Biochemical Pharmacology, Vol. 30, No. 16, pp. 2273–2277, 1981.
Printed in Great Britain.

0006-2952/81/162273-05 $02.00/0

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON ALDEHYDE DEHYDROGENASE ACTIVITY IN THE BABOON

M. E. LEBSACK,* ELLEN R. GORDON and CHARLES S. LIEBER
Alcohol Research Center, Bronx Veterans Administration Medical Center, and the Mount Sinai School of Medicine (CUNY), New York, U.S.A.

(*Recieved* 14 *August* 1980; *accepted* 21 *January* 1981)

Abstract—Baboon liver has detectable aldehyde dehydrogenase (AlDH) activity in the mitochondrial, microsomal and soluble fractions. Based on kinetic data, the mitochondrial and soluble fractions each appear to contain two forms of AlDH, one with a high, and another with a low, K_m for acetaldehyde. In the microsomes there was activity only with millimolar concentrations of acetaldehyde. In the baboon liver, about 75 per cent of total AlDH activity resides in the mitochondria and 20 per cent in the soluble faction. Chronic ethanol consumption decreased total and low K_m mitochondrial AlDH activity in baboon liver. In rats, ethanol consumption also resulted in decreased mitochondrial low K_m AlDH activity.

It has been postulated that acetaldehyde, the first metabolite of ethanol, plays an important role in the toxic and pharmacologic effects of alcohol [1, 2]. It is generally agreed that the elimination of acetaldehyde in mammals is almost exclusively by oxidation to acetate as catalyzed by liver aldehyde dehydrogenase (EC 1.2.1.3, AlDH). The properties and subcellular distribution of this enzyme have been studied extensively in the rat [3–5], but only limited studies have been carried out in primates, primarily in human autopsy samples [6–9]. These studies yielded conflicting results and suggested the existence of one [6, 7], two [8], or four [9] forms of AlDH in human liver. The methods of cell fractionation and AlDH assay conditions varied widely.

Alterations in acetaldehyde-oxidizing capacity may arise after chronic consumption of ethanol. There is evidence that alcoholics have higher blood acetaldehyde levels than nonalcoholic subjects when blood ethanol concentrations are maintained at the same level [10]. Lieber and DeCarli [11] have developed a primate model for alcoholic liver injury consisting of baboons ingesting a liquid diet containing quantities of ethanol comparable to those consumed by alcoholics. Pikkarainen *et al.* [12] have demonstrated higher plasma free acetaldehyde levels in ethanol-fed baboons than in pair-fed controls when these animals were given ethanol by intravenous infusion. The mechanism for these increased levels, specifically the respective roles of increased ethanol oxidation versus decreased acetaldehyde catabolism, has not been determined.

The present investigation was undertaken to determine the subcellular distribution of AlDH activity in the livers of healthy primates. We were able to obtain sufficient quanitities of surgical biopsy samples from baboons whose food and drug intake and other environmental variables were closely regulated. We are also able to determine the effect of prolonged ethanol consumption on AlDH activity in this primate and to compare this effect with that seen in the rat.

* Address correspondence to: Dr. M. E. Lebsack, Alcohol Research Center, Veterans Administration Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, U.S.A.

METHODS

Animals. Liver samples were obtained from surgical biopsies of baboons under ketamine anesthesia. Unanesthetized male Sprague–Dawley rats were decapitated and livers were perfused with 0.9% NaCl before removal.

Animals in AlDH distribution and characterization studies had free access to animal chow and water.

To test the effect of chronic ethanol consumption, animals were pair-fed liquid diets with ethanol or isocaloric carbohydrates as described by Lieber and DeCarli [13]. Baboons were pair-fed ethanol as 50% of total calories for 4 months and rats were maintained on the liquid diet regimen for 2 months with 36% of total calories as ethanol.

Subcellular fractionation. Liver samples were homogenized in ice-cold sucrose solution (0.25 M sucrose containing 10 mM Tris–HCl, pH 7.4, and 1 mM sodium EDTA), and mitochondria were isolated as described by Hasumura *et al.* [14]. The mitochondria were resuspended in the sucrose solution (4 ml/g wet weight liver) containing sodium deoxycholate at a final concentration of 0.2% and were then centrifuged for 1 hr at 38,000 *g*. The resulting supernatant fractions were used for AlDH determinations.

The supernatant fractions from above the mitochondrial pellets were centrifuged at 100,000 *g* for 1 hr. These supernatant fractions were used for cytosolic AlDH assays. The 100,000 *g* pellets were washed with 1.15% KCl and centrifuged for 30 min at 100,000 *g*. The washed pellets were resuspended

in KCl solution (1 ml/g liver) containing deoxycholate and treated as described for the mitochondrial fractions.

Mitochondrial recoveries and contamination of the cytosolic fraction by mitochondria were estimated from the glutamate dehydrogenase activity in subcellular fractions and homogenates (25–45 per cent in baboons, 20–35 per cent in rats). Enzyme activity was measured as described by Tottmar *et al.* [3]. Recovery of microsomes was estimated in baboons (55–65 per cent) from the cytochrome P-450 levels in homogenates and microsomal fractions [15]. Microsomal recoveries in rats (35–55 per cent) were estimated from glucose-6-phosphatase activities in the subcellular fractions [16].

AlDH assays. AlDH activity was measured spectrophotometrically by monitoring the rate of NADH formation at 340 nm as described previously [17]. Acitivity was measured with 0.5 mM NAD at 37° at pH 7.4 in 50 mM potassium phosphate buffer containing 1.15 mM $MgCl_2$. In the mitochondrial fractions, 2 μM rotenone was added to inhibit NADH oxidase. Assay mixtures of microsomal and cytosolic fractions contained 200 μM 4-methylpyrazole to inhibit alcohol dehydrogenase.

Since the apparent K_m values for interaction of baboon liver AlDH forms with acetaldehyde were well below 5 mM, "total" AlDH activity of each liver fraction was measured with 5 mM acetaldehyde as substrate. "Low K_m" activity was estimated as activity with 50 μM acetaldehyde as substrate. "High K_m" activity was calculated by subtracting the low K_m from the total activity [17]. Concentrations of acetaldehyde from 20 μM to 5 mM were used to determine K_m values for enzyme in the mitochondrial and cytosolic fractions.

Proteins were determined as described by Lowry *et al.* [18]. Differences between means of control and ethanol-fed animals were compared by Student's *t*-test.

RESULTS

Both the mitochondrial and cytosolic fractions of baboon liver exhibited biphasic kinetics with acetaldehyde. Apparent K_m values, one in the micromolar and another in the millimolar range, were calculated from the two linear portions of the Lineweaver–Burk plots of the mitochondrial and cytosolic fractions of baboon liver (Table 1). With 5 mM (but not with 50 μM) acetaldehyde as substrate, some AlDH activity was detected in the microsomal fraction of baboon liver. For comparison, the apparent K_m values for the AlDH–acetaldehyde interaction in mitochondrial and cytosolic rat liver fractions were also determined.

Table 1. Apparent K_m of AlDH for acetaldehyde in baboon and rat liver subcellular fractions

Animal	Mitochondria Low K_m (μM)	Mitochondria High K_m (μM)	Cytosol Low K_m (μM)	Cytosol High K_m (μM)
Baboon	11	150	45	550
Rat	6	900	120	1100

Table 2. Subcellular distribution of hepatic AlDH in the baboon*

Subcellular fraction	AlDH activity Total	Low K_m	High K_m
Mitochondria	1630	1190	440
	1610	1360	250
Microsomes	90	Trace	90
	78	Trace	78
Cytosol	595	383	212
	357	231	126
Sum of fractions	2320	1570	742
	2050	1590	450

* Activities, expressed in nmoles NADH produced · min^{-1} · g $liver^{-1}$, are values from two baboons. Total activity was measured with 5 mM acetaldehyde, low K_m activity with 50 μM, and high K_m activity was calculated by subtracting the low K_m from the total activity.

In the baboon liver, about 70–80 per cent of the total AlDH activity was located in the mitochondrial fraction (Table 2). Almost all of the remaining total activity was in the cytosol with only 4 per cent having been in the microsomes. The mitochondria also contained about 80 per cent of the low K_m AlDH activity with the other 20 per cent having been in the cytosolic fraction. Using 50 μM acetaldehyde as substrate, AlDH was barely detectable in the microsomal fraction. The high K_m activity was more evenly distributed among the three fractions.

In rat liver (Table 3), the major part of both the total and low K_m AlDH activity was found in the mitochondria. In contrast to the baboon, the hepatic microsomal activity in rat constituted more than one-third of the high K_m activity and contributed 20 per cent to the total hepatic AlDH activity.

Chronic ethanol consumption by the baboons led to a 22 per cent decrease in total hepatic mitochondrial AlDH activity expressed per g weight liver. This decrease resulted from a 25 per cent decrease in low K_m activity (Table 4). As illustrated by Table 5, about a 20 per cent decrease in total hepatic and total mitochondrial AlDH activity resulted from chronic ethanol consumption by the rat. Hepatic low K_m activity was 31 per cent lower and mitochondrial low K_m activity decreased 35 per cent after 2 months of ethanol consumption. The high K_m AlDH activity

Table 3. Subcellular distribution of hepatic AlDH in the rat*

Subcellular fraction	AlDH activity Total	Low K_m	High K_m
Mitochondria	2110 ± 340	1430 ± 120	683 ± 354
Microsomes	657 ± 37	6 ± 5	651 ± 36
Cytosol	558 ± 47	120 ± 9	439 ± 39
Sum of fractions	3320 ± 560	1550 ± 180	1770 ± 350

* Activity [nmoles · min^{-1} · (g $liver)^{-1}$] is expressed as mean NADH produced ± S.E.M. (N = 4). Total AlDH activity was measured with 5 mM acetaldehyde, low K_m activity with 50 μM, and high K_m activity was calculated as total minus low K_m activity.

Table 4. Effect of ethanol consumption on AlDH in baboon liver subcellular fractions*

Subcellular fraction	AlDH activity		
	Total	Low K_m	High K_m
Ethanol-fed			
Mitochondria	1450 ± 30	1240 ± 90†	213 ± 42
Cytosol	593 ± 109	450 ± 83	113 ± 38
Microsomes	76 ± 16	5 ± 3	71 ± 10
Sum of fractions	2090 ± 110	1700 ± 120	398 ± 61
Pair-fed controls			
Mitochondria	1860 ± 250	1660 ± 170	207 ± 71
Cytosol	414 ± 76	308 ± 56	110 ± 11
Microsomes	84 ± 33	9 ± 4	76 ± 25
Sum of fractions	2370 ± 160	1970 ± 110	393 ± 61

* Activity [nmoles·min^{-1}·(g liver)$^{-1}$] is expressed as mean NADH produced ± S.E.M. (N = 3). Total activity was measured with 5 mM and low K_m with 50 μM acetaldehyde as substrate. High K_m activity was calculated as the difference between the total and low K_m activities.

† $P < 0.05$, compared to control

was not altered significantly by ethanol consumption in either baboon or rat.

In both animals, AlDH specific activity [nmoles·min^{-1}·(mg protein)$^{-1}$] of the liver fractions exhibited the same pattern of change with ethanol consumption as seen in activity expressed per g liver. Mitochondrial specific activity was decreased significantly when measured with either 50 μM or 5 mM acetaldehyde.

As illustrated by Table 6, the liver weights of ethanol-fed rats and their pair-fed controls were not different after 8 weeks of ethanol consumption. Total mitochondrial AlDH activities were 33 and 26 μmoles·min^{-1}·liver^{-1} in control and ethanol-fed animals respectively. Mitochondrial activities with 50 μM acetaldehyde were 28 and 19 μmoles·min^{-1}·liver^{-1}, respectively, in control and ethanol-fed animals, indicating that ethanol consumption caused a 34 per cent decrease in activity per total liver. The liver volumes of the baboons used in this study have been estimated by roentgenologic methods*. The liver volumes of the ethanol-fed animals were approximately 90 per cent of those of their pair-fed controls. Therefore, the decrease in low K_m mitochondrial activity after ethanol feeding should be at least as great if not greater when expressed per liver or per animal.

The body weights of rats fed ethanol chronically were only 90 per cent of those of the pair-fed controls

* P. Jauhonen, personal communication (1981).

Table 6. Effect of ethanol consumption on liver and body weights of rats*

	Liver wt (g)	Body wt (g)	Liver/body wt (g/100 g)
Pair-fed controls	9.93 ± 0.44	326 ± 13	3.06 ± 0.15
Ethanol-fed	10.2 ± 0.5	290 ± 5	3.53 ± 0.18

* Values are means ± S.E.M. with three animals in each group.

Table 5. Effect of chronic ethanol consumption on AlDH in the subcellular fractions of rat liver*

Subcellular fraction	AlDH activity		
	Total	Low K_m	High K_m
Ethanol-fed			
Mitochondria	2560 ± 390†	1840 ± 270†	720 ± 133
Cytosol	435 ± 55	290 ± 29	145 ± 31
Microsomes	763 ± 37	2 ± 3	761 ± 38
Sum of fractions	3760 ± 380†	2130 ± 270†	1630 ± 140
Pair-fed controls			
Mitochondria	3330 ± 60	2850 ± 270	485 ± 245
Cytosol	563 ± 41	258 ± 67	306 ± 100
Microsomes	876 ± 111	6 ± 4	870 ± 109
Sum of fractions	4770 ± 100	3110 ± 250	1640 ± 330

* Acitivity [nmoles·min^{-1}·(g liver)$^{-1}$] is expressed as mean NADH produced ± S.E.M. (N = 3). Total activity was measured with 5 mM and low K_m with 50 μM acetaldehyde as substrate. High K_m activity was calculated as the difference between the total and low K_m activities.

† $P < 0.05$, compared to control.

(Table 6). In a similar manner, the baboons fed alcohol weighed less than the controls. Therefore, the ratio of liver to body weight was much greater in ethanol-fed animals and AlDH activity per g body weight was not lower, and may have been even higher, in ethanol-fed animals.

DISCUSSION

In our experiments, AlDH activity was distributed in the mitochondria, endoplasmic reticulum, and cytosol of the baboon liver. The distribution of hepatic AlDH in baboon was very similar to that reported by Koivula [19] for human liver obtained by surgical biopsy. In both primates, high and low (for substrate) K_m forms of AlDH were found in both the cytosol and mitochondria and only minor, high K_m activity was found in the microsomes. In the baboon liver most of the total activity and 80 per cent of the low K_m activity was located in the mitochondria. Koivula [19] found that at least one-half of the total and more than 60 per cent of the low K_m AlDH activity in the human liver was in the mitochondria.

Harada *et al.* [9], however, using human autopsy samples, reported a greater contribution of cytosolic AlDH to liver acetaldehyde metabolism. Since AlDH activity in the mitochondria and microsomes of rat liver decreased much more than that in the cytosol when liver samples were kept overnight at room temperature, Pietruszko *et al.* [20] concluded that autopsy samples may not provide actual subcellular distributions of AlDH.

Unlike the controversy regarding the primate, it is generally accepted that in rat liver the mitochondrion is the primary site of acetaldehyde oxidation [21, 22]. In the present study, the majority of the total and almost all of the *in vitro* low K_m AlDH activity were contained in the mitochondrial fraction of the rat liver, and the distribution of AlDH corresponded to that in other studies on rats [3, 4] using different subcellular fractionation and AlDH assay condition. Therefore, the fractionation and AlDH assay conditions used in the studies of baboon liver should have produced adequate results since these procedures were as similar as possible to those with rats.

The present results may, in part, explain the mechanism whereby ethanol consumption leads to increased blood acetaldehyde levels after acute ethanol administration [10, 12]. Chronic ethanol consumption diminishes total *in vitro* AlDH activity in baboon liver. A reduced low K_m mitochondrial AlDH activity is responsible for this decrease. Jenkins and Peters [23] found that AlDH activity in liver needle-biopsy specimens was considerably lower in alcoholics than in control subjects. Enzyme activity, however, as measured with 8.3 mM acetaldehyde as substrate, was lower in the cytosolic fraction as well as in the mitochondrial fraction. The AlDH activities of Jenkins and Peters [23] are expressed only in relative units; therefore direct comparison with the changes seen in baboon liver is not possible.

The diminution in AlDH activity does not appear to be species specific. In the present study, ethanol consumption by rats caused a decrease in hepatic AlDH activity. As we found in the baboon, this decrease was apparently due to decreased activity of the low K_m mitochondrial form of the enzyme. This form of AlDH in rat liver has been reported to be more sensitive than the high K_m form to inhibition by chloral hydrate and disulfiram [5] and pargyline [17] and by the dietary factor described by Marchner and Tottmar [24]. Inhibition of protein synthesis by cycloheximide led to a more rapid decrease in the activity of the low K_m mitochondrial form than of the other mitochondrial form of AlDH [25]. The reasons for the possible higher sensitivity of this form of AlDH are not known.

A decrease in AlDH activity as seen in the present study would explain the lowered acetaldehyde-oxidizing capacity of isolated rat liver mitochondria from ethanol-fed animals reported by Hasumura *et al* [14]. In this earlier study [14], however, low K_m AlDH activity in disrupted mitochondrial fractions was not different in the ethanol and control groups when the assays were conducted at pH 8.5 and 25° with 180 μM acetaldehyde as substrate. The variables that decreased (the oxidizing capacity of undisrupted mitochondria in the earlier study and the *in vitro* AlDH activity of the present study) were both measured at a more physiological pH of 7.4 and temperature of 37°. It is also possible that alterations can be detected in the intact mitochondria before *in vitro* AlDH activity is altered. In the previous study, ethanol consumption continued for only 4–5 weeks compared to 8 weeks in the present study. It should also be noted that Horton and Barrett [26] have reported the induction of hepatic mitochondrial AlDH in rats 3 hr after gastric intubation of ethanol. This effect may further complicate comparison between different experiments.

The mechanism by which ethanol consumption causes decreased AlDH activity is not known. Administration of carbon tetrachloride to rats also causes lower than normal hepatic AlDH activity [27] indicating that the decrease may be due to a more general alteration in the liver rather than to a specific effect of ethanol. A decreased ability to oxidize acetaldehyde coupled with the reported increased capacity of the liver to oxidize ethanol after chronic alcohol consumption [28, 29] could lead to elevated acetaldehyde levels and the toxicity that this compound may produce.

Acknowledgements—We would like to thank Ms. R. Goodblatt and Ms. N. Lowe for their technical assistance and Dr. M. Arai who performed the glutamate dehydrogenase assays. This work was supported by the Medical Research Service of the Veterans Administration and Grants AA-07275, AA-03508 and AA-00224, and was presented, in part, at the 1980 meeting of the Federation of American Societies for Experimental Biology [30].

REFERENCES

1. M. J. Walsh, in *Biological Aspects of Alcohol* (Eds, M. K. Roach, W. M. McIssac and P. J. Creaven), p. 233. University of Texas Press, Austin (1971).
2. C. S. Lieber and L. M. DeCarli, in *Metabolic Aspects of Alcoholism* (Ed. C. S. Lieber), p. 31. University Park Press, Baltimore (1977).
3. S. O. C. Tottmar, H. Pettersson and K-H. Kiessling, *Biochem. J.* **135**, 577 (1973).

4. T. Koivula and M. Koivusalo, *Biochim. biophys. Acta* **397**, 9 (1975).
5. C. Siew, R. A. Deitrich and V. G. Erwin, *Archs Biochem. Biophys.* **176**, 638 (1976).
6. R. J. Kraemer and R. A. Deitrich, *J. biol. Chem.* **243**, 6402 (1968).
7. A. H. Blair and F. H. Bodley, *Can. J. Biochem.* **47**, 265 (1969).
8. N. J. Greenfield and R. Pietruszko, *Biochim. biophys. Acta* **483**, 35 (1977).
9. S. Harada, D. P. Agarwal and H. W. Goedde, *Life Sci.* **26**, 1773 (1980).
10. M. A. Korsten, S. Matsuzaki, L. Feinman and C. S. Lieber, *New Engl. J. Med.* **292**, 386 (1975).
11. C. S. Lieber and L. M. DeCarli, *J. med. Primatol.* **3**, 153 (1974).
12. P. H. Pikkarainen, M. P. Salaspuro and C. S. Lieber, *Alcoholism* **3**, 259 (1979).
13. C. S. Lieber and L. M. DeCarli, *Fedn Proc.* **35**, 1232 (1976).
14. Y. Hasumura, R. Teschke and C. S. Lieber, *J. biol. Chem.* **251**, 4908 (1976).
15. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
16. A. E. Harper, in *Methods of Enzymic Analysis* (Ed. H. U. Bergmeyer), p. 1788. Academic Press, New York (1963).
17. M. E. Lebsack, D. R. Petersen, A. C. Collins and A. D. Anderson, *Biochem. Pharmac.* **26**, 1151 (1977).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. T. Koivula, *Life Sci.* **16**, 1563 (1975).
20. R. Pietruszko, N. J. Greenfield and C. R. Edson, in *Alcohol and Aldehyde Metabolizing Systems Vol. 2*, (Eds. R. G. Thurman, J. R. Williamson, H. R. Drott and B. Chance), Vol. 2, p. 195. Academic Press, New York (1977).
21. R. Parrilla, K. Ohkawa, K. O. Lindros, U. U. P. Zimmerman, K. Kobayashi and J. R. Williamson, *J. biol. Chem.* **249**, 4926 (1974).
22. D. L. Cinti, S. R. Keyes, M. A. Lemelin, H. Denk and J. B. Schenkman, *J. biol. Chem.* **251**, 1571 (1976).
23. W. J. Jenkins and T. J. Peters, *Lancet* **1**, 628 (1980).
24. H. Marchner and O. Tottmar, *Acta pharmac. tox.* **38**, 59 (1976).
25. M. E. Lebsack and A. D. Anderson, *Res. Commun. Chem. Path. Pharmac.* **26**, 263 (1979).
26. A. A. Horton and M. C. Barrett, *Biochem. J.* **156**, 177 (1976).
27. H. Kono, M. Fujii, T. Sokabe and J. Kaneshige, *Enzyme* **24**, 142 (1979).
28. C. S. Lieber and L. M. DeCarli, *J. Pharmac exp. Ther.* **181**, 279 (1972).
29. L. Videla, J. Bernstein and Y. Israel, *Biochem. J.* **134**, 507 (1973).
30. M. E. Lebsack, E. R. Gordon and C. S. Lieber, *Fedn Proc.* **39**, 540 (1980).