Calcium exerts an indirect effect on ATP-dependent proteolysis of rat liver mitochondria

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The ATP-dependent proteolysis of rat liver mitochondria prepared in electrolyte-poor sucrose media requires the presence of Ca^{2+}. Lanthanum, an inhibitor of Ca^{2+} uptake, inhibits the proteolysis. In contrast, proteolysis of mitochondria prepared in a salt medium does not require Ca^{2+}, nor is it inhibited by lanthanum. It is concluded that Ca^{a+} exerts its effect in an indirect manner, by causing swelling and thereby increasing the accessibility of the membrane proteins of the inner mitochondrial membrane.

ATP-dependent proteolysis *Rat liver mitochondria* *Ca^{2+}, effect on proteolysis*

1. INTRODUCTION

In [1–4] an ATP-dependent proteolytic system of rat liver mitochondria was described and characterized which does not require a preceding attack by lipoxygenase but appears to be calcium-dependent.

Here we report experiments which indicate that calcium exerts its effects in an indirect manner, presumably by causing swelling of the mitochondria, and that mitochondria incubated in a salt medium no longer exhibit a calcium requirement.

2. MATERIALS AND METHODS

Adult male rats (250–300 g) were decapitated and their livers immediately removed. Mitochondria were prepared as in [3] with isolation solutions containing sucrose (250 mM), Tris–HCl, pH 7.4 (70 mM) and EGTA (1 mM). After preparing mitochondria in this isolation solution the final mitochondrial pellet was resuspended in an incubation solution containing sucrose (250 mM), Tris–HCl, pH 7.6 (70 mM), EGTA (1 mM) and $MgCl_2$ (5 mM). For experiments in a salt medium the mitochondria were isolated in a medium containing sucrose (250 mM) and EGTA (1 mM) and incubated in a medium containing KCl (130 mM), HEPES, pH 7.6 (3 mM), KH_2PO_4 (2 mM) and $MgCl_2$ (5 mM) with a final concentration of about 10–20 mg protein/ml suspension.

For the experiments, 1 ml mitochondrial suspension was used. To compensate for differences of incubation volume caused by additives, 2–4 ml of the corresponding incubation solutions were added to a final volume of 5.6 ml.

ATP, $CaCl_2$, lanthanum nitrate and antimycin A were used in final concentrations of 5.7 mM, 1.6 mM, 0.2 mM and 0.005 mM, respectively. Usually the incubations were carried out for 2 h at 37°C and pH 7.6. Proteolysis was measured by the lysine method [5] and was expressed in terms of lysine liberation from mitochondrial proteins during the incubation period.

3. RESULTS AND DISCUSSION

The data summarized in fig.1 demonstrate the role of Ca^{2+} for the ATP-dependent proteolysis of rat liver mitochondria prepared and incubated in a sucrose medium. In the presence of EGTA, there is only little protein breakdown. Lanthanum, a specific inhibitor of mitochondrial Ca^{2+} uptake [6,7], completely inhibits the Ca^{2+}-stimulatable, ATP-dependent proteolysis.

The data in fig.2 demonstrate the lack of pro-

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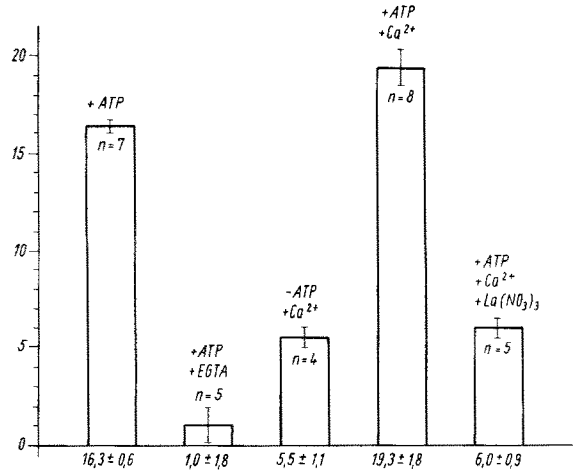

Fig.1. Effects of Ca^{2+}, EGTA, and lanthanum on ATP-dependent proteolysis of rat liver mitochondria. Final concentrations: EGTA, 1.0 mM; free Ca^{2+}, 0.58 mM; lanthanum, 0.22 mM.

teolysis in the presence of Ca^{2+} without ATP. Thus Ca^{2+}-dependent proteolysis [8] is of minor if any importance. Preincubation under conditions which do not permit Ca^{2+} influx (column 3) inhibits the ATP-dependent proteolysis. After preincubation with Ca^{2+}, lanthanum does not affect the ATP-dependent protein breakdown.

The data in fig.3 demonstrate the effects of incubation in a salt medium. Under these conditions the ATP-dependent proteolysis is, on the one hand, much larger than in a sucrose medium and, on the other hand, no longer inhibited by lanthanum. One may conclude therefore that it is no longer Ca^{2+}-dependent. Ca^{2+} ions again have only little effect on the rate of proteolysis.

From these results one may conclude that the ATP-dependent proteolytic system of rat liver mitochondria depends on Ca^{2+} in an indirect manner.

The indirect stimulatory effect of Ca^{2+} is indicated by several types of evidence:

(i) The ATP-dependent, Ca^{2+}-stimulated proteolysis of mitochondria in a sucrose medium is inhibited by lanthanum, a specific inhibitor of mitochondrial Ca^{2+} uptake;

(ii) After a preincubation with Ca^{2+} in a sucrose medium, lanthanum is unable to decrease ATP-dependent protein breakdown;

(iii) ATP-dependent proteolysis in a salt medium is larger than in sucrose media and is independent of Ca^{2+}.

Both the incubation of isolated mitochondria in salt media with high concentrations of monovalent cations (such as K^+ and Na^+) at slightly alkaline pH and 37°C in the absence of sucrose [9–11], and

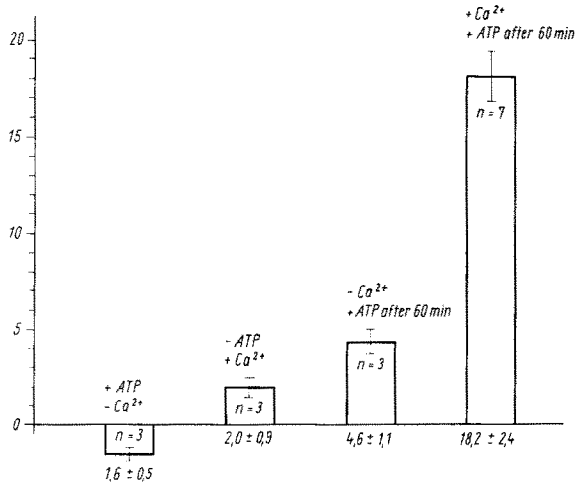

Fig.2. Effect of 60 min preincubation with and without Ca^{2+} on ATP-dependent proteolysis in the presence of EGTA. After 60 min preincubation lanthanum nitrate was added to all samples.

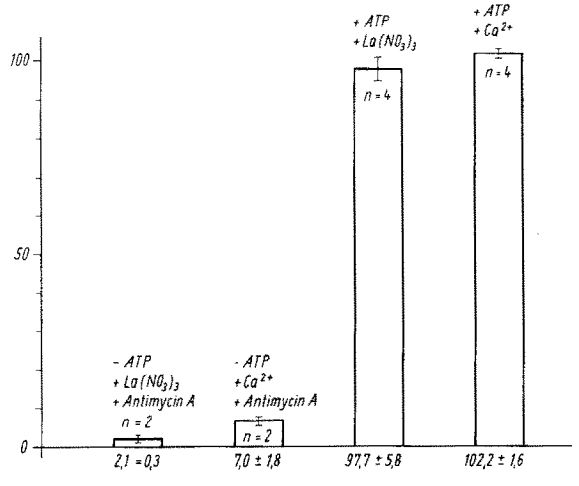

Fig.3. The effects of a salt medium on ATP-dependent proteolysis of rat liver mitochondria. Isolation and incubation of mitochondria were performed as described in section 2.

the incubation in the presence of Ca^{2+} [6,7,12–15] lead to the swelling of mitochondria. In salt media the volume changes of mitochondria are not affected by the presence of ATP [11], whereas additions of sucrose to swollen mitochondria produce rapid osmotic contractions [10]. Ca^{2+}-induced swelling is energy-dependent [7,13,14] and accompanied by changes of permeability of the inner mitochondrial membrane [6,14] connected with a decrease of the respiratory control ratio and the enhancement of enzyme activities such as pyruvate dehydrogenase, isocitrate dehydrogenase and α-oxoglutarate dehydrogenase [16], the enzymes of mitochondrial fatty aid oxidation [15], and is inhibited by lanthanum and ruthenium red [6,7]. Furthermore, it was shown that there exists a mitochondrial proteolytic activity increasing with swelling of the organelles, which was believed to produce the swelling of mitochondria [17]. Our results demonstrate that the ATP-dependent proteolysis of rat liver mitochondria is elevated by swelling of mitochondria both in a salt medium and after the accumulation of Ca^{2+}.

The effects demonstrated may be ascribed to a facilitated accessibility of membrane proteins of the inner membrane of mitochondria owing to changes of lipid–protein interactions produced by swelling. Increased susceptibility of swollen mitochondria to externally-added proteases has been observed [18]. Similarly, it has been shown that the susceptibility of rat liver mitochondria to the attack by lipoxygenase is enhanced by hypotonic sucrose media and is decreased by conditions which produce a condensed state [19].

Therefore one may surmise that in vivo under physiological conditions old preswollen mitochondria rather than young ones are subject to a selective degradation by the ATP-dependent proteolytic systems. Under pathological conditions matters would be different.

Cell death by ischemia, chemicals, viruses, radiation or toxins is connected with massive Ca^{2+}-influx into the cells [20]. The consequence of an elevated intracellular Ca^{2+} concentration would be the accumulation of Ca^{2+} by mitochondria with a consequent massive and indiscriminate breakdown of young and old mitochondria.

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