 were then preincubated with respiratory inhibitors (1 μM antimycin A and 1 μg/mL oligomycin) for 20 sec. The cells in Fig. 5 were preincubated for 20 min with 5 mM glucose and the respiratory inhibitors. The cells in Fig. 6 were preincubated for 20 min with either 1 μM antimycin A and 5 mM glucose, or 5 mM 2-DG. Control samples without the inhibitors were suspended in a medium containing 5 mM glucose. (For details of the incubation procedures, see legends to figures.)

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† Abbreviations: BSS, buffered salt solution; 2-DG, 2-deoxy-D-glucose.

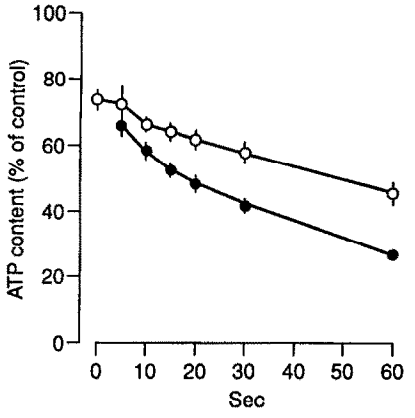

Fig. 1. Effect of compound 48/80 on the ATP content of mast cells pretreated with both glycolytic and respiratory inhibitors. Abscissa scale: time of incubation of the cells without (○) or with (●) compound 48/80 (sec). Ordinate scale: the cellular content of ATP as a percentage of control value from cells incubated without the metabolic inhibitors: 1.70 ± 0.11 pmol/10^3 cells (mean value and SEM, N = 5). Histamine secretion was 50.3 ± 2.9%, and the spontaneous secretion was 3.6 ± 0.5% (mean value and SEM, N = 5). Mean values from five experiments are shown; vertical lines show SEM.

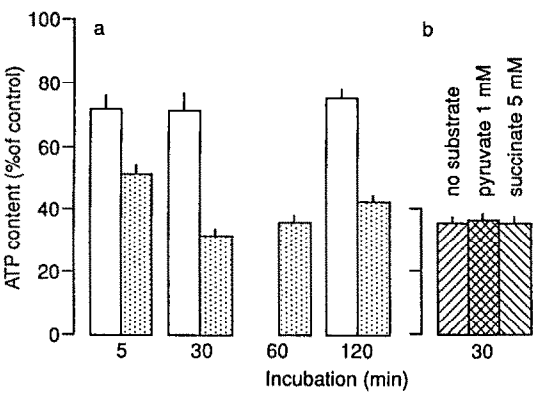

Fig. 3. ATP content of the mast cells. (a) After a preincubation period with 2-DG, the cells were incubated for 5–120 min without (open columns) or with 1 μg/mL compound 48/80 (dotted columns). (b) In control experiments with respiratory substrates. The cell suspensions contained either 1 mM pyruvate or 5 mM succinate and the cells were preincubated with 5 mM 2-DG for 20 min and then incubated with compound 48/80 for 30 min. Abscissa scales: time of incubation with compound 48/80 (min). Ordinate scales: ATP content of the mast cells. 100 on the ordinates represents control values from cells incubated without 2-DG and substrates and without compound 48/80: 1.64 ± 0.05 pmol/10^3 cells (N = 15; Fig. 3a) and 1.46 ± 0.10 pmol/10^3 cells (N = 4; Fig. 3b) (mean values and SEM). Mean values from seven (5 min) and three experiments (Fig. 3a) and mean values from four experiments (Fig. 3b) are shown; vertical lines show SEM.

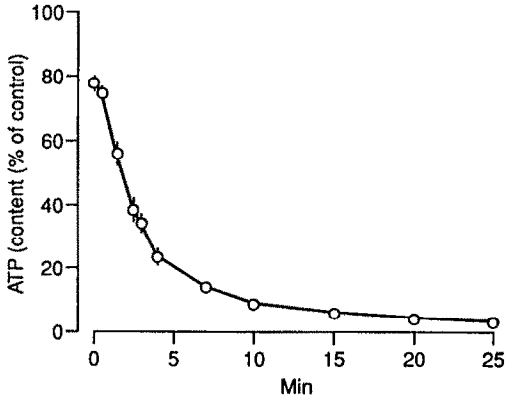

Fig. 2. Time course of ATP decrease of 2-DG-treated mast cells caused by incubation with respiratory inhibitors (1 μM antimycin A, 1 μg/mL oligomycin). After preincubation with 2-DG the cells were incubated with the respiratory inhibitors (abscissa). Ordinate scale: the cellular content of ATP as a percentage of control value from cells incubated without the metabolic inhibitors: 1.70 ± 0.06 pmol/10^3 cells (mean ± SEM, N = 5). Mean values from five experiments are shown; vertical lines show SEM.

Determination of histamine secretion, ATP content, lactate production and Na^+/K^+*-pump activity.* Histamine was determined by the fluorometric method [7] and the ATP content of the cells by the bioluminescence technique using luciferin-luciferase from firefly tails [8]. The accumulation of lactate was measured according to the method described by Lowry and Passonneau [9] (for details, see [5]). The activity of the Na^+/K^+-pump was measured as the ouabain-sensitive K^+-uptake [6]. The cellular uptake of K^+ was calculated on the basis of the uptake of the radioactive tracer, $^{86}Rb^+$, which was used as a K^+-analogue. The radioactive concentration was kept constant at 56 MBq/L. The specific activity was 11.8 MBq/mmol K^+. The Rb^+ concentration was 137 ± 8 μM (mean and SEM, N = 4). The radioactive concentration of $^{86}Rb^+$ in relation to the sum of the concentrations of K^+ and Rb^+ in the extracellular medium was used to calculate the cellular K^+ ($^{86}Rb^+$)-uptake. Samples containing BSS and no cells were run in parallel in order to determine the radioactivity not associated with the cells, and this was subtracted before the calculation of the cell specific K^+($^{86}Rb^+$)-uptake.

Materials. Human serum albumin was supplied by AB Kabi (Stockholm, Sweden), antimycin A, oligomycin, 2-DG, bovine serum albumin, histamine, compound 48/80, firefly lanterns, L-lactic acid, glutamic-pyruvic transaminase (EC 2.6.1.1.), 2-amino-2-methyl-1-propanol, and glutamate monosodium by Sigma Chemical Company (St Louis, MO, U.S.A.), lactate dehydrogenase (EC 1.1.1.27) and NAD^+ by Boehringer-Mannheim GmbH (Mannheim, Germany), Percoll by Pharmacia Fine Chemicals (Sweden), scintillation liquid (Ecoscint) by BN plastics (Helsinge, Denmark), $^{86}Rb^+$ (specific activity 1–12 mCi/mg Rb^+) by Amersham (Amersham, U.K.), ouabain and diethylether by Merck (Germany), D-glucose monohydrate by Fluka

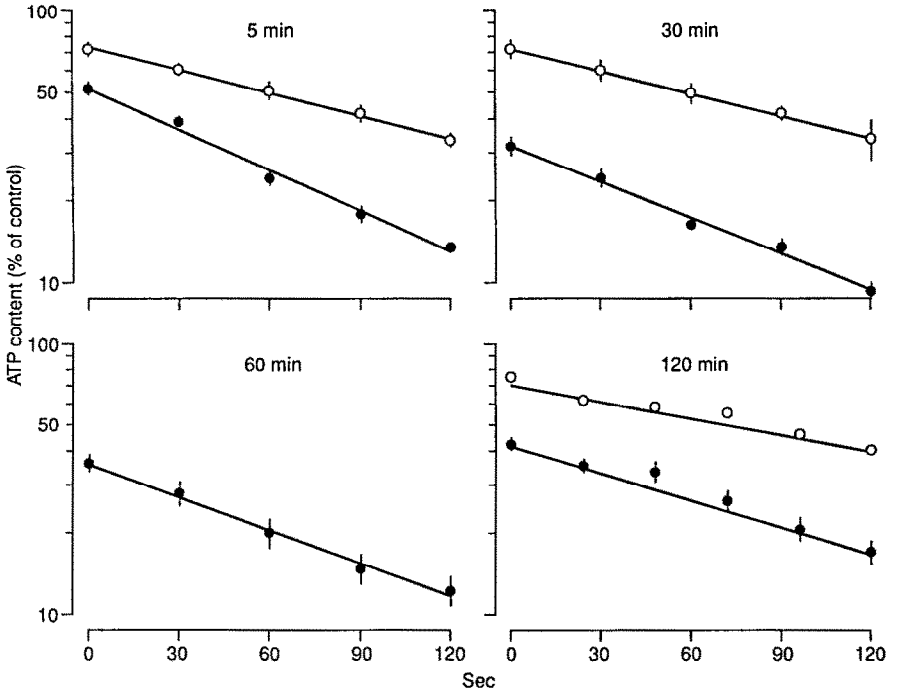

Fig. 4. Rate of ATP depletion. After preincubation with 2-DG the cells were incubated with (●) or without (○) 1 μg/mL compound 48/80 for 5, 30, 60 and 120 min. Then respiratory inhibitors 1 μM antimycin A and 1 μg/mL oligomycin were added for 20–120 sec (abscissa scale). Ordinate scale: the cellular content of ATP (log scale). 100 on the ordinate represents the control value from mast cells incubated without inhibitors and without compound 48/80: 1.64 ± 0.05 pmol/10^3 cells. Histamine secretion from 2-DG-treated cells was 54.8 ± 1.6% after correction for the spontaneous secretion: 2.4 ± 0.2%, (mean values and SEM, N = 13). Mean values from seven (5 min) and three experiments are shown; vertical lines show SEM.

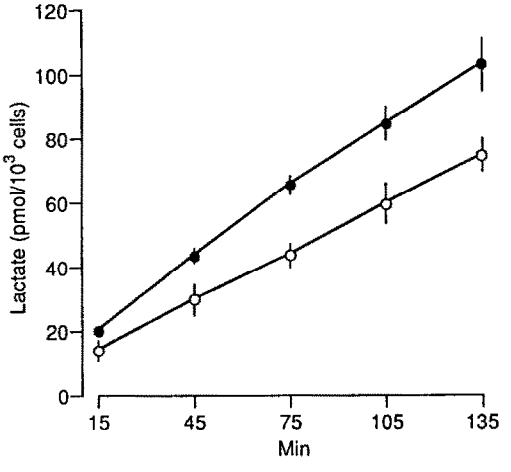

Fig. 5. Rate of lactate production. After preincubation with respiratory inhibitors and glucose, the cells were incubated without (○) or with (●) 1 μg/mL compound 48/80 for 15–135 min (abscissa scale). Ordinate scale: lactate production. Histamine secretion was 68.5 ± 7.2% after correction for the spontaneous secretion, 3.1 ± 1.1% (mean values and SEM). Mean value from four experiments; vertical lines show SEM.

(Switzerland), Diluid "azide free" from J.T. Baker Chemicals (Holland), Heparine from SAD (Denmark). All other chemicals were of analytical grade.

A stock solution of 5 mM antimycin A was made in ethanol 96% (v/v). Antimycin A was diluted in BSS to a final concentration of 1 μM. The final concentration of ethanol in the cell suspension was 0.02%, and this does not influence the ATP content of the cells neither the secretion of histamine nor the Na^+/K^+-pump activity.

The BSS 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, serum albumin 1 mg/mL, pH 7.1–7.2 (room temperature).

Presentation of data. Statistical analysis was performed by use of Student's *t*-test. $P < 0.05$ was considered statistically significant. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

The secretion of histamine was completed after 15 sec incubation of the cells with compound 48/80, and then the secretion was 48.8 ± 5.1%. The spontaneous secretion in the absence of compound 48/80 was 4.7 ± 1.2%. Histamine secretion from samples incubated without metabolic inhibitors was

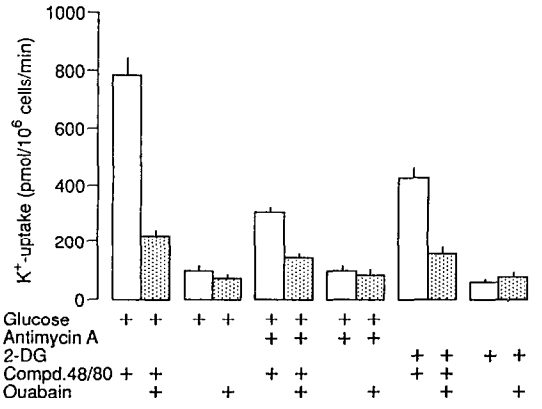

Fig. 6. Na^+/K^+-pump activity of the mast cells measured 30 min after histamine secretion. The cells were preincubated for 20 min at 25° with either 1 μM antimycin A or 5 mM 2-DG. In the presence of antimycin A, the glycolysis of the cells was supported by adding 5 mM glucose to the medium. Control samples without the inhibitors were suspended in a medium containing 5 mM glucose. After the preincubation period, the cells were stimulated with 1 μg/mL compound 48/80 and 30 min later the cellular uptake of K^+ was measured by incubation of the cells for 10 min with the radioactive tracer $^{86}Rb^+$. The ouabain-sensitive K^+ uptake was calculated from data for the cellular K^+ uptake that occurred in the absence and presence of 1 mM ouabain. Ouabain was added to the cell suspensions before the cells were preincubated. Histamine secretion and the spontaneous release in the absence of ouabain were 65.9 ± 3.7% and 2.7 ± 0.3% (glucose alone), 62.4 ± 4.0% and 2.1 ± 0.6% (antimycin A and glucose), 59.3 ± 4.4% and 2.4 ± 0.4% (2-DG alone), respectively (mean and SEM, N = 4). Mean values from four experiments are shown; vertical lines show SEM.

52.3 ± 3.0%, and the spontaneous secretion was 4.5 ± 0.9% (mean ± SEM, N = 4). In the absence of compound 48/80 there was a time-dependent decrease of the cellular ATP content. The rate of the ATP decrease was enhanced when compound 48/80 was added to the cell suspension (Fig. 1), and there was a significant difference between the ATP content of the cells after incubation for 10 sec or more in presence and absence of compound 48/80 ($P < 0.02$ by Student's t-test for paired data, degree of freedom 4). When the secretory process was completed, the ATP decrease due to compound 48/80 was equivalent to 12% of the control value (Fig. 1), i.e. a decrease of 0.20 pmol ATP/10^3 cells in 15 sec. Figure 2 shows that by addition of respiratory inhibitors (1 μM antimycin A and 1 μg/mL oligomycin) to 2-DG-treated mast cells, the ATP content decreased to a value of 9% or less of the control value from untreated cells after incubation for 10 min or more with the inhibitors.

The cellular ATP content decreased to 73% (mean value) of the control value from untreated cells by incubation with 2-DG (Fig. 3a). This level was very stable up to 120 min incubation (the longest period of observation). Upon addition of compound 48/80 the cellular ATP content decreased to a minimum level of 32% of the control value followed by a small increase in the ATP level up to a value of 42% of control value. Pyruvate or succinate had no effect on the ATP content of mast cells exposed to compound 48/80 (Fig. 3b).

Addition of respiratory inhibitors (antimycin A and oligomycin) to 2-DG-treated cells caused a rapid depletion of the cellular ATP content. Apparently, the decay curves 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 0.9893 ± 0.0116 (mean value and SD, N = 29, range: 0.9510–0.9993, $P < 0.05$ or lower). The time period for 50% decrease of the ATP content ($t_{\frac{1}{2}}$) was 110, 111 and 146 sec (Table 1) after incubation for 5, 30 and 120 min, respectively, in the absence of compound 48/80. In presence of compound 48/80 there was an initial decrease of $t_{\frac{1}{2}}$ followed by a gradual increase up to 90 sec after 120 min incubation.

The lactate production of mast cells treated with respiratory inhibitors (antimycin A and oligomycin) and glucose was linear related to time. The rate of the lactate production in the absence of compound 48/80 was 0.51 pmol/10^3 cells/min (mean value), and in the presence of compound 48/80 it was 0.69 pmol/10^3 cells/min (mean value) (Fig. 5).

The Na^+/K^+-pump activity was increased after the secretion of histamine (Fig. 6). While in the non-stimulated cells the pump activity was negligible, 30 min after the cells had been stimulated with compound 48/80 the pump activity was increased to (in pmol $K^+/10^6$ cells/min) 566 (glucose), 163 (antimycin A and glucose) and 269 (2-DG). The ouabain-resistant K^+-uptake was increased from mean 79 (range 75–84) in non-stimulated cells to mean 175 (range 146–222) in stimulated cells, which is likely to be related to an increased cell surface caused by the exocytotic process [4].

DISCUSSION

Incubation of the mast cells with 5 mM 2-DG and high concentrations of the respiratory inhibitors (antimycin A, oligomycin) is likely to block the synthesis of ATP in the mast cells during the secretion of histamine (Fig. 2) [3, 10]. The observed ATP decrease (0.20 pmol ATP/10^3 cells in 15 sec) in compound 48/80-treated mast cells may thus be caused by an increased cellular utilization of ATP associated with the secretory process. These data confirm previous observations at 37° [11].

The ATP decay curves obtained in presence of both glycolytic and respiratory 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. 4). The rate of the oxidative ATP synthesis in the 2-DG-treated cells was, therefore, calculated by use of the following equations:

$$\ln C_t = \ln C_0 - k \times t \quad (1)$$

$$k = \ln 2 \times (t_{\frac{1}{2}})^{-1} \times \text{sec}^{-1}. \quad (2)$$

C_0 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

Table 1. Rate of cellular ATP synthesis after histamine secretion

	ATP depletion ($t_{\frac{1}{2}}$)		ATP synthesis pmol/10^6 cells/min	
Incubation (min)	−48/80	+48/80	−48/80	+48/80
5	110 ± 4	58 ± 3	0.42 ± 0.04	0.57 ± 0.06*
30	111 ± 11	69 ± 3	0.50 ± 0.03	0.36 ± 0.03†
60		74 ± 3		0.34 ± 0.02
120	146 ± 4	90 ± 5	0.35 ± 0.03	0.32 ± 0.01‡

Mean ± SEM; *$P < 0.01$(degree of freedom 13); †$P < 0.02$ (degree of freedom 5); ‡no statistical significance (t-test, two groups of data). For details of the experiments, see legend to Fig. 4. $t_{\frac{1}{2}}$ is the time period (in sec) for 50% decrease of the cellular ATP content.

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 in presence of compound 48/80 was lower than (30 min) or identical with (120 min) the values observed in the absence of compound 48/80 (Table 1). In order to estimate the rate of ATP synthesis this method requires a stable level of cellular ATP. This was, however, not the case concerning the ATP value observed after 5 min incubation of the cells with compound 48/80 (Fig. 3a). Since the ATP content was gradually decreasing, the small but statistically significant ($P < 0.01$, degree of freedom 13) increase of the rate of the oxidative ATP synthesis after 5 min incubation with compound 48/80 may be an overestimation. The levels of the rates of aerobic ATP synthesis in stimulated as well as unstimulated cells (about 0.3–0.4 pmol/10^6 cells/min) are similar to previous observations (0.3 pmol/10^6 cells/min) in unstimulated cells at 25° [12], and we have no explanation for the somewhat higher rate of ATP synthesis in unstimulated cells at 30 min in this investigation.

The late and persistent decrease of the cellular ATP content after the completion of the secretory process (Fig. 3a) confirms previous observations with mast cells incubated both without and with metabolic inhibitors [3, 8]. The lack of an increased rate of oxidative ATP synthesis subsequent to the secretory activity may explain the persistent ATP decrease. The possibility of an uncoupling of the oxidative phosphorylation in relation to the histamine secretion is not likely due to the fact that the ATP content of compound 48/80-treated cells was very stable even when the cells were pretreated with 2-DG and thus without glycolytic ATP supply (Fig. 3a: 30–120 min). Although the cells were incubated in a substrate-free medium this does not explain the persistent ATP decrease, since addition of respiratory substrates (Fig. 3b) or glucose in the absence of metabolic inhibitors [8] had no effect on the level of the ATP content of the cells. Similar observations have been reported in relation to anaphylactic histamine secretion [10], i.e. a persistent decrease of the cellular ATP content after completion of the secretory process and no increased rate of oxidative ATP synthesis 30–120 min after the secretion had taken place.

Considering the lack of an increased rate of ATP synthesis in 2-DG-treated cells it may be speculated that there is no increased requirement for an accelerated rate of ATP synthesis after completion of the secretory event. This is, however, not a likely explanation. In addition to morphological evidence for cell recovery that might require ATP [13], we have recently reported a large increase in the activity of the Na^+/K^+-pump after histamine secretion at 37° induced by compound 48/80 or induced by the antigen-antibody reaction [4]. This indicates a demand for adjustment of the intracellular Na^+ concentration after the secretory event, since this ion determines the pump activity if the outside stimulation with K^+ is maximal [14]. The increased pump activity lasted for about 2 hr during which there is an increased requirement for ATP in the mast cell. The present data of an increased Na^+/K^+-pump activity after 30 min incubation of the cells with compound 48/80 at 25° was almost identical with the value observed previously [4]. The rate of ATP utilization necessary for support of the pump activity in the present study is 0.28 pmol/10^3 cells/min.

While the aerobic pathway for ATP synthesis does respond to a change in incubation temperature from 25° to 37° by a 2-fold increase in the rate of ATP synthesis [12], the possibility exists that this pathway does not respond to the increased ATP requirement associated with the increased Na^+/K^+-pump activity. This is in accordance with the recent observation that the Na^+/K^+-pump activity in cells from sheep Purkinje fibres is preferentially fuelled by ATP from the glycolysis [15]. Similarly, we observed an increased rate of lactate production of 0.18 pmol/10^3 cells/min under anaerobic conditions reflecting an increased rate of glycolytic ATP synthesis. This value was measured at 25°, and it corresponded, therefore, to our previous observation of 0.40 pmol/10^3 cells/min obtained at 37° [11]. The increased pump activity under anaerobic conditions requires 0.08 pmol ATP/10^3 cells/min, thus utilizing half of the increased rate of glycolytic ATP synthesis.

Incubation of the cells with compound 48/80 caused a large increase in the activity of the Na^+/K^+-pump when fuelled with ATP from either the anaerobic or the aerobic pathway for ATP synthesis. However, the Na^+/K^+-pump activity was only 1/3 and 1/2, respectively, of the Na^+/K^+-pump

activity observed in cells incubated with glucose alone, i.e. without 2-DG or antimycin A.

The increased activity of the Na^+/K^+-pump is related quantitatively to the secretory response [4]. The different levels of Na^+/K^+-pump activities were, however, not explained by variation in the secretion of histamine. Rather, they may be explained by differences in the cellular supply of ATP, which may thus be a limiting factor determining the level of the increase in Na^+/K^+-pump activity. Furthermore, the increased requirement of ATP to support the Na^+/K^+-pump is a likely explanation for the long lasting ATP decrease observed after histamine secretion.

In conclusion, the present data indicate an increased requirement for ATP after the secretory response associated with increased activity of the Na^+/K^+-pump, and this may explain the long lasting ATP decrease observed after histamine secretion. The anaerobic, but not the aerobic, pathway for ATP synthesis is able to respond to the increased ATP requirement. Apparently, the ATP requirement to support the pump is only partly satisfied by the glycolytic or the oxidative pathway, and a decreased rate of ATP synthesis in the presence of 2-DG or antimycin A may be a limiting factor for the extent of increase in Na^+/K^+-pump activity following histamine secretion.

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