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Role of Cytosolic Rat Liver Aldehyde Dehydrogenase in the Oxidation of Acetaldehyde during Ethanol Metabolism *in vivo*

By C. J. PETER ERIKSSON,* MARIOS MARSELOS† and TIMO KOIVULA‡
**Research Laboratories of the State Alcohol Monopoly (Alko), SF-00101 Helsinki 10, Finland,*
†Department of Physiology, University of Kuopio, SF-70101 Kuopio 10, Finland, and
‡Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

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The activity of a high-K_m aldehyde dehydrogenase in the liver cytosol was increased by phenobarbital induction. No corresponding increase in the oxidation rate of acetaldehyde *in vivo* was found, and it is concluded that cytosolic aldehyde dehydrogenase plays only a minor role in the oxidation of acetaldehyde during ethanol metabolism.

It has been proposed that aldehyde dehydrogenase activity might be the main regulator for acetaldehyde oxidation during ethanol metabolism in rat liver (Eriksson, 1973). Metabolic studies have shown that acetaldehyde oxidation has little effect on the cytosolic redox state (Lindros *et al.*, 1972) and a relatively large effect on the mitochondrial redox state (Eriksson, 1973; Parilla *et al.*, 1974). A number of investigations have shown the existence of a low-K_m (<10μM for acetaldehyde) aldehyde dehydrogenase in the mitochondria and high-K_m (in the molar range) enzymes in the cytosol, microsomal fraction and the mitochondria (Deitrich, 1966; Marjanen, 1972; Tottmar *et al.*, 1973; Koivula & Koivusalo, 1975). These data, together with the fact that the acetaldehyde concentrations vary between 100 and 200nmol per g wet wt. of liver during ethanol metabolism (Eriksson, 1973), strongly support the importance of the mitochondrial oxidation of ethanol-derived acetaldehyde.

Redmond & Cohen (1971) showed that phenobarbital treatment increased the aldehyde dehydrogenase activity in mouse liver. Deitrich (1971) and Deitrich *et al.* (1972) studied this effect more extensively in the rat and found that the induction of the aldehyde dehydrogenase occurred in the supernatant fraction, and that it was genetically determined and inherited as an autosomal dominant character. Marselos & Hänninen (1974) obtained similar results by administering several common inducers of drug metabolism.

Most of the ethanol-derived acetaldehyde is immediately oxidized further in the liver, so that even a small change in the activity of the oxidizing enzymes could have a relatively large effect on the liver output of acetaldehyde. The aim of this study was to determine the role *in vivo* of the cytosolic aldehyde dehydrogenase in the oxidation of acetaldehyde during ethanol metabolism. The phenobarbital induction effect was used as a means of obtaining two rat groups with different cytosolic aldehyde dehydrogenase activities.

Experimental

Male albino rats of the Wistar/Af/Han/Mol/(Han 67) strain, purchased as specific pathogen-free from Møllegaard Avlslaboratorier (Ejby, Denmark), were used. The animals were given standard laboratory diet and water *ad libitum*. At the age of 3 months they were given phenobarbital (E. Merck A.G., Darmstadt, Germany), 80mg/kg intraperitoneally as single doses for 4 consecutive days. At 24h after the last phenobarbital administration the animals underwent an open liver biopsy under light ether anaesthesia, during which part of the left hepatic lobe (about 2g) was excised. The tissue removed was homogenized in 3vol. (w/v) of ice-cold 0.25M-sucrose solution and centrifuged at 10000*g* for 15min. The supernatant was re-centrifuged at 120000*g* for 60min and from this supernatant aldehyde dehydrogenase activity was spectrophotometrically assayed (acetaldehyde concentration 35mM) as described previously (Marselos & Hänninen, 1974). The symbols rr and RR, introduced by Deitrich (1971), were used for non-reactors and reactors to the phenobarbital induction respectively. In the rr rats ($n = 6$) the aldehyde dehydrogenase activity (0.39±0.20μmol of NADH/min per g wet wt. of liver; mean±S.D.) was close to that of six untreated controls (0.37±0.07), whereas the RR rats ($n = 6$) showed an almost 10-fold increase in the enzyme activity (3.04±0.50).

After 1 month, when they had completely recovered, the operated animals were subjected to a second 4 days of phenobarbital treatment. At 24h after the last phenobarbital administration the rats were injected with 1.5g of ethanol/kg intraperitoneally as a 10% (w/v) solution in 0.9% NaCl. Blood samples were taken from the tip of the tail 30, 60, 90 and 120min after the ethanol adminis-

tration and pipetted into ice-cold 0.6M-$HClO_4$ containing 25mM-thiourea (pro analysi; E. Merck A.G.). They were then shaken and the precipitates centrifuged down at 4000*g* for 15min at 4°C. Ethanol and acetaldehyde were measured in the supernatants by the head-space gas-chromatography technique described by Eriksson *et al.* (1975).

After the last blood sample the rats were anaesthetized with 40mg of pentobarbital (Nembutal R; Abbot S.A., Brussels, Belgium)/kg intraperitoneally, given as a 1% (w/v) solution in 0.9% NaCl. At 15min after the pentobarbital injection liver pieces were frozen *in situ* by means of aluminium clamps precooled in liquid N_2. The frozen liver pieces were pulverized in a mortar, and 1g of the liver powder was suspended in 10ml of ice-cold 0.6M-$HClO_4$. The mixture was shaken and the precipitate centrifuged down at 4000*g* for 15min at 4°C. These supernatants were used directly for analysis of acetoacetate and 3-hydroxybutyrate by head-space gas chromatography as described by Eriksson (1972). Lactate and pyruvate were assayed enzymically in the neutralized supernatants by a method of Hohorst *et al.* (1959). Enzymes and coenzymes were supplied by C. F. Boehringer (Mannheim, West Germany). Ethanol and acetaldehyde were measured in supernatants, to which 25mM-thiourea was added, by the same method as was used for the blood supernatants.

Immediately after the freeze-stop samples had been taken the remaining livers were excised and divided into two parts. One part, weighing about 3g, was homogenized in 0.25M-sucrose solution containing 10mM-sodium phosphate and 2mM-2-mercaptoethanol (E. Merck). Subcellular fractionation and spectrophotometric measurements of alcohol dehydrogenase and aldehyde dehydrogenase activities in the isolated fractions were done as described previously (Koivula *et al.*, 1975). The samples, frozen immediately after subcellular fractionation, were kept for 1 week at −80°C before the enzyme activities were measured.

The other parts of the livers were immediately placed at −20°C, stored for 3 days at this temperature, and then homogenized in 0.25M-sucrose containing 1% (v/v) Triton X-100 (BDH Chemicals, Poole, Dorset, U.K.). The rate of NAD^+-dependent acetaldehyde removal by the liver homogenates was determined by a gas-chromatographic method described by Koivula *et al.* (1975).

Results and discussion

The NAD^+-dependent acetaldehyde-oxidation capacity of the homogenates of the freeze-clamped livers is shown in Fig. 1. The reactor animals displayed a significantly higher aldehyde dehydrogenase activity at all the concentrations used. The difference in activity between the RR and rr rats increased with the acetaldehyde concentration, showing the marked influence of a high-K_m enzyme. When plotted as double-reciprocal forms of Lineweaver & Burk (1934) ($1/v$ versus $1/s$), curved plots still remained, indicating the action of more than one enzyme.

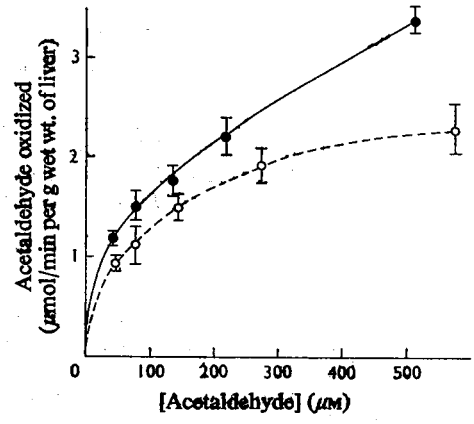

Fig. 1. *NAD^+-dependent acetaldehyde-oxidation capacity in crude liver homogenate*

Detergent-treated liver homogenates were incubated at 37°C in 50mM-sodium–potassium phosphate buffer, pH7.4, supplemented with 4mM-NAD^+, 0.16mM-4-methylpyrazole and initial acetaldehyde concentrations of 50–800μM. The results are given as means±S.D. of the acetaldehyde disappearance between 2 and 4min. ○, rr animals ($n=6$); ●, RR animals ($n=5$).

The subcellular-distribution data showed that the enzyme activity differences found in the crude liver homogenates were mainly due to a higher cytosolic aldehyde dehydrogenase activity in the RR rats. With 18mM-acetaldehyde as substrate, means±S.D. of 2.81±0.80 (5) and 0.42±0.06 (6) μmol of acetaldehyde oxidized/min per g wet wt. of liver were obtained for the RR and rr groups respectively. No significant activity differences were found with a low acetaldehyde concentration (0.12mM), the cytosolic activities being negligible in this case. Nor were there any significant differences in the alcohol dehydrogenase activities in this cell compartment. The apparent K_m value of this cytosolic enzyme (acetaldehyde concentrations from 0.12 to 18mM, 1.33mM-NAD^+) was found to be 22.7±4.6 (mean ±S.D.) and 21.3±9.4mM for the RR ($n=5$) and rr ($n=6$) groups respectively. The mitochondrial fraction data showed no significant differences with 0.12mM-acetaldehyde, the activities being about 0.20μmol/min per g wet wt. of liver. With 18mM-acetaldehyde a small but significant ($P<0.005$ according to the Student's t distribution) difference

Table 1. *Hepatic ethanol- and acetaldehyde-oxidation rate, acetaldehyde concentration and cytosolic and mitochondrial redox state after intraperitoneal ethanol injection*

Rats were treated and analyses made as described under 'Experimental'. The rates of ethanol elimination were calculated from the extrapolated time when ethanol would be completely eliminated. The approximation that all ethanol oxidation occurs in the liver was used. The rates of acetaldehyde oxidation were calculated by subtracting the hepatic acetaldehyde concentration × blood flow rate through the liver [0.79 ml/min per g according to Spector (1956)] from the rates of ethanol oxidation. The results are given as means±S.D. *P* values (**P* < 0.025 and ***P* < 0.005) were calculated by means of the Student's *t* distribution.

	Cytosolic redox state	Mitochondrial redox state	Ethanol-oxidation rate	Hepatic acetaldehyde	Acetaldehyde-oxidation rate
	Lactate/pyruvate	3-Hydroxybutyrate/acetoacetate	(μmol/min per g wet wt. of liver)	(nmol/g wet wt. of liver)	(μmol/min per g wet wt. of liver)
rr (6)	51±16	1.11±0.58	3.43±0.63	173±18	3.29±0.63
RR (4)	120±32**	0.71±0.16	3.19±0.17	136±22*	3.08±0.16

occurred, the RR and rr animals displaying activities of 0.86±0.06 and 0.71±0.05 μmol/min per g wet wt. of liver respectively. In Deitrich's (1971) study, in which he discovered the cytosolic induction, no differences were found in the mitochondria. Considering the great difference in the cytoplasmic aldehyde dehydrogenase activities, it seems possible that even a very small part of the induced enzyme bound to the isolated mitochondrial fraction may account for the difference observed at the high aldehyde concentration. In the microsomal fraction no significant differences were found with 18 mM-acetaldehyde, the activities being about 0.90 μmol/min per g wet wt. of liver, and with 0.12 mM-acetaldehyde the activities were negligible. These subcellular data strongly support findings (Tottmar *et al.*, 1973; Koivula & Koivusalo, 1975) that indicated the importance of the mitochondrial low-K_m enzyme in the oxidation of ethanol-derived acetaldehyde.

A strong indication of a cytosolic effect is the higher lactate/pyruvate ratio in the RR animals (Table 1). This significant ($P < 0.005$) difference suggests that there is another system besides ethanol oxidation in the RR animals that contributes to an increase in the cytosolic free NADH. In the mitochondria an opposite, but not significant, redox effect could be seen. Previously it has been shown (Eriksson, 1973) that the intramitochondrial redox state could reflect the mitochondrial acetaldehyde oxidation during ethanol metabolism. If that were true in this case a lower redox ratio in the mitochondria of the RR group would indicate a lower mitochondrial acetaldehyde oxidation in these animals compared with the rr rats. Thus the redox ratios obtained here can be interpreted as indicating a change in the compartmentation of acetaldehyde oxidation when cystosolic aldehyde dehydrogenase has been induced. In the non-reactor animals with no induced aldehyde dehydrogenase the acetaldehyde is almost exclusively oxidized in the mitochondria, but in the reactor rats the induced cytosolic aldehyde dehydrogenase could cause an increased cytosolic acetaldehyde oxidation, which could partly decrease the mitochondrial oxidation.

The hepatic acetaldehyde concentration during ethanol oxidation, with the RR rats showing a 25% lower concentration than the rr animals (Table 1), represents the difference between acetaldehyde formation and elimination in this organ. Acetaldehyde formation includes the hepatic ethanol oxidation and the amount of acetaldehyde coming into the liver. This amount of acetaldehyde, due to extrahepatically oxidized ethanol or acetaldehyde that has not been oxidized during circulation, or both, is not known. However, considering the results obtained here, with more than 95% of the ethanol-derived acetaldehyde being oxidized before leaving the liver (Table 1), and the fact that the ethanol oxidation is almost exclusively located in the liver, the amount of acetaldehyde entering the liver by the portal vein must be negligible compared with the amount of acetaldehyde formed during the hepatic ethanol oxidation. The hepatic acetaldehyde elimination includes acetaldehyde oxidation and the amount of acetaldehyde that leaves the liver through the hepatic vein.

From the pharmacological point of view it is important to realize that highly significant differences in blood (about 30 and 20 nmol/ml of blood for rr and RR rats respectively) and liver acetaldehyde concentrations can be caused by very small changes in either ethanol- or acetaldehyde-oxidation rates.

Because the effect on the total acetaldehyde oxidation was significant, despite the large increase in the cytosolic aldehyde dehydrogenase activity in the RR group, it can be concluded that the cytosolic aldehyde dehydrogenase plays only a minor role in the regulation of the rate of total acetaldehyde metabolism during ethanol oxidation. However, changes in the cytosolic aldehyde dehydrogenase activity might be important for the compartmentation of the acetaldehyde oxidation in the liver cell.

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