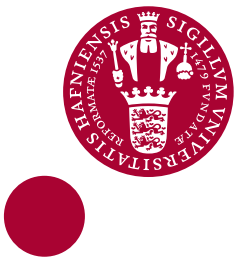

Oxidation rate of cytosolic NADH in isolated rat liver parenchymal cells and in perfused rat liver.

Quistorff, Bjørn; I.D. Thieden, Niels

Published in:
Regulation of Heparic Metbolism

Publication date:
1974

Document version
Publisher's PDF, also known as Version of record

1973

Oxidation Rate of Cytosolic NADH in Isolated Rat-Liver Parenchymal Cells and in Perfused Rat-Liver

Niels Grunnet, Bjørn Quistorff & Herluf I. D. Thieden

When ethanol is used to generate cytosolic NADH, the rate of ethanol disappearence may reflect the rate of oxidation of NADH in the cytoplasm. The oxidation of cytosolic NADH may be limited by e. g. the availability of reducible substrates in the cytosol, by transfer of reducing equivalents across the mitochondrial membrane or by the capacity of the respiratory chain.

The ethanol oxidation rate in isolated rat liver parenchymal cells may be compared to that of the isolated perfused liver by assuming that the perfused liver contains approximately 60 % parenchymal cells on a wet weight basis (Bücher *et al.* 1972).

As shown in Table I, the unstimulated ethanol oxidation rate in perfused rat liver (Ylikahri *et al.* 1971, Damgaard *et al.* 1974) is about twice that of isolated cells. In perfused rat liver, no 'fructose effect' is found (Ylikahri *et al.* 1971, Damgaard *et al.* 1973), and the rate of ethanol oxidation corresponds to the fructose-stimulated rate in isolated cells.

The ethanol oxidation by isolated liver cells exhibits a remarkable pyrazole inhibition pattern (Fig. 1). In the absence of fructose, low concentrations of pyrazole (below approximately 50 μM) do not inhibit the rate of ethanol oxidation, indicating a lack of correlation between the alcohol dehydroge-

Table I. Ethanol oxidation rate in isolated rat liver parenchymal cells and in isolated perfused rat liver.

Addition	Isolated Cells (μmol · min^{-1} · g^{-1} wet wt.)	Perfused Liver (μmol · min^{-1} · g^{-1} wet wt.)
None	0.9	~ 2.0
Fructose	2.0	~ 2.0

»Regulation of Hepatic Metabolism«, Munksgaard, Copenhagen.

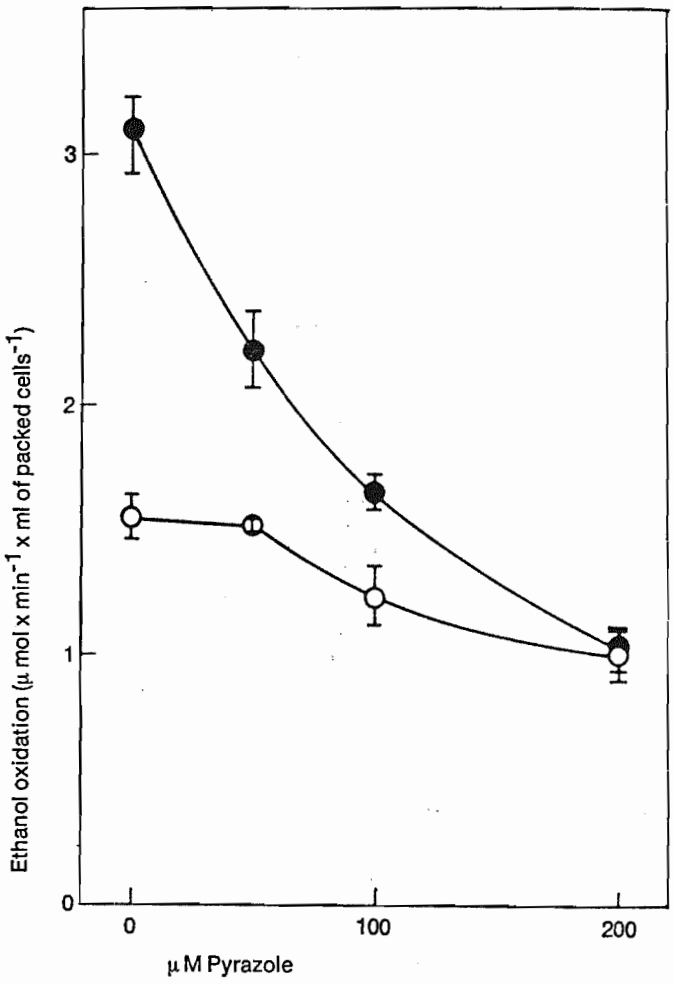

Fig. 1. Pyrazole inhibition of ethanol oxidation by isolated rat-liver parenchymal cells. Cells were isolated as described (Quistorff *et al.* 1973) and incubated with 4 mM ethanol (0) or 4 mM ethanol plus 8 mM fructose (●) (Grunnet *et al.* 1973). Bars indicate S.E. (four experiments).

nase activity and the ethanol oxidation rate. Upon addition of fructose, the rate is increased about 2.5 times and the ethanol metabolism now appears sensitive to even low concentrations of pyrazole (Fig. 1), indicating that in the presence of fructose, the maximal activity of alcohol dehydrogenase is rate-limiting for ethanol metabolism.

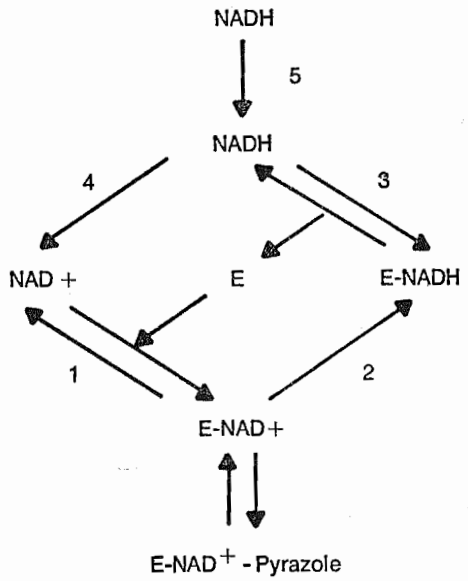

Fig. 2. Alcohol dehydrogenase catalyzed ethanol oxidation. Reactions 1–3 symbolize the Theorell-Chance mechanism for oxidation of ethanol to acetaldehyde (Theorell & Chance 1951), reaction 4 represents oxidation of cytosolic NADH and reaction 5 production of cytosolic NADH by reactions other than those catalyzed by the alcohol dehydrogenase.

These results may be explained in the following way (Fig. 2). The alcohol dehydrogenase catalyzed reaction most probably is limited by dissociation of the enzyme-NADH complex (Theorell & Chance 1951), which is very sensitive to inhibition by NADH concentrations within a physiological range (Grunnet *et al.* 1973), and therefore may be regulated by the rate of oxidation of cytosolic NADH (Fig. 2, reaction 4). We conclude that the unstimulated ethanol oxidation rate in isolated cells is limited by the rate of oxidation of cytosolic NADH and that this rate is lower in unstimulated isolated cells than in the unstimulated perfused liver (cf. Table I). Fructose addition to the isolated cells increases the oxidation rate of cytosolic NADH to a level not lower than that of the perfused liver.

REFERENCES

Bücher, Th., Brauser, B., Conze, A., Klein, F., Langguth, O. & Sies, H. (1972) State of oxidation-reduction and state of binding in the cytosolic NADH-system as disclosed

by equilibration with extracellular lactate/pyruvate in hemoglobin-free perfused rat liver. *Europ. J. Biochem. 27,* 301–317.

Damgaard, S. E., Sestoft, L. & Lundquist, F. (1974) Fate of ethanol carbon during fructose stimulated ethanol metabolism in the perfused pig and rat liver. In *Regulation of Hepatic Metabolism,* Alfred Benzon Symposium VI, ed. Lundquist, F. & Tygstrup, N., pp. 530–540. Munksgaard, Copenhagen.

Grunnet, N., Quistorff, B. & Thieden, H. I. D. (1973) Rate-limiting factors in ethanol oxidation by isolated rat-liver parenchymal cells. Effect of ethanol concentration, fructose, pyruvate and pyrazole. *Europ. J. Biochem. 40,* 275–282.

Quistorff, B., Bondesen, S. & Grunnet, N. (1973) Preparation and biochemical characterization of parenchymal cells from rat liver. *Biochim. biophys. Acta. 320,* 503–516.

Theorell, H. & Chance, B. (1951) Studies on liver alcohol dehydrogenase II. The kinetics of the compound of horse liver alcohol dehydrogenase and reduced diphosphopyridine nucleotide. *Acta chem. scand. 5,* 1127–1144.

Ylikahri, R. H., Hassinen, I. E. & Kähönen, M. T. (1971) Metabolic interactions of fructose and ethanol in perfused liver of normal and thyroxine-treated rats. *Metabolism 20,* 555–567.

DISCUSSION

LINDROS: We find that in a liver perfused in the absence of any added substrate you get the rate of ethanol oxidation down to 0.9 μmol/g per min, which is the same as you found in the cells in the absence of substrate.

GRUNNET: Yes, I should have said that these cells are always incubated in the presence of glucose but no other substrate. And the reason for this low oxidation rate of cytosolic NADH, of course, could be substrate depletion, although we find reasonable levels of malate and glutamate in the cells.

SCHIMASSEK: Dr. Lindros, how can you make a perfusion without substrates or substrate supply because the liver immediately produces amino acids?

LINDROS: This is not a problem with the once-through perfusion technique that we used.

BERRY: I think it is important to stress the different experimental conditions. In the perfused liver there is lactate in the medium. Lactate stimulates ethanol oxidation in liver cells. I think basically there isn't any significant difference between the two preparations in regard to ethanol oxidation. However, my own data does not agree with your findings on pyrazole and even low concentrations of pyrazole, I find, do inhibit the basal ethanol oxidation and I cannot explain the discrepancy. The effects of pyrazole is much more dramatic with fructose (Berry 1971) or with pyruvate, but it certainly gives an inhibition with the basal rate, also.

GRUNNET: I cannot explain that either, if you, too, have used pyrazole concentrations as low as 50 μM. But I think we agree that the capacity to oxidize ethanol is the same in the two preparations.

J. R. WILLIAMSON: What Michael Berry said certainly agrees with our data using methylpyrazole. We haven't used fructose but we have used dihydroxyacetone and glycerol as substrates and the methylpyrazole is 95 % or so effective in inhibiting ethanol uptake.

GRUNNET: It is more easy to make a titration with a less strong inhibitor, I think.

Berry, M. N. (1971) The action of fructose on ethanol metabolism by intact isolated liver cells. *Biochem. J. 123,* 40P.