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LIVER ALDEHYDE AND ALCOHOL DEHYDROGENASE ACTIVITIES IN RAT STRAINS GENETICALLY SELECTED FOR THEIR ETHANOL PREFERENCE

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Abstract Rat strains raised by genetical selection for either high (AA strain) or low (ANA strain) voluntary ethanol consumption were compared with respect to their hepatic alcohol and aldehyde dehydrogenase activities. Liver alcohol dehydrogenase activity was lower in both sexes in the AA strain compared with the ANA strain. The NAD-dependent aldehyde dehydrogenase activity was higher in the mitochondrial and microsomal fractions and lower in the soluble fraction in the AA strain than in the ANA strain. These differences were more pronounced in females than in males. The NAD-dependent utilization of acetaldehyde in liver homogenates was higher in the AA strain in both sexes, when the initial acetaldehyde level was 0·40 mM, but there was no difference at 0·13 mM acetaldehyde. It is concluded that the higher activities in the AA strain are due mainly to those aldehyde dehydrogenases of mitochondrial and microsomal fractions, which have K_m-values for aldehydes in the millimolar range. The higher alcohol dehydrogenase and lower aldehyde dehydrogenase activity in livers of rats of the ethanol-avoiding ANA strain may contribute to the previously found higher acetaldehyde levels in blood and liver of rats of this strain after ethanol administration.

Strains of mice and rats have been found to differ in their level of alcohol consumption when given a choice between water and a dilute ethanol solution (for a review see Rodgers[1]). It is clearly established that genetic factors are involved in these differences, but the underlying biochemical basis is still partly unknown. The ethanol-avoiding mouse strains have been reported to have generally lower liver alcohol dehydrogenase activity than the ethanol-preferring strains [1]. Sheppard *et al.*[2, 3] have demonstrated, using inbred strains of mice, that liver aldehyde dehydrogenase activity was significantly lower in ethanol-avoiding animals; these animals also had a higher acetaldehyde level in the blood after ethanol administration than the ethanol-preferring mice. It was suggested, that the lower enzyme activity might result in an acetaldehyde-induced avoidance of ethanol.

In two strains of rats raised by genetic selection for either high (AA strain) or low (ANA strain) voluntary ethanol consumption by Eriksson[4, 5], the ethanol-preferring AA strain was also found to have higher acetaldehyde levels in blood and liver [6]. The present work was undertaken to determine if differences in liver aldehyde and alcohol dehydrogenase activities between the AA and ANA strains could contribute to the differences in acetaldehyde levels. It has been shown recently that there are several types of aldehyde dehydrogenases in rat liver, which differ in their subcellular distribution, K_m-values for aldehydes and some other properties [7, 8]. We have therefore measured aldehyde dehydrogenase activities separately in the soluble, mitochondrial and microsomal fractions prepared from liver tissue of both strains.

MATERIALS AND METHODS

Chemicals

NAD^+ and glucose-6-phosphate were purchased from Boehringer, Mannheim, Germany. Pyrazole was obtained from Aldrich-Europe, Beerse, Belgium, 4-methyl-pyrazole from Labkemi AB, Göteborg, Sweden, and *p*-iodonitrotetrazolium violet (INT) from Sigma Chemical Co., St. Louis, MO, U.S.A. Propionaldehyde, acetaldehyde and all other chemicals were obtained from E. Merck, Darmstadt, Germany.

Animals

Rats weighing 150–250 g of the AA (Alko, Alcohol) and ANA (Alko, Non-Alcohol) strains were used in these experiments. These strains have been developed in an outbreeding program in which animals were selected for their ethanol preference [4]. The ANA strain prefers water to a 10% (v/v) ethanol solution; the AA strain prefers the ethanol solution. The rats were given a standard laboratory diet (Astro-Ewos, Astra AB, Södertälje, Sweden) and water *ad lib.* No ethanol had been given to the animals previously.

Two series of experiments were conducted. In the first 16 female AA and 16 female ANA rats were used. In the second both males and females of both strains were used.

Subcellular fractionation

The rats were killed by decapitation, and the livers excised and homogenized with a Potter-Elvehjem type Teflon–glass homogenizer in 0·24 M sucrose containing 10 mM Na-phosphate buffer, pH 7·4, and 2 mM mercaptoethanol to make a 10% (w/v) homogenate. The homogenate was centrifuged in a Sorvall

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SS-1 centrifuge for 5 min at 700 *g*. The pellet was washed once with 20 ml of the buffered sucrose solution and centrifuged again as above. The combined supernatants were centrifuged for 10 min at 4500 *g*. The pellet was then washed once with 20 ml of the buffered sucrose solution and centrifuged for 10 min at 12,500 *g*. The pellet, containing the mitochondrial fraction, was suspended in 5 ml 0·158 M KCl.

The postmitochondrial supernatant was centrifuged for 60 min at 105,000 *g* in a Spinco Model L Ultracentrifuge. The supernatant was used as the soluble fraction and the pellet which contained the microsomal fraction was suspended in 10 ml 0·158 M KCl.

Aldehyde dehydrogenase activity was liberated from the mitochondria in the first series of experiments by freezing the mitochondrial suspension rapidly in an ethanol solid CO_2 mixture, and then thawing it. This was repeated three times and the suspension was then centrifuged for 60 min at 105,000 *g*. The resulting supernatant was used for the enzyme assays. In the second series sodium deoxycholate was added to the mitochondrial and microsomal suspensions to a final concentration of 0·2% (w/v). The enzyme activities were then measured directly from the clear solutions without centrifuging.

Assay methods

Aldehyde dehydrogenase. Enzyme activity was measured spectrophotometrically by following the NADH production at 340 nm with a Gilford model 2000 attachment for the Beckman DU monochromator. The cuvette holder was maintained at 25°. The reaction mixture contained 70 mM sodium pyrophosphate buffer pH 8·0, 1·33 mM NAD^+ and 1·67 mM pyrazole. The reaction was started by the addition of aldehyde, bringing the total volume to 3 ml. Two concentrations of aldehyde were used, either 4·5 mM propionaldehyde or 0·5 mM acetaldehyde. The kinetic constants for acetaldehyde and propionaldehyde are of the same magnitude for the different rat liver aldehyde dehydrogenases (Koivula and Koivusalo, unpublished results). Blanks without substrate were measured simultaneously. One unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μmole of NADH per min under the above conditions.

Alcohol dehydrogenase. The assay mixture contained 70 mM NaOH–glycine buffer, pH 9·6, 0·67 mM NAD^+, and 10 mM ethanol. Total volume was 3 ml. The reaction was started by addition of the coenzyme and the initial rates of NADH production were measured spectrophotometrically as in the aldehyde dehydrogenase assay.

Glucose-6-phosphatase. Activities were measured at 25° in the subcellular fractions and in deoxycholate-treated total homogenate (0·2% w/v final concentration) by the method of Harper [9]. An average yield of 70% was found for the microsomal fraction. About 25% of the total glucose-6-phosphatase activity was found in the mitochondrial fraction and only a negligible amount in the soluble fraction.

Succinate-INT-reductase. Succinate-INT-reductase was used as a marker enzyme for mitochondria. Its activities were determined in the subcellular fractions and in deoxycholate-treated (0·2% final concn) total homogenate by the method of Pennington[10]. An average yield of 45% was found for the mitochondrial fraction. There were only negligible activities in the soluble and microsomal fractions.

Calculation of aldehyde dehydrogenase activities. The activities are expressed as μmoles per min per g liver wet wt. The yield and purity of the mitochondrial and microsomal fractions were assessed on the basis of succinate-INT-reductase and glucose-6-phosphatase activities in these fractions, when compared with the activities in deoxycholate-treated (0·2% final concn) total homogenate. The final values are corrected on this basis.

Protein. The biuret method of Gornall *et al.*[11] was used with dry bovine serum albumin as standard (Armour Pharmaceutical Co. Ltd., Eastbourne, England). Corrections for the effects of sucrose and deoxycholate in the samples were made by adding these in the corresponding amounts to the protein standards.

Determination of NAD-dependent acetaldehyde utilization. The capacity of liver homogenates to utilize acetaldehyde in the presence of NAD was determined by a modification of the method described by Marjanen[12]. Samples from deep-frozen livers were homogenized with a Potter-Elvehjem homogenizer in 4 parts 10 mM Na-K-phosphate buffer, pH 7·4, containing 0·25 M sucrose, 2 mM mercaptoethanol and 1% Triton X-100. Acetaldehyde uptake rates were determined at 37° in 50 mM Na-K-phosphate buffer, pH 7·4, supplemented with 4 mM NAD, 0·16 mM 4-methyl-pyrazole and an initial acetaldehyde concentration of 0·40 or 0·13 mM. The reaction was started by addition of 0·2 ml homogenate and the final volume was 3·2 ml. Samples were withdrawn at 2-min intervals, pipetted into serum bottles containing 0·6 M perchloric acid and analyzed for their content of acetaldehyde by a Perkin Elmer F 40 gas chromatograph using the head-space technique as described previously [6]. Blanks with NAD omitted from the reaction mixture were subtracted from the results. The uptake was usually linear for at least 6 min.

Statistical treatment of results. Results are expressed as means ± S.E.M. The statistical comparison between the strains were made with Student's *t*-test.

RESULTS

In the first series of experiments the mitochondrial aldehyde dehydrogenase activity was liberated by several freezing–thawing cycles, a method which tends to release primarily the low-K_m enzyme (Koivula and Koivusalo, unpublished results). With this technique, no statistically significant differences between the strains could be observed in the aldehyde dehydrogenase activities of the liver soluble (0·502 ± 0·016 and 0·512 ± 0·019 μmoles/min per g liver wet wt in AA and ANA strain, respectively) or mitochondrial (0·559 ± 0·033 and 0·591 ± 0·031 μmoles/min per g liver wet wt in AA and ANA strains, respectively) fractions. Microsomal activity was not measured. Alcohol dehydrogenase activity was significantly higher ($P < 0·005$) in the soluble fraction from livers of the ANA strain (Table 1). Preliminary experiments

Table 1. Alcohol dehydrogenase activities in liver of AA and ANA strain rats

	AA strain	ANA strain
	(μmoles/min per g liver wet wt)	
Females (n = 16 in both groups) series I	2·14 ± 0·10	2·87 ± 0·22*
Females (AA:n = 10, ANA:n = 8) series II	2·14 ± 0·05	2·98 ± 0·09*
Males (n = 6 in both groups) series II	1·23 ± 0·25	1·83 ± 0·27*

Values are means ± S.E.M. The activity was assayed in the soluble fraction as described in Methods.
* $P < 0.005$ for difference between AA and ANA strains.

in this series on brain aldehyde dehydrogenase activity in the soluble and crude mitochondrial fractions indicated no difference between the strains.

In the second series sodium deoxycholate was used to release aldehyde dehydrogenase activity in the mitochondrial and microsomal fractions. Addition of sodium deoxycholate to give a final concentration of 0·2% (w/v) has been found to liberate more of the high-K_m enzymes located in these fractions (Koivula and Koivusalo, unpublished results).

The aldehyde dehydrogenase activities measured with a relatively high aldehyde concentration (4·5 mM propionaldehyde) as substrate are presented in Table 2. Female rats of the AA strain had significantly higher activity in both mitochondrial ($P < 0.025$) and microsomal ($P < 0.005$) fractions than those of the ANA strain, but in the soluble fraction the aldehyde dehydrogenase activity was higher ($P < 0.025$) in ANA rats. The total activity in female rats (calculated as the sum of the activities in the three fractions) was higher ($P < 0.05$) in the AA strain. In male rats there was the same trend as in females but the differences were statistically significant ($P < 0.025$) only in the microsomal fraction, where the AA rats had again higher activity.

When the same preparations were assayed for aldehyde dehydrogenase activities using a low concentration of aldehyde (0·5 mM acetaldehyde) as substrate (Table 3), the measured activities were considerably lower in all fractions than with a higher aldehyde level. The activity was higher in the soluble fraction prepared from livers of ANA rats both in females ($P < 0.005$) and males ($P < 0.025$). In the microsomal fraction, however, AA rats had higher activity ($P < 0.005$) in both sexes than ANA rats. There were no significant differences in the mitochondrial or total activities.

Determinations of K_m-values for propionaldehyde and acetaldehyde using pooled soluble and mitochondrial liver fractions indicated no significant differences

Table 2. Aldehyde dehydrogenase activities in liver of AA and ANA strain rats assayed with 4·5 mM propionaldehyde as substrate

	(μmoles/min per g liver wet wt)			
	Females		Males	
	AA strain (n = 10)	ANA strain (n = 8)	AA strain (n = 6)	ANA strain (n = 6)
Soluble fraction	0·39 ± 0·02	0·49 ± 0·03†	0·50 ± 0·03	0·48 ± 0·01
Mitochondrial fraction	1·03 ± 0·07	0·82 ± 0·05†	0·94 ± 0·12	0·81 ± 0·05
Microsomal fraction	0·86 ± 0·05	0·65 ± 0·03‡	0·78 ± 0·05	0·59 ± 0·06†
Sum of activities in soluble, mitochondrial and microsomal fractions	2·27 ± 0·13	1·96 ± 0·09*	2·22 ± 0·19	1·88 ± 0·11

The assays are described in Methods.
The values are from experimental series II and are expressed as means ± S.E.M.
* $P < 0.05$, † $P < 0.025$, ‡ $P < 0.005$ for differences between AA and ANA strains.

Table 3. Aldehyde dehydrogenase activities in liver of AA and ANA strain rats assayed with 0·5 mM acetaldehyde as substrate

	(μmoles/min per g liver wet wt)			
	Females		Males	
	AA strain (n = 10)	ANA strain (n = 8)	AA strain (n = 6)	ANA strain (n = 6)
Soluble fraction	0·093 ± 0·007	0·142 ± 0·012†	0·119 ± 0·004	0·141 ± 0·007*
Mitochondrial fraction	0·351 ± 0·034	0·299 ± 0·025	0·359 ± 0·064	0·366 ± 0·038
Microsomal fraction	0·083 ± 0·005	0·061 ± 0·004†	0·090 ± 0·009	0·059 ± 0·003†
Sum of activities in soluble, mitochondrial and microsomal fractions	0·527 ± 0·037	0·502 ± 0·033	0·568 ± 0·070	0·566 ± 0·040

The assays are described in Methods.
The values are from experimental series II and are expressed as means ± S.E.M.
* $P < 0.025$, † $P < 0.005$ for differences between AA and ANA strains.

either in the high-K_m or low-K_m enzymes between the two strains. Further work with more purified enzyme preparations is in progress in order to study this aspect in more detail.

The glucose-6-phosphatase activity of the liver microsomal fraction was also measured in the second series in all males of both strains. No difference was found between them: the values were 4·88 ± 0·28 and 4·85 ± 0·20 μmoles/min per g liver wet wt for AA and ANA strains, respectively. The ratio of microsomal aldehyde dehydrogenase to glucose-6-phosphatase activities was significantly higher ($P < 0·005$) in the rats of AA strain (0·158 ± 0·007) than in ANA strain (0·121 ± 0·008).

The strain differences were the same if the aldehyde dehydrogenase activities of the fractions were expressed per mg of protein.

The strains were compared also with respect to the capacity of total liver homogenates to utilize acetaldehyde in the presence of added NAD. These experiments were performed at physiological pH (7·4) and temperature (37°) and at acetaldehyde levels close to those observed during ethanol oxidation in the intact liver [6, 13]. With 0·40 mM acetaldehyde as substrate the NAD-dependent acetaldehyde utilization was significantly higher in preparations from AA rats than in those from ANA rats both in females ($P < 0·001$) and in males ($P < 0·05$) (Table 4). At the lower initial acetaldehyde level of 0·13 mM, however, there was no difference between the strains in the utilization rates.

DISCUSSION

The aldehyde oxidizing capacity with indoleacetaldehyde as substrate was reported by Sheppard *et al.*[2] to be 2–3 fold higher in liver homogenates from the ethanol-preferring C57BL/6J inbred strain of mice than from the ethanol-avoiding inbred DBA/2J strain. The same authors also found 2–3 fold differences in the K_m-constant for aldehyde substrates when partially purified liver aldehyde dehydrogenase preparations from the two strains were compared [3]. In contrast to this result Eriksson and Pikkarainen[14] were unable to detect any significant difference in aldehyde oxidizing capacity of liver preparations from C57BL and CBA/Ca (ethanol-avoiding) strains of mice. This discrepancy might be explained by the fact that the latter authors measured the activity only in the soluble fraction.

Table 4. NAD-dependent acetaldehyde utilization of liver homogenates from AA and ANA rats

	(n)	Utilization of acetaldehyde (μmoles/g liver wet wt per min) conen of acetaldehyde 0·40 mM	0·13 mM
Female rats			
AA	(19)	2·82 ± 0·07	1·84 ± 0·05
ANA	(11)	2·25 ± 0·13†	1·83 ± 0·09
Male rats			
AA	(6)	2·87 ± 0·09	1·78 ± 0·06
ANA	(6)	2·55 ± 0·08*	1·70 ± 0·15

Samples from detergent-treated liver homogenates were incubated at 37° in the presence of NAD and an initial acetaldehyde concentration of 0·40 or 0·13 mM. Acetaldehyde disappearance was determined by gas chromatography. For further details see Methods. Values are means ± S.E.M.

* $P < 0·05$. † $P < 0·001$ for difference between AA and ANA strains.

In the present study differences were found between liver aldehyde dehydrogenase activities of the ethanol-preferring AA strain of rats and the ethanol-avoiding ANA strain, but the differences were not nearly as large as the differences in aldehyde oxidizing capacity found by Sheppard *et al.*[2] in mice. We consistently found the microsomal aldehyde dehydrogenase activity to be significantly higher in the AA strain. The mitochondrial activity was also generally higher in the AA strain. These differences were in general more pronounced in female rats.

According to recent investigations [7, 8] there are several types of rat liver aldehyde dehydrogenases. An enzyme with relatively high-K_m for aldehydes (in the millimolar range) is found both in the mitochondrial and microsomal fractions; the mitochondria also have an additional aldehyde dehydrogenase with a low-K_m for aldehydes (in the micromolar range). In the soluble fraction at least one different enzyme is found with a high-K_m for aldehydes. When analyzed with 4·5 mM propionaldehyde the total aldehyde dehydrogenase activity of rat liver is distributed between the subcellular fractions so that about 40% is found in the mitochondria, 40% in the microsomal fraction and the rest in the soluble fraction (Table 2). At a lower aldehyde concentration, 0·5 mM acetaldehyde, the distribution is shifted so that only 15–20% of the activity is confined to the microsomal fraction (Table 3). This heterogeneity of liver aldehyde dehydrogenase activity necessitates the use of clearly defined tissue preparations and substrate concentrations in the activity determinations especially with crude preparations and makes the comparison of old results from different laboratories difficult. Using low aldehyde concentrations only the activity of the mitochondrial low-K_m enzyme is determined, and with increasing concentrations of aldehyde the measured activity increases due to the high-K_m enzymes. If the aldehyde concentration used is too high, however, substrate inhibition appears.

A comparison of the activities found in the mitochondrial fractions when assayed at low and high aldehyde levels (Tables 2–4) indicates that there is no or little difference between the strains in the activity of the mitochondrial low-K_m enzyme. When the mitochondrial aldehyde dehydrogenase activity was liberated with repeated freezing thawing cycles there was no difference between the strains but after deoxycholate treatment a significant difference was found. Control experiments with unselected Wistar rats demonstrated that the freezing thawing procedure liberated mainly low-K_m activity and that with deoxycholate treatment relatively more high-K_m activity could be found (Koivula and Koivusalo, unpublished). This also indicates that the strain difference in the mitochondrial fraction is due to the high-K_m enzyme.

The difference between the strains in microsomal aldehyde dehydrogenase activity was not due to any general difference in microsomal enzyme activities as

indicated by the absence of any difference in glucose-6-phosphatase activity.

Our finding that the liver alcohol dehydrogenase activity is significantly lower in the ethanol preferring rats is opposite to studies in mice, where the higher activities have been found in the ethanol-preferring strains [1, 2, 14]. The liver alcohol dehydrogenase activities did not correlate with the previously reported ethanol elimination rates [6].

According to Eriksson[6], after ethanol administration, the acetaldehyde concentrations in blood and liver of the ANA rats were higher than in the AA rats; this effect was more pronounced in females. Acetaldehyde has also been reported to accumulate in blood following ethanol administration in mouse strains with low preference for ethanol [3, 15].

The differences in liver aldehyde dehydrogenase activities between the ethanol-avoiding and ethanol-preferring rat strains found in this work were rather small and apparently restricted to the enzymes with high-K_m-values for aldehydes. These enzymes, may, however, be important in the regulation of aldehyde levels because their activity increases with increasing aldehyde concentration, while the low-K_m enzymes are fully active already at very low aldehyde levels. Lower aldehyde dehydrogenase activity together with higher alcohol dehydrogenase activity in the livers of the ethanol-avoiding ANA rat strain may, at least in part, be responsible for the higher blood and tissue acetaldehyde levels found in this strain after administration of ethanol [6]. The higher acetaldehyde level in the tissues of ethanol-avoiding animal strains after ingestion of ethanol might then be a reason for their avoidance of ethanol, possibly through effects on brain metabolism as has been suggested previously [1, 3, 6, 15].

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