THE EFFECT OF CA^{2+} ON THE OXIDATION OF EXOGENOUS NADH BY RAT LIVER MITOCHONDRIA

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Received September 15,1975

SUMMARY

The respiratory rate of rat liver mitochondria in the presence of NADH as exogenous substrate is enhanced by the addition of $CaCl_2$ (> 50 μM) when inorganic phosphate is present in the medium. The Ca-induced oxidation of NADH is inhibited by rotenone but is not affected by uncoupling agents. EDTA, which does not reverse the swelling of mitochondria which occurs in the presence of Ca^{2+} and phosphate, is able to inhibit reversibly the Ca-stimulated NADH oxidation. A stimulation of the rate of oxidation of NADH by Ca^{2+} is also observed in mitochondria partially swollen in a hypotonic medium.

INTRODUCTION

Externally added NADH is not readily oxidized by mammalian mitochondria, due to the impermeability of the mitochondrial membrane to NADH (1-2). In these mitochondria NADH is oxidized at a high rate only when either the mitochondria membrane is damaged or cytochrome C is added (1). In this last case the oxidation of NADH occurs through a non phosphorylating external pathway, and is mediated by a rotenone insensitive NADH-cytochrome C reductase located in the outer membrane of mitochondria (2,3). Externally added NADH can be easily oxidized by yeast and plant mitochondria and its oxidation is accompanied by phosphorylation (4,5). Since mammalian, as well as yeast and plant mitochondria, are impermeable to NADH and NAD (2,6,7) it has been suggested that yeast and plant mitochondria contain two different and separate NADH dehydrogenase, one located on the outer surface and one on the inner surface of the inner

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Abbreviations used are: EDTA: ethylendiaminetetracetic acid; HEPES: N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; DNP: 2,4,dinitrophenol; SMP: submitochondrial particles

membrane (6,7) whereas mammalian mitochondria have only one dehydrogenase, on the matrix side of the inner membrane (8).

A stimulation by Ca^{2+} of the oxidation of NADH has been observed in microsomal membranes and in plant mitochondria (3,5,9). Recent studies have also shown the importance of Ca^{2+} on the oxidation of NAD-linked substrate by mitochondria and it has been observed that Ca^{2+} induces an inhibition of the respiration, accompanied by a depletion of the endogenous pyridine nucleotides (10,11).
In this paper it is shown that Ca^{2+} stimulates the oxidation of endogenously added NADH in partially swollen mitochondria. The Ca^{2+} stimulated oxidation is sensitive to rotenone and is blocked reversible by EDTA.

MATERIALS AND METHODS

Liver mitochondria were prepared from albino rats fasted for 12 hours. The liver was homogenized in 0.25M sucrose, 5 mM HEPES pH 7.0 and 0.5 mM Na-EDTA, and washed once with the same medium. A medium without EDTA was used for the last washing and for the final suspension of mitochondria. The protein content of the suspension was measured by the biuret method. Submitochondrial particles were prepared as described by C.P. Lee and L. Ernster (12). Oxygen consumption was measured polarographically with a Clark type oxygen electrode. Swelling was measured by the change in absorbance at 540 nm in a DB/GT Beckman spectrophotometer or an Aminco DW-2UV/VIS spectrophotometer. In some experiments the oxygen consumption and swelling were measured simultaneously in a stirred cuvette in which an oxygen electrode was inserted. All the experiments were carried out at 25°C. The NADH solution at pH 8.0 was prepared fresh every day.

RESULTS AND DISCUSSION

The respiratory rate of rat liver mitochondria in the presence of NADH is low, and is not stimulated by the addition of ADP or uncouplers (Fig. 1A). However, when Ca^{2+} at a concentration higher than 50 μM is added to the system, a rotenone-sensitive increase in the rate of respiration is observed. The effect can not be duplicated by other divalent cations like Mg^{2+}, Sr^{2+}, or Mn^{2+}, although these cations can prevent the stimulation by Ca^{2+} when added at high concentrations (Fig. 1B). Since the Ca-induce oxidation of NADH occurs only in a medium containing inorganic phosphate, which could promote the swelling of mitochondria, the changes in light scattering

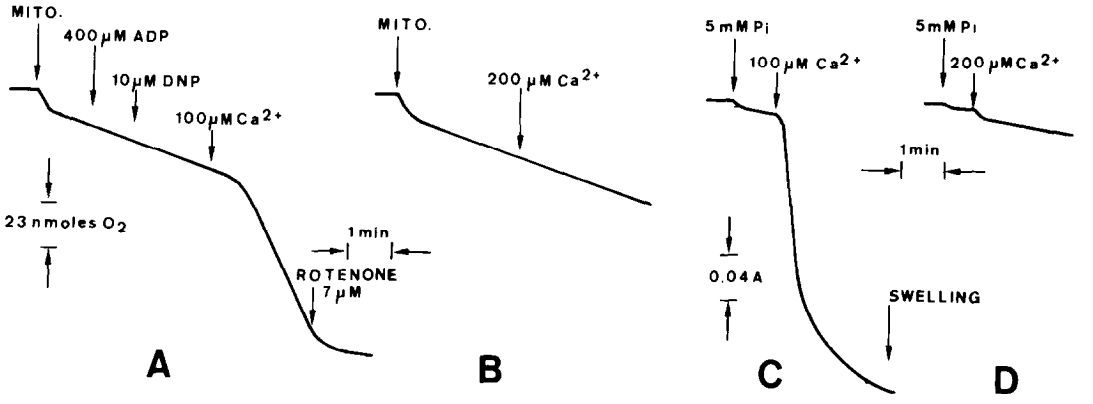

Fig. 1. The effect of Ca^{2+} on the respiration and swelling of mitochondria in the presence of NADH as exogenous substrate. The medium contains 0.15 M sucrose, 0.05M KCl, 5 mM HEPES, pH 7.0, 4 mM NADH, 5 mM phosphate buffer pH 7.0 (only in A and B), and 1mg prot./ml. The medium was supplemented with 5 mM $MgCl_2$ or $SrCl_2$ (in B and D). Other additions are indicated in the figures.

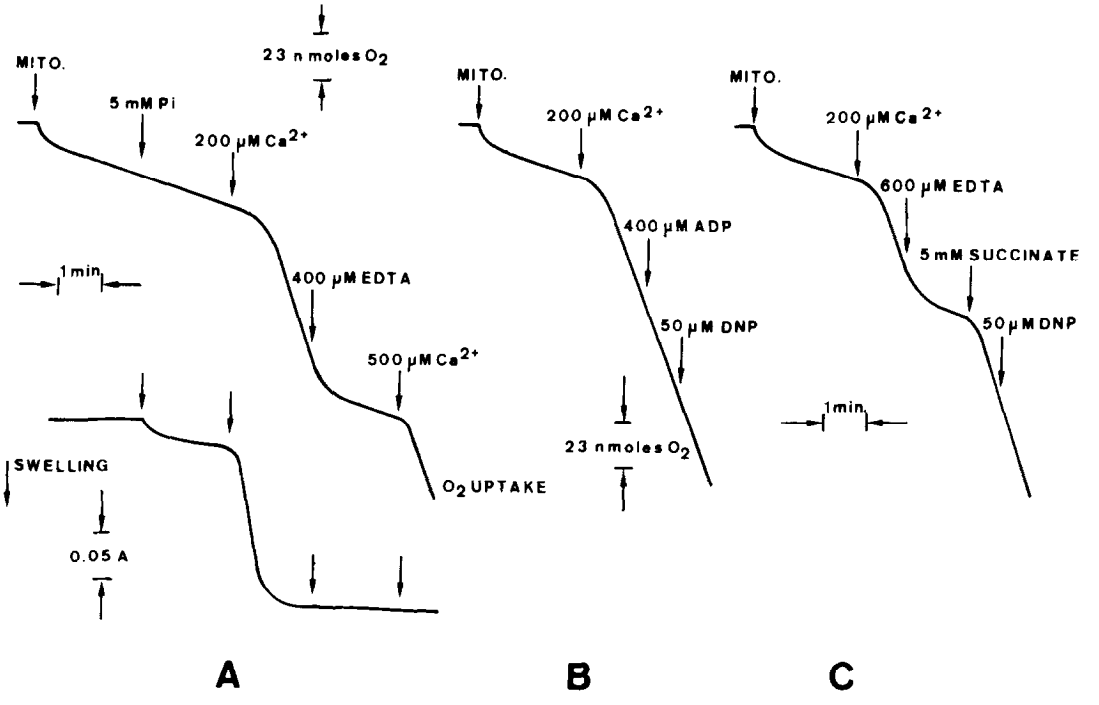

Fig. 2. Effect of EDTA on the Ca-stimulated oxidation of NADH and on the swelling of mitochondria. Basic medium as described in Fig. 1, phosphate buffer was present only in B and C. Other additions are indicated in the figures.

of the suspensions were tested. Fig. 1C shows that Ca^{2+} indeed increases the swelling induced by phosphate, and almost no swelling occurs when the medium contains $MgCl_2$ or $SrCl_2$ (Fig. 1D). The Ca-stimulated oxidation of NADH is inhibited by EDTA, the inhibition is reversed by excess Ca^{2+} (Fig. 2A) but not by other divalent cations. EDTA, however, does not reverse the swelling of mitochondria. Therefore, Ca^{2+} seems to be specifically required for the oxidation of NADH when mitochondria are swollen. The swelling induced by Ca^{2+} seems to cause uncoupling of mitochondria. Infact, the rate of oxygen uptake during the Ca^{2+}-stimulated NADH oxidation is not enhanced by the addition of ADP or DNP (Fig. 2 B), and as shown in Fig. 2 C, the oxidation of succinate after removal of Ca^{2+} with EDTA is not stimulated by the addition of uncouplers. The role of Ca^{2+} and other cations on the oxidation of NADH has also been studied using mitochondria partially swollen in hypotonic buffer without phosphate. Again, Ca^{2+} stimulates the oxidation of NADH and the stimulation is inhibited by EDTA (Fig. 3A). A slight stimulation is observed also with Sr^{2+} (not shown). The possibility of a direct effect of Ca^{2+} on the NADH dehydrogenase has been studied using inside-out submitochondrial particles, which should

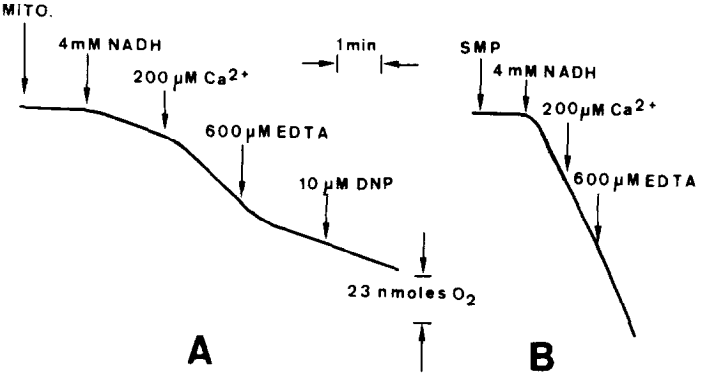

Fig. 3. Polarographic traces of the oxidation of NADH by hypotonically swollen mitochondria and by submitochondrial particles. In A the reaction medium contains 6 mM HEPES, 4 mM sucrose and 1mg of mitochondrial protein/ml. Mitochondria were preincubated in the reaction medium for 3 minutes before adding NADH.
The reaction medium in B is as described in the legend of Fig. 1, but instead of mitochondria 1 mg/ml of submitochondrial particles were added.

have the dehydrogenase exposed on the outside. In this case Ca^{2+} did not stimulate but rather inhibited slightly the oxidation of NADH (Fig. 3B).

The data presented suggest that when the permeability of the mitochondrial membrane is changed as it is in swollen mitochondria, Ca^{2+} favours the transport of NADH to the site in the inner membrane where the dehydrogenase is located. This could occur through the formation of a NADH-Ca complex, which could be transported into the hydrophobic core of the membrane as has been recently proposed by Vinogradov and Scarpa (10). The possibility of a direct effect of Ca^{2+} on the dehydrogenase is excluded, since Ca^{2+} does not change the activity of the dehydrogenase when it is exposed to the outside as in the submitochondrial particles. The data are also in agreement with the finding that Ca^{2+} induces an increased permeability of the mitochondrial membrane to endogenous NADH and NAD, in this way inhibiting the oxidation of NAD-dependent substrates (10, 11).

ACKNOWLEDGEMENT

The Author wishes to thank Professor E. Carafoli for his interest and valuable criticism.

REFERENCES

1. Lehninger, A.L., (1951) J. Biol. Chem., 190, 345
2. Lehninger, A.L., (1954-55) Harvey Lectures p. 17, Academic Press, New York
3. Sottocasa, G.L., (1968) Membrane Models and Formation, Bolis, L., and Pethica, B.A., eds. p. 229, North Holland Publishing Co., Amsterdam
4. Ohnishi, T., Kawaguchi, L. and Hagiharab, T., (1966) J. Biol. Chem. 241, 1797
5. Hackett, D.P. (1961) Plant Physiol. 36, 445
6. von Jagow, G. and Klingenberg, M., (1970) Eur. J. Biochem. 12, 583
7. Palmer, J.M., and Passam, H.C. (1970) Biochem. J. 122, 16 p
8. Klingenberg, M., and von Jagow, G. (1970) Electron Transport and Energy Conservation, Tager, J.M., Papa, S., Quagliariello, E., Slater, E.C., eds. p. 281, Adriatica Editrice, Bari.
9. Coleman, J.O.D., and Palmer, J.M. (1971) FEBS Letters 17, 203
10. Vinogradov, A., Scarpa, A., and Chance, B. (1972) Arch. Biochem. Biophys. 152, 646
11. Eboli, M.L., Cittadini, A. and Terranova, T. (1974) Experientia 30, 1125
12. Lee, C.P., and Ernster, L. (1967) Methods in Enzymology vol. X, p. 543, Colowick, S.P. and Kaplan, N.O., eds. Academic Press, New York.