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ALCOHOL METABOLISM AT HIGH ALCOHOL CONCENTRATIONS

Herluf I.D. Thieden, Bjørn Quistorff, and Niels Grønnet

Department of Biochemistry A, Panum Institute
University of Copenhagen, DK-2200 Copenhagen, Denmark

ABSTRACT

The incorporation rate of tritium from (R,S)ethanol-1-³H, (R)ethanol-1-³H and (S)ethanol-1-³H in lactate and β-hydroxybutyrate is investigated by means of a GLC method.

Preliminary results show no decrease in incorporation of ³H from (R)ethanol during the period of labelling (15 min). These results indicate that either a shift in the isotope effect occurs at high concentrations of ethanol, or the results may indicate that ethanol does not exhibit a substrate inhibitory effect on alcohol dehydrogenase in the liver cells. Furthermore, the fraction of acet-aldehyde metabolized in the cytosol is determined.

Ethanol is eliminated from the body mainly by metabolism in the liver. All evidence indicate that alcohol dehydrogenase is the principal enzyme responsible for ethanol oxidation, but alcohol dehydrogenase is not the only enzyme catalyzing the oxidation

of ethanol to acetaldehyde. Specific inhibitors of alcohol dehydrogenase such as pyrazole or 4-methylpyrazole have been used to inhibit that part of ethanol metabolism and by comparing the inhibited and control rates one may get a possible measure of the relative importance of the alternative ethanol oxidizing pathways, such as catalase (Thurman, Ley, and Scholz, 1972) or the microsomal ethanol oxidizing system (Lieber and DeCarli, 1970). A number of studies suggest that the rate of ethanol oxidation depends on the concentration of ethanol.

In liver slices we found higher rates of ethanol oxidation when the ethanol concentration was raised from 4 to 80 mM (Thieden, 1971) and these results have been corroborated by experiments with isolated liver cells (Fig. 1) and perfused rat liver (Damgaard, Sestoft, and Lundquist, 1975). This increase seems to be due to ethanol oxidation by pathway(s) different from alcohol dehydrogenase and with the K_m which is about one order of magnitude higher than that of alcohol dehydrogenase. This interpretation is supported by the lower inhibitory effect of pyrazole or 4-methylpyrazole on ethanol elimination at high concentrations of ethanol (Fig. 1).

It must, however, be noticed that this effect has not been observed in in vivo experiments (see Khanna and Kalant in this Symposium).

In this work we have used tritium-labelled ethanol as substrate. The purpose is to estimate the extent of participation of alcohol dehydrogenase in ethanol oxidation at high concentrations of ethanol and also to determine the intracellular location of acetaldehyde oxidation.

Methods

(S)ethanol-1-³H and (R)ethanol-1-³H was prepared enzymically by reduction of acetaldehyde. Other methods and preparation of liver cells from 24 h fasted female Wistar rats as in Grunnet, Thieden, and Quistorff, 1976.

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Fig. 1. Ethanol oxidation rate versus ethanol concentration.

The dotted lines represent values in the absence and the full lines in the presence of inhibitor (pyrazole or 4-methylpyrazole). The filled symbols are values from experiments with cells from fed rats (Selmer and Grunnet, 1976); the open symbols with cells from fasted rats (Grunnet, Thieden, and Quistorff, 1976).

Results and discussion

The extent of incorporation of the tritium from ethanol into lactate and β -hydroxybutyrate will depend on the intracellular generation of (^3H)NADH and thus on the participation of different pathways in ethanol oxidation (Fig. 2). The two hydrogen atoms on C-1 are different with respect of the products formed. The S-atom of (R,S)ethanol-1- ^3H is retained at the C-1 in acetaldehyde formed whether the oxidation is catalyzed by aldehyde dehydrogenase, catalase or the microsomal oxidation system. Further oxidation of acetaldehyde will result in formation of (^3H)NADH.

Fig. 2

The R-atom is transferred to NAD only if the reaction is catalyzed by alcohol dehydrogenase. The oxidation of ethanol by an alternative pathway will generate tritium-labelled water.

It follows from these considerations that incorporation of tritium into lactate will depend on the formation of tritium-labelled NADH in the cytosol which may occur either through the action of alcohol dehydrogenase or cytoplasmic aldehyde dehydrogenase.

Fig. 3. Incorporation of tritium into lactate from (R,S)ethanol.

Triangles are the values actually measured and circles these values corrected for the detritiation of 2-³H-L-lactate. Open and filled symbols represent values in the presence and absence of 4-methylpyrazole (1.7 mM). The liver cells were incubated in buffer containing 25 mM HEPES and 3 mM pyruvate (Grunnet, Thieden, and Quistorff, 1976).

In experiments where 65 mM (R,S)ethanol-1-³H is used as substrate (Fig. 3) and 3 mM pyruvate is present in the incubation medium the labelling rate of lactate declines with time. The rate declines with the decrease in pyruvate concentration in the incubation medium and the concomitant fall in lactate production. The rate of tritium incorporated into lactate is 1.5 $\mu\text{mol} \times \text{min}^{-1} \times \text{ml}$ packed cells⁻¹ in the absence and 0.23 $\mu\text{mol} \times \text{min}^{-1} \times \text{ml}$ packed cells⁻¹ in the presence of 4-methylpyrazole. In these experiments the alcohol dehydrogenase-mediated ethanol oxidation rate was

determined to $3.18 \pm 0.19 \mu\text{mol} \times \text{min}^{-1} \times \text{g wet wt}^{-1}$ (N=5) indicating that less than 20 % of the acetaldehyde is oxidized in the cytoplasm.

This conclusion is supported by experiments where 65 mM ethanol specifically labelled in R-position or S-position was used as substrate (Table 2). The total activity incorporated into lactate using (S)ethanol was only 5 % of that using (R)ethanol as substrate, indicating that about 5 % of the acetaldehyde formed may be metabolized in the cytoplasm.

Using (R)ethanol as substrate a rather constant fraction of tritium is incorporated into lactate (Table 1), indicating that this incorporation is proportional to the formation of (^3H)NADH in this compartment, even in case lactate and NADH in the cytosolic compartment should be in isotopic equilibrium (Cronholm, 1974). The fraction of tritium incorporated into lactate both from (R)ethanol and (S)ethanol is somewhat higher than found by Corral, Havre, Margolis, Kong, and Landau (1975) in liver slices; these workers did not add pyruvate to the incubation medium.

Table 1. Incorporation of tritium into lactate from (R)ethanol.

Liver cells from 24 h fasted rats were incubated in Krebs-Ringer bicarbonate buffer containing 3 mM pyruvate with 95 % O_2 - 5 % CO_2 as gas phase.

Time min	2- ^3H -L-lactate formed (nmol x ml packed cells $^{-1}$)	relative incorporation (%)
1	820 $\pm$ 215 (4)	39 $\pm$ 13
3	2299 $\pm$ 546 (4)	40 $\pm$ 4
5	2915 $\pm$ 361 (4)	37 $\pm$ 2
15	4549 $\pm$ 493 (4)	36 $\pm$ 3

In Table 2 the results from experiments with high and low concentrations of ethanol are summarized. It is remarkable that the incorporation of tritium into lactate from (R)ethanol does not decrease at high concentrations of ethanol. All depending on the isotope effect of the alcohol dehydrogenase reaction one of the two following explanations may be correct. Providing the same isotope effect is present at high and low concentrations of ethanol this implies that in liver cells no substrate inhibition of the alcohol dehydrogenase occurs, an effect which is quite contrary to in vitro substrate inhibition of alcohol dehydrogenase by ethanol (Dalziel and Dickinson, 1966). Substrate inhibition of alcohol dehydrogenase has been used to explain the different effect of high and low ethanol concentrations on metabolite levels in rat liver (Guynn and Pieklik, 1975).

Table 2. Incorporation rate of tritium into lactate and β -hydroxybutyrate from tritium labelled ethanol. Incubation time is 15 min and the incubation rate is expressed as % of ethanol used during the same period. Experimental conditions as in Table 1.

Ethanol isomer	Ethanol concentration	
	6 mM	65 mM
β -hydroxybutyrate (R)ethanol	0.4 ± 0.2 (4)	0.2 ± 0.1 (3)
lactate (R)ethanol	19 ± 2 (4)	21 (2)
β -hydroxybutyrate (S)ethanol	0.7 ± 0.2 (4)	0.4 (2)
lactate (S)ethanol	5.1 ± 0.3 (4)	1.3 (2)

In experiments with isolated liver alcohol dehydrogenase it has been found that the isotope effect of the alcohol dehydrogenase reaction depends on the acetaldehyde level, being 3.8 in the absence of acetaldehyde and 1.2 in the presence of 70 μ M acetaldehyde (Damgaard, in preparation). The steady-state acetaldehyde concentration in the liver cells might be higher at high ethanol levels (Lindros, Vihma, and Forsander, 1972).

A lower isotope effect at high concentrations of ethanol will cause an increased incorporation of tritium into lactate and unchanged incorporation rate into lactate might thus be compatible with a decreased ethanol oxidation by the cellular alcohol dehydrogenase reaction.

The decrease of incorporation of tritium into β -hydroxybutyrate at high levels of ethanol is not significant and does not necessarily have connection with the decreasing reduction of mitochondrial redox levels observed at high concentrations of ethanol (Grunnet and Thieden, 1972; Guynn and Pieklik, 1975).

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