DEMONSTRATION OF A MITOCHONDRIAL ENERGY-DEPENDENT PYRIDINE NUCLEOTIDE TRANSHYDROGENASE REACTION

Lennart Danielson and Lars Ernster

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden.

Received December 3, 1962

Studies reported in the preceding communication (Danielson and Ernster, 1962) have led to the conclusion that pyridine nucleotide-linked dismutations in rat liver mitochondria require a supply of high-energy intermediates in order to proceed at maximal rate. The present paper describes direct evidence for an energy-dependent pyridine nucleotide transhydrogenase reaction, involving a reduction of TPN by DPNH, catalyzed by submitochondrial preparations from both rat liver and beef heart.

Experimental.- "Phosphorylating" submitochondrial particles were obtained by sonication of mitochondria from rat liver according to Kielley and Bronk (1958), and from beef heart according to Linnane and Ziegler (1958) with the modifications of Löw and Vallin (1963)[1]. All incubations were performed at 30°C, using a final volume of 3 ml. The composition of the reaction mixtures is specified in the figure legends. Pyridine nucleotide reduction was recorded at 340 mμ with a Beckman DK-2 spectrophotometer.

Results.- Fig. 1 summarizes typical data on the energy-dependent reduction of TPN by DPNH. Rat liver or beef heart particles were incubated in a buffered Mg^{++}--containing medium in the presence of KCN, ethanol, alcohol dehydrogenase and a relatively small amount of DPN. When the reduction of DPN by the alcohol dehydrogenase system was completed, 0.7 μmole of TPN was added. No appreciable increase in A_{340} occurred. When 6 μmoles of ATP were now added, A_{340} increased at a linear rate. No similar increase in A_{340} was observed if DPN or TPN was omitted. ADP,

[1] We are indebted to Dr. Hans Löw for kindly supplying us with the beef heart particles.

when substituted for ATP, had no effect in the case of liver particles; it did have an effect in the case of heart particles but only after a lag period. The ATP-induced reduction of TPN was completely inhibited by oligomycin. It was insensitive to Amytal, Rotenone and dinitrophenol (0.1 mM). Similar results to those shown in Fig. 1 were obtained when DPNH rather than DPN was added at the start, together with ethanol and alcohol dehydrogenase, in order to maintain DPN in the reduced state. In the case of the liver particles, ethanol and alcohol dehydrogenase could be replaced by β-hydroxybutyrate since the particles contained a sufficiently active β-hydroxybutyric dehydrogenase.

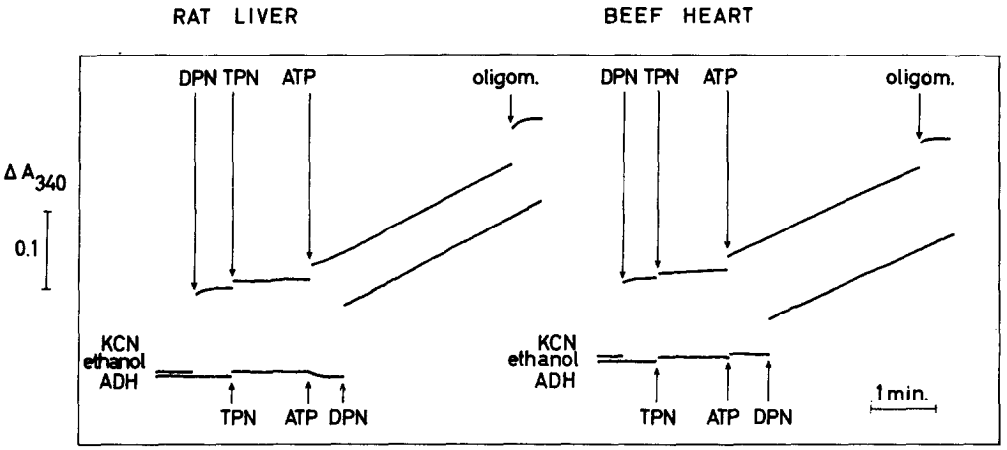


Fig. 1. ATP-dependent reduction of TPN by DPNH.

The basic reaction mixture consisted of 50 mM tris buffer (pH 8.0), 6 mM $MgCl_2$, and 250 mM sucrose. Liver or heart particles containing 0.65 mg protein. Additions, when indicated, were: 1 mM KCN, 53 mM ethanol, 0.1 mg alcohol dehydrogenase (ADH), 0.033 mM DPN, 0.233 mM TPN, 2 mM ATP, 3 μg oligomycin.

The particles also catalyzed the converse reaction, i.e. reduction of DPN by TPNH, but this required no added ATP (Fig. 2).

Low et al. (1961,1962) have demonstrated that beef heart particles catalyze an ATP dependent reduction of DPN by succinate. The same reaction was observed here with liver particles (Fig. 3a). The formation of DPNH was ascertained by adding acetoacetate which caused a rapid drop in A_{340}. When TPN was added together with DPN (Fig. 3b), the same rate of pyridine nucleotide reduc-

tion was observed as with DPN alone. However, in this case, addition of acetoacetate caused no drop in A_{340}, indicating that TPNH rather than DPNH was the reaction product. This reduction of TPN by succinate proceeded via DPN, as shown by the fact that, when DPN was omitted, no reduction of TPN occurred (Fig. 3c). The ATP-dependent succinate-linked pyridine nucleotide reduction was, in accordance with earlier observations (Chance and Hollunger, 1961; Löw et al., 1961, 1962, 1963), sensitive to Amytal, Rotenone and oligomycin, as well as to dinitrophenol (41 % inhibition by 0.02 mM, and 100 % by 0.1 mM dinitrophenol).

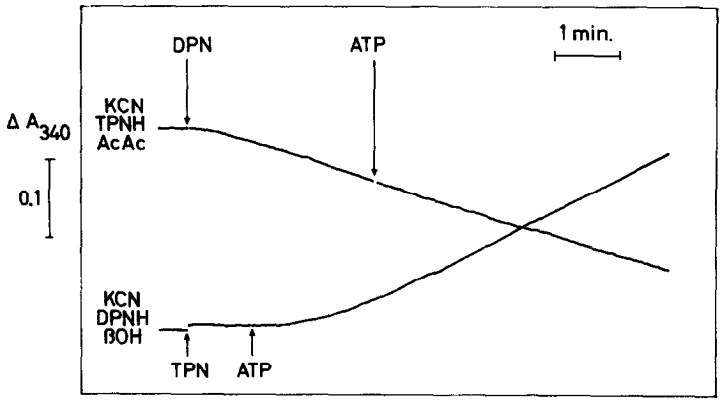


Fig. 2. ATP-dependent reduction of TPN by DPNH and ATP-independent reduction of DPN by TPNH.

The basic reaction mixture was as in Fig. 1. Liver particles containing 0.55 mg protein. Additions, when indicated, were: 1 mM KCN, 0.67 mM acetoacetate (AcAc), 3.3 mM DL-β-hydroxybutyrate (βOH), 0.167 mM DPNH, 0.233 mM TPNH, 0.167 mM DPN, 0.233 mM TPN, 2 mM ATP.

Energy-dependent reduction of TPN by DPNH could also be achieved under aerobic conditions (Fig. 4). In this system, addition of ATP was not required, the energy being supplied by the aerobic oxidation of DPNH. When the latter was blocked, e.g. by Rotenone, TPN reduction was abolished. Addition of succinate to the Rotenone-blocked system restored TPN reduction, which again could be abolished upon further addition of cyanide. As could be anticipated, the TPN reduction under these conditions was sensitive to dinitrophenol (30 % inhibition by 0.02 mM, and 95 % by 0.1 mM). It was unaffected, or even stimulated, by oligomycin, indicating that the energy-transfer did not involve P_i. The non-involvement of P_i could be demonstrated directly by preparing liver partic-

les in tris rather than in phosphate buffer, thus avoiding as much as possible the presence of trace amounts of P_i during the incubation. This procedure did not affect the ability of the particles to exhibit energy-linked reduction of TPN by DPNH. Moreover, arsenate, in the presence of oligomycin, did not interfere with the reaction. These features are similar to those of the succinate--linked DPN reduction observed with intact mitochondria (Ernster, 1961,1962,1963; Snoswell, 1962) where high-energy compounds generated by the aerobic oxidation of succinate serve as the source of energy.

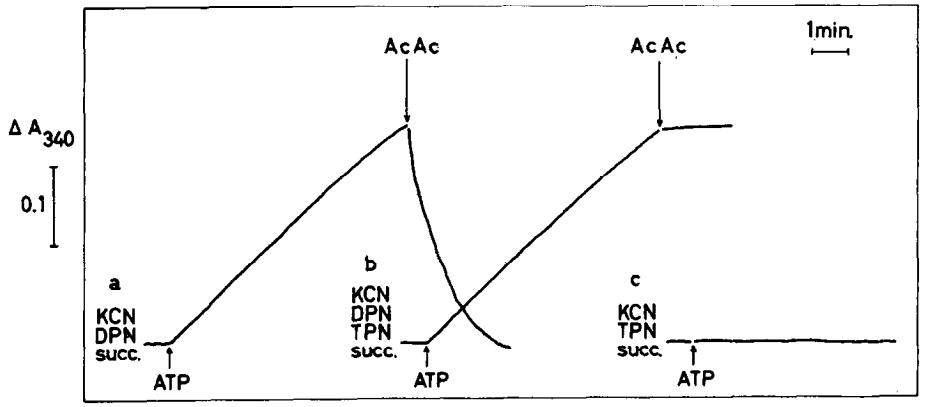


Fig. 3. ATP-dependent reduction of DPN and TPN by succinate.

The basic reaction mixture was as in Fig. 1. Liver particles containing 0.65 mg protein. Additions, when indicated, were: 1 mM KCN, 3.3 mM succinate, 0.167 mM DPN, 0.233 mM TPN, 2 mM ATP, 0.67 mM acetoacetate (AcAc).

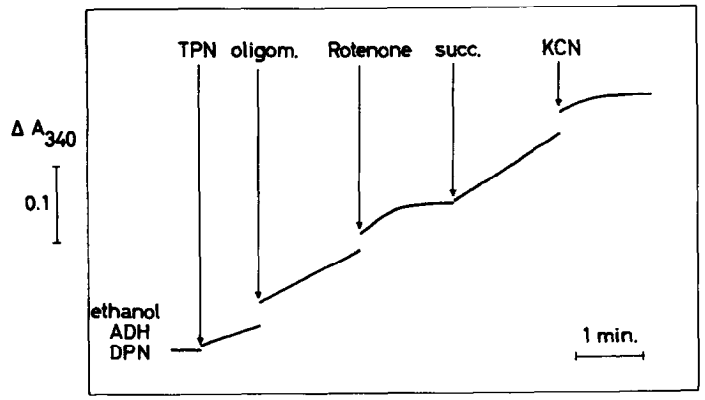
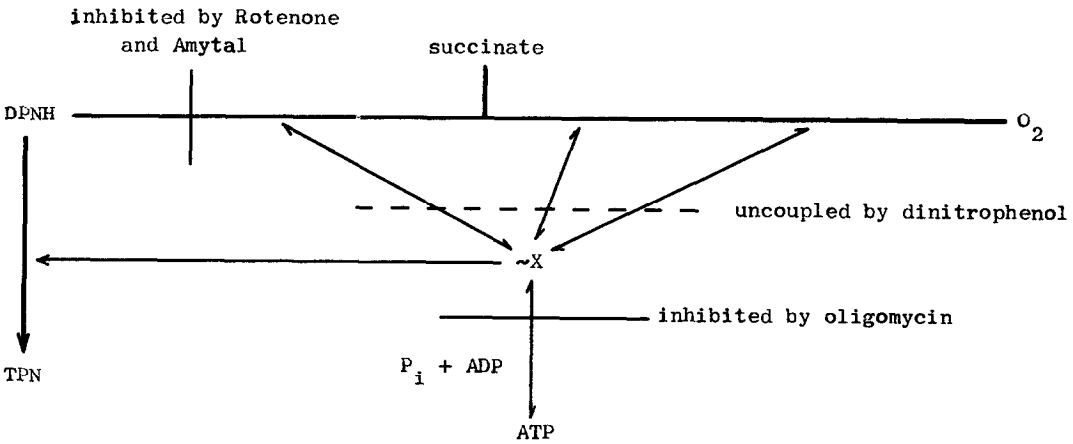


Fig. 4. Energy-dependent reduction of TPN by DPNH under aerobic conditions.

The basic reaction mixture was as in Fig. 1. Liver particles containing 0.65 mg protein. Additions, when indicated, were: 53 mM ethanol, 0.1 mg alcohol dehydrogenase (ADH), 0.033 mM DPN, 0.233 mM TPN, 3 μg oligomycin, 2 μg Rotenone, 3.3 mM succinate, 1 mM KCN.

Discussion.- The present results demonstrate the occurrence of an energy-dependent reduction of TPN by DPNH in mitochondria. Indications of such a reaction have in the recent past been reported by Klingenberg and Schollmeyer (1962) and by Estabrook (1963). This reaction is different from the transhydrogenase reaction between TPNH and DPN (Kaplan et al., 1953) which shows no requirement for high-energy compounds.

Our tentative interpretation of the results reported above may be summarized in the following scheme:



It is evident from the data that the energy-dependent TPN-reduction by DPNH utilizes high energy intermediates (here denoted by the collective symbol ~X) which can be generated either by the respiratory chain or by added ATP. It appears highly probable that the energy-dependent transhydrogenase reaction involves an activated form of either DPNH or TPN. It is conceivable that such an activated pyridine nucleotide is an intermediate in the energy-dependent dismutations recently described (Tager, 1962; Danielson and Ernster, 1962). The possible connection of the present observations with those of Purvis (1958,1960) and Griffiths and Chaplain (1962) on an activated derivative of DPN or DPNH is the subject of current studies in this laboratory.

This work has been supported by a grant from the Swedish Cancer Society. We thank Miss Kerstin Nordenbrand for excellent technical assistance.

REFERENCES.

Chance, B., and Hollunger, G. (1961) J.Biol.Chem., 236, 1534, 1555, 1562, 1577.

Danielson, L., and Ernster, L. (1962) Biochem.Biophys.Research Communs. 10, 85

Ernster, L. (1961) in "Biological Structure and Function", First IUB/IUBS Symp., Stockholm, 1960, Academic Press, London, 2, 139.

Ernster, L. (1962) in "Symposium on Intracellular Respiration", Proc.Vth Internatl Congr.Biochem., Moscow, 1961, Pergamon Press, Oxford, 5, 115.

Ernster, L. (1963) in "Die funktionelle und morphologische Organisation der Zelle' Symp.Ges.deut.Naturforsch.u.Ärtzte, Rottach-Egern, 1962, Springer-Verlag, Heidelberg, in press.

Estabrook, R.W. (1963) in "Die funktionelle und morphologische Organisation der Zelle", Symp.Ges.deut.Naturforsch.u.Ärtzte, Rottach-Egern, 1962, Springer -Verlag, Heidelberg, in press.

Griffiths, D.E., and Chaplain, R.A. (1962) Biochem.Biophys.Research Communs., 8, 497,501; Biochem.J., 85, 20P.

Kaplan, N.O., Colowick, S.P., and Neufeld, E.F. (1953) J.Biol.Chem., 205, 1.

Kielley, W.W., and Bronk, J.R. (1958) J.Biol.Chem., 230, 521.

Klingenberg, M. and Schollmeyer, P. (1962) in "Symposium on Intracellular Respiration", Proc.Vth Internatl.Congr.Biochem., Moscow, 1961, Pergamon Press, Oxford, 5, 46.

Linnane, A.W., and Ziegler, D.M. (1958) Biochim.Biophys.Acta, 29, 630.

Löw, H., Krueger, H., and Ziegler, D.M. (1961) Biochem.Biophys.Research Communs., 5, 231.

Löw, H., and Vallin, I. (1962) Biochem.Biophys.Research Communs., 9, 307.

Löw, H., and Vallin, I. (1963) Biochim.Biophys.Acta, in press.

Purvis, J.L. (1958) Nature, 182, 711.

Purvis, J.L. (1960) Biochim.Biophys.Acta, 38, 435.

Snoswell, A.M. (1962) Biochim.Biophys.Acta, 60, 143.

Tager, J.M. (1962) Biochem.J., 84, 64P.