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BRAIN ACETALDEHYDE AFTER ETHANOL ADMINISTRATION

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Abstract—Gas chromatographic determination of blood and brain levels of acetaldehyde was made by two methods in C57Bl/6J and DBA/2J mice after a single injection of ethanol (3 g/kg, i.p.). Ninety min after the administration of ethanol, blood levels of acetaldehyde were 70 nmoles/ml and 200 nmoles/ml for the C57 and DBA mice respectively. Corresponding brain levels, after correcting for blood content, were 6.4 nmoles/g and 17.3 nmoles/g for C57 and DBA mice respectively. These results indicate that blood acetaldehyde levels after acute administration of ethanol do not reflect those found in brain. The results further suggest that brain may be protected, at least in part, from concentrations of acetaldehyde found in blood, by an effective metabolic barrier.

Certain physiologic effects noted after after ingestion of ethanol have been attributed to the action of the ethanol metabolite, acetaldehyde, in the central nervous system (for review, see Ref. 1). Recent work [2–4] on the pharmacology of ethanol "addiction" has also led to speculation that acetaldehyde may be involved in induction of the ethanol dependence syndrome. Before a critical evaluation of the effects of ethanol and acetaldehyde *in vivo* can be made, one should determine the concentrations of these agents at their proposed site of action. Few investigators [5–7], however, have measured both brain and blood ethanol or acetaldehyde levels after ethanol administration and the published reports are contradictory. In many cases, blood levels of ethanol or acetaldehyde have been assumed to reflect the levels of these compounds in the CNS. However, a recent report by Sippel [8] has indicated that no acetaldehyde was present in rat brain tissue at blood acetaldehyde levels of less than 200 nmoles/ml.

Much current work in our laboratory and those of others has been directed toward elucidating the effects of ethanol administration (for reviews, see Refs. 9 and 10). We, therefore, measured the blood and brain levels of ethanol and acetaldehyde after ethanol administration and compared two methods used for the determination of acetaldehyde in brain [6, 8]. We also determined the rate at which brain tissue may metabolize acetaldehyde to determine whether metabolism of the acetaldehyde delivered to brain may be an effective barrier [8] to the accumulation of this ethanol metabolite in the CNS. Due to the popularity of C57Bl/6J and DBA/2J mice in evaluating both the behavioral [11–13] and biochemical [14–16] effects of ethanol, these animals were chosen for the present study.

MATERIALS AND METHODS

Male C57Bl/6J and DBA/2J mice were obtained from Jackson Laboratories, Bar Harbor, Me., and were housed in our facilities for at least 7 days before use. The mice were given access to Purina Lab Chow and water, *ad lib.*, and maintained at a constant temperature of 22 ± 1°, with a 6:00 a.m.–6:00 p.m. light cycle. All mice used in these experiments were between 55 and 65 days of age. Absolute ethanol was purchased from U.S. Industrial Chemical Co., and sealed ampules of acetaldehyde were obtained from Matheson, Coleman & Bell and redistilled prior to use. Mice were injected intraperitoneally with a 20% (v/v) aqueous solution of ethanol (3 g/kg). Ethanol and acetaldehyde levels in blood and brain were determined at various times thereafter.

Ethanol and acetaldehyde levels in blood were determined by the paper disc method as described by Coldwell *et al.* [7]. Twenty μl of blood was collected in heparinized capillary tubes from the tip of the tail or from mixed arterial and venous flow after decapitation and placed on a sodium fluoride-impregnated filter paper disc. The disc was contained in a 25-ml flask which was immediately sealed. Alternatively, 20 μl of blood was added to 1 ml of ice-cold 0.6 M perchloric acid containing 25 mM thiourea [17] in a 25-ml flask, and the flask was immediately sealed. After an equilibration period of a minimum of 30 min at room temperature, 1 or 2 ml of the gas volume from the flasks was injected into the port of a Beckman GC-65 gas chromatograph equipped with a flame ionization detector. The ethanol and acetaldehyde were chromatographed on Porapak Q (Walters Associates, Inc.) packed in a glass column (6 ft × 4 mm) and quantified by use of standards prepared by adding known amounts of ethanol or acetaldehyde at appropriate concentrations to heparinized blood and carrying these standards through the procedures. Operating parameters of the gas chromatograph were as follows: nitrogen (carrier gas) flow rate 40 cc/min; hydrogen gas flow 45 cc/min; air flow 300 cc/min; inlet, column, and detector temperatures were 150°, 115° and 250° respectively.

Ethanol and acetaldehyde levels in brain tissue were determined by a modification of the method of Duritz and Truitt [6]. Brains were removed quickly, blotted, weighed and added to 0.25 ml of ice-cold 5% zinc sulfate. Cold distilled water was added such that the total volume was 1.75 ml. Brain tissue was homogenized using a Tekmar Tissuemizer; 0.25 ml of 0.3 N

barium hydroxide was added to the homogenate and 1 ml of the resultant mixture was pipetted into a 25-ml flask which was quickly sealed. Flasks were gently shaken in a water bath for approximately 30 min at 30°, or allowed to stand at room temperature for 4–5 hr. Alternatively, brain tissue was homogenized in 2 ml of ice-cold 0.6 M perchloric acid containing 25 mM thiourea [8]. One ml of the homogenate was pipetted into 25-ml flasks. The flasks were sealed and equilibrated before determination of ethanol and acetaldehyde by gas chromatography as described above. One or 2 ml of gas from the headspace of flasks containing the homogenized brain tissue was chromatographed using Porapak Q. Standards consisted of known amounts of ethanol or acetaldehyde added to brain tissue from saline-injected animals and processed in a similar manner as brain from ethanol-injected mice. Under our assay conditions, the retention times for acetaldehyde and ethanol were 3.5 and 6.6 min respectively.

Aldehyde dehydrogenase activity in brain was measured by following the decrease in the concentration of acetaldehyde in reaction mixtures. Reaction mixtures contained NAD^+ (1 mM), nicotinamide (5 mM), acetaldehyde (0.1 mM), and mouse brain homogenate (50 mg tissue), in 25 mM sodium phosphate, pH 7.4. Incubations were performed in a 2 ml total volume at 23° in sealed 10-ml flasks. Control incubation mixtures contained all ingredients except that brain homogenate had been kept at 100° for 10 min before being added to these mixtures. At 5-min time intervals, 10 μl of incubation mixture was withdrawn using a Hamilton μl syringe and transferred onto a sodium fluoride-impregnated disc contained in a sealed 25-ml flask. After a 30-min equilibration period at room temperature, 1 ml of headspace gas was chromatographed on Porapak Q as described above.

The amount of blood present in brain was estimated by measuring the relative hemoglobin content of brain and blood. Blood was collected in heparinized capillary pipettes and hemolyzed in 5 ml of 0.4% ammonium hydroxide. Sodium hydrosulfite ($Na_2S_2O_4$) was added to an aliquot of the hemolyzed blood to convert oxyhemoglobin to free hemoglobin [18] and a differential spectrum of oxyhemoglobin (reference) vs deoxygenated hemoglobin (sample) was obtained using a Beckman Acta III recording spectrophotometer. Such a spectrum contained a peak at 555–557 nm followed by a trough at 576–578 nm. The optical density difference between peak and trough was found to be proportional to the hemoglobin present in the lysate.

Brain was obtained in a manner similar to that used for obtaining tissue for determination of ethanol and acetaldehyde levels and was disintegrated in distilled H_2O (4 ml total volume). Particulate material was removed by centrifugation at 17,300 *g* for 30 min at 4°. Ammonium hydroxide was added to the resultant supernatant such that the final concentration was 0.4% and a differential spectrum was obtained after addition of sodium hydrosulfite to an aliquot of the supernatant. Blood content of brain was calculated from the optical density difference between peak (555–557 nm) and trough (576–578 nm) of the differential spectrum for brain supernatant. In other experiments, blood was added to brain before disintegration to monitor recovery of hemoglobin as measured by this method.

RESULTS

Blood ethanol levels in C57Bl/6J mice measured by the paper disc method [7] reached peak concentrations of 102 ± 19 μmoles/ml (n = 9) within 30 min after injection. The blood levels of ethanol* at 60 and 90 min after injection measured by the same method [7] were 82 ± 8 (n = 9) and 72 ± 9 (n = 5), respectively, and declined thereafter. Ethanol levels became undetectable 4.5 hr after injection. Brain ethanol levels were found to peak earlier (within the first 10 min) reaching levels of 120 ± 9 μmoles/g and declined thereafter. C57Bl/6J mice lost their righting reflex 2–7 min after the injection of ethanol and regained the righting reflex in 24.8 ± 6.8 min after injection (n = 8). Blood acetaldehyde levels in C57Bl/6J mice increased during the first 10–20 min after ethanol injection (Fig. 1). For 120 min thereafter the mean blood acetaldehyde levels were maintained at approximately 60–70 nmoles/ml. A secondary peak in blood acetaldehyde became evident after 150 min with levels rising to approximately 100 nmoles/ml. Similar blood acetaldehyde levels were found when tail blood was assayed by either the paper disc method described by Coldwell *et al.* [7] or with the use of perchloric acid containing 25 mM thiourea [8, 17] (Table 1). Blood obtained from the neck after decapitation of C57Bl/6J mice was also assayed for acetaldehyde. The values obtained 90 min after ethanol injection (71 ± 15 nmoles/ml of blood; n = 3) did not differ significantly from levels found in blood taken from the tail. Blood acetaldehyde levels in DBA/2J mice were found to be significantly higher than levels found in C57Bl/6J mice (Table 1).

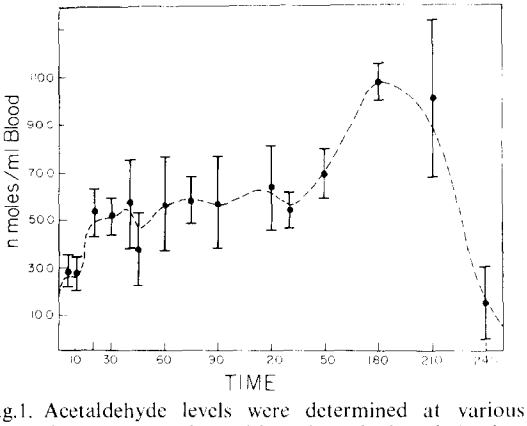

Fig. 1. Acetaldehyde levels were determined at various times after an intraperitoneal injection of ethanol (3 g/kg). Blood acetaldehyde levels were determined by the method of Coldwell *et al.* [7]. Four to six animals were used at each time point, and values are the mean ± standard deviation.

* When blood ethanol levels were measured by the method of Sippel [8], the resultant levels appeared lower [e.g. at 60 min after injection blood ethanol was calculated to be 71 ± 11 μmoles/ml (n = 6)]. However, these differences were not statistically significant.

Table 1. Blood and brain acetaldehyde levels determined by various methods

Acetaldehyde levels*	Methods: Paper disc†	Methods: Perchlorate + thiourea‡
Blood		
(C57Bl/6J mice)	60 ± 14 (6)	73 ± 12 (6)
(DBA/2J mice)		199 ± 38 (4)
	$ZnSO_4$ + $Ba(OH)_2$§	Perchlorate + thiourea‡
Brain		
(C57Bl/6J mice)		
30-min equilibration	29.4 ± 8.3	6.4 ± 2.2 (11)
4- to 5-hr equilibration	130.3 ± 23	4.2 ± 1.2 (5)
Brain		
(DBA/2J mice)		
30-min equilibration		17.3 ± 6.8 (4)

* Acetaldehyde levels were determined 90 min after an intraperitoneal injection of 3 g/kg of ethanol and are expressed as nmoles/ml of blood or nmoles/g of brain ± 1 standard deviation. The number of experiments is in parentheses.

† Blood was placed on a paper disc impregnated with sodium fluoride and contained in a sealed flask. Headspace was analyzed for acetaldehyde content. See text and Ref. 7 for further details.

‡ Blood was placed in 1 ml of 0.6 M perchloric acid containing 25 mM thiourea in a sealed flask. Brains were homogenized in 2 ml of 0.6 M perchloric acid containing 25 mM thiourea, and 1 ml of homogenate was placed in a sealed flask. Headspace was analyzed for acetaldehyde. See text and Ref. 8 for further details. Brain acetaldehyde levels were adjusted for the contribution of acetaldehyde contained in blood.

§ Brains were homogenized in $ZnSO_4$ and $Ba(OH)_2$ was added to the mixture. The mixtures were contained in a sealed flask and headspace was assayed for acetaldehyde. See text and Ref. 6 for further details. Brain acetaldehyde levels were adjusted for the contribution of acetaldehyde contained in blood.

Due to the presence in brain supernatant of compounds which interfered with an accurate determination of hemoglobin by direct spectral analysis, we utilized the differential spectrum produced by treatment of a portion of the supernatant with sodium hydrosulfite for determination of this protein. By this method, the amount of blood present in brain tissue was found to be 13.4 ± 1.9 μl/g (n = 8). This value was corrected for an 89 per cent recovery of blood hemoglobin from brain tissue.

Brain acetaldehyde levels were determined at a time that blood acetaldehyde levels were at a steady state (Fig. 1). Since brain tissue used in our experiments contained blood, acetaldehyde levels found in brain tissue were adjusted by subtracting blood-borne acetaldehyde from acetaldehyde found in brain tissue. Ninety min after the injection of ethanol, brain acetaldehyde levels, determined by the method of Duritz and Truitt [6], were approximately 29 nmoles/g of brain. These levels were obtained using a headspace equilibration period of approximately 30 min. However, if samples were allowed to equilibrate for several hr, acetaldehyde levels in the assay mixtures increased to 130 nmoles/g of brain (Table 1). On the other hand, brain acetaldehyde levels (corrected for the presence of blood in brain) assayed in the presence of 25 mM thiourea were found to be quite low, and there were no significant differences between values obtained after a 30-min incubation and those from a 5-hr incubation of samples (Table 1). Under our assay conditions for acetaldehyde in brain, the limit of sensitivity was approximately 2 nmoles acetaldehyde/g of tissue.

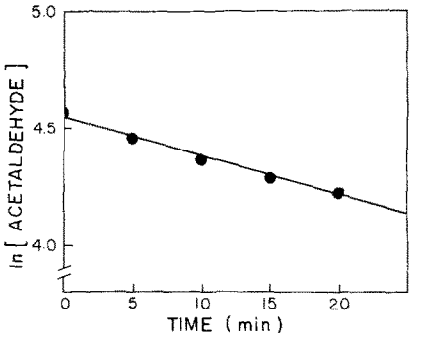

Fig. 2. Decline in acetaldehyde concentration (μM) was monitored in incubation mixtures containing mouse brain homogenate, acetaldehyde (100 μM) and NAD^+ (1 mM). Results show one typical experiment. Rate constants and initial velocities were calculated from the first-order plots of such regression lines.

The metabolism of acetaldehyde by C57Bl/6J mouse brain homogenates was determined to be 104 ± 54 nmoles/min/g of brain (n = 3) when assayed at 23°. The rate determined within each experiment followed first-order kinetics (Fig. 2). No acetaldehyde metabolism was witnessed with brain homogenates which were preheated to 100° for 10 min before being cooled and used in incubation mixtures.

DISCUSSION

A lack of agreement exists in the literature as to the relative concentrations of acetaldehyde in blood and brain after ethanol administration. Duritz and Truitt [6] reported higher levels of acetaldehyde in rat brain compared to blood after ethanol administration. Ridge [5] found no differences in rat blood and brain levels of acetaldehyde, but reported significant synchronized variation in blood and brain acetaldehyde concentrations over time. Wiberg *et al.* [19], on the other hand, reported that blood and brain acetaldehyde levels did not oscillate in a synchronous manner. Truitt [20] has noted that acetaldehyde concentrations found after ethanol administration may be erroneous due to "release" of endogenous acetaldehyde by ethanol, and recently it has been proposed that this "released" acetaldehyde is actually formed non-enzymatically from ethanol [17]. The addition of thiourea to homogenates of tissue samples was shown to prevent this spurious formation of acetaldehyde during assay procedures [8, 17].

Our results (Table 1) showed an increase in assayed acetaldehyde levels in brain homogenates, as a function of the sample equilibration time, when the method similar to that described by Duritz and Truitt [6] was used. These results indicated that acetaldehyde could be produced during the assay procedure described by Duritz and Truitt [6]. On the other hand, the levels of acetaldehyde detectable in

mouse brain tissue were not altered by sample equilibration time if thiourea was present in the homogenizing medium (Table 1). The levels of acetaldehyde found in brains of both C57Bl/6J and DBA/2J mice were considerably lower than blood acetaldehyde levels in these animals. DBA/2J mice, however, were found to have higher brain levels of acetaldehyde as well as higher blood acetaldehyde levels when compared to C57Bl/6J mice. Sippel [8] found that acetaldehyde was not detectable in rat brain until blood acetaldehyde levels exceeded 200 nmoles/ml. His values were corrected for the presence of 75 μl blood, containing acetaldehyde, per g of brain. Such results led Sippel [8] to hypothesize that the capacity of rat brain to metabolize acetaldehyde was between 100–180 nmoles/min/g. We found the amount of blood present in mouse brain tissue was only ~ 13 μl, and brain tissue of these animals contained low but detectable levels of acetaldehyde even at blood acetaldehyde concentrations of ~ 70 nmoles/ml. The low brain acetaldehyde levels found in our study would, however, still be consistent with an active metabolism of this compound by brain tissue.

The capacity of mouse brain to metabolize acetaldehyde at levels of acetaldehyde approximating those in blood 90 min after the injection of ethanol was found to be > 100 nmoles/min/g of brain. However, due to the volatility of acetaldehyde, the experiments were perfomed at 23° and one may expect enzyme activity to be greater at physiologic temperature.* The metabolic rate would, of course, be dependent on several factors, such as: the accessibility of acetaldehyde to aldehyde dehydrogenase in brain tissue, Michaelis constants for acetaldehyde with brain aldehyde dehydrogenase(s) [21, 22], and the levels of NAD^+ in brain after ethanol administration as well as the presence of other enzymes capable of metabolizing acetaldehyde. NAD^+ levels in rat brain have been shown to be approximately 0.35 mM [23]. These levels are significantly above the K_m for NAD^+, determined for liver aldehyde dehydrogenase from C57Bl/6J mice [15]. Since little change in brain NAD^+/NADH ratios occurs even after anesthetic doses of ethanol [24], the availability of cofactor should not be a rate-limiting factor for the activity of brain aldehyde dehydrogenase. In addition to the NAD^+-dependent aldehyde dehydrogenase, a lower amount of $NADP^+$-dependent aldehyde dehydrogenase has been found in brain tissue, and may also metabolize acetaldehyde [12, 25]. Using near saturation levels of acetaldehyde, we found that mouse brain tissue can metabolize acetaldehyde at a faster rate than the rate at which acetaldehyde would be delivered to brain by blood. Assuming a blood flow of 0.8 ml/min/g of brain [26], one can estimate that acetaldehyde would be delivered to brain at a rate of approximately 50 nmoles/min/g of brain. Blood flow through brain has been shown to be little affected by ethanol administration [27]. As mentioned above, however, the rate of metabolism would depend on the availability of the substrate.

*The Q_{10} for liver aldehyde dehydrogenase has been determined to be 2.8 (R. A. Dietrich, personal communication). Q_{10} is used to indicate the fold increase in enzymatic activity for every 10° rise in temperature.

The reported competitive inhibition by acetaldehyde of the metabolism of 5-hydroxyindoleacetaldehyde by brain aldehyde dehydrogenase *in vitro* [28] would suggest that the production of 5-hydroxyindoleacetic acid (5-HIAA) from serotonin should be diminished if significant amounts of acetaldehyde were being metabolized in brain. However, previous work using push–pull perfusions of localized brain areas [29] and analysis of whole brain 5-HIAA levels [14] demonstrated that 5-HIAA levels actually increased in brain after ethanol administration. On the other hand, we have demonstrated that ethanol administration inhibits the exit of 5-HIAA from brain [30, 31]. Thus, an inhibition in the production of 5-HIAA may be overlooked due to accumulation of this metabolite in a system where transport from brain is also inhibited. The presence of acetaldehyde in brain and the participation of acetaldehyde in the CNS effects of ethanol by competing with endogenous aldehydes for metabolic enzymes should, therefore, not be ignored.

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