Pergamon

Biochemical Pharmacology, Vol. 48, No. 8, pp. 1551–1558, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00337-8

HEPATIC ALDEHYDE AND ALCOHOL DEHYDROGENASES IN ALCOHOL-PREFERRING AND ALCOHOL-AVOIDING RAT LINES

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(*Received* 5 *January* 1994; *accepted* 28 *July* 1994)

Abstract—The alcohol-avoiding ANA (Alko, Non-Alcohol) and alcohol-preferring AA (Alko, Alcohol) rat lines are known to differ in their acetaldehyde metabolism and were originally found to differ in hepatic alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities in the 1970s. At the beginning of the 1980s, these rat lines were revitalized and some previously found line differences were lost. Thus, the purpose of this study was to determine whether these enzymatic line differences still exist and, if so, to study them further at the isoenzyme level. ADH and ALDH activities were measured from liver homogenates and different subcellular fractions of the rats. The ANA rats were found to have lower hepatic ALDH and higher ADH activities than AA rats, in accordance with the previous study. The line difference in ALDH activity was observed in all fractions, but was more apparent with millimolar than micromolar substrate concentrations and generally more pronounced in females than in males. The line difference in the microsomal ALDH activity was found to be quantitative, and it seemed to concern both microsomal ALDH isoenzymes. A qualitative line difference concerning mitochondrial high K_m ALDH isoenzyme was found, and three different cytosolic ALDH isoenzyme patterns were observed, the frequencies of which were found to be different in the two lines. In conclusion, the results of the present study support the assumption that line differences in hepatic ADH and ALDH activities may be relevant to the acetaldehyde accumulation and the particularly low ethanol consumption of the ANA rats.

Key words: genetics; selection; rat aldehyde dehydrogenase; acetaldehyde metabolism; alcohol drinking

The most important enzymes catalysing the conversion of ethanol to acetate in mammals are ADH† (EC 1.1.1.1) and ALDH (EC 1.2.1.3 and 1.2.1.5). Both enzymes are NAD^+-dependent and are mainly located in the liver. The oxidation of ethanol to acetate occurs via acetaldehyde. It is far more toxic than ethanol itself, but most of it is usually immediately eliminated by ALDH so that appreciable amounts of acetaldehyde do not accumulate in blood.

In mammals, liver ALDH exists as several isozymes which differ in their subcellular location, electrophoretic mobility, isoelectric point, kinetic properties, molecular size and substrate specificity. These isozymes are coded by different gene loci, but are usually divided into two broadly defined groups: high K_m ALDH and low K_m ALDH based upon their Michaelis constant for acetaldehyde. In both humans and rats, mitochondrial low K_m ALDH (ALDH2) is believed to be the most important enzyme in oxidizing ethanol-derived acetaldehyde. The ethanol-oxidizing ADH is located mainly in the cytosolic fraction.

Goedde *et al.* [1] first demonstrated that approx. 50% of Japanese and Chinese lack liver ALDH2 activity. This deficiency in ALDH activity has been found to be a consequence of a structural mutation leading to the synthesis of an enzymatically-inactive protein [2]. The mutation consists of glutamic acid in position 487 being replaced with lysine [3, 4]. Nowadays, this mutation is known to be the underlying cause of the alcohol sensitivity symptoms which are a consequence of the ingestion of moderate amounts of alcohol common in Oriental populations. The accumulation of acetaldehyde in the blood is probably the immediate reason for these symptoms.

Individuals with ALDH2 deficiency have been found to consume less alcohol than individuals with normal ALDH isozymes. Because of this relationship, which has been confirmed in several studies [5–8], the possibility has been raised that ALDH2 deficiency could protect individuals from alcoholism.

An atypical form of ADH with a catalytic activity several times higher than that of normal ADH has also been found in humans [9]. However, there does not seem to be any significant difference in the ethanol elimination rate of normal and atypical ADH phenotype carriers [10], and there is no evidence that this polymorphism is associated with alcohol drinking behavior.

The possible association between the liver ethanol metabolizing enzymes, especially ALDH, and alcohol drinking behavior has also been studied with alcohol-avoiding and alcohol-preferring rodents. The cytosolic ALDHs of alcohol-preferring C57BL/6J and alcohol-avoiding DBA/2J mouse strains have

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† Abbreviations: ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase; AA, Alko, Alcohol; ANA, Alko, Non-Alcohol.

been found to exhibit different kinetic properties [11–13]. There also seems to be a genetically-determined difference in the response of hepatic ALDH activity to ethanol exposures between C57BL/6J and alcohol-avoiding BALB/c and 129/ReJ strains [14, 15]. Furthermore, the activity of ADH-A_2, the main class I ADH in mouse liver, is found to be 2-fold higher in livers of C57BL/6 mice than in DBA/2J mice [16]. Alcohol-preferring P and -nonpreferring NP rat lines are also found to have a single amino acid difference in ALDH2 [17, 18].

The alcohol-preferring AA (Alko, Alcohol) and alcohol-avoiding ANA (Alko, Non-Alcohol) rat lines have been found to differ in their acetaldehyde metabolism: the blood acetaldehyde levels during ethanol metabolism are considerably higher in ANA than in AA rats [19–21]. The underlying cause of this line difference is still unknown. However, it seems probable that line differences in hepatic ethanol and acetaldehyde metabolizing enzymes play some role. The difference in liver ALDH activity was first proposed by Eriksson [19] and later confirmed by Koivula *et al.* [22]. In the latter study, ANA rats were found to have lower hepatic ALDH and higher ADH activities than AA rats.

In 1983 the AA and ANA rat lines (F_{37}) were revitalized (they were crossed with F_1-hybrids from Lewis × Brown Norwegian lines) and some previously found line differences were lost. The line difference in blood acetaldehyde levels during ethanol metabolism remained [20, 21], but the differences in ADH and ALDH activities have not been studied after revitalization. Thus, the initial purpose of this study was to examine whether the previously found line differences in these enzymes still exist. Hepatic ALDH activities were measured from the liver homogenate and the mitochondrial, microsomal, and cytosolic fractions; ADH activity was measured from the cytosolic fraction. The isoelectric focusing patterns of mitochondrial and cytosolic ALDH isozymes and of cytosolic ADH were determined. Microsomal ALDH was studied by western blot analysis; microsomal ALDH II was purified from both lines and their K_m values for acetaldehyde, propionaldehyde, and NAD^+ were determined.

MATERIALS AND METHODS

Animals. AA and ANA rat lines have been developed in Alko Biomedical Research Center for high and low voluntary ethanol consumption by selective outbreeding, as described elsewhere [23]. The rats used in these experiments were from three generations. ALDH and ADH activities and isoelectric patterns were studied using 3.5–4.5 month old rats from generation F_{60} (13 males and 12 females per line). The rats used in the western blot analysis were of the same age but from generation F_{62} (seven to nine males and females per line). The microsomal ALDH isoenzyme II was purified from 3.5 month old male AA and ANA rats from generation F_{65}. The F_{62} and F_{65} animals had no experience with ethanol whereas the F_{60} animals had been injected twice i.p. with 1.5 g/kg ethanol as a 12% (w/v) solution 4–6 weeks before decapitation. All animals were housed in groups of four to six and had free access to Ewos R3 rat chow (Ewos AB, Södertälje, Sweden) and tap water.

Subcellular fractionation of rat liver. Rats were killed by decapitation and the livers removed immediately. Ice-cold sucrose medium containing 0.25 M sucrose, 5 mM Tris, 0.5 mM EDTA and 2 mM 2-mercaptoethanol (pH 7.2) was added to the liver tissue in amounts making the liver constitute 20% (w/v) of the total. It was homogenized at 800 rpm and the homogenate fractionated by differential centrifugation. All steps were carried out from 0 to +4°. The homogenate was centrifuged for 10 min at 1000 *g*. The pellet which represented the nuclear fraction was discarded, and the mitochondria were isolated by centrifugation at 10,000 *g* for 10 min. The pellet was resuspended in sucrose medium and washed twice at 10,000 *g* for 10 min following one centrifugation at 20,000 *g* for 10 min. The final pellet was resuspended in 20 mL of sucrose medium and designated the mitochondrial fraction.

The supernatant fraction was centrifuged twice at 10,000 *g* for 10 min and once at 20,000 *g* for 10 min. The pellets were discarded, and the supernatant obtained was first centrifuged at 105,000 *g* for 60 min, thereafter both the supernatant and the resuspended pellet were washed once at 120,000 *g* for 60 min. The final pellet, resuspended in 20 mL of sucrose medium, and the final supernatant were designated the microsomal and cytosolic fractions, respectively. All the fractions were stored in small aliquots at −80°.

Enzyme assays. All the assays of dehydrogenase enzymes were performed spectrophotometrically by following the formation of NADH or NAD^+ at 340 nm. ADH was measured at 37° and all the other enzymes at 25°. All reactions were started by addition of the substrate. One unit of enzyme activity is defined as the amount of enzyme catalysing the formation of 1 μmol of coenzyme per min. The enzyme activities are expressed as specific activities (U/mg protein).

ALDH was assayed using acetaldehyde as substrate. Before activity measurements, sodium deoxycholate was added to the samples at a concentration of 0.3% following incubation at +4° for at least 20 min. Homogenate samples were centrifuged after incubation to remove debris, and the supernatant was used in the assay. Other samples were used without centrifugation. The assay mixture contained 0.5 mM NAD^+, 0.1 mM 4-methylpyrazole, 2 μM rotenone, and 0.1 or 10 mM acetaldehyde in 60 mM sodium pyrophosphate buffer, pH 8.8. Blank cuvettes without substrate were run simultaneously, and the results were corrected for blank reactions.

ADH was assayed in a mixture containing 5 mM NAD^+ and 5 mM ethanol in 9 mM glycine–120 mM sodium pyrophosphate buffer, pH 8.8. Blank cuvettes which contained 1 mM 4-methylpyrazole instead of ethanol were run simultaneously, and the results were corrected for blank reactions.

Glutamate dehydrogenase was assayed in a mixture containing 0.2 mM NADH, 1.2 mM ADP,

118 mM sodium acetate, and 9 mM alpha-keto-glutarate in 58 mM TEA–3 mM EDTA-buffer, pH 8.0.

The assay of lactate dehydrogenase was performed in a mixture containing 0.2 mM NADH and 1.6 mM sodium pyruvate in 67 mM Tris–167 mM NaCl buffer, pH 7.35.

Glucose 6-phosphatase was assayed following a two-step method. In the first step, 0.25 mL of sample dilution was incubated with 0.2 mL of 0.3 M beta-dimethylglutarate–12.5 mM EDTA-buffer, pH 6.4, and 50 μL of 50 mM glucose 6-phosphate for 30 min. The reaction was stopped with 0.75 mL of 6% TCA, and the samples were centrifuged at 5000 rpm for 10 min. Blanks were prepared for each sample by adding TCA before the addition of the substrate. In the second step, 0.25 mL of supernatant was used for the assay of inorganic phosphorus. It was incubated with 1.25 mL of 0.25% ammonium-molybdic acid and 0.25 mL of 5.7% sodium hydrogen sulfite–0.1% 1-amino-2-naphthol-4-sulfonic acid for 60–90 min; absorbance in 625 nm was measured. Potassium dihydrogen phosphate was used as a standard.

Proteins were assayed using the Bio-Rad Protein Assay method (Bio-Rad, Richmond, CA, U.S.A.) according to the instructions of the manufacturer. Bovine serum albumin was used as the standard protein.

Isoelectric focusing. Isoelectric focusing was performed as described previously [21]. Samples were treated with 0.2% Triton X-100 and 1 mM dithiothreitol before focusing. The mitochondrial high K_m ALDH was studied in gels with a pH range of 5–8 and the cytosolic ALDH and ADH isoenzymes in gels with a pH range of 3.5–9.5. The electrode solutions for the former gels were 0.5 M sodium hydroxide and 0.5 M phosphoric acid and for the latter 25 mM lysine–25 mM arginine–2 M ethylendiamine and 25 mM glutamic acid–25 mM aspartic acid.

The gels were stained for ALDH activity with an agarose overlay technique as described previously [24]. ADH activity staining was performed by preparing an agarose overlay containing 1% agarose, 3.6 mM NAD^+, 0.95 mM MTT, 100 μM Meldola blue and 0.15 M ethanol in 100 mM sodium pyrophosphate buffer, pH 9.0. Blanks were prepared by omitting the substrate. The pIs were determined using LKB pI-marker range: 5.65–8.30 and 4.70–10.60 (LKB, Bromma, Sweden).

Western blot analysis of microsomal ALDH isoenzymes. Isolated microsomal preparations corresponding to 4 μg (microsomal ALDH I) or 10 μg (microsomal ALDH II) protein, which were found to be in the linear part of the dose–response curve, were subjected to SDS–PAGE which was performed on a slab gel of 9% acrylamide–0.2% bis-acrylamide with a stacking gel of 4% acrylamide–0.1% bisacrylamide. After electrophoresis, the proteins were electrotransferred to a nitrocellulose membrane and probed with either microsomal ALDH I- or II-specific antibody [25], which were generously provided by Dr Ronald Lindahl. An immunoreactive protein was detected by the alkaline phosphatase method using bromochloroindolyl phosphate/nitro blue tetrazolium as substrate. The relative amounts of protein from the western blots were quantified using a UVP GDS2000 video camera gel documentation system.

Purification and characterization of microsomal ALDH II. Lindahl and Evces [26] have purified both microsomal isoenzymes. They found that microsomal ALDH I does not bind to DEAE-cellulose whereas microsomal ALDH II does. In our experiments, over 90% of the microsomal ALDH activity was found to bind to DE52-cellulose and only this enzyme was purified.

Microsomal ALDH II was purified from AA and ANA lines by the method described by Lindahl and Evces [26]. The purification steps were DE52-anion exchange chromatography and 5'-AMP-Sepharose 4B affinity chromatography. The purity of the preparation was determined using ExcelGel SDS, gradient 8-18 polyacrylamide gels, and ExcelGel SDS buffer strips (Pharmacia LKB, Uppsala, Sweden) according to the manufacturer's instructions. Molecular weight standards were from the same manufacturer. Isoelectric focusing of the purified enzyme was performed as described above. Both SDS–PAGE and IEF gels were stained with Brilliant blue G.

Michaelis constants were determined under the same activity assay conditions as described above. K_m values for acetaldehyde and propionaldehyde were determined at 1 mM NAD^+ and for NAD^+ at 5 mM propionaldehyde. K_m values were determined from standard double-reciprocal Lineweaver–Burk plots.

Statistics. The results are expressed as means ±SE Student's *t*-test was used for statistical comparison of the two lines. The G-test was used to determine statistical significance between the frequencies of different cytosolic ALDH patterns in AA and ANA lines. Pearson correlation coefficients between the high K_m microsomal ALDH activities and the microsomal content of microsomal ALDH isoenzymes I and II were calculated using computer programs by SAS Institute, Inc.

RESULTS

Subcellular distribution of the marker enzymes

The distribution of the marker enzymes is shown in Fig. 1. Approximately 96% of the total lactate dehydrogenase activity was found in the cytosolic fraction, whereas only traces of this enzyme were located in mitochondrial and microsomal fractions. The cytosolic fraction contained less than 2.5% of total glutamate dehydrogenase and glucose-6-phosphatase activities but some cross-contamination was observed between mitochondrial and microsomal fractions. About 13% of total glutamate dehydrogenase activity was found in the microsomal fraction and nearly 23% of the total glucose-6-phosphatase activity was found in the mitochondrial fraction.

Cytosolic ADH activity

The ANA rats had significantly higher cytosolic ADH activities than the AA rats. This line difference was observed both in males and females (Table 1). In the ANA line, there was a significant difference between sexes, males having higher activities

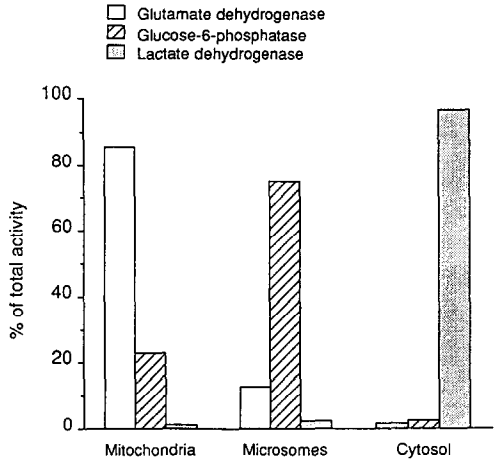

Fig. 1. Subcellular distribution of the marker enzymes. The percentage of the activity in the individual fractions is compared to the total activity in all fractions.

Table 1. Cytosolic ADH activities in AA and ANA rat lines

Line	Sex	ADH activity
AA	Male	33.0 ± 1.4†
ANA	Male	43.3 ± 0.7
AA	Female	30.7 ± 0.7*
ANA	Female	36.3 ± 1.3

Results are expressed as mU/mg protein.
Means ± SE are indicated. N = 12–13.
* $P < 0.01$ and † $P < 0.001$ denote line difference significances.

($P < 0.0001$) whereas no significant sex difference was found in the AA line.

ALDH activity

ALDH activities were measured using two different acetaldehyde concentrations (10 mM and 0.1 mM) as substrate. The activities measured with 0.1 mM acetaldehyde represent low K_m enzyme activities, and the difference between the activities measured with 10 mM acetaldehyde and those measured with 0.1 mM acetaldehyde are regarded as high K_m enzyme activities. The results of the measurements are shown in Table 2. AAs having higher activities than ANAs, line differences were found in both low and high K_m enzyme activities although they were more apparent in the latter. The differences were generally more pronounced in females than in males.

Among females, there were significant line differences in the high K_m enzyme activity of the homogenate and the mitochondrial and microsomal fractions, while in the low K_m enzyme activity there were significant differences in the homogenate and the microsomal fraction. Among males, high K_m enzyme activity line differences were observed in the homogenate and the microsomal fraction, and low K_m enzyme activity line differences in the microsomal and cytosolic fractions. There were no line differences in the protein content of the liver or in the liver weight/body weight ratio.

Isoelectric focusing studies

Isoelectric focusing studies were performed to study the number and the focusing patterns of ALDH and ADH isozymes in AA and ANA rats. The mitochondrial and cytosolic fractions were examined in these studies. Only ADH isozymes from the cytosolic fraction were studied. No band formation was observed in blank gels. In the mitochondrial fraction, two major zones of ALDH activity with pIs of 5.1–5.2 and 6.1–6.4 were found

Table 2. Hepatic ALDH activities in different subcellular fractions in AA and ANA rat lines

Fraction	AA Male	ANA Male	AA Female	ANA Female
Homogenate				
(a)	8.4 ± 0.2	8.0 ± 0.2	7.0 ± 0.1‡	5.5 ± 0.2
(b)	27.0 ± 0.6‡	22.1 ± 0.5	23.3 ± 0.5‡	16.8 ± 0.4
Microsomes				
(a)	5.6 ± 0.2*	4.7 ± 0.3	5.1 ± 0.2‡	3.7 ± 0.2
(b)	62.6 ± 2.0†	53.5 ± 1.8	59.3 ± 1.6‡	42.9 ± 2.1
Mitochondria				
(a)	27.0 ± 0.9	28.9 ± 1.5	25.1 ± 0.9	23.8 ± 1.3
(b)	27.3 ± 0.7	25.3 ± 1.0	35.1 ± 1.2‡	27.1 ± 0.5
Cytosol				
(a)	2.6 ± 0.7†	1.7 ± 0.5	1.9 ± 0.4	1.9 ± 0.5
(b)	1.6 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2

Results are expressed as mU/mg protein. Means ± SE are indicated. N = 12–13.
(a) ALDH activity measured using 0.1 mM acetaldehyde as substrate.
(b) ALDH activity measured using 10 mM acetaldehyde as substrate with (a) subtracted.
* $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ denote line difference significances.

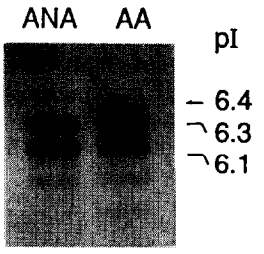

Fig. 2. The different IEF banding patterns of mitochondrial high K_m ALDH in the AA and ANA rat lines.

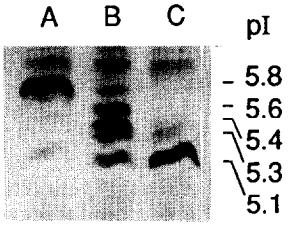

Fig. 3. Three different IEF patterns of cytosolic ALDH isozymes found in the AA and ANA rat lines.

Table 3. The frequencies of the different cytosolic ALDH isoenzyme patterns in AA and ANA rat lines

	Pattern		
Line	A N(%)	B N(%)	C N(%)
AA	5(20)	12(48)	8(32)
ANA	1(4)	4(16)	20(80)

$G^2 = 12.41$, df = 2, $P < 0.01$.

in both lines. The anodic zone represents low K_m ALDH (ALDH2) and the cathodic one high K_m ALDH, which possibly is glutamic-gamma-semialdehyde dehydrogenase (EC 1.5.1.12) [27]. In high K_m ALDH, a distinct difference in banding pattern between the AA and ANA lines was observed; all the AA rats had a pattern with five close but clearly distinct bands of almost equal intensity with pIs of 6.1–6.4, whereas 84 % of the ANA rats showed two equal bands with pIs 6.1 and 6.3 (Fig. 2). In ALDH2, three different banding patterns could be distinguished, but the lines did not differ in the frequencies of these patterns. This polymorphism of ALDH2 in AA and ANA rats is discussed in another report [21].

In the cytosolic fraction, three different ALDH isoenzyme patterns were observed (Fig. 3). Two of these patterns were similar to those previously found in the cytosolic fraction of Wistar rats [28]: the A-pattern with a major ALDH activity band with pI 5.8 and the B-pattern with a triplet of isoenzymes with pIs 5.3, 5.4 and 5.6. The B-pattern also had a minor band with pI 5.1. Besides these two patterns, one additional pattern was observed in which the anodic band at pI 5.1 was clearly predominant. This pattern is referred to here as C-pattern. All three patterns were found in both AA and ANA lines, but their frequencies were different in different lines (Table 3). No differences in cytosolic ALDH activity were observed between different phenotype carriers.

No interindividual variation was observed in the IEF pattern of cytosolic ADH. All the animals had two bands of ADH activity with pIs 8.8 and 9.0, which is comparable to the previously reported focusing pattern of rat liver ADH [29].

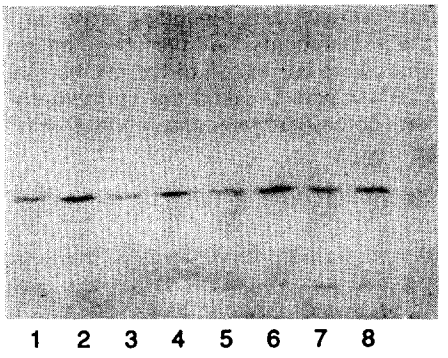

Fig. 4. A representative immunoblot analysis of the microsomal ALDH II in the liver microsomes of AA and ANA rats. Microsomal preparations containing 10 μg protein were separated on SDS–PAGE, transferred electrophoretically to nitrocellulose, and microsomal ALDH II identified by western blot analysis. Lanes 1,3,5,7: ANA rats. Lanes 2,4,6,8: AA rats.

Table 4. The correlation between the content of the microsomal ALDH isoenzymes I and II and microsomal high K_m ALDH activity, given as Pearson correlation coefficients

Sex	I	II
Males	0.624*	0.672*
Females	0.642*	0.706*

N = 16–17, *$P < 0.01$.

Western blot analysis of the microsomal ALDH isoenzymes

Both antibodies were found to recognise one major band of molecular mass 54,000 Da (Fig. 4). A significant line difference in the relative amount of microsomal ALDH II was observed in females [AA: 98.6 ± 16.1 (arbitrary units ± SD); ANA: 66.6 ± 15.7, $P < 0.01$)] whereas the corresponding line difference in the relative amount of isoenzyme I did not reach significance (AA: 100.8 ± 11.9; ANA: 84.5 ± 18.6, $P = 0.056$). No significant

differences were observed in males. However, when the relative amounts of the two microsomal isoenzymes were correlated to the microsomal high K_m ALDH activities, significant correlations were found with both isoenzymes and in both sexes (Table 4).

Characterization of the purified microsomal ALDH II

There were no line differences in the characteristics of the purified microsomal ALDH II. The isoelectric focusing produced only one band with pI 5.7. The K_m values of the purified enzyme were 2.4 mM for acetaldehyde, 0.7 mM for propionaldehyde, and 34 μM for NAD^+. The subunit molecular mass of the purified ALDH was 54,000 Da. SDS–PAGE also revealed that the preparation was not completely homogeneous: one additional band of molecular mass 130,000 Da was detected.

DISCUSSION

Hepatic acetaldehyde metabolism may be associated with ethanol aversion in the ANA rat line [19, 30]. This difference in acetaldehyde accumulation between the AA and ANA rat lines was first reported in the F_{17} generation [19] and was confirmed after revitalization in the F_{40} and F_{43} generations [20] as well as in the F_{60} generation [21]. The existence of this line difference after revitalization suggests a relevant association to alcohol drinking behavior in these lines.

The present results are in accordance with those of previous studies performed before [22] and after [21] revitalization, supporting the assumption that ALDH and ADH enzyme differences are related to line differences regarding acetaldehyde levels and ethanol drinking. In these studies, ANAs were found to have higher hepatic ADH and lower ALDH activities than AAs. In the present study the most remarkable line difference in ALDH activity was found in the microsomal fraction. In this fraction, the line difference was observed both with micromolar and millimolar acetaldehyde concentrations and in both sexes. The mitochondrial ALDH line difference was more specific, being pronounced only at higher acetaldehyde concentrations and apparent only in females, as observed previously [21, 22]. It has been suggested that sex hormones can affect liver ALDH activities both in mice [31, 32] and rats [33]. Thus, it is conceivable that the sex-dependent line difference in mitochondrial ALDH activity could be due to a line difference in sex hormones. The previously found line difference in cytosolic ALDH activity, ANAs having higher activities than AAs, was not observed here. Instead, a significant line difference in the opposite direction in low K_m ALDH activity of this fraction was observed in males. The isoelectric focusing studies revealed that at least two mitochondrial enzymes with ALDH activity, the isozyme with high K_m for acetaldehyde and the low K_m ALDH2, are active in both AA and ANA lines. However, there was a distinct line difference in the isoelectric focusing pattern of the high K_m enzyme. The possible association of this difference with acetaldehyde metabolism will be studied further with F_2-generation hybrids from reciprocal crosses of the AA and ANA lines. Several previous studies have reported another mitochondrial ALDH isozyme with high K_m for acetaldehyde in pI region 6.6–6.7 [26, 28]. However, neither the AA nor the ANA line had any ALDH activity in this pI region.

A correlation between voluntary alcohol consumption and cytosolic ALDH isoenzyme patterns has been found in Wistar rats [34]. In this study all the high drinkers were found to have only one isoenzyme at either pI 5.9 or 6.2 while the low drinkers always had three isoenzymes with pIs 5.9, 6.0 and 6.2. These IEF patterns were somewhat different from those observed in this study with AA and ANA rats. Although there were marked line differences in the frequencies of the three IEF patterns, no conclusions can be drawn on this basis concerning the association of the cytosolic isoenzyme pattern with alcohol drinking behavior in these lines. These differences, like the difference in the mitochondrial high K_m isoenzyme pattern, may also reflect a random association between drinking behavior and the genetic differences underlying these IEF patterns in the breeding populations of AA and ANA rats because of genetic drift or founder effects. Thus, future studies with F_2-generation hybrids will also resolve whether this line difference is of any importance.

In this study, attention was paid mainly to microsomal ALDH isoenzymes because of the most remarkable ALDH activity difference between the lines in this fraction. Two microsomal ALDH isoenzymes have been characterized [26]; we determined their contents in AA and ANA liver microsomes by western blot analysis in order to study which one is responsible for the line difference in catalytic ALDH activity. The contents of both isoenzymes were found to correlate significantly with microsomal high K_m ALDH activity, indicating that the line difference in microsomal ALDH activity is quantitative and probably due to some factor(s) which regulate synthesis, either at the transcriptional or translational level, posttranslational processes, or the degradation of both enzyme proteins. The lack of qualitative line differences in microsomal ALDH II, the major microsomal ALDH isoenzyme, was confirmed by the characterization of the purified enzyme.

It is well-known that the bulk of hepatic acetaldehyde oxidation is carried out by low K_m mitochondrial ALDH in the rat [35] and, thus, the relevance of the present line differences regarding high K_m ALDH enzymes may be questioned. However, the fact that the hepatic efficacy of the oxidation of ethanol-derived acetaldehyde is above 95% means that even small changes in hepatic acetaldehyde oxidation will result in the several-fold magnification of the subsequent change in hepatic acetaldehyde output and blood acetaldehyde concentration [35, 36]. Thus, every hepatic (as well as extrahepatic) acetaldehyde oxidation system may play a relevant role in the regulation of acetaldehyde concentrations during ethanol oxidation. This was also demonstrated in a previous study in which the induction of a high K_m cytosolic ALDH substantially

reduced acetaldehyde levels without excessively increasing the overall rate of hepatic acetaldehyde oxidation [36].

The significantly higher hepatic ADH activity in ANA than in AA rats could contribute to higher acetaldehyde levels in the ANA rats. In addition to ADH, ethanol is known to be metabolized by two other hepatic mechanisms, the microsomal ethanol oxidizing system (MEOS) and catalase. In principle, line differences in these systems could also affect the rate of hepatic acetaldehyde formation. Catalase from AA and ANA rat lines has never been measured and its possible importance thus remains open. However, levels of cytochrome P450 2E1 have been measured from both lines but no difference was observed (Koivisto, unpublished results). Extrahepatic mechanisms may also contribute to the overall metabolism of ethanol. Whether or not line differences exist regarding such mechanisms (e.g. the gastric ADH system [37]) remains to be studied.

The present results reveal that the differences between AAs and ANAs in hepatic ADH and ALDH activities are now, 10 years after revitalization, almost identical to the line differences observed in the 1970s, nearly 10 years before revitalization. The consistent findings, therefore, are in agreement with the hypothesis that low hepatic ALDH activity together with high ADH activity may explain acetaldehyde accumulation in the ANA line and contribute to the aversion to ethanol shown by this rat line. Studies with F_2-generation hybrids, which will be performed in the near future, will hopefully settle the relevance of these interrelations.

Acknowledgements—The authors thank Dr Ronald Lindahl for providing the microsomal ALDH I- and II-specific antibodies, Drs J. David Sinclair and Kai O. Lindros for their useful comments on the manuscript, and Ms Hilkka Salohalla and Ms Arja Mikkelinen for their excellent technical assistance.

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