THE POSSIBLE ROLE OF THE MITOCHONDRIAL BOUND CREATINE KINASE IN REGULATION OF MITOCHONDRIAL RESPIRATION

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It was shown recently that skeletal and heart muscle mitochondria contain bound creatine kinase; the bound enzyme is different from the cytoplasmic one (Jacobs, Heldt and Klingenberg, 1964). Because this enzyme is able, in the presence of creatine, to regenerate ADP continuously as phosphate acceptor for the respiratory chain it might have a role in the control of mitochondrial respiration in response to the requirements of muscle contraction.

This paper presents quantitative data on the role of bound creatine kinase in mitochondrial respiration and gives evidence that extra mitochondrial adenine nucleotides are required for the function of this acceptor system which does not act as an acceptor for intramitochondrial ATP.

Experimental

Pigeon breast muscle mitochondria were isolated by a method developed recently in this laboratory; its details will be described elsewhere. The preparations exhibited some ATPase activity, as judged from the acceleration of respiration after adding ATP. Respiration was measured with the vibrating platinum electrode using the Gilson oxygraph. The standard reaction medium contained 200 mM mannitol, 5 mM $MgCl_2$, 25 mM tris-Cl, 21 mM KCl, 5 mM potassium phosphate, 10 mM sucrose, 2, 5.10^{-5} M cytochrome c, and the final pH was 7.4. Substrates for respiration were either 5 mM glutamate or 5 mM α-ketoglutarate, this latter in the

presence of 1 mM malonate. The final volume was 2.8 ml, the final mitochondrial protein concentration was about 0.8 mg/ml, and the temperature 24°C. Varying amounts of creatine were added to the medium before adding the mitochondria and the adenine nucleotide concentration was altered during the course of respiratory measurement by successive additions of ATP or ADP.

Results and Discussion

Pigeon breast muscle mitochondria exhibit respiratory control: their respiration is increased several fold on adding ADP to the medium (State 3) and when the ADP is phosphorylated, the respiratory rate diminishes again (State 4). The rate after conversion of ADP to ATP is always somewhat higher than before its addition; this is a consequence of the ATPase activity in the preparation (Fig. 1A). When the medium contained creatine, the rate before adding ADP was similar to that without creatine. The ADP-stimulated rate was also the same with or without added creatine. In the presence of creatine, however, the respiratory rate did not return to the State 3 level (Fig. 1 B and C).

The mitochondria responded slightly, depending on its concentration, to added ATP (Fig. 2A, apparent ATPase activity). In the presence of creatine, ATP was a powerful stimulator of respiration. The respiratory rate was dependent on the concentrations of both the creatine and ATP (Fig. 2 B and C). The respiratory rate reflected the steady state concentration of ADP which was continuously expended by the mitochondrial respiratory chain and regenerated by the mitochondrial creatine kinase. In Fig. 3 the respiratory rate is plotted against the ATP concentration at different creatine concentrations. Apparently the ATP added was sufficient to saturate the system but the added highest creatine concentration probably failed to do so. The calculated half-maximal stimulation of respiration (in the presence of 214 μ M ATP) was at 7×10^{-3} M creatine concentration. In muscular

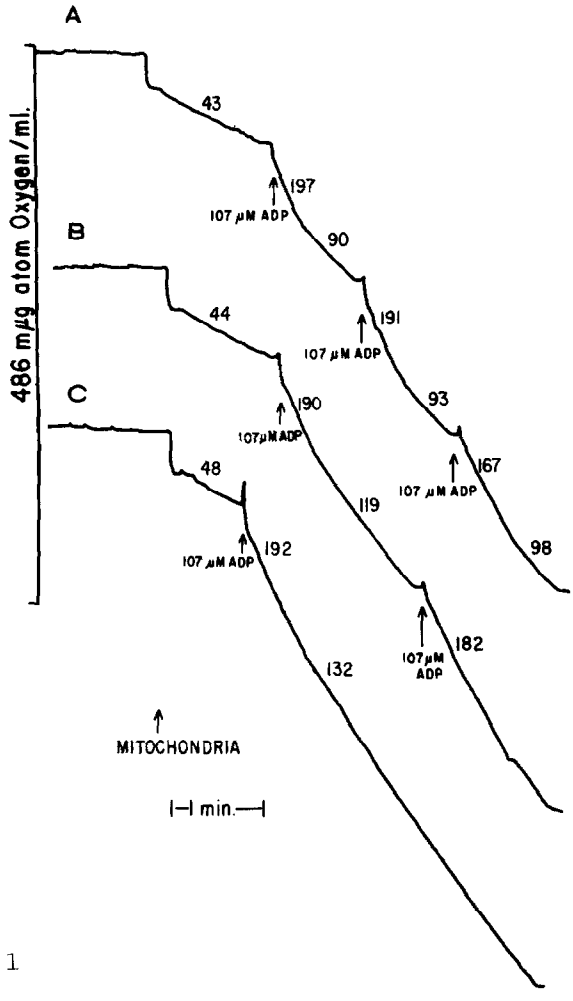

Figure 1

Respiratory control in pigeon breast muscle mitochondria in the absence and presence of creatine. A: control; B: 10 mM creatine; C: 20 mM creatine. 0.70 mg mitochondrial protein/ml; the numbers indicate m μg atom oxygen consumption/min. To avoid stirring artifacts, the pen of the oxygraph was turned off during the additions.

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tissue, the concentration of free creatine plus phosphocreatine is 20×10^{-3} M. This means that physiological changes in the free creatine concentration resulting from muscle contraction may trigger the change in respiratory rate.

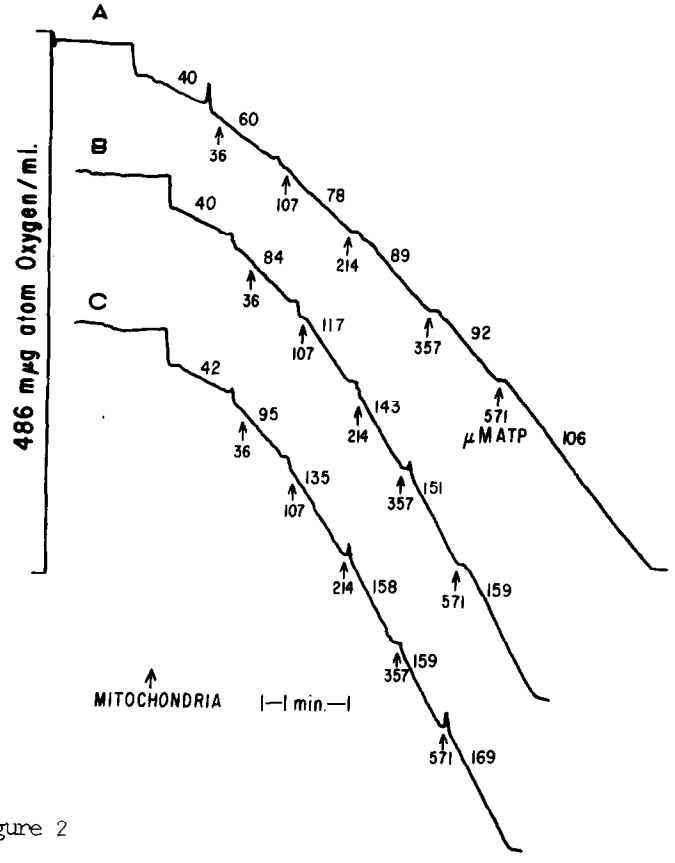

Figure 2

Mitochondrial respiration with different concentrations of creatine and ATP. A: control; B 10 mM creatine; C: 20 mM creatine. The numbers at arrows indicate the final concentration of ATP added in the reaction chamber; the numbers above the tracings are the oxygen consumption in m μg atoms/min. 0.68 mg mitochondrial protein/ml.

In the presence of 10 or 20 mM creatine, half-maximal stimulation occurred at about 60-80 μM ATP added. Even at the highest creatine and ATP concentrations employed, the respiratory rate was less than the State 3 respiration produced by 214 μM ADP. The steady state concentration of ADP produced through the mitochondrial bound creatine kinase system even at maximum physiological concentrations

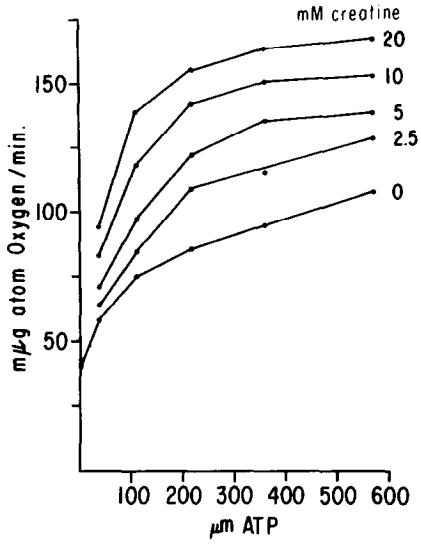

Figure 3

The dependence of mitochondrial respiration on ATP and creatine.

of substrate was lower than optimal for acceptor effect. It seems probable, therefore, that the bound creatine phosphokinase system can only partially activate the respiratory chain.

It is significant, that mitochondrially bound creatine kinase did not react appreciably with the intramitochondrially bound nucleotides. It required for its action the addition of adenine nucleotides. In the reverse reaction, the reduction of mitochondrial pyridine nucleotide driven by the phosphocreatine-creatine phosphokinase-ADP-system, Klingenberg (1963) demonstrated a dependency on added ADP. It seems probable therefore, that mitochondrial nucleotides participate only in certain specific transphosphorylation reactions. These facts are contradictory to the supposed role of bound creatine phosphokinase in a phosphate bound shuttle between the extra-and-intramitochondrial compartments. Its role might be rather the feed-back regulation of respiraton in response to muscular activity.

REFERENCES

1. Jacobs, H., Heldt, H.W. and Klingenberg, M., Biochem. Biophys. Res. Comm. 16, 516 (1964)

2. Klingenberg, M. in Energy-linked Functions of Mitochondria, (B. Chance, Ed.) Academy Press, New York, London, 1963 p. 141