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RAPID EFFLUX OF CA^{2+} FROM HEART MITOCHONDRIA IN THE PRESENCE OF INORGANIC PYROPHOSPHATE

Anibal Vercesi[1] and Albert L. Lehninger[2]

Department of Physiological Chemistry
The Johns Hopkins University School of Medicine
725 N. Wolfe Street, Baltimore, MD 21205

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SUMMARY: Inorganic pyrophosphate (PP_i) in the intracellular concentration range causes rapid efflux of Ca^{2+} from rat heart mitochondria oxidizing pyruvate + malate in a low Na^+ medium. Half-maximal rates of Ca^{2+} efflux were given by 20 μM PP_i. During and after PP_i-stimulated Ca^{2+} efflux the mitochondria retain their structural integrity and complete respiratory control. Carboxyatractyloside inhibits PP_i-stimulated Ca^{2+} efflux, indicating PP_i must enter the matrix in order to promote Ca^{2+} efflux. Heart mitochondria have a much higher affinity for PP_i uptake and PP_i-induced Ca^{2+} efflux than liver mitochondria.

Inorganic pyrophosphate (PP_i)[3] is generated at significant rates during fatty acid activation, biosynthesis of nucleic acids, proteins, lipids, and glycogen, and the formation of urea (1-3). Although animal tissues contain active pyrophosphatases (4), the steady-state concentration of PP_i in the tissues appears to be out of equilibrium with inorganic phosphate (1) and can become quite high, particularly during ATP-dependent activation of acetate, which largely occurs in the cytosol (5). PP_i levels are also influenced by endocrine state (3). These and other observations (3) suggest active metabolic role(s) for PP_i other than or in addition to its rapid hydrolysis by pyrophosphatases for the purpose of "pulling" biosynthetic reactions to completion (6,7).

[1] Present address: Departamento de Bioquimica, Inst. Biologia, UNICAMP, C.P. 1170, Campinas, SP-Brazil.

[2] To whom reprint requests should be sent.

[3] Abbreviations: PP_i, inorganic pyrophosphate; AdN, adenine nucleotides; AdN-T, adenine nucleotide translocator; P_i, orthophosphate; FCCP, carboxylcyanide trifluoromethoxyphenylhydrazone.

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PP_i has been shown (8) to enter rat liver mitochondria on the adenine nucleotide translocase (AdN-T) in exchange for matrix adenine nucleotides (AdN). However, in liver mitochondria this process requires high external PP_i concentrations, it is quite slow, and it results in loss of Ca^{2+} and other cations, as well as some structural degradation of rat liver mitochondria (8-10). The observations described here show that PP_i uptake and consequent Ca^{2+} efflux in heart mitochondria is a much more active process than in liver mitochondria and occurs without loss of respiratory control.

EXPERIMENTAL DETAILS

Rat heart mitochondria were prepared according to (11). Changes in Ca^{2+} concentration were recorded with a Ca^{2+}-sensitive electrode (Radiometer, F2112) calibrated with internal standards. O_2 uptake was monitored with a Clark-type electrode.

RESULTS

Effect of PP_i on Ca^{2+} retention and efflux. Figure 1A shows the effect of 100 μM PP_i on the uptake and retention of Ca^{2+} coupled to the oxidation of pyruvate + malate by rat heart mitochondria (11), in a medium containing 6.7 μM Ca^{2+} and 2.0 mM P_i. In the control without PP_i, Ca^{2+} uptake proceeded rapidly until the concentration remaining in the medium was about 0.3 μM. In the presence of 100 μM PP_i the initial rate of Ca^{2+} uptake was unaffected but only about 70 percent of the Ca^{2+} was taken up, followed by net Ca^{2+} efflux. Ca^{2+} efflux was accompanied by a slow and limited mitochondrial swelling, (lower traces), presumably caused by an increasing rate of Ca^{2+} cycling.

Figure 1B shows more directly that the failure of the mitochondria to retain Ca^{2+} in the presence of PP_i was primarily due to stimulation of Ca^{2+} efflux. In this experiment, otherwise identical to that in Figure 1A, ruthenium red was added to both systems after accumulation of most of the Ca^{2+}, to inhibit further Ca^{2+} influx on the Ca^{2+} uniporter. Net Ca^{2+} efflux then ensued, which was much faster in the presence of PP_i than in its absence. Neither system in the presence of ruthenium red showed significant swelling, as expected, since Ca^{2+} cycling was prevented by ruthenium red. Ca^{2+} efflux induced by PP_i is not due to its hydrolysis

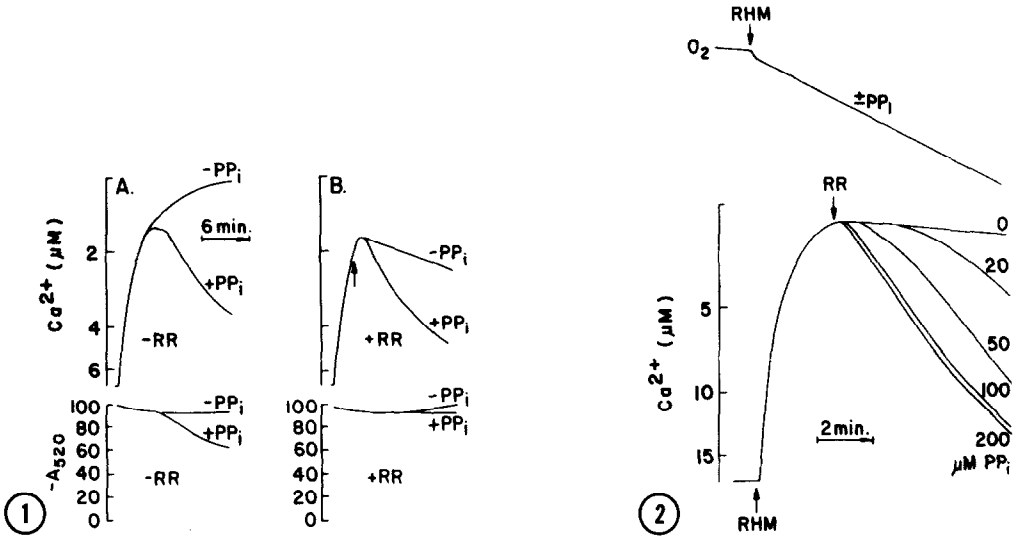

Figure 1. Effect of PP_i and ruthenium red on respiration-coupled Ca^{2+} uptake and release. The test system (1.8 ml; 25°) contained 130 mM KCl, 3.0 mM K^+HEPES pH 7.2, 2.0 mM P_i, 0.5 mM pyruvate, 0.5 mM malate, 100 μM PP_i. In A no ruthenium red was added; in B 0.1 μM ruthenium red was added at the arrow. Initial Ca^{2+} was 6.7 μM at zero time. The reactions were initiated by addition of rat heart mitochondria (1.0 mg protein).

Figure 2. Effect of PP_i concentration on rate of Ca^{2+} efflux and acceptor control. The test system was as shown in Figure 1, with PP_i concentrations as indicated. ADP was present at 800 μM in the O_2 uptake experiments. Reactions were started by addition of rat heart mitochondria (1.0 mg).

to P_i, which is well-known to cause efflux of Ca^{2+} from rat liver mitochondria (review, 12), since the systems in Figure 1 already contained 2.0 mM P_i. The Na^+ concentration was very low (< 2.0 mM) in all experiments described here; PP_i was added in the form of the K^+ salt.

Effect of PP_i concentration on Ca^{2+} efflux. Figure 2 shows that very low concentrations of PP_i stimulate Ca^{2+} efflux from heart mitochondria respiring on pyruvate + malate. After Ca^{2+} uptake had proceeded to near-completion in each system, ruthenium red was added to block the Ca^{2+} uniporter and the rates of Ca^{2+} efflux were recorded. In the absence of PP_i no Ca^{2+} efflux took place, showing the capacity of the mitochondria to maintain a constant Ca^{2+} set-point in the medium at about 1.0 μM under these conditions. The maximum rate of Ca^{2+} efflux, about 2.5 nmol Ca^{2+}/min/mg was given by 100 μM PP_i. At 20 μM PP_i, about the normal intracellular concentration (1,3,5), the rate of Ca^{2+} efflux, after a lag

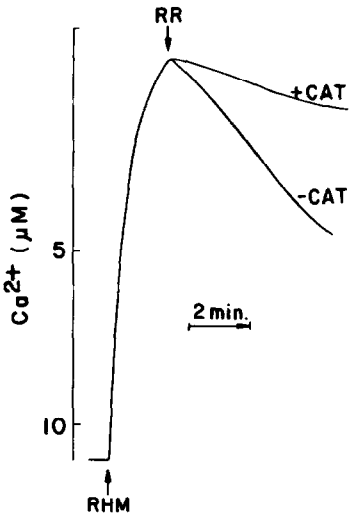

Figure 3. Inhibition of PP_i-induced Ca^{2+} efflux by carboxyatractyloside. The test system was as in Figure 2, with 100 μM PP_i and 1.0 μM carboxyatractyloside (CAT) also present. Ruthenium red (0.1 μM) was added at the point shown.

of about 2 min, was approximately half-maximal at ∿ 2 nmol/ min·mg, close to the rate of Ca^{2+} cycling in rat liver mitochondria maintaining a set-point of ∿0.3 μM (12). The O_2 uptake traces of these systems were identical (Figure 2, top); all showed a respiratory jump on addition of the mitochondria, corresponding to very rapid uptake of Ca^{2+}, followed by return to the pre-Ca^{2+} state 4 rate. Thus Ca^{2+} efflux stimulated by PP_i was not preceded or accompanied by loss of respiratory control, the most sensitive indicator of membrane potential. Addition of FCCP at the end of the experiment gave the expected large increase in O_2 uptake (not shown).

Involvement of the adenine nucleotide translocase in Ca^{2+} efflux induced by PP_i. Figure 3 shows that stimulation of Ca^{2+} efflux from rat heart mitochondria by PP_i in the presence of ruthenium red is blocked by carboxyatractyloside, indicating that this effect of PP_i requires its entry into the matrix, presumably in exchange for matrix ADP or ATP on the AdN-T. Ca^{2+} efflux thus may be caused by loss of adenine nucleotides from the matrix or, alternatively, it may be caused by matrix PP_i *per se*, with loss of matrix AdN relevant only because it is obligatory for the entry of PP_i.

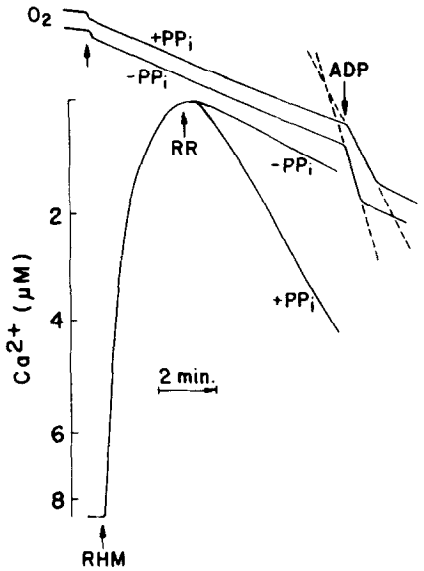

Figure 4. Effect of PP_i on Ca^{2+} retention and oxidative phosphorylation. The test system (1.8 ml; 25°) contained 130 mM KCl, 3.0 mM K^+HEPES pH 7.1, 1.0 mM pyruvate, 1.0 mM malate, 1.0 mM P_i, 100 μM PP_i where shown, and 0.1 μM ruthenium red, where shown. The reaction was begun by adding rat heart mitochondria (1.0 mg). The initial Ca^{2+} concentration was 8.3 μM. ADP (300 nmol) was added at the point shown to initiate phosphorylation (state 3).

Effect of PP_i-induced Ca^{2+} efflux on oxidative phosphorylation. In the experiments in Figure 4, rat heart mitochondria were allowed to take up Ca^{2+} from the medium in the presence or absence of 100 μM PP_i. After Ca^{2+} uptake was almost complete, ruthenium red was added. In the system containing PP_i, Ca^{2+} efflux was greatly stimulated, as in the preceding experiments. Again, the rate of O_2 consumption was identical to that in the control without PP_i. After much of the accumulated Ca^{2+} had been discharged by the PP_i, ADP was added to both systems to test their capacity for oxidative phosphorylation. Both systems immediately responded with large increases in the rate of O_2 uptake; after the added ADP was phosphorylated both systems returned precisely to the pre-ADP controlled rate. The ADP/O ratio in both the PP_i system and the control was 2.7. It will be noted, however, that the rate of state 3 O_2 uptake was about 30 percent lower in the system containing PP_i; a similar effect of PP_i was observed in rat liver mitochondria (8-10). The decrease in the ADP-induced state 3

respiration is probably caused by the decreased intramitochondrial AdN levels and consequent lowering of the AdN translocation rate.

Other observations. PP_i does not compete effectively with ADP for entry into rat heart mitochondria, since 100 μM PP_i does not inhibit state 3 respiration when added together with ADP. It is therefore more likely that PP_i enters by competing with medium ATP. Addition of Mg^{2+} significantly inhibits the action of PP_i in inducing Ca^{2+} efflux, presumably by forming a $MgPP_i$ complex which is not translocated. PP_i is much less effective in inducing Ca^{2+} efflux from rat heart mitochondria when succinate (in the presence of rotenone), rather than pyruvate + malate, is the respiratory substrate, suggesting that PP_i-induced Ca^{2+} efflux is favored by a relatively oxidized steady state of mitochondrial NADP, as has been shown for liver mitochondria (13).

DISCUSSION

The experiments reported here indicate that PP_i exchanges with matrix adenine nucleotide via the AdN-T of rat heart mitochondria at a much higher rate and affinity than has been reported for rat liver mitochondria (9-11). This observation may reflect a basic difference between mitochondria from excitable vs. non-excitable tissues. The former are known to have an extremely active Na^+/Ca^{2+} exchange as the main route for Ca^{2+} efflux (12,14) and a less active H^+/Ca^{2+} exchange than mitochondria from rat liver and other non-excitable tissues. All the experiments described here were carried out in a medium in which the Na^+ concentration (< 2 mM) was substantially below K_M for Na^+ (12,14), so that PP_i induced Ca^{2+} efflux may have occurred in part or wholly by Ca^{2+}/H^+ exchange. Stimulation of Ca^{2+} efflux from heart mitochondria by PP_i does not bring about irreversible collapse of membrane potential, since there was complete retention of respiratory control during and after Ca^{2+} efflux.

Because PP_i is generated metabolically not only in the cytosol but also within mitochondria, largely during activation of fatty acids, it appears possible that PP_i translocation across the inner membrane of heart

mitochondria is a factor controlling the relative concentrations of Ca^{2+} in the cytosolic and matrix compartments of the heart, particularly the Ca^{2+} level of the matrix, which recent studies indicate may be the primary function of the Ca^{2+} influx and efflux systems of heart mitochondria (15). Moreover, PP_i translocation may also be a factor controlling shifts of adenine nucleotides, particularly ATP, between the cytosol and matrix compartments and thus their relative phosphorylation potentials.

ACKNOWLEDGEMENTS

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