Biochemical Pharmacology, Vol. 30, No. 7, pp. 799–802, 1981.
Printed in Great Britain.

0006-2952/81/070799-04 $02.00/0

DETERMINANTS OF PLASMA FREE ACETALDEHYDE LEVELS DURING THE OXIDATION OF ETHANOL

EFFECTS OF CHRONIC ETHANOL FEEDING*

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(*Received* 2 *June* 1980; *accepted* 21 *September* 1980)

Abstract—Plasma free acetaldehyde levels were measured by an improved method in baboons fed ethanol chronically and in pair-fed controls during an intravenous infusion of ethanol. After an appropriate loading dose, ethanol was infused at the rate of its elimination to achieve a steady state at one of three different blood ethanol levels (50 ± 10 mM, 10 ± 2 mM, or 5 ± 1 mM). The rate of production of acetaldehyde was calculated from the rate of ethanol elimination by subtracting losses into urine and expired air. Liver mitochondrial aldehyde dehydrogenase (AlDH) activity was measured in surgical biopsy samples with 50 μM acetaldehyde as substrate. Chronic ethanol administration resulted in both higher plasma free acetaldehyde levels and faster acetaldehyde production at each level of blood ethanol. When the blood level of ethanol was increased from 5 to 50 mM, the level of plasma free acetaldehyde also rose in both groups of animals. The rate of acetaldehyde production, however, increased only in alcohol-fed baboons. Plasma free acetaldehyde had a significant positive correlation with production rate of acetaldehyde ($r = 0.69$) and a significant negative correlation with liver mitochondrial AlDH specific activity ($r = -0.59$). When these two parameters were combined (acetaldehyde production rate/AlDH activity), a correlation coefficient of 0.84 resulted, suggesting that, in addition to increased production, decreased catabolism may contribute to the higher acetaldehyde levels seen after chronic consumption.

Acetaldehyde, the product of ethanol oxidation, is considered to be toxic because of its reactivity [1]. It has been shown that during ethanol oxidation, blood acetaldehyde levels are higher in alcoholics than in non-alcoholic subjects [2]. This could exacerbate the neurologic, hepatic, and cardiac complications of alcoholism. Even subjects having alcoholic parents or siblings have been reported to have elevated blood acetaldehyde concentrations after a moderate dose of alcohol [3]. Recent methodological advances have provided evidence that the concentration of free acetaldehyde in the plasma is lower than the total levels reported in these earlier studies [4]. Therefore, we re-investigated the question of factors that could regulate the level of acetaldehyde in the blood. Acetaldehyde levels were compared with the rate of acetaldehyde production and the activity of hepatic aldehyde dehydrogenase.

MATERIALS AND METHODS

Experimental animals. Twenty baboons were pair-fed either an ethanol-containing (50% of total calories) liquid diet or a diet in which ethanol was replaced isocalorically by carbohydrate as described previously [5]. Controlled feeding was carried out for 3 months (four pairs), 2 years (four pairs), 6 years (one pair), or 7 years (one pair) at the Laboratory for Experimental Medicine and Surgery in Primates, Tuxedo, NY. All animals fed ethanol for 3 months, as well as one animal fed ethanol for 2 years, had only fatty livers. The remainder of the ethanol-fed animals had either fibrosis (4) or cirrhosis (1), in addition to fatty infiltration, of the liver.

Experimental design. In all experiments the ethanol-fed baboons and their controls were studied on the same day. Individual experiments on the same pair were carried out at least 1 week apart.

On the day preceding each experiment, the ethanol or control diets were withdrawn from the animals at 4:00 p.m. Each of the baboons then received one-third of their average daily intake of control diet. In addition, at midnight, a dextrin–maltose solution equivalent to one-third their caloric intake was given to avoid the effect of fasting. At 8:00 a.m., under light ketamine anesthesia, a urinary catheter and two indwelling intravenous catheters, one for blood sampling and the other for infusion, were inserted. During the experiment, a 5% glucose solution was infused to prevent hypoglycemia in the animals. To obtain an initial blood ethanol level of 50 ± 10 mM, 10 ± 2 mM or 5 ± 1 mM, a loading dose of 1.7, 0.4 or 0.2 g of ethanol per kg body weight, respectively, was given over 30–45 min.

To maintain blood ethanol at a constant level, a 10% solution of ethanol in saline was continuously infused during the subsequent 4 hr with a Harvard

* This research was supported by the Medical Research Service of the Veterans Administration, USPHS Grants AA 03508, 07275 and 00224, and presented, in part, at the Eleventh Annual NCA/AMSA/RCA Medical-Scientific Conference, Seattle, WA. (P. Pikkarainen, E. R. Gordon, M. E. Lebsack and C. S. Lieber, [6]).

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Apparatus infusion pump model 975. The infusion rate was based on the rate of ethanol elimination previously determined after a single dose of ethanol in each individual animal. Samples for blood ethanol determination (0.5 ml) were taken every 15 min. After blood ethanol levels reached the desired plateau (60–90 min from the start of the experiment), a 2-hr urine sample was collected, and its volume and ethanol concentration were measured. Samples for plasma free acetaldehyde determination were taken 275 and 300 min after the start of the experiment.

The rate of ethanol elimination equals the rate of ethanol infusion when blood ethanol levels remain constant. Since blood ethanol concentrations sometimes deviated from the plateau level, a correction factor, calculated as described previously [7], was subtracted from the infusion rate to give the actual elimination rate. The rate of change of the blood ethanol concentration was determined in each experiment. An estimated volume of distribution of ethanol was obtained by multiplying 0.7 times the body weight of the animal in kilograms. The correction factor was then calculated by multiplying the slope by the volume of distribution, and in each group of animals the average correction was less than 10 per cent of the ethanol elimination rate. In addition, even assuming an error as large as 10 per cent in the volume of distribution, the error in the calculated rate of ethanol elimination would be less than 1 per cent.

The rate at which ethanol is oxidized to acetaldehyde is the ethanol elimination rate less the rate of excretion of unmetabolized ethanol. The urinary rate of ethanol excretion was measured as described [7]. The rate of ethanol exhalation was calculated from the partition ratio of blood to breath ethanol of 2100 and the respiratory minute volume of 100 ml/g per min for baboons reported by Haglin [8]. The pulmonary elimination of ethanol was approximately 5 per cent at 50 mM, 1 per cent at 10 mM, and 0.5 per cent at the 5 mM level of blood ethanol.

Analytical methods. A Perkin–Elmer F-40 gas chromatograph equipped with an automatic sampling device was used to measure blood ethanol [2] and plasma free acetaldehyde [4] as described previously. For plasma free acetaldehyde determination, heparinized blood was immediately centrifuged. Plasma (0.5 ml) was placed in sealed vials and incubated for 15 min at 37°, and the acetaldehyde in the vapor was measured. The concentration of acetaldehyde was determined by comparing peak heights to acetaldehyde standards (0.5 to 10 μM) prepared in distilled water. Under these conditions, the recovery was 72 ± 2 per cent, and the sensitivity was 0.5 ± 0.1 μM.

Aldehyde dehydrogenase assay. Liver samples were obtained from surgical biopsies taken under ketamine anesthesia from 2 to 4 weeks after the infusion study. Samples were homogenized in 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris (pH 7.4). Mitochondrial fractions were separated by differential centrifugation [9]. After solubilization with 0.2% (final concentration) sodium deoxycholate, the mitochondrial preparations were centrifuged for 1 hr at 38,000 g, and the supernatant fraction was used in aldehyde dehydrogenase (EC 1.2.1.3) assays. The rate of NADH production at 37° was measured spectrophotometrically at 340 nm. Reaction mixtures contained 0.5 mM NAD, 2 μM rotenone, 50 mM potassium phosphate buffer (pH 7.4) containing 1.15 mM $MgCl_2$, and 50 μM acetaldehyde as substrate. Mitochondrial protein was determined as described by Lowry *et al.* [10].

Statistical analysis. Matched-pair t-tests were used to evaluate the differences between ethanol-fed and control animals.

RESULTS

Plasma free acetaldehyde levels. As shown in Table 1, the plasma free acetaldehyde levels at 275 and 300 min were similar within each group, indicating that, when blood ethanol levels were at a steady state, plasma free acetaldehyde levels also remained relatively constant. At all three blood ethanol levels, 5, 10 or 50 mM, the plasma free acetaldehyde levels were significantly higher in the ethanol-fed baboons compared to their respective controls. In both groups of baboons, the plasma level of free acetaldehyde was significantly higher at 50 mM than at 5 mM ethanol.

Rate of acetaldehyde production. The rates of acetaldehyde production at the different blood ethanol levels are shown in Table 2. Baboons chronically fed ethanol had significantly higher rates of acetaldehyde production than their pair-fed controls at each level of blood ethanol investigated. In alcohol-fed baboons the rate of acetaldehyde production was significantly higher at 50 mM than at 5 mM blood ethanol levels. In control baboons there was no change in the rate of acetaldehyde production with increasing blood concentrations.

Mitochondrial aldehyde dehydrogenase activity. A significant difference was observed between the ethanol-fed and control baboons in the specific

Table 1. Effect of chronic ethanol consumption on plasma free acetaldehyde levels during ethanol infusion*

Blood ethanol	Pair-fed controls 275 min	Pair-fed controls 300 min	Ethanol-fed 275 min	Ethanol-fed 300 min
5 mM (9)	1.2 ± 0.2	1.0 ± 0.2	3.1 ± 0.8†	2.9 ± 0.6†
10 mM (6)	1.3 ± 0.2	1.3 ± 0.2	3.8 ± 1.2†	4.0 ± 1.2†
50 mM (10)	1.8 ± 0.2	1.9 ± 0.2	5.0 ± 1.0†	5.1 ± 1.0†

* Values (μM) are means ± S.E. The number of animals in each group is given in parenthesis.
† Significantly different from control value ($P < 0.01$).

Table 2. Effect of chronic ethanol consumption on acetaldehyde production rates*

Blood ethanol	Pair-fed controls	Ethanol-fed
5 mM (9)	2.47 ± 0.14	3.02 ± 0.12†
10 mM (6)	2.43 ± 0.10	3.23 ± 0.12‡
50 mM (10)	2.59 ± 0.22	3.41 ± 0.20§

* Values are means ± S.E. expressed as mmoles/kg body weight per hr. The number of pairs of animals in each group is given in parenthesis.
† Significantly different from controls ($P < 0.05$).
‡ Significantly different from controls ($P < 0.001$).
§ Significantly different from controls ($P < 0.005$).

activity of the low K_m hepatic mitochondrial AlDH activity. The mean activity ± the standard error in nmoles of NADH formed · min^{-1} · (mg protein)$^{-1}$ was 41.4 ± 4.0 and 52.5 ± 2.2 in the alcohol-fed and controls respectively ($P < 0.05$).

Correlation of plasma acetaldehyde with production rate and AlDH activity. The range of plasma free acetaldehyde was greater in ethanol-fed baboons than in their controls (Fig. 1). Within the alcohol-fed group there was a positive correlation between the plasma free acetaldehyde level and the rate of production of acetaldehyde at the 50 mM blood ethanol level ($r = 0.65$; $P < 0.05$). When the data from all twenty baboons were included, the correlation coefficient was 0.69 ($P < 0.001$). No significant correlation was found at the lower blood ethanol levels.

Plasma free acetaldehyde levels had a significant negative correlation with mitochondrial AlDH activity when data from all twenty baboons were used ($r = -0.59$; $P < 0.01$) (Fig. 2). A more highly significant correlation was obtained when the plasma free acetaldehyde levels were correlated with the ratio of the rate of acetaldehyde production divided by *in vitro* AlDH activity (Fig. 3). In alcohol-fed baboons, the correlation coefficient was 0.80 ($P < 0.01$) and including the controls the correlation coefficient was 0.84 ($P < 0.001$).

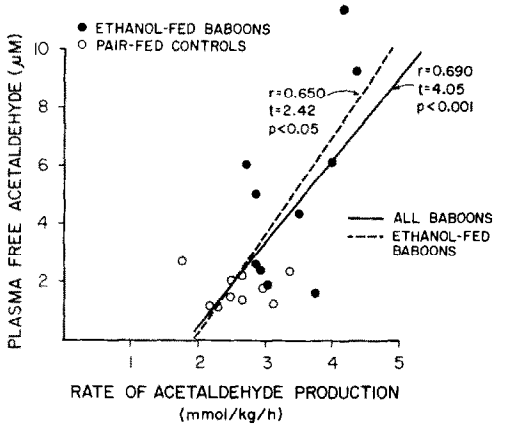

Fig. 1. Correlation between plasma free acetaldehyde concentration and the rate of acetaldehyde production. Blood ethanol levels were maintained at 50 mM.

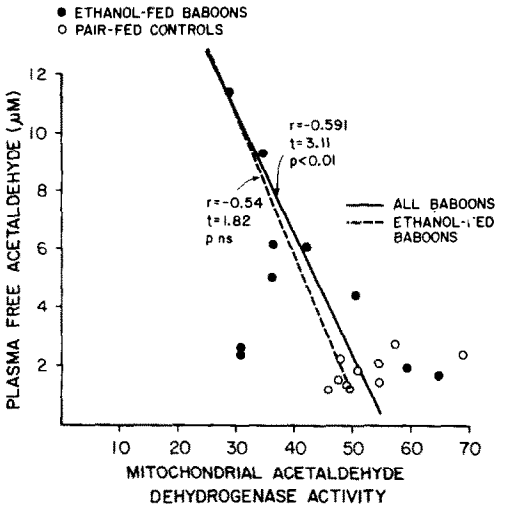

Fig. 2. Correlation between plasma free acetaldehyde concentrations and liver mitochondrial aldehyde dehydrogenase activity. AlDH activity is expressed in nmoles NADH formed per min per mg protein.

DISCUSSION

This study reveals that plasma free acetaldehyde levels are positively correlated with the rate of acetaldehyde production and negatively with *in vitro* hepatic AlDH activity. The plasma free acetaldehyde levels observed in the present study are lower than blood acetaldehyde levels reported previously in human subjects with similar blood ethanol levels [2]. This difference probably is the result of a change in methods. In the present study, the new method used to measure the concentration of free acetaldehyde in plasma circumvented the spontaneous production of acetaldehyde from ethanol in blood samples during the precipitation of proteins. Although the absolute levels of acetaldehyde detected were very

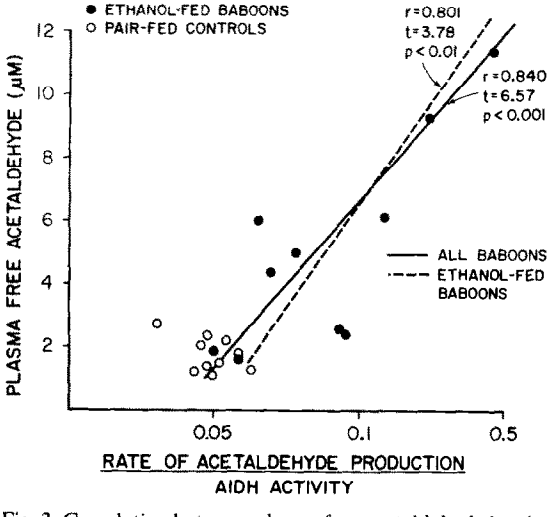

Fig. 3. Correlation between plasma free acetaldehyde levels and the ratio of the acetaldehyde production rate relative to liver mitochondrial AlDH activity.

low, the observed relative changes agree with earlier increases reported in alcoholic subjects [2]. Ethanol-fed baboons, with blood levels of 50 mM ethanol, had plasma acetaldehyde levels two and one-half times greater than their respective pair-fed controls at the same blood ethanol level.

This response was observed in all baboons that had been maintained on the ethanol containing diet for at least 3 months. This included baboons that exhibited only the early stage of alcoholic liver injury (a fatty liver) as well as those maintained for from 2 to 7 years and exhibiting injury ranging from mild fibrosis to incomplete cirrhosis.

In this primate model, we have examined two systems that may regulate blood acetaldehyde levels, namely ethanol oxidation rate and AlDH activity. The data suggest that the difference in plasma free acetaldehyde levels between ethanol and control baboons can be explained, in part, by a greater rate of acetaldehyde production in the ethanol-fed animals. In addition, data from our laboratory indicate that the subcellular forms of AlDH in the liver of the baboon are very similar to those reported in the rat [11] and human [12] and that at micromolar acetaldehyde concentrations mitochondrial AlDH activity may predominate. The mean specific AlDH activity of mitochondrial fractions with 50 μM acetaldehyde was significantly lower in the ethanol-fed baboons compared to their controls. It is not known, however, whether *in vivo* acetaldehyde oxidizing capacity is impaired after chronic ethanol consumption since factors other than enzyme activity may be rate limiting. Furthermore, total hepatic AlDH activity cannot be calculated because the liver weight of these animals is not known.

In summary, plasma levels of free acetaldehyde correlated positively with the rate of production of acetaldehyde and negatively with mitochondrial AlDH activity. Since the most significant correlation was obtained when blood acetaldehyde levels were compared to a combination of an *in vivo* production rate and an *in vitro* AlDH activity (Fig. 3), it is possible that a decrease in acetaldehyde oxidation as well as increased acetaldehyde production may contribute to the increased blood acetaldehyde levels seen after chronic ethanol consumption.

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