ION REGULATION OF ATP HYDROLYSIS IN MITOCHONDRIA

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1. Introduction

Some years ago Wadkins and Lehninger reported that the ATPase activity of submitochondrial particles diminished when the respiratory carriers were kept in the reduced state by adding succinate to the reaction mixture [1]. More recently it was found that DNP* at relatively high concentrations inhibited mitochondrial respiration and ATPase activity by competitively inhibiting the uptake of substrates and ATP into the mitochondria [2, 3]. On the other hand, Fonyo reported that succinate inhibited the DNA stimulated ATPase activity by acting on some step of the ATPase reaction [4], while van Dam and Slater [5] showed that 7 mM succinate inhibited the DNP stimulated ATPase activity by competing with the uptake of DNP. However, as McGivan et al. [6] found that the translocation of ATP into the mitochondria is electrogenic it was considered possible that succinate, by nature of its anionic character, once on the inner side of the mitochondria could increase the internal negative charge and thereby inhibit the translocation of ATP and its subsequent hydrolysis. The results of this paper indicate that influx of ATP, as measured by its hydrolysis on the inner side of the mitochondria is controlled by the internal charge of the mitochondria.

2. Methods

Rat liver mitochondria were prepared as described previously [7]. ATPase activity was measured in the indicated incubating conditions, the reaction was stopped with 6% trichloroacetic acid final concentration and inorganic phosphate was measured in the supernatant according to Sumner [8]. Oxygen uptake was determined polarographically and mitochondrial swelling was followed by measuring the decrease in optical density at 520 nm. Mitochondrial protein was determined according to Murphy and Kies [9].

* DNP = 2,4-dinitrophenol.

3. Results

The results of table 1 (exp. 1) show that the ATPase activity of mitochondria incubated with DNP is inhibited by succinate. According to van Dam and Slater [5], the succinate induced inhibition of uncoupler stimulated ATPase activity could be due to an inhibition in the uptake of the uncoupler into the mitochondria, but as DNP induced maximal respiratory rates in mitochondria incubated under identical conditions (50 natoms min^{-1} mg^{-1}), it may be concluded that the entrance of DNP into the mitochondria was not significantly impaired and that other factors must be involved.

The hydrolysis of ATP on the inner side of the mitochondria involves the following exchange, where out and in refer to the outer and inner side of the mitochondria.

$$\underset{\text{out}}{ATP^{4-}} \dashrightarrow \underset{\text{in}}{ATP^{4-}}$$

$$\underset{\text{out}}{ADP^{3-}} + \underset{\text{out}}{H_2PO_4^-} \dashleftarrow \underset{\text{in}}{ADP^{3-}} + \underset{\text{in}}{H_2PO_4^-}$$

Therefore it is possible that once on the inside of the mitochondria the negatively charged succinate shifted the equilibrium of the exchange and thus inhibited the

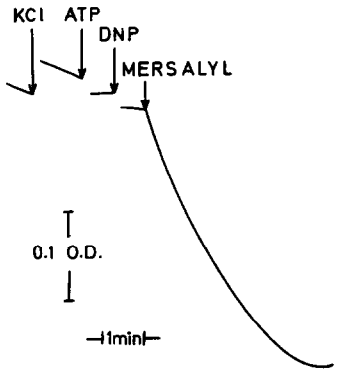

Fig. 1. Mersalyl induced mitochondria swelling. Mitochondria (4 mg of protein) were added to 3 ml of a mixture that contained 20 mM succinate, 0.16 mM sucrose 10 mM Tris-HCl, pH 7.3. KCl (100 mM), ATP (3.6 mM), DNP (50 μM) and mersalyl (96 nmoles) were added at the arrows, the temperature was 25°.

Table 1
The effect of anions and mersalyl on the ATPase stimulated by DNP, Ca^{2+} and valinomycin.

Experiment	Additions	ATPase activity (μmoles P_i formed)
1	DNP	5.2
	DNP + succinate	0.8
2	DNP	6.1
	DNP + succinate	0.5
	DNP + mersalyl	5.3
	DNP + mersalyl + succinate	2.6
3	Valinomycin	6.9
	Valinomycin + succinate	5.1
4	DNP	5.5
	DNP + pyruvate	3.7
	Valinomycin	6.9
	Valinomycin + pyruvate	6.7
5	$CaCl_2$	6.7
	$CaCl_2$ + succinate	6.3

8 mg of mitochondrial protein were incubated for 10 min at 25°C in 2 ml of a medium that contained 100 mM sucrose, 3.4 mM ATP; 20 mM Tris-HCl pH 7.3 and as indicated, 50 μM DNP, 10 mM succinate or pyruvate; 4 mM $CaCl_2$; 0.3 μg valinomycin and 92 nmoles mersalyl. In experiments 2 and 3 and 4, sucrose was replaced by 50 mM KCl.

translocation of ATP and its hydrolysis. Theoretically this possible impairment could be partially compensated by the influx of K^+. This possibility was tested with the use of mersalyl in the experiments outlined in fig. 1 and exp. 2 of table 1.

Tyler [10] reported that mersalyl prevents the efflux of phosphate derived from ATP hydrolysis. Fig. 1 shows that important mitochondrial swelling in the presence of ATP, succinate K^+ and DNP occurs upon the addition of mersalyl; that is phosphate generated from ATP, once it was trapped on the inner side of the mitochondria provided a negative potential which resulted in cation influx, simultaneous to the increase in K^+ influx a high DNP stimulated ATPase activity took place (exp. 2 of table 1). Therefore these findings are highly suggestive that the uptake of K^+ partially reverses the inhibiting effect of succinate, most probably by compensating, the internal negative charge.

Further evidence for the postulation that internal negative charges control ATPase activity is provided in experiments 3 to 5 of table 1. The valinomycin stimulated ATPase activity is less sensitive to succinate than the DNP stimulated ATPase activity (exp. 3), and hardly affected by pyruvate (exp. 4) which has only one negative charge. As valinomycin simultaneously stimulates ATPase activity and promotes the influx of K^+ and the release of H^+, at an H^+/K^+ ratio of less than 1.0 [11], the internal negative charges due to the presence of the negatively charged oxidizable substrate should be compensated by the uptake of K^+. Thus in the presence of valinomycin a higher influx and hydrolysis of ATP occurs regardless of the presence of succinate or pyruvate.

Along the same line, pyruvate with only one negative charge is less effective than succinate in inhibiting the DNP stimulated ATPase activity. This would be expected since the internal negative charge would be less with pyruvate than with succinate assuming equal substrate uptake.

It has been shown that mitochondria have the capacity to accumulate massive amounts of Ca^{2+} in the presence of ATP [12], at a ratio of H^+ ejected per Ca^{2+} taken in the presence of succinate of about 1.0 [13] thus in the presence of succinate and Ca^{2+} a diminution in the internal negative charge of the mitochondria would be expected. As shown in exp. 5, the Ca^{2+} stimulated ATPase activity is hardly inhibited

by succinate. That is the accumulation of $^{5}Ca^{2+}$ compensated for the internal charges due to succinate and thus no inhibitory action of succinate was observed on the Ca^{2+} stimulated ATPase activity.

4. Discussion

The experiments of McGivan et al. [6] indicate that the transport of ADP and ATP in mitochondria is electrogenic and compensated by the movement of phosphate. Indeed McGivan et al. [6] showed that the inhibition of phosphate translocation inhibited the ADP–ATP exchange and ATPase activity. Furthermore, it has been recently shown that the influx of ATP into the mitochondria may occur in symport with K^+ [14, 15]. From the presently described experiments and in agreement with Pfaff and Klingenberg [16], it may be concluded that the translocation of ATP is controlled by the internal charge of the mitochondria. Furthermore the results indicate that the inward movements of cations facilitate the influx of ATP by diminishing the internal negative charge of the mitochondria.

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