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Brain Aldehyde Dehydrogenase Activity In Rat Strains with High and Low Ethanol Preferences

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INOUE, K., M. RUSI AND K. O. LINDROS. *Brain aldehyde dehydrogenase activity in rat strains with high and low ethanol preferences*. PHARMAC. BIOCHEM. BEHAV. 14(1) 107–111, 1981.—The activity of aldehyde dehydrogenase in subcellular fractions of whole brain homogenates from the AA and ANA rat strains developed respectively for high and low ethanol preferences has been studied. No significant strain or sex differences between naive AA and ANA rats were found. In ethanol-experienced rats some strain and sex differences were found, the most consistent being higher enzyme activity in AA females than in males both with aliphatic and aromatic aldehyde substrates. However, contrary to previous findings no relation between brain aldehyde dehydrogenase activity and drinking behavior was found in the AA and ANA rat strains.

Brain aldehyde dehydrogenase Drinking behaviour Preference Acetaldehyde Rat strains

RECENTLY, much interest has been focused on the possible contribution of acetaldehyde, the reactive metabolite from ethanol, to pharmacological and behavioral effects of ethanol (for reviews, see Lindros [19] and Amir *et al.* [4]). Experimental evidence for both aversive and reinforcing properties of acetaldehyde in experimental animals exists [5,8], but these results seem incompatible with the near-absence of acetaldehyde in brain tissue during ethanol intoxication [20, 25, 26, 28].

What appears to be a relationship between acetaldehyde and alcohol drinking behavior may, however, be only a reflection of a primary relationship between alcohol drinking and differences in tissue capacity to oxidize acetaldehyde, with the metabolic differences than affecting acetaldehyde levels. In fact, several recent studies by Amir [1–3] and from our laboratory [24] indicate a direct relationship between brain aldehyde dehydrogenase activity and voluntary alcohol consumption. In order to further test this possiblity, the AA and ANA rat strains, which have been developed in our laboratory by selective outbreeding for respectively high and low voluntary alcohol intake [11,12], were compared. Low ethanol intake by ANA rats has previously been shown to be associated with higher blood acetaldehyde [7] and lower liver aldehyde dehydrogenase activities [17]. In the present study brain aldehyde dehydrogenase activities were assayed using both aromatic and aliphatic aldehyde substrates. Since the selection procedure for the AA and ANA strains is preceded by habituation, giving the animals only alcohol solution to drink, the possibility that the previous ethanol experience affects brain aldehyde oxidizing capacity or drinking behaviour cannot be excluded. Therefore, both young naive animals, and rats with ethanol experience were tested.

Although a large difference in alcohol intake and some differences in brain aldehyde dehydrogenase acitivity were observed, the results do not indicate a relation between brain aldehyde dehydrogenase activity and alcohol preference in the AA and ANA rat strains.

METHOD

Animals

We used adult male and female rats of the AA (Alko, Alcohol) and ANA (Alko, Non-Alcohol) strains developed for high and low voluntary ethanol consumption by selective outbreeding, as described elsewhere [11,12]. Each generation has typically been exposed to a 7 day habituation period with only 10% (v/v) ethanol as the drinking fluid [10]. Subsequently, a free choice between 10% ethanol and tap water is offered. The order of the bottles is changed weekly. The fluid intake is recorded daily and consumption of food (from Astra-Ewos, Södertälje, Sweden) and body weights once a week.

The free choice experiments are carried out in a room

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TABLE 1

SUBCELLULAR DISTRIBUTION OF BRAIN ALDEHYDE DEHYDROGENASE IN NAIVE AA AND ANA RATS

Subcellular fraction	Substrate	nmoles/min per g tissue Males AA(6)	Males ANA (6)	Females AA (6)	Females ANA (6)
Total homogenate	Acetaldehyde 50 μM	39.8 ± 9.2	46.2 ± 10.5	42.6 ± 11.8	44.8 ± 11.1
	Benzaldehyde 50 μM	18.7 ± 3.8	20.3 ± 7.4	19.7 ± 7.2	22.9 ± 6.9
	Benzaldehyde 2 mM	83.1 ± 6.4	89.0 ± 10.1	97.1 ± 14.5	93.3 ± 18.2
Mitochondrial Fr.	Acetaldehyde 50 μM	14.7 ± 1.3	14.4 ± 1.9	14.4 ± 2.3	14.1 ± 1.7
	Benzaldehyde 50 μM	4.6 ± 0.2	4.8 ± 0.6	5.0 ± 0.8	5.2 ± 0.8
	Benzaldehyde 2 mM	17.9 ± 1.5	20.0 ± 1.2	20.1 ± 3.7	23.2 ± 4.5
Nuclear Fr.	Benzaldehyde 2 mM	27.2 ± 3.5	27.6 ± 7.2	30.4 ± 1.6	31.6 ± 3.8
Microsomal Fr.	Benzaldehyde 2 mM	12.3 ± 0.6	12.7 ± 0.6	11.7 ± 0.7	13.4 ± 0.9*
Cytosol	Benzaldehyde 2 mM	12.4 ± 1.1	13.1 ± 1.5	13.8 ± 2.0	13.4 ± 2.3

Values are expressed as means ± SD with the number of animals in parentheses.
*$p<0.01$ for difference between the corresponding AA and ANA group.

with a 12 hr light/12 hr dark cycle, the temperature maintained between 22–24°C and the relative humidity 50–55%.

In the present experiments, 4 months old rats of the 35th generation were subjected to this procedure, with 3 weeks of free choice (the first free-choice period). After 4 months on only water, the same animals were again given a free choice for two weeks in the same manner, but without habituation (the second free choice period). After this the rats received water for 3 days and were then killed for the determination of brain enzyme activity. Enzyme activity was also determined in 3 months old naive AA and ANA rats (F 36) with no ethanol experience.

Brain Subcellular Fractionation

Rats were killed by decapitation and the brains were immediately excised. The brains were homogenized with 3 volumes of 0.32 M sucrose solution containing 10 mM sodium phosphate buffer (pH 7.4) and 1 mM glutathione (reduced form). A crude subcellular fractionation was carried out [21]. The mitochondrial and nuclear pellets were washed twice and resuspended in one volume of 10 mM phosphate (pH 7.4) buffer with 1 mM glutathione and 1% Trition X-100. The microsomal pellet was resuspended without washing in the same manner. Original homogenate was also treated with 1% (final concentration) Trition X-100 to determine total activity. For a measurement of total activity alone, the brains were homogenized with the medium used for resuspension of particulate fractions.

Assay Methods

Aldehyde dehydrogenase activity was measured spectrophotometrically at 30°C using a scale expander by following the reduction of NAD to NADH. Samples were preincubated with an assay medium conssiting of 50 mM sodium phosphate (pH 7.4), 1 mM NAD and 1 mM MgCl for 10 min, initiating the reaction by the addition of aldehyde. The reaction velocity was linear and recorded for 15 min. Succinate dehydrogenase was determined as a marker for the mitochondrial fraction [22]. Acetaldehyde and benzaldehyde were from BDH Chemicals, England, and indole-3-acetaldehyde bisulfite salt from Sigma, USA. Free indole-3-acetaldehyde was obtained as described by Deitrich [6] and its concentration was determined enzymatically using rat liver mitochondrial aldehyde dehydrogenase.

RESULTS

Brain Aldehyde Dehydrogenase Activities

Young, naive (without alcohol experience) male and female AA and ANA rats were tested for total brain aldehyde dehydrogenase activity. Homogenates and mitochondrial fractions were assayed using acetaldehyde (50 μM) and benzaldehyde (50 μM and 2 mM) as substrate and nuclear, microsomal and cytosolic fractions using only 2 mM benzaldehyde as substrate. Neither in the total homogenate nor in the subcellular fractions were any statistically significant differences observed (Table 1). Only in the microsomal fraction of female ANA rats was there a slightly higher ($p<0.01$) activity observed than in the corresponding AA group. Neither were any significant differences between the sexes observed.

Brains from older animals, that had been in the free-choice twice situation, were analyzed without subcellular fractionation using a high (0.7–2 mM) and low (50 μM) concentration of acetaldehyde, benzaldehyde or indole-3-acetaldehyde as substrates. In these animals some strain and sex differences were observed (Table 2). In males, the AA animals exhibited lower activities with all three substrates, the differences being statistically significant with acetaldehyde benzaldehyde. In females, however, the AA rats tended to have slightly higher activities than the ANAs, but the difference was significant only with 50 μM benzaldehyde as substrate. In the AA strain a clear sex difference was observed: higher activities were observed in brains from females with all the substrates tested. In the ANA strain, however, females had a statistically lower activity with benzaldehyde, but higher activity with 2 mM indole-3-acetaldehyde.

Voluntary Alcohol Intake

The AA rats consumed significantly more alcohol, measured as g absolute ethanol per kg body weight, than the ANA animals during both the first and second free choice period

TABLE 2

BRAIN ALDEHYDE DEHYDROGENASE ACTIVITY OF ETHANOL-EXPERIENCED AA AND ANA RATS

Substrate		nmoles/min per g tissue Males AA(8)	Males ANA (8)	Females AA(8)	Females ANA (8)
Acetaldehyde	50 μM	64.6 ± 5.0	77.4 ± 5.7***	76.6 ± 9.5††	73.4 ± 8.8
	2 mM	112.4 ± 6.2	125.5 ± 14.9*	128.1 ± 10.9††	121.4 ± 14.4
Benzaldehyde	50 μM	20.5 ± 5.3	26.9 ± 2.5**	26.5 ± 3.7†	21.6 ± 1.2†††
	2 mM	69.6 ± 4.7	81.5 ± 7.7**	78.3 ± 7.2††	71.0 ± 10.2†
Indole 3-	50 μM	31.7 ± 5.0	36.8 ± 7.6	37.7 ± 4.7†	37.1 ± 3.1
aldehyde	0.7 mM	167.9 ± 11.2	171.2 ± 18.7	178.0 ± 16.6	199.7 ± 27.4†

Values are expressed as means ± SD with the number of animals in parentheses.
***$p<0.001$, **$p<0.01$, *$p<0.05$ for differences between corresponding AA and ANA groups.
†††$p<0.001$, ††$p<0.01$, †$p<0.05$ for sex differences within strains.

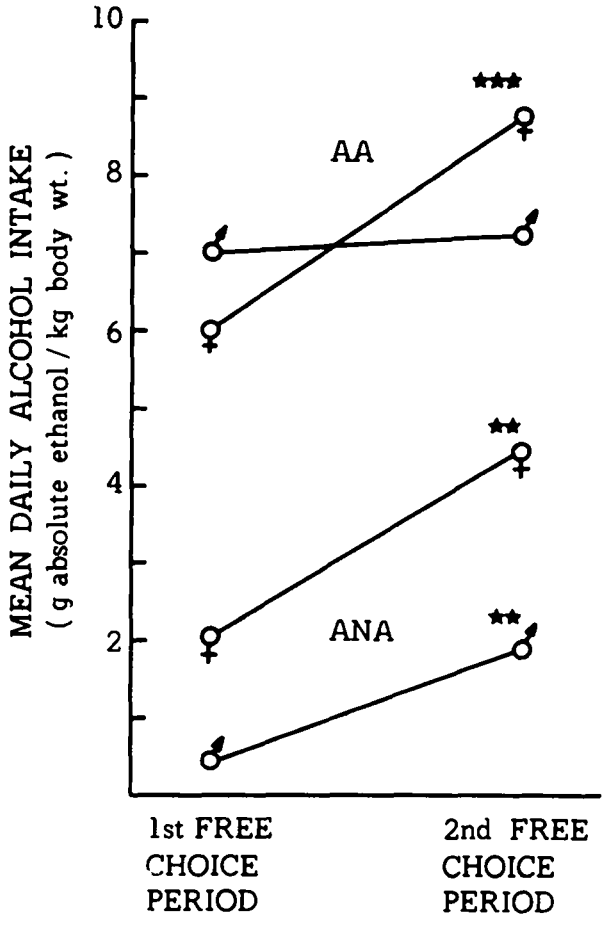

FIG. 1. Voluntary ethanol consumption of AA and ANA rats during two separate free-choice periods. The mean daily consumption of 10% (v/v) ethanol during the last week of the test period is given. The interval between the first and second free-choice period was 4 months. Strain differences were highly significant during both test periods. **$p<0.01$; ***$p<0.001$; for difference between the first and second period for the same group.

(Fig. 1). Females of the ANA strain drank significantly ($p<0.01$) more alcohol than males during free-choice periods, but in the AA strain males drank significantly ($p<0.01$) more than females during the first period, and significantly ($p<0.05$) less than females during the second period.

DISCUSSION

An association between ethanol preference in experimental animals and the activity of brain aldehyde dehydrogenase has been found in several recent studies. Amir found a relation between aldehyde dehydrogenase and alcohol intake from a correlation study in Wistar rats [1], when comparing three different rat strains [3], and after electrical brain stimulation or lesions [2]. Sinclair and Lindros [24] administered the aldehyde dehydrogenase inhibitor cyanamide, which profoundly depressed preference and caused ethanol-derived acetaldehyde accumulation. However, subsequent administration of the alcohol dehydrogenase inhibitor 4-methylpyrazole failed to affect voluntary intake, although it completely removed acetaldehyde accumulation. These data suggest that a deficiency in aldehyde dehydrogenase activity is associated with abstention from alcohol while higher enzyme levels are seen in animals with a preference for alcohol. Brain aldehyde dehydrogenase may influence the metabolism of biogenic aldehydes formed via MAO oxidation from their respective amines either directly or in combination with a modulation of the proposed enzymatic barrier [25] preventing circulating acetaldehyde in cerebral capillaries from penetrating into the brain tissue proper.

However, in the present study, there was no consistent relationship between strain differences in brain aldehyde dehydrogenase, and the differences in drinking behaviour. In naive AA and ANA rats we were unable to detect any significant differences in enzyme activity from the brain homogenate. Similarly, no difference was seen by measuring the rate of acetaldehyde uptake in crude brain homogenates [9]. Since the physiologically crucial low-Km brain aldehyde dehydrogenase is mainly confined to the mitochondrial fraction [15], which also contains most of the synaptosomes, special interest was focused on this fraction. However, we found no strain difference in this or in any of the other subcellualr fractions studied.

In AA and ANA rats with previous ethanol experience some significant strain and sex differences in enzyme activities were observed, but these did not seem to relate to drinking behavior. As previously observed [3, 12, 13, 23], females consumed more alcohol than males. This occurred in both AA and ANA rats, but in AA rats females had higher enzyme activities while in the ANA strain females had lower activities with benzaldehyde and higher activities with indole-3-acetaldehyde as substrate. With respect to strain comparisons, slightly higher enzyme activities were found in ANA rats in spite of much lower alcohol intake. No significant correlations between voluntary alcohol intake and brain enzyme activity were seen when tested for within each group of animals. Thus, in contrast to Amir [1–3] we were unable to detect any consistent individual, strain, or sex relation between high brain aldehyde dehydrogenase activity and elevated alcohol intake. It reamins to be seen whether this discrepancy is explained by different methods used. For instance, in the assay used by Amir non-aldehyde dehydrogenase mediated conversion of indole-3-acetaldehyde to indole acetic acid is also measured.

An increase in alcohol consumption with age is generally observed [10, 14, 23, 27], although the opposite has been found [3, 16, 29]. The absolute increases are of similar magnitude in all groups except in AA males. Relatively seen, however, the increase in ANA animals is very pronounced (2-fold in females and 4-fold in males). If an aversive mechanism causes the low ethanol intake in this strain considerable desensitation of this mechanism must have occurred, either with age or because of the previous alcohol experience. The smaller increase observed especially in the AA males may have a metabolic basis, since these animals already had such a high alcohol consumption during the first period that it approached their capacity to metabolize the ingested alcohol. Unfortunately these drinking effects cannot be related to possible differences in brain aldehyde dehydrogenase activities of young and old animals, since the brain samples were prepared by slightly different methods.

On the basis of these results it is suggested that brain aldehyde dehydrogenase activity does not limit alcohol intake in the AA and ANA rats. Analysis of total brain may of course, not reveal functionally important local differences, but it has, on the other hand, been reported that the enzyme is rather uniformly distributed at least in bovine brain [15]. Thus it seems more probable that during the selection of the AA and ANA strains other genes than those coding for brain aldehyde dehydrogenase have been co-selected with alcohol drinking behavior. At present, the previously observed strain differences in blood acetaldehyde [7] and liver aldehyde dehydrogenase activity [17] still seem to give the best clues for metabolic differences related to the drinking behavior of the strains. As previously pointed out, circulating acetaldehyde penetrating into the brain should affect the oxidation of biogenic aldehydes by competitive inhibition of brain aldehyde dehydrogenase [15], which by this mechanism could affect drinking [7]. This hypothesis is, however, somewhat difficult to accept since very little, if any acetaldehyde has been detected in the brain of intoxicated animals [25, 26, 28] in spite of blood acetaldehyde levels considerably higher than those expected to occur during normal voluntary alcohol intake.

Finally, it is notable that other strains selected for high and low ethanol preference do not differ with respect to their acetaldehyde metabolism [18], suggesting that different biochemical correlates to alcohol drinking behavior have emerged in the different strain development programs.

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