ATP opens an electrophoretic potassium transport pathway in respiring yeast mitochondria

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Abstract In the presence of KCl and only at low phosphate concentrations, ATP stimulated state 4 of the respiration of isolated yeast mitochondria. This effect could be related to a partial collapse of the transmembrane potential which was created by the respiratory chain or the F_0F_1-ATPase. Sodium and lithium could not replace potassium ion. Atractyloside prevented the opening of this K^+ pathway, suggesting that only matricial ATP operated. All these effects were inhibited by increasing phosphate concentration, or by adding propranolol, quinine, Zn^{2+} or Mg^{2+}.

Key words: Respiration; ATP; K^+ transport; Yeast mitochondrion

1. Introduction

The maintaining of a large proton motive force for ATP synthesis during the oxidative phosphorylation process requires the inner mitochondrial membrane to be impermeable to ions. However, a number of cation and anion transport pathways have been characterized both in mammalian [1–4] and in yeast [5,6]. Apart from the K^+/H^+ exchange in yeast mitochondria [5], these transport pathways are not spontaneously active and they are observed only after Mg^{2+} or nucleotide depletion, or at alkaline pH. Recently, Prieto et al. [7] reported that external ATP induced a proton permeability pathway in mitochondria from *Saccharomyces cerevisiae*. In addition, phosphate was shown to inhibit this proton permeability and it was suggested that the phosphorylation efficiency could depend on the opening state of this channel. On the other hand, Guerin et al. [8] showed that external ATP opened an unspecific channel which was inhibited by ADP, phosphate, Mg^{2+}, Zn^{2+} and propranolol. Additionally, the conditions under which oxidative phosphorylation is usually measured (low salt concentration, phosphate) should not allow this channel to be opened. This is in accordance with the fact that ATP does not uncouple oxidative phosphorylations [9].

Recently, we observed that when oxidative phosphorylation was measured both in the presence of physiological KCl concentrations and at high phosphate concentrations, K^+ can enter the matrix by electrophoretic pathways without uncoupling respiration from ATP synthesis (Manon et al., submitted). However, when phosphate transport was inhibited by mersalyl, KCl addition induced an uncoupling of oxidative phosphorylation, as revealed by a decrease in the ATP/O ratio, showing that the stimulation of ATP synthesis by KCl resulted from a stimulation of phosphate transport. The observations reported by Prieto et al. [7] and Guérin et al. [8] prompted us to investigate if ATP by itself could also have an uncoupling effect under these conditions. In the present paper, it is shown that, at low phosphate concentrations, ATP opens a K^+ transport pathway in respiring yeast mitochondria.

2. Materials and methods

The diploid wild-type strain Yeast Foam was grown aerobically in a 1% yeast extract (Gibco), 0.12% $(NH_4)_2SO_4$, 0.1% KH_2PO_4 medium supplemented with 2% DL-lactate (adjusted at pH 5.0 with NaOH). Cells were harvested in the mid-exponential growth phase and mitochondria were isolated as previously described [10]. Mannitol and sorbitol used in preparation buffers were routinely deionized. Mitochondria were frozen as small beads in liquid nitrogen and could be stored at −80°C for weeks without significant alteration of their phosphorylating properties. Proteins were measured by the biuret method with bovine serum albumin as a standard. Oxygen consumption was measured at 28°C in a 2 ml thermostatically controlled chamber equipped with a Clark electrode. The basal medium for all the experiments was 0.65 M mannitol, 0.36 mM EGTA, 10 mM Tris-maleate (pH 6.8) and 0.3% bovine serum albumin. Mitochondria were suspended at 0.5 mg/ml. When 100 mM KCl was added to the medium, the mannitol concentration was decreased to maintain a constant osmolarity.

Mitochondrial transmembrane potential was evaluated from the absorbance red shift of rhodamine 123 by dual wavelength spectrophotometry at 516 nm minus 495 nm [11], using a DW2000 Aminco Chance spectrophotometer.

All the experiments reported were done with 40 mM ethanol as the respiratory substrate.

3. Results

3.1. ATP stimulates respiration in KCl medium at low phosphate concentration

In the absence of KCl, and whatever the phosphate concentration, ATP did not change the respiration rate (Fig. 1, traces a,b), which is opposite to the effect observed by Prieto et al. [6]. Addition of KCl at a constant osmolarity stimulated state 4 of respiration as described previously (Manon et al., submitted). Subsequent addition of ATP was without effect at 5 mM phosphate (Fig. 1, trace c), but at 0.5 mM phosphate, ATP induced a marked increase in the respiration rate (Fig. 1, trace d). This stimulation was still observed in the presence of oligomycin (at a concentration which abolished state 3 respiration in KCl-containing medium, both at 0.5 mM and 5 mM phosphate; Fig. 1, trace e), showing that contaminating ADP could not be implicated in the stimulatory effect. Nevertheless, all the experiments reported herein were done in the presence of oligomycin.

Fig. 2a shows that in the presence of 100 mM KCl and at 0.5 mM phosphate, stimulation of the respiration was maximal at 2 mM ATP; on the other hand this stimulation was dependent

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Abbreviations: CCCP, *p*-chloro *m*-carbonylcyanide hydrazone.

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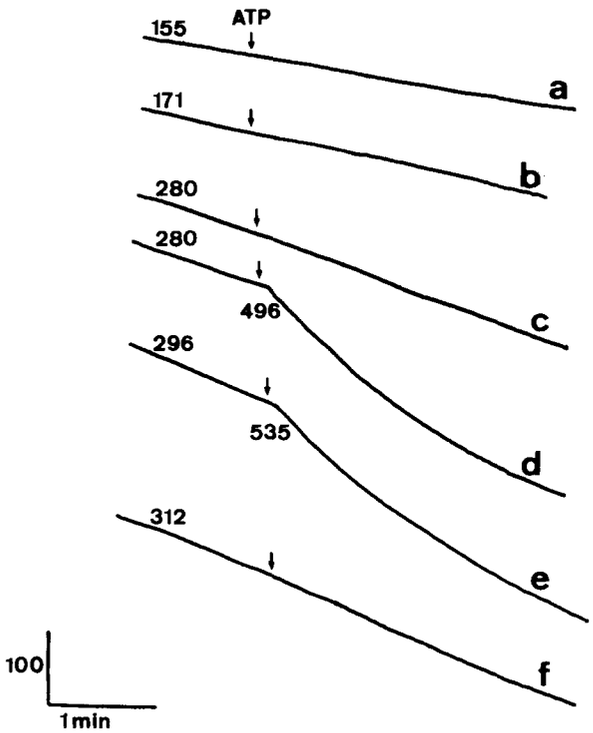

Fig. 1. Stimulation of respiration by ATP in a KCl-containing medium. Mitochondria (0.5 mg/ml) were suspended in respiration buffer without KCl (a,b) or supplemented with 100 mM KCl (c,d,e,f), at 0.5 mM (b,d,e,f) or 5 mM (a,c) phosphate. Arrows indicate the addition of 2 mM ATP. In (e), the experiment was done in the presence of 20 μg/mg protein oligomycin, and in (f) in the presence of 35 μM atractyloside. The respiration rates are given in nmol O/min/mg protein. The vertical bar represents an oxygen content of 100 nmol O/ml.

on phosphate concentration and fully prevented by 4 mM phosphate (Fig. 2b).

This effect of ATP in a KCl-containing medium suggested the opening of a cation channel, and the dependence towards ATP and phosphate was reminiscent of the ATP-induced unspecific channel described by Guérin et al. [8]. Indeed, the opening of this ion channel could explain the stimulation of the respiration rate by decreasing the proton motive force, following K^+ entry into the matrix space. However, atractyloside (at a concentration which prevents state 3 respiration in a KCl-containing medium) completely inhibited the effect of ATP (Fig. 1, trace f); therefore, opposite to the ATP-induced unspecific channel [8], only matricial ATP stimulated the respiration rate in the presence of KCl.

We assayed different monovalent cations for their ability to replace K^+ in this effect; Table 1a reports the values of the initial respiration rates after ATP addition in KCl-, RbCl-, NaCl- and LiCl-containing respiration buffers. It appeared that ATP induced a significant stimulation in the presence of KCl and RbCl only.

3.2. *ATP-induced stimulation of respiration rate is due to a partial collapse of the transmembrane potential*

Since ATP is able to change the kinetic characteristics of the respiratory chain, namely at the cytochrome *c* oxidase level [9], we measured the effect of ATP and KCl on $\Delta\Psi$ to ensure that the stimulation of respiration was actually due to the opening of a K^+ electrophoretic transport pathway.

Whatever the phosphate concentration, ATP addition did not induce any change of the transmembrane potential of mitochondria respiring in the absence of KCl (not shown). In the presence of 100 mM KCl and at low phosphate concentrations, ATP induced a decrease in $\Delta\Psi$ (Fig. 3, trace a); this effect was reversed by adjusting the phosphate concentration to 5 mM. This showed that the stimulation of respiration by ATP was a consequence of a decrease in the transmembrane potential. We

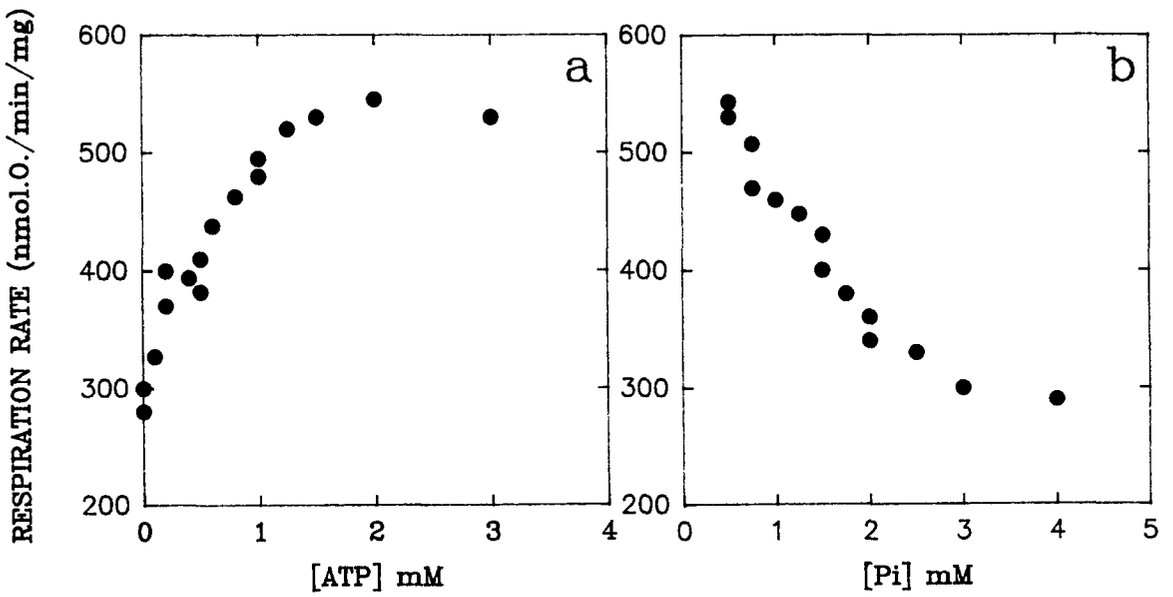

Fig. 2. Dependence of the stimulation of the respiration rate on ATP (a) or phosphate (b) concentrations. Mitochondria were suspended in 100 mM KCl-containing respiration buffer, supplemented with 20 μg/mg protein of oligomycin. In (a), the buffer contained 0.5 mM phosphate; in (b), stimulation of respiration was induced by 2 mM ATP.

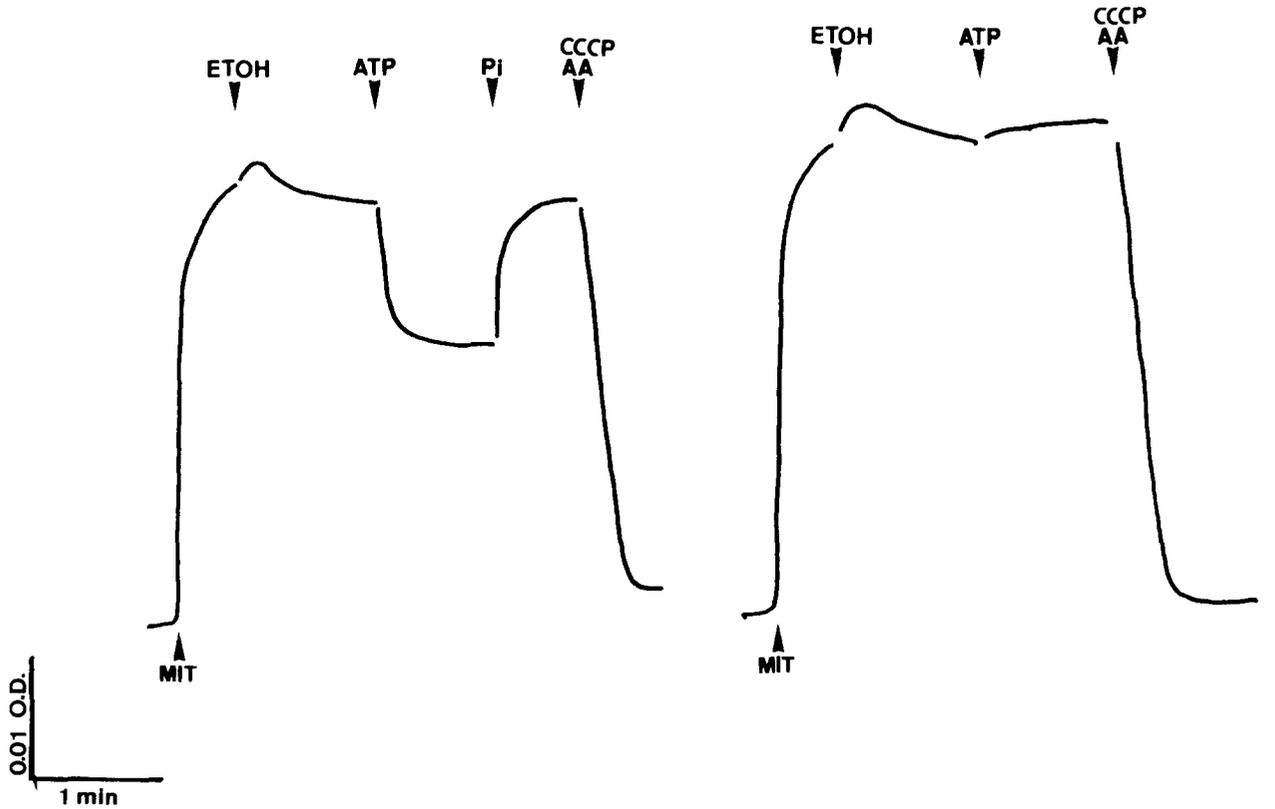

Fig. 3. Effect of ATP on the mitochondrial transmembrane potential created by the respiratory chain. The transmembrane potential of mitochondria was monitored by rhodamine 123 (2 μg/ml). Where noted, mitochondria (0.15 mg/ml) were suspended in respiration buffer containing 100 mM KCl and 20 μg/mg protein of oligomycin. Mitochondria were energized by ethanol, and the membrane potential collapsed by addition of 5 μM CCCP and 2.5 μg/mg protein of antimycin A. Experiments were done with 0.5 mM (a) or 5 mM phosphate (b). Where noted, 2 mM ATP was added. Trace a, reversal of the ATP effect by 5 mM phosphate.

verified that atractyloside prevented this decrease in $\Delta\Psi$ (not shown). As expected from results reported in Fig. 1, the presence of 5 mM phosphate before ATP addition prevented the $\Delta\Psi$ decrease (Fig. 3, trace b).

We also assayed the effect of ATP on the transmembrane potential in NaCl and LiCl respiration buffers (not shown); we noted a slight decrease in the presence of NaCl, and no change in the presence of LiCl. This was in accordance with the weak stimulation of respiration by Na^+, and the absence of stimulation by Li^+ (see Table 1a).

3.3. KCl prevents mitochondrial membrane energization by ATPase

To confirm that the effect of ATP was due to the opening of a K^+ electrophoretic transport pathway independently on any kinetic effect of ATP on the respiratory chain, we looked at the effect of KCl on the $\Delta\Psi$ created by the F_0F_1-ATPase.

Fig. 4 (trace a) shows that in the absence of KCl, ATPase supported a transmembrane potential; a few seconds after the DCCD addition, $\Delta\Psi$ was collapsed and was further completely abolished by CCCP. On the other hand, in the presence of 100

Table 1
Modulation of the ATP effect on respiration by different salts and divalent cations

a	+ KCl		+ RbCl		+ NaCl		+ LiCl	
	− ATP	+ ATP	− ATP	+ ATP	− ATP	+ ATP	− ATP	+ ATP
Respiration rate (nmol O/min/mg)	276	503	301	479	290	320	287	280

b	$MgCl_2$		$ZnCl_2$		$CaCl_2$	
	−	+	−	+	−	+
Respiration rate (nmol O/min/mg)	479	280	482	270	509	312

Mitochondria (0.5 mg/ml) were suspended in respiration buffer in the presence of 100 mM of the indicated salt (a) or 100 mM KCl (b). In all cases, 20 μg/mg protein of oligomycin and 0.5 mM phosphate were present, and ATP was used at 2 mM. (a) Effect of the addition of ATP in the presence of different salts. (b) Effect of divalent cations; concentrations were 5 mM for $MgCl_2$, 0.4 mM for $ZnCl_2$ and 1 mM for $CaCl_2$. Respiration rates are given in nmol O/min/mg protein.

mM KCl, ATPase was unable to maintain a high $\Delta\Psi$ (Fig. 4, trace b) unless 5 mM phosphate was present (Fig. 4, trace c). We concluded that whatever the way the inner mitochondrial membrane was energized, ATP partially dissipated the transmembrane potential in respiration buffer supplemented with KCl.

3.4. Inhibitors of the ATP uncoupling effect

Since ATP seemed to open an electrophoretic K^+ pathway, we tried to prevent the ATP uncoupling effect by inhibitors known to affect ionic pathways of the inner mitochondrial membrane. Inhibitors were assayed both in transmembrane potential (Fig. 5) and in respiration measurements (Table 1b).

3.4.1. Effect of amphiphilic amines. Guérin et al. [8] have reported that propranolol but not quinine, inhibited the ATP-induced unspecific channel. We noted that both inhibitors reversed the ATP-induced $\Delta\Psi$ decrease, and we reported in Fig. 1 (trace a) the effect of quinine. They also both prevented the ATP-induced stimulation of respiration (not shown). The inhibitory effect of quinine showed that the effect we observed was not due to the opening of the unspecific channel reported by Guérin et al. [8].

3.4.2. Effect of divalent cations. Zn^{2+} is a potent inhibitor of the K^+/H^+ exchange [5], and of the ATP-induced unspecific channel [8]. Fig. 5 (trace b) shows that it also prevented the ATP uncoupling effect. On the other hand, Mg^{2+} and Ca^{2+}, which had no effect on the K^+/H^+ exchange [5], had the same inhibitory effect as Zn^{2+} on the stimulation of respiration by ATP (Table 1b).

4. Discussion

In this paper, we report the existence of an ATP-induced $K^+(Rb^+)$ transport pathway in yeast mitochondria. In the presence of KCl and only at low phosphate concentrations, ATP induced a stimulation of state 4 respiration; this stimulation resulted from a decrease in the transmembrane potential. This decrease was observed whenever $\Delta\Psi$ was created by the respiratory chain or by the F_0F_1-ATPase. The opening of this electrophoretic transport pathway is therefore expected to have an uncoupling effect on oxidative phosphorylation.

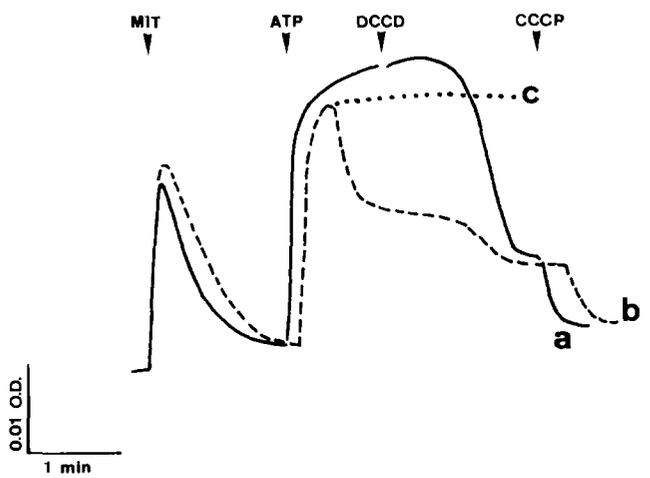

Fig. 4. Effect of KCl on the transmembrane potential created by the F_0F_1-ATPase. Mitochondria (0.15 mg/ml) were suspended in respiration buffer supplemented with 4 μg/mg protein of antimycin, and in the absence (a) or the presence of 100 mM KCl (b,c). Phosphate concentrations were 0.5 mM (a,b) or 5 mM (c). After total inhibition of the respiratory chain, 10 mM ATP, 20 μg/mg protein of DCCD (except in trace c), and 5 μM CCCP were added sequentially.

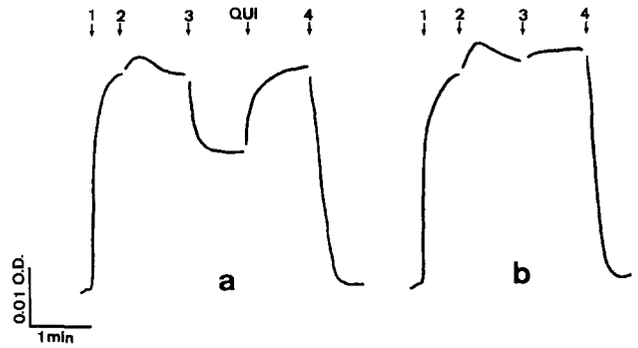

Fig. 5. Effect of quinine on the ATP-induced $\Delta\Psi$ decrease. The experiments were done under the same conditions as in Fig. 3, trace b. The arrows indicate: 1 for mitochondria (0.15 mg/ml), 2 for ethanol (40 mM), 3 for 2 mM ATP, 4 for 5 μM CCCP plus 2.5 μg/mg protein of antimycin A. Trace a, addition of 0.2 mM quinine (arrow noted QUI). Trace b, 0.4 mM $ZnCl_2$ was added in buffer.

The regulation by ATP and phosphate was reminiscent of the ATP-induced unspecific channel studied by Guérin et al. [8]; but we noted some obvious differences. First, only K^+ induced a significant uncoupling of respiration, whereas the ATP-induced unspecific channel can transport K^+ as well as Na^+; it is interesting to note that such a selectivity was previously reported for the electrophoretic pathway implicated in the non-energetic swelling of mitochondria suspended in KCl at alkaline pH [6]. Second, in contrast to the unspecific channel, only matricial ATP caused the K^+ channel to be open. The third difference was seen with the effect of inhibitors: the ATP-induced unspecific channel was not sensitive to quinine, opposite to the ATP-induced uncoupling effect in the presence of KCl. Further investigations are underway to find out the way by which ATP opens this $K^+(Rb^+)$ transport pathway.

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