*Br. J. Pharmac.* (1979), **65**, 103–109

# UTILIZATION OF ADENOSINE TRIPHOSPHATE IN RAT MAST CELLS DURING HISTAMINE RELEASE INDUCED BY THE IONOPHORE A23187

TORBEN JOHANSEN

Department of Pharmacology, Odense University, J. B. Winsløws Vej 19, DK-5000 Odense C, Denmark

**1** The rôle of endogenous adenosine triphosphate (ATP) in histamine release from rat mast cells induced by the ionophore A23187 *in vitro* has been studied.

**2** The amount of histamine released by calcium from rat mast cells primed with the ionophore A23187 was dependent on the ATP content of the mast cells.

**3** In aerobic experiments a drastic reduction in mast cell ATP content was found during the time when histamine release induced by A23187 takes place.

**4** Anaerobic experiments were performed with metabolic inhibitors (antimycin A, oligomycin, and carbonyl cyanide *p*-trifluorometroxyphenylhydrazone), which are known to block the energy-dependent calcium uptake by isolated mitochondria. The mast cell ATP content was reduced during A23187-induced histamine release under anaerobic conditions in the presence of glucose. This indicates an increased utilization of ATP during the release process.

**5** The observations are consistent with the view that energy requiring processes are involved in ionophore-induced histamine release from rat mast cells although part of the ATP reduction in the aerobic experiments may be due to an uncoupling effect of calcium on the oxidative phosphorylation.

## Introduction

The 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 rat isolated mast cells (Foreman, Mongar & Gomperts, 1973).

Results of a study of the dependence of A23187-induced histamine release on calcium and temperature, and a time course similar to the anaphylactic histamine release, indicate that the same mechanism may be involved in anaphylactic and A23187-induced histamine release (Johansen, 1978).

Anaphylactic histamine release is dependent on mast cell ATP content and there is an increased utilization of ATP during the release (Johansen & Chakravarty, 1975). A23187-induced histamine release has also been shown to be blocked by preincubation of the cells with metabolic inhibitors (Foreman *et al.*, 1973).

In the present investigation the relation between mast cell ATP content and A23187-induced histamine release under aerobic and anaerobic conditions has been studied in order to evaluate the dependence of the histamine release mechanism on endogenous ATP.

## Methods

### *Isolation of rat mast cells*

Male Sprague–Dawley rats, 285 to 550 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. This

0007-1188/79/010103-07 $01.00

fraction always consisted of more than 96% mast cells.

*Incubation procedure*

Mast cell suspensions pooled from 2 to 6 rats were divided into samples of the same cell density in a final volume of 0.5 ml both for determination of the ATP content of the mast cells and for the histamine release experiments. The cell density from different experiments varied from $0.8 \times 10^5$ to $2.6 \times 10^5$ cells per ml. The cell suspensions were prewarmed in a 37°C bath for 10 min and the incubation was continued with A23187 (10 μmol/l) for 1 to 60 min. In the anaerobic experiments (Figures 3 and 4) mast cells were preincubated for 20 min with different metabolic inhibitors (antimycin A, 1 μmol/l; oligomycin, 1 to 2 μg/ml; carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) 5 μmol/l), glucose (1 or 5 mmol/l) and A23187 (10 μmol/l) in calcium-free Krebs–Ringer solution, and the incubation was continued with calcium (1 mmol/l) for 15 s to 5 min both for histamine release experiments and for determination of mast cell ATP content. Samples with and without glucose were included for determination of the control ATP value. Control samples for spontaneous histamine release and for A23187-induced release were always included. In the experiments presented in Figure 1 the ATP content was determined after incubation of the cells in calcium-free medium with antimycin A (10 nmol/l) for different periods of time (5, 7.5, 10, and 15 min). A23187 was present for the last 5 min of the incubation period. In the histamine release experiments the cell suspensions were preincubated in calcium-free medium with antimycin A for 5 to 15 min. A23187 was present for the last 5 min of the preincubation period. The cells were then incubated with calcium (1 mmol/l) for 20 min to induce histamine release. Control samples without antimycin A and A23187 and control samples for spontaneous and A23187-induced histamine release were included. For further details of the experimental conditions see legends to figures and results.

*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 fluorometric method (Shore, Burkhalter & Cohn, 1959). Ionophore A23187, ethanol, glucose, antimycin A, oligomycin or FCCP 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 lantern as described earlier (Johansen & Chakravarty, 1975). None of the reagents interfered with the ATP determination.

Human serum albumin was supplied by AB KABI (Stockholm, Sweden), antimycin A and oligomycin by Sigma Chemical Company (St. Louis, U.S.A.), FCCP (carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone) by Pierce (Illinois, U.S.A.) and glucose by BDH. All other chemicals were of Analar quality. 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).

The 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. In calcium-free Krebs–Ringer solution, NaCl was 143.3 mmol/l.

## Results

*Correlation between the ATP content of mast cells and histamine release induced by A23187*

ATP content and the histamine release induced by calcium were studied in samples of the same suspension of mast cells after preincubation with antimycin A and A23187 in calcium-free medium. ATP was reduced to 15 to 58% of the normal value from control samples after the preincubation and histamine release was reduced to 7 to 87% of control values.

There was a linear correlation (coefficient: 0.96, $P < 0.001$) between the ATP content of the mast cells at the time when histamine release was initiated by addition of calcium to the cell suspension and the amount of histamine released as shown in Figure 1. By extrapolation of the correlation curve about 25% reduction of mast cell ATP content seems to be compatible with normal histamine release.

*Changes in ATP content of mast cells following incubation with A23187 for different periods of time*

Histamine release in the presence of calcium, 1 mmol/l, was complete after 10 min incubation with A23187 with 90% released after 5 min, but only 25% during the first minute (Johansen, 1978). The time

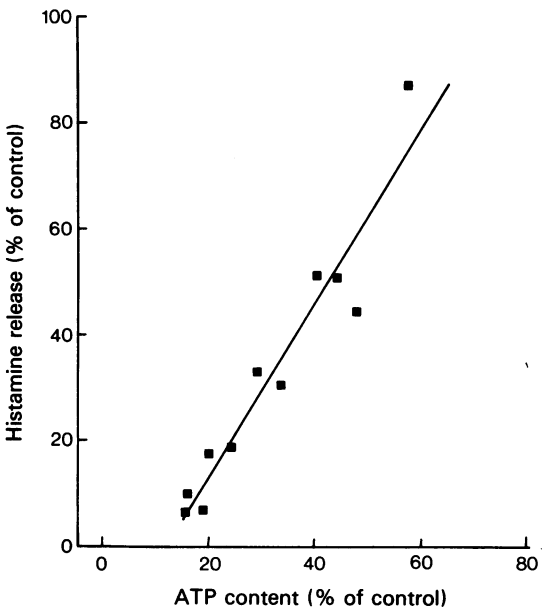


**Figure 1** Correlation between ATP content of the mast cells and histamine release induced by the ionophore A23187. Abscissa scale: ATP content of mast cells after incubation with antimycin A and A23187. 100% on the abscissa scale represents ATP content in control samples with cells incubated without antimycin A and without ionophore: 1.86 ± 0.07 pmol/$10^3$ cells (mean ± s.d., $n$ = 3). Ordinate scale: histamine release induced by calcium from cells preincubated with antimycin A and A23187. 100% on the ordinate scale represents histamine release induced by A23187 from mast cells incubated in the absence of antimycin A in Krebs-Ringer solution with calcium (1 mmol/l): 73.0% ± 6.8% (mean ± s.d., $n$ = 3). Mast cells: 96.9% of cell population (mean value). Individual results from three experiments.

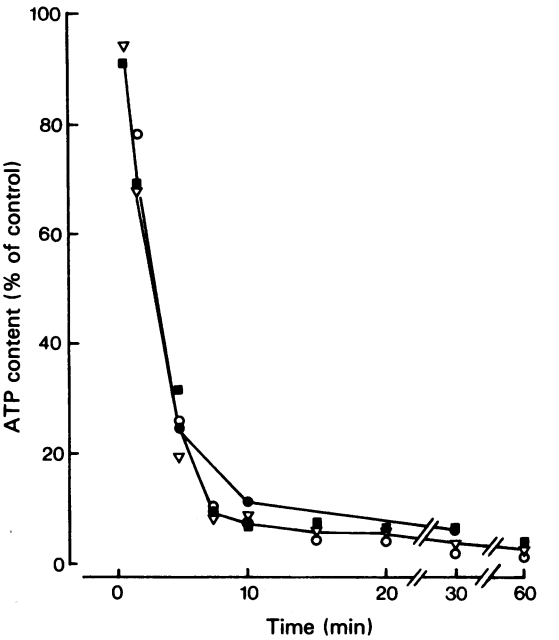


**Figure 2** Changes in mast cell ATP content after incubation with ionophore in the presence (●) or absence of glucose (5 mmol/l) in calcium containing Krebs-Ringer solution. Abscissa scale: time of incubation with ionophore. Ordinate scale: mast cell ATP content. 100% represents ATP content of mast cells incubated without ionophore in presence or absence of glucose: 1.54 ± 0.09 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 15) or 2.00 ± 0.05 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 26), respectively. Mast cells: 98.9% of cell population (mean value). Individual results from three experiments without glucose (■,▽,○) and mean ± s.e. mean from four (2, 5, 10 min) and three (30 min) experiments with glucose.

curve of ATP depletion of the mast cells after exposure to A23187 is similar whether the mast cells were suspended in a glucose containing or substrate-free Krebs–Ringer solution with calcium (1 mmol/l) (Figure 2). In both cases the ATP content was reduced 50% after incubation for 3.5 min.

*Changes in mast cell ATP content during histamine release induced by calcium from A23187-primed mast cells incubated under anaerobic conditions*

Antimycin A is known to block the respiratory chain. Antimycin A, 0.2 μmol/l, has been shown to block completely $^{14}CO_2$-production from rat isolated mast cells incubated with $^{14}$C-labelled pyruvate (Diamant, Norn, Felding, Olsen, Ziebell & Nissen, 1974). In the present experiments antimycin A, 1 μmol/l, was used to block the oxidative ATP synthesis in order to study ionophore-induced histamine release under anaerobic conditions. Mast cells suspended in calcium-free Krebs–Ringer solution were preincubated for 20 min with antimycin A in the presence of glucose and ionophore. The time course of histamine release induced by calcium is shown in Figure 3. Histamine release (mean 58%, $n$ = 3) was completed after 2 min incubation with calcium, 1 min produced 90% of this release. Changes in ATP content of the mast cells after incubation with calcium are shown in Figure 4. After 15 s incubation there was a slight reduction of ATP in all three experiments (mean 9%) when histamine release had just started (mean 10%). When histamine release was completed (2 min) there

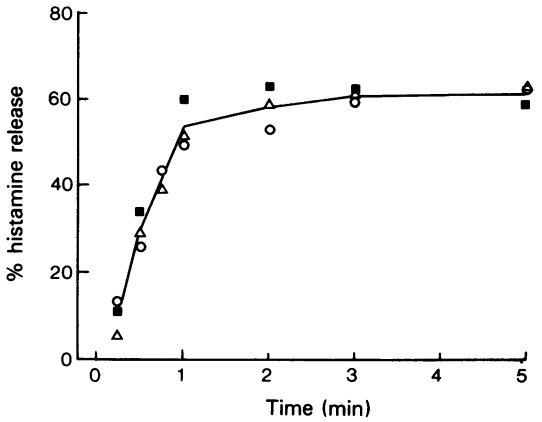


**Figure 3** Time course of histamine release induced by calcium from mast cells primed with ionophore and incubated under anaerobic conditions with antimycin A and glucose. Abscissa scale: time of incubation with calcium. Ordinate scale: histamine release as percentage of the total mast cell histamine content. Spontaneous histamine release (mean 3.5%) deducted. Mast cells: 98.1% of cell population (mean value). Individual results from three experiments.

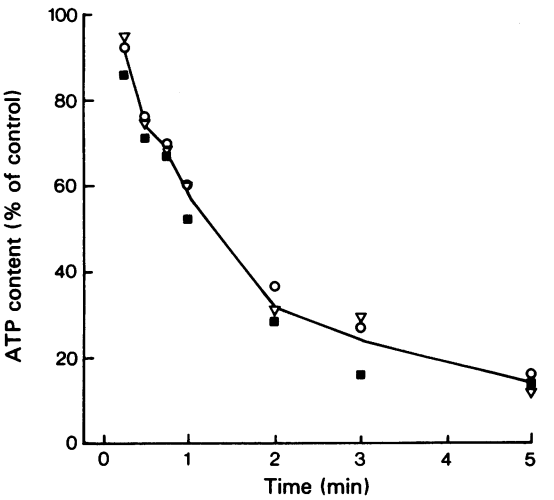


**Figure 4** Changes in mast cell ATP content after incubation with calcium for 15 s to 5 min. Abscissa scale: time of incubation with calcium. Ordinated scale: ATP content as percentage of control value from cells incubated in calcium-free medium with antimycin A in the presence of glucose and ionophore: $0.71 \pm 0.04$ pmol/$10^3$ cells (mean $\pm$ s.e. mean, $n = 3$), which was 52.6% (mean value) of the ATP content of mast cells treated with glucose alone: $1.35 \pm 0.07$ pmol/$10^3$ cells (mean $\pm$ s.e. mean, $n = 3$). No difference in mast cell ATP content in the presence or absence of glucose was observed. Individual results from three experiments.

was a 68% reduction of ATP. The ATP content was further reduced by 86% at 5 minutes.

In similar experiments oligomycin as well as antimycin A were added during the preincubation period in order to block ATP-dependent calcium uptake by mitochondria (Bielawski & Lehninger, 1966). The time courses of histamine release and reduction in ATP were virtually the same as those reported above with antimycin A alone.

Figure 5 shows the effect of antimycin A on the ATP content of mast cells in the presence and absence of glucose and ionophore. There was an appreciable decrease in the ATP content after incubation for 1 min with antimycin A (mean 23%) followed by a drastic reduction with 10 min incubation when the ATP content was 17% of control and less than 5% at 90 min. When mast cells suspended in calcium-free Krebs–Ringer solution were incubated for 10 to 30 min with antimycin A together with glucose and ionophore, ATP was reduced to 50 to 55% of the control value for cells incubated with glucose alone. After incubation for 60 and 90 min the ATP content was 40% and 35%, respectively, of the control value. In parallel experiments, addition of oligomycin with the antimycin A had little effect on the ATP content of the mast cells. After incubation for 10 to 30 min the ATP content was 44 to 46% of the control value with glucose alone. After incubation for 60 min ATP was 38% of control value.

*Changes in mast cell ATP content during histamine release induced by calcium from ionophore-primed mast cells in the presence of FCCP, oligomycin and glucose*

The time course of calcium-induced histamine release and changes in ATP content of mast cells after incubation with calcium in the presence of an uncoupler of oxidative phosphorylation (FCCP) was investigated. Mast cells, suspended in calcium-free Krebs–Ringer solution, were preincubated with FCCP for 20 min. Glucose (5 mmol/l), oligomycin and ionophore were added at the same time. Essentially the same time course of histamine release and of reduction in ATP content as those reported with antimycin A (Figures 3 and 4) was observed after calcium was added to the cells. However, in the presence of FCCP there was a lag period of 30 s with only 6% histamine release and 5% reduction in ATP. After 2 min incubation with calcium the release of histamine was completed (mean 44%, $n = 4$) and the ATP reduction was 54%. From 2 to 5 min a slow histamine release of 9% occurred and after 5 min the ATP reduction was 64%.

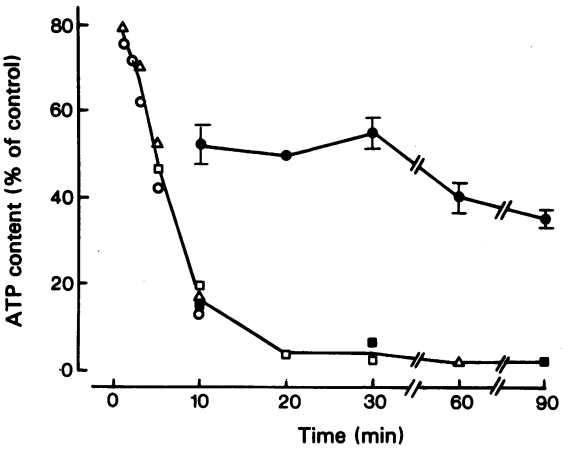


**Figure 5** Changes in mast cell ATP content after incubation for 1 to 90 min with antimycin A (1 μmol/l) in the absence (lower curve) or in the presence (upper curve, calcium-free medium) of ionophore (10 μmol/l) and glucose (1 mmol/l). Abscissa scale: time of incubation. Ordinate scale: mast cell ATP content as percentage of control value from cells incubated with glucose: 1.61 ± 0.07 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 24, control for upper curve) or without glucose: 1.45 ± 0.06 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 15, control for lower curve). No essential difference in ATP level in control samples incubated for short and long time interval was observed. Figure shows results from two separate groups of experiments: mean ± s.e. mean from 3 to 5 experiments (upper curve) and individual results from four experiments (lower curve). Mast cells: 98.1% of cell population (mean value from the two groups of experiments).

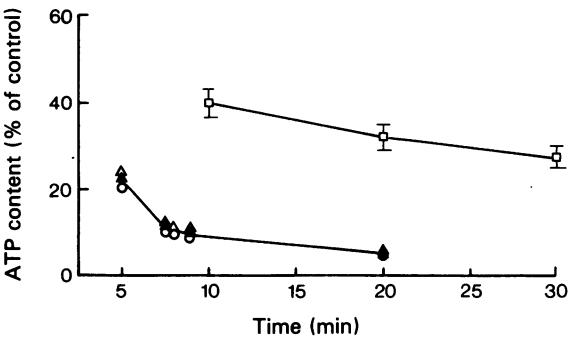


**Figure 6** Changes in mast cell ATP content after incubation for 5 to 30 min in calcium-free medium with FCCP in the absence (lower curve) or presence (upper curve) of oligomycin, glucose and ionophore. Abscissa scale: time of incubation. Ordinate scale: ATP content as percentage of control value from cells incubated with glucose: 1.40 ± 0.03 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 12, control for upper curve) or without glucose: 1.56 ± 0.07 pmol/$10^3$ cells (mean ± s.e. mean, $n$ = 8, control for lower curve). Figure shows results from two separate groups of experiments. Mean ± s.e. from four experiments (upper curve) and individual results from three experiments (lower curve). Mast cells: 97.1% of cell population (mean value from the two groups of experiments).

The effect of FCCP in the presence and absence of oligomycin, glucose, and A23187 on mast cell ATP is shown in Figure 6. FCCP alone reduced the ATP content to 5% of the normal value after 20 min incubation. When mast cells were incubated for 10 min with FCCP in the presence of oligomycin, glucose and ionophore, but without calcium, mast cell ATP content was 40% of control value with glucose alone and during the next 20 min incubation a slight further reduction (mean 12%) was observed. After incubation for 20 and 30 min the ATP content was 32% and 28%, respectively.

## Discussion

When oligomycin was used to inhibit mast cell ATP synthesis, a linear correlation was reported between the ATP content of the mast cells and the amount of histamine released by compound 48/80 (Johansen & Chakravarty, 1972) or induced by antigen–antibody (anaphylactic) reaction *in vitro* (Johansen & Chakravarty, 1975). A similar correlation is now demonstrated between the ATP content of the mast cells and histamine release induced by calcium from cells pretreated with A23187. By extrapolation of these results it seems that about 25% reduction of the ATP content was compatible with normal histamine release induced by calcium and A23187. An almost identical observation was reported for compound 48/80-induced histamine release (Johansen & Chakravarty, 1972), but from the observations with anaphylactic histamine release, some inhibition of the release seemed to occur with oligomycin even when the ATP content was normal, suggesting an effect of oligomycin on the release apart from inhibition of ATP synthesis. The correlation between the ATP content of the mast cell and the amount of histamine released by calcium in the presence of ionophore shows its dependence on endogenous ATP.

The drastic reduction in the ATP content of the mast cells in the presence or absence of glucose in the aerobic experiments may be due to an uncoupling of the oxidative phosphorylation by A23187 in addition to an increased consumption of ATP for the release of histamine. A23187 has been shown to uncouple the oxidative phosphorylation of rat liver

mitochondria by a calcium-requiring mechanism (Reed & Lardy, 1972). In similar experiments with anaphylactic or compound 48/80-induced histamine release only a moderate reduction in the ATP content was observed after histamine release was completed. However, when the ATP synthesis was partially inhibited by preincubation of the cells with 2-deoxyglucose, a decrease in the ATP content could be demonstrated in direct time relation to both anaphylactic and compound 48/80-induced histamine release (Johansen & Chakravarty, 1975). The metabolic response due to incubation with A23187 was different from that caused by compound 48/80 or antigen–antibody reaction. The difference may be explained by an uncoupling of the oxidative phosphorylation by A23187 and calcium. The rate of ATP reduction was greater with A23187 and calcium than the rate of ATP reduction caused by blocking the respiration with antimycin A (Figures 2 and 5). This may be explained by the combined effect of a block of the oxidative phosphorylation and an increased energy demand of the cell during A23187-induced histamine release.

It has been shown that isolated mitochondria from most eucaryotic cells accumulate calcium by an energy-dependent mechanism (Lehninger, Carafoli & Rossi, 1967; Lehninger, 1970; Carafoli & Lehninger, 1971), and in liver mitochondria, calcium uptake takes precedence over oxidative phosphorylation in utilizing respiratory energy (Rossi & Lehninger, 1964). Histamine release induced by A23187 seems to be initiated by transport of calcium into the cell (Foreman *et al.*, 1973; Johansen, 1978). An increase in cytosolic calcium concentration by A23187 may possibly uncouple the oxidative phosphorylation in the mast cell in addition to the effect on the histamine release mechanism. The energy-dependent calcium uptake by isolated mitochondria is supported by either the oxidation of respiratory substrates, or by the hydrolysis of added ATP (Rossi & Lehninger, 1963; Brierley, Murer & Bachmann, 1964; Bielawski & Lehninger, 1966; Malmström & Carafoli, 1977). In the first case the process is inhibited by respiratory inhibitors and not by oligomycin (Vasington & Murphy, 1962; Brierley, Murer & Green, 1963; Brierley *et al.*, 1964), in the second, by oligomycin, and not by respiratory inhibitors (Rossi & Lehninger, 1963; Brierley *et al.*, 1964; Bielawski & Lehninger, 1966). In both cases the transport is abolished by uncouplers of oxidative phosphorylation (Vasington & Murphy, 1962; Brierley *et al.*, 1963; 1964; Bielawski & Lehninger, 1966). The role of endogenous mast cell ATP in the release of histamine by A23187 and extracellular calcium has also been studied under anaerobic conditions by the use of antimycin A, which blocks the respiration and thus the mitochondrial calcium uptake. The reduction in the ATP content of the mast cells observed during histamine release under anaerobic conditions is unrelated to uncoupling of the oxidative phosphorylation by the calcium requiring mechanism and thus may be largely related to the release process. However, the possibility exists that ATP generated by glycolytic (or anaerobic) phosphorylation and substrate-linked phosphorylation is utilized by the mitochondrial calcium-accumulating mechanism in the anaerobic experiments. However, the almost identical time course of ATP reduction during histamine release from mast cells pretreated with oligomycin, which blocks the calcium-induced mitochondrial ATP hydrolysis (Bielawski & Lehninger, 1966), in addition to antimycin A supports the interpretation above that the ATP reduction seems to be related to the release process and not to mitochondrial calcium accumulation. Furthermore, support has been obtained from experiments in which the mitochondrial calcium uptake was abolished by FCCP. In these experiments oligomycin was added to the cells in order to block uncoupler stimulated ATP hydrolysis by mitochondrial ATPase (Huijing & Slater, 1961; Lardy, Connelly & Johnson, 1964; Slater & Ter Welle, 1969). Huijing & Slater (1961) reported that rather high concentrations of oligomycin were necessary to inhibit the dinitrophenol-stimulated ATPase activity of isolated rat liver mitochondria. In relation to the protein content of the mast cells (Diamant & Lowry, 1966) the oligomycin concentration in the present experiments used to block the uncoupler-induced ATPase activity was over 20 times the value used for the mitochondrial preparation.

These observations are consistent with the view that energy requiring processes are involved in ionophore-induced histamine release from rat mast cells, although part of the ATP reduction in the aerobic experiments may be due to an uncoupling effect of calcium on oxidative phosphorylation.

I am grateful to Professor N. Chakravarty for criticism of the manuscript. The technical assistance of Mrs Annette Kragh Rasmussen and Mrs Susanne Nielsen is gratefully acknowledged. The ionophore A23187 was kindly supplied by Eli Lilly & Co. (Indiana, U.S.A.). This work was supported by funds obtained from Mr. F. E. M. Gaardon and Mrs. Lydia Gaardon's Foundation and F. L. Smidth's Jubilee Foundation.

## References


BIELAWSKI, J. & LEHNINGER, A.L. (1966). Stoichiometric relationship in mitochondrial accumulation of calcium and phosphate supported by hydrolysis of adenosine triphosphate. *J. biol. Chem.*, **241**, 4316–4322.

BRIERLEY, G.P., MURER, E. & GREEN, D.E. (1963). Participation of an intermediate of oxidative phosphorylation in ion accumulation by mitochondria. *Science, N.Y.* **140**, 60–62.

BRIERLEY, G.P., MURER, E. & BACHMANN, E. (1964). Studies on ion transport. III. The accumulation of calcium and inorganic phosphate by heart mitochondria. *Archs Biochem. Biophys.*, **105**, 89–102.

CARAFOLI, E. & LEHNINGER, A.L. (1971). A survey of the interaction of calcium ions with mitochondria from different tissues and species. *Biochem. J.*, **122**, 681–690.

CHAKRAVARTY, N. (1965). Glycolysis in rat peritoneal mast cells. *J. cell Biol.*, **25**, 123–128.

CHAKRAVARTY, N. & ZEUTHEN, E. (1965). Respiration of peritoneal cells. *J. cell Biol.*, **25**, 113–121.

DIAMANT, B. & LOWRY, O.H. (1966). Dry weight determination of single lyophilized mast cells of the rat. *J. Histochem. Cytochem.*, **14**, 519–524.

DIAMANT, B., NORN, S., FELDING, S., OLSEN, N. ZIEBELL, A. & NISSEN, J. (1974). ATP level and $CO_2$ production of mast cells in anaphylaxis. *Int. Archs Allergy appl. Immun.*, **47**, 894–908.

FOREMAN, J.C., MONGAR, J.L. & GOMPERTS, B.D. (1973). Calcium ionophores and movements of calcium ions following the physiological stimulus to a secretory process. *Nature, Lond.*, **245**, 249–251.

HUIJING, F. & SLATER, E.C. (1961). The use of oligomycin as an inhibitor of oxidative phosphorylation. *J. Biochem. (Tokyo)*, **49**, 493–501.

JOHANSEN, T. (1978). Mechanism of histamine release from rat mast cells induced by ionophore A23187: effects of calcium and temperature. *Br. J. Pharmac.* **63**, 643–649.

JOHANSEN, T. & CHAKRAVARTY, N. (1972). Dependence of histamine release from rat mast cells on adenosine triphosphate. *Naunyn-Schmiedebergs Arch. Pharmac.*, **275**, 457–463.

JOHANSEN, T. & CHAKRAVARTY, N. (1975). The utilization of adenosine triphosphate in rat mast cells during histamine release induced by anaphylactic reaction and compound 48/80. *Naunyn-Schmiedebergs Arch. Pharmac.*, **288**, 243–260.

LARDY, H.A., CONNELLY, J.L. & JOHNSON, D. (1964). Antibiotics as tools for metabolic studies. II. Inhibition of phosphoryl transfer in mitochondria by oligomycin and aurovertin. *Biochemistry*, **3**, 1961–1968.

LEHNINGER, A.L. (1970). Mitochondria and calcium ion transport. *Biochem. J.*, **119**, 129–138.

LEHNINGER, A.L., CARAFOLI, E. & ROSSI, C.S. (1967). Energy-linked ion movements in mitochondrial systems. *Adv. Enzymol.*, **29**, 259–320.

MALMSTRÖM, K. & CARAFOLI, E. (1977). The interaction of $Ca^{2+}$ with mitochondria from human myometrium. *Archs Biochem. Biophys.*, **182**, 657–666.

REED, P.W. & LARDY, H.A. (1972). Antibiotic A23187 as a probe for the study of calcium and magnesium function in biological systems. In *The Role of Membranes in Metabolic Regulation.* ed. Mehlman, M.A. & Hanson, R.W. pp. 111–131. New York and London: Academic Press.

ROSSI, C.S. & LEHNINGER, A.L.(1963). Stoichiometric relationships between accumulation of ions by mitochondria and the energy-coupling sites in the respiratory chain. *Biochem. Z.*, **338**, 698–713.

ROSSI, C.S. & LEHNINGER, A.L. (1964). Stoichiometry of respiratory stimulation, accumulation of $Ca^{++}$ and phosphate, and oxidative phosphorylation in rat liver mitochondria. *J. biol. Chem.*, **239**, 3971–3980.

SHORE, P.A., BURKHALTER, A. & COHN, V.H. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmac. exp. Ther.*, **127**, 182–186.

SLATER, E.C. & TER WELLE, H.F. (1969). Applications of oligomycin and related inhibitors in bioenergetics. In *Inhibitors, Tools in Cell Research.* ed. Bücher, T. & Sies, H. pp. 258–278. Berlin, Heidelberg and New York: Springer–Verlag.

VASINGTON, F.D. & MURPHY, J.V. (1962). $Ca^{++}$ uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. *J. biol. Chem.* **237**, 2670–2677.


*(Received May 25, 1978. Revised July 24, 1978.)*