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ADENINE NUCLEOTIDE CONTROL OF HEART MITOCHONDRIAL OSCILLATIONS

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SUMMARY

Oscillations of heart mitochondria were analyzed by measuring changes in transmittance, H^+, K^+, respiration rate and morphology. It has been demonstrated that an oscillatory flow of ADP and ATP may be an important synchronizing, as well as a controlling, factor of energy flows in this sytem.

The phenomenon of oscillatory transport of ions in mitochondria[1–6] is valuable for elucidating aspects of mitochondrial temporal organization, for investigating possible synchronizing mechanisms between mitochondria, and as a sensitive measure of their functional integrity. In the present investigation, the specific system used was developed for heart mitochondria by CHANCE AND YOSHIOKA[1]. It has several significant features. It involves the cyclic transport of H^+ and K^+, both parameters which can be easily measured and quantified. Further, this system has very little damping compared to other mitochondrial systems.

Mitochondria were isolated from heart tissue of pigeons. Isolation was similar to the method of CHANCE AND YOSHIOKA[1]. The final suspending medium was 0.25 M sucrose. The oscillation medium consisted of 0.25 M sucrose, 2.7 mM KCl, 2.0 mM K_2HPO_4, 2.0 mM glutamic acid and 2.0 mM malic acid, and the pH was brought to 6.25 using Tris base. The oscillations were initiated by the addition of valinomycin. All experiments were done at 25° using 0.8 mg mitochondrial protein per ml.

The various parameters used to measure the oscillations are shown in Fig. 1. Photometric means are the easiest and most sensitive way of measuring oscillations. The initial increase in transmittance is usually interpreted as swelling of the inner mitochondrial compartment[7]. The trace indicates a damped train of swelling and contraction cycles. A final abrupt decrease of transmittance is coincident with the depletion of O_2 in the system. The H^+ and K^+ concentrations in the medium are measured by selective ion glass electrodes. Initially K^+ is taken up by the mitochondria and associated with this is a H^+ expulsion. The respiration rate as a function of time was measured by electronically differentiating the signal from an O_2 electrode. Electron microscopy was used as a fifth parameter. By a rapid fixation procedure using 2% glutaraldehyde, the structural states of the mitochondria can be trapped at any given point of the oscillation[6]. Before the initiation of the oscillation the matrix material appears highly condensed between the cristae (contracted state). Many mitochondria can be shown to be entirely filled with matrix material at the first photo-

metric peak (swollen state). Some damaged mitochondria can also be observed, and as time goes on their number increases. The population of intact mitochondria seems to go through the swelling and contraction phases in correspondence to the photometric trace.

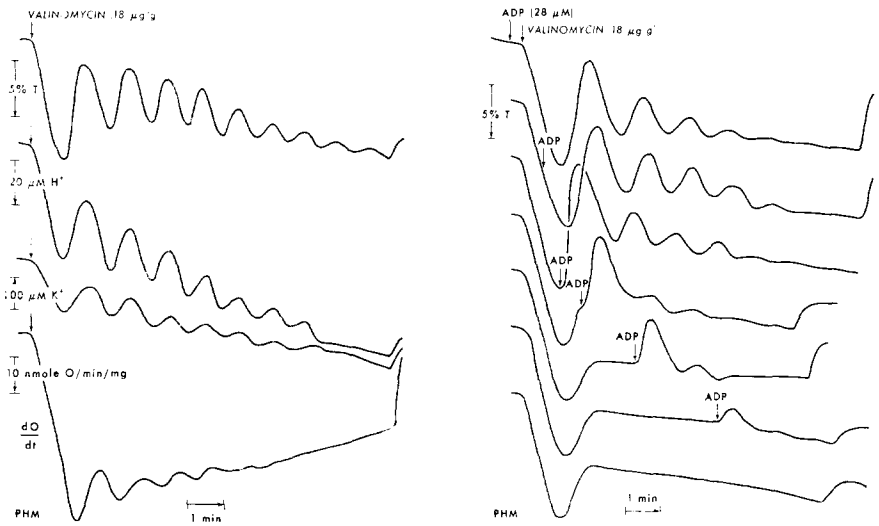

Fig. 1. Oscillations of pigeon heart mitochondria (PHM) as measured by changes of transmittance (T), H^+ and K^+ in the medium using glass electrodes and respiration rate (dO/dt) by differentiating a signal from the O_2 electrode. The direction of the arrow indicates an increase of the measured parameter. The initial O_2 concentration was 550 μM. Other experimental conditions are specified in the text, with the exception that 280 μM ADP was included in the medium.

Fig. 2. Conformational changes measured by transmittance as a function of added ADP at various times. Initial O_2 concentration was 550 μM. Other conditions are specified in the text.

ADP has an extremely important role in these oscillations. Without ADP generally only one rebound or a highly damped oscillation was observed. A very small amount of added ADP can greatly aid in sustaining the oscillation (Fig. 2). A similar effect has been reported by BOITEUX AND CHANCE[8]. This effect seems to quickly reach a threshold at about 25 μM ADP, above which more ADP has very little effect. ATP shows the same effect. 5'-AMP, which can be converted to ADP by the mitochondrial adenylate kinase, also shows the same effect. However, the nucleotides 2'-AMP and GTP have no effect. Further, it can be shown that the stimulating effect induced by ADP, ATP or 5'-AMP can be inhibited by the addition of oligomycin (Fig. 3). In the presence of oligomycin the oscillations are always reduced to a single rebound. The evidence thus indicates that a reversible ATPase reaction is a major factor in controlling these oscillations.

Atractyloside, a known inhibitor of ADP–ATP transport also reduces the stimulating effects of the nucleotides to one rebound as shown in Fig. 4. Pyrophosphate, which may be involved in the transport mechanism of ADP–ATP[9], does not cause a sustaining effect. Furthermore, pyrophosphate in the presence of ADP results in a more highly damped oscillation than with ADP alone. These experiments demonstrate the important role of the transport of these nucleotides in these oscillations.

The important controlling influence of ADP is demonstrated by adding this nucleotide at different times (Fig. 2). ADP always induces contraction relative to the response without ADP. This is commensurate with two possibilities. First, that in the

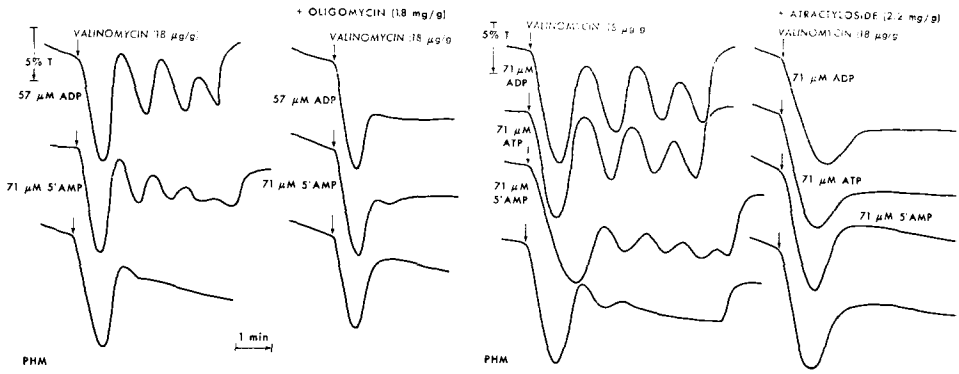

Fig. 3. The influence of oligomycin on the stimulating effects induced by ADP, 5'-AMP and the absence of nucleotide, in the reaction medium. The arrows indicate the point where valinomycin was added. The initial O_2 concentration was 240 μM. Other conditions are specified in the text.

Fig. 4. The influence of atractyloside on the stimulating effects induced by ADP, ATP, 5'-AMP and the absence of nucleotide in the reaction medium. The arrows indicate the point where valinomycin was added. The initial O_2 concentration was 240 μM. Other conditions as specified in the text.

presence of ADP, energy from the electron transport chain preferably supports phosphorylation, thus allowing ionic gradients that were being maintained by electron transport to collapse, which would correspond to the observed transmission change. Another possibility involves the known fact that such an ionic gradient in itself is a storage of energy. It has been shown that in the presence of ADP, such a gradient can collapse yielding its energy to the formation of ATP[10]. It is quite possible that both of these mechanisms may play a significant role.

Hence, ADP and ATP have a strong controlling influence on the functional and morphological state of mitochondria. Oscillations of ADP and ATP across the membrane are probably occurring because only small amounts of either ADP or ATP are required and since a reversible ATPase reaction is needed. Release of such nucleotides to the medium could have a strong synchronizing effect. Damping in most other mitochondrial systems is believed to be largely due to the loss of synchrony between individually oscillating mitochondria. It is concluded that the oscillations described here are so well sustained because of a strong synchronizing effect of oscillating concentrations of ADP and ATP in the medium.

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