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IN VIVO ACETALDEHYDE IN THE BRAIN OF THE RAT TREATED WITH ETHANOL*

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Abstract—Ethanol, 4.5 g/kg, was administered intragastrically to rats to determine if acetaldehyde could be detected in brain interstitial fluid. Samples from both blood and brain were collected at half-hour intervals. Brain interstitial fluid samples were collected from both the caudate nucleus and the thalamus–hypothalamus region using the push–pull perfusion technique. The ethanol and acetaldehyde concentrations in these samples were determined by a head space gas chromatographic technique. Blood ethanol levels typically ranged from 200 to 400 mg/100 ml, while acetaldehyde levels ranged from 15 to 40 μM in blood and 5 to 20 μM in brain fluid. When disulfiram was given to the rats 20 hr prior to ethanol administration, blood acetaldehyde increased to 70–280 μM and brain interstitial fluid acetaldehyde increased to between 25 and 120 μM. Whole brain acetaldehyde levels were also measured after an ethanol dose was given. No acetaldehyde could be detected in whole brain unless the animal had first been treated with disulfiram. These data demonstrate that acetaldehyde does enter the brain, coming into direct contact with the brain cells bathed in the interstitial fluid. The acetaldehyde concentration in the interstitial fluid is higher than that in the brain cells, probably due to its rapid oxidation in the cells catalyzed by aldehyde dehydrogenase.

Though the presence of ethanol in the body could be responsible for many of the physiological effects associated with abusive drinking, it is conceivable that acetaldehyde, the first metabolite derived from ethanol, may also produce some of the adverse effects caused by ethanol drinking [1, 2]. The blood concentration of acetaldehyde averages between 20 and 50 μM in humans after consumption of an intoxicating dose of ethanol [3], while in rats the level ranges between 10 and 100 μM after a dose of ethanol [2]. At mM levels acetaldehyde will inhibit Na^+, K^+ and Mg^{2+} activated ATPase [4], protein synthesis [5] and the release of biogenic amines [6], yet at lower, potentially physiological levels, it does not affect many biological reactions.

The amount of acetaldehyde found in various organs and tissues, especially blood and brain, after ethanol is administered to the animal has been investigated by numerous workers [2,7]. Prior to the early 1970s it was reported that the concentration of acetaldehyde in brain was at least as high as in other organs [2]. It was shown later, however, that spontaneous, nonenzymatic formation of acetaldehyde occurs when biological samples containing ethanol are heated [8], a step required in the gas chromatographic determination of the compound. Thus, it is thought that the high level of brain acetaldehyde, earlier reported to exist in brain, was due not to endogenous acetaldehyde, but rather to its formation during the preparation of tissue for analysis. Thiourea has been shown to prevent this spontaneous formation from occurring [8].

Using thiourea in the analytical procedure, various workers have redetermined the concentration of acetaldehyde in brain after the administration of ethanol. However, in two recent studies the final conclusions differed. In one, no acetaldehyde was found in the brain of the rat until the blood level reached approximately 200 μM [9]. Such a high concentration of acetaldehyde is not normally obtained during ethanol metabolism and was achieved in that study only by a supplemental dose of acetaldehyde given intraperitoneally. In the other study utilizing mice, the brain acetaldehyde level averaged 6 nmoles/g when the concentration in blood was approximately 70 μM [10].

Different theories have been presented to explain why the acetaldehyde level is lower in brain than in blood. Eriksson and Sippel [9] hypothesized that all of the acetaldehyde is metabolized in the capillaries and hence none actually enters the brain. Tabakoff *et al.* [10] argued that acetaldehyde entering the brain is metabolized so rapidly that it begins to accumulate in brain only when the blood level is so high that aldehyde dehydrogenase cannot metabolize it at the same rate as it passes through the blood–brain barrier. A study by Pettersson and Kiessling [11] indicated the importance of a low K_m aldehyde dehydrogenase in maintaining the low level of brain acetaldehyde during ethanol metabolism.

In the previous studies, brain acetaldehyde was measured after rapid removal of the brain, at intervals following the administration of a dose of ethanol to an animal. Inasmuch as it is possible that a low level of acetaldehyde might be detectable in the interstitial fluid of the brain, an alternative technique was used to determine the concentration of acetaldehyde in the brain of a live animal. By employing the 'push–pull' perfusion technique [12], it is possible to remove a sample of the interstitial fluid which

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bathes the cells of the brain. In this manner it is possible to determine whether there is acetaldehyde present in the brain of a conscious animal metabolizing ethanol.

MATERIALS AND METHODS

Male and female Wistar rats from the Purdue University Biochemistry Department Rat Colony, weighing between 275 and 425 g, were used. The animals were given free access to Wayne Lab-blox and water until 12 hr prior to surgery when they were deprived of both food and water. Before surgery, each rat was given 0.1 ml atropine (0.4 mg/ml) intramuscularly followed 10 min later by 40 mg/kg sodium pentobarbital intraperitoneally. After the head of the animal was shaved, it was positioned in a stereotaxic instrument. A 20 gauge stainless steel cannula guide was implanted as described by Myers [12] in either the caudate nucleus (AP 7.2; lat 3; hv 4) or in the thalamus (AP 4.6; lat 1, hv 5.0) or hypothalamus (AP 5.0; lat 1, hv 7.5) [13]. The cannula was held in place by cranioplast cement packed around the stainless steel anchor screws inserted in the calvarium. The rats were allowed to recover for 4–14 days prior to use.

Push–pull perfusion procedure. Following the recovery period, the standard push–pull perfusion procedure [14] was utilized to minimize tissue damage. The concentrically positioned push and pull cannulae were connected by polyethylene tubing to calibrated 1.0 ml glass syringes (Hamilton) which were mounted on a Harvard perfusion pump. After the cannulae were lowered into the guide cannula of a rat, the perfusion was begun. The reciprocating pump was precalibrated to deliver the perfusate at a flow rate of 20 μl/min. After the site had been perfused for 10 min, the push–pull cannulae were removed, and the 200-μl sample which had collected in the polyethylene tubing was immediately ejected into a glass vial. The vial was kept on dry ice to freeze the perfusate immediately. The vial was sealed with a Teflon septum. After 3–5 min elapsed, the diencephalic or caudate site was perfused again. The total volume of 400 μl, collected during the two perfusions, was pooled and considered as one sample. An additional 10 μl of a solution, consisting of 0.63 M methanol and 2 M thiourea, was added to each sample to provide an internal standard for the gas chromatographic analysis of acetaldehyde and ethanol as well as to prevent the spontaneous formation of acetaldehyde [8].

The perfusing solution was an artificial cerebrospinal fluid (CSF) which consisted of the following salts: NaCl, 7.5 g/l; KCl, 1.9 g/l; $MgCl_2$, 0.19 g/l and $CaCl_2$, 0.14 g/l. The zero time sample of perfusate was used to determine the background in the gas chromatograph. The rat was then given ethanol intragastrically (25%, v/v), administered in a dose of 4.5 g/kg. Four additional samples were collected at 0.5, 1.0, 1.5 and 2.0 hr after injection. The rat was kept on a heating pad during each experiment so that its temperature could be maintained at 35–37°. In cases of blood contamination of the perfusate, the samples were discarded.

Blood samples. After the first 200 μl of the brain perfusate were collected, a 100-μl blood sample was taken from the tail of the rat in a heparinized glass capillary pipette. The blood sample was placed in a cold centrifuge tube along with 0.5 ml of an internal standard solution which was comprised of 50 mM thiourea and 32 mM methanol in water. The vial was sealed with a Teflon septum, and 0.2 ml of 0.3 M $ZnSO_4$ and 0.2 ml of 0.2 M $Ba(OH)_2$ were added to the vial to precipitate the protein. The solution was kept on ice for up to 30 min and then subjected to centrifugation to remove the precipitated protein. A portion of the resulting supernatant fraction (0.4 ml) was removed with a syringe and injected into a 1-dram vial which was sealed with a Teflon septum.

Administration of disulfiram. Recrystallized disulfiram was suspended in 1.0% carboxymethyl cellulose at a concentration of 200 mg/ml [15]. Each disulfiram-treated animal was given 600 mg/kg of the drug by gavage 12 hr prior to the administration of ethanol.

Whole brain acetaldehyde determination. Each brain was removed rapidly from the animal 1 hr after administration of 4.5 g/kg ethanol. The cerebellum was dissected away and the remaining brain was placed in a preweighed vial, frozen at −78° and weighed. Approximately 5 ml of the internal standard solution were added per g of brain. The solution was homogenized in a 4° cold room using an all glass, hand held homogenizer. A 600-μl sample of the homogenate was removed with a syringe and injected into a sealed centrifuge tube. To precipitate the protein, 0.2 ml of 0.3 M $ZnSO_4$ and 0.2 ml of 0.2 M $Ba(OH)_2$ were added to the tube which was then centrifuged for 5 min at 4°. A 0.4-ml portion of the resulting supernatant fraction was removed and placed into a 1-dram vial which was sealed with a Teflon septum. An additional portion of the brain homogenate was used to determine, by the method of Klein [16], the amount of blood contaminating the tissues. We estimate that less than 5 per cent of the brain acetaldehyde was lost by these procedures, as determined by adding a known amount of acetaldehyde directly to a rat brain prior to homogenization.

Gas chromatographic analysis of ethanol and acetaldehyde. A modified method of the head space technique developed by LeBlanc [17] was used. The samples were heated to 40° for 30 min and 200 μl of the head space vapor were injected into a Varian 3700 gas chromatograph equipped with flame ionization detectors. A 6 ft glass column packed with 0.2% carbowax 1500 on 80/100 Carbopak was used. The conditions for analysis were: 120° injector temperature, 40° column temperature and 150° detector temperature. Air flow was 100 cm^3/min, hydrogen flow was 10 cm^3/min and nitrogen flow was 10 cm^3/min. The attenuator setting was 1×10^{-12} for acetaldehyde and 4×10^{-11} for methanol and ethanol. The chromatogram was recorded on a Linear chart recorder and peak heights were used to calculate the concentration of the components. Representative tracings are shown in Fig. 1. The ratios of acetaldehyde and ethanol to the methanol internal standard were compared to ratios obtained from standard curves made under the same conditions. The sensitivity of the analysis allowed for accurate

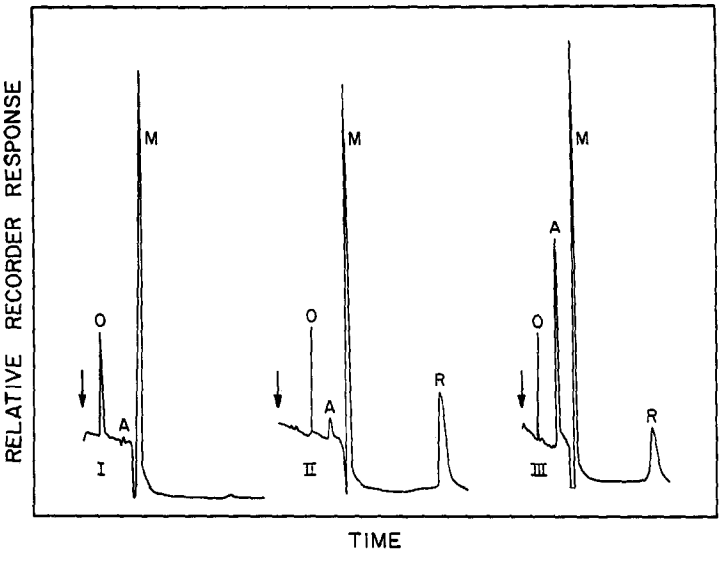

Fig. 1. Representative gas chromatograph tracings of perfusate samples. Arrow indicates sample injection. Key: (O) air peak; (A) acetaldehyde; (M) methanol (internal standard) and (R) ethanol. Curve I: brain perfusate sample obtained prior to ethanol administration. A small peak with the same retention time as acetaldehyde was always found. Curve II: brain perfusate sample obtained 30 min after the administration of a 4.5 g/kg dose of ethanol. In this sample the acetaldehyde concentration was 0.45 μM and ethanol was 3.1 mM. These correspond to 10 μM acetaldehyde and 69 mM ethanol in brain fluid after correcting for perfusion exchange efficiency. Curve III: brain perfusate sample obtained 1 hr after a 4.5 g/kg dose of ethanol was administered to a rat previously treated with disulfiram. In this sample the acetaldehyde concentration was 5.4 μM and ethanol was 1.6 mM. These correspond to 155 μM acetaldehyde and 46.5 mM ethanol in the brain fluid.

determinations of acetaldehyde concentrations as low as 0.3–0.5 μM. Due to the dilution factor, this corresponded to a level of approximately 5.0 μM in the blood or 5 nmoles/g in samples of whole brain. The accurate lower limit of detectability in the brain perfusate was calculated to correspond to 4–8 μM in the brain fluid itself.

Histology. The anatomical position of the cannulae in the brain was verified by standard histological procedures for selected animals [18]. Upon completion of the final push–pull perfusion, the rat was given an overdose of sodium pentobarbital after which 0.9% saline followed by 10% formalin was perfused through the heart. Following fixation, the brain was removed from the skull, kept in formalin for 1 day, and then blocked. The brain was washed and sectioned at 100 μm thickness, and then stained with cresyl violet. The anatomical location of the site was verified by light microscopy.

RESULTS

Spontaneous formation of acetaldehyde. When the artifical CSF was perfused through the brain tissue of the rat prior to the administration of ethanol, a small peak having the same retention time as acetaldehyde was detected by means of gas chromatography. The size of this presumed artifactual peak was found to be constant with repeated perfusions and was similar for all animals. To determine whether the mere passage of ethanol through the perfusion apparatus or through the brain tissue itself would produce acetaldehyde, the following experiments were performed and the height of this component peak was monitored. A 10 mg/100 ml ethanol solution in the perfusion medium, which would be comparable to the concentration ultimately recovered in perfusates obtained from an animal given ethanol, was perfused through different sites in the brain tissue of four rats. An analysis of the collected perfusates revealed that there was no increase in the height of this peak. This indicates that acetaldehyde was not being produced by the simple passage of ethanol through the brain or through the perfusion apparatus. Inasmuch as these perfusates were subjected to the head space analytical technique, the lack of acetaldehyde production revealed that no spontaneous formation of acetaldehyde was occurring.

Media exchange during perfusion. During any push–pull perfusion, approximately 5 per cent of the fluid recovered is actual brain fluid, the remainder being the perfusion fluid itself [12]. In order to quantitate the concentration of acetaldehyde in the brain, it was necessary to determine the precise extent of this exchange of interstitial fluid with the perfusing medium. One method for determining this exchange is to measure the blood and perfusate concentrations of an administered compound which has free access to the brain. Following a period of equilibration, the concentration of the compound should be equal in both blood and brain fluids. By comparing the concentration in perfusate vs blood, the percent of exchange can be measured.

Ethanol is an excellent compound to use for measuring exchange since it (1) diffuses freely across the blood–brain barrier, rapidly reaching equilibrium

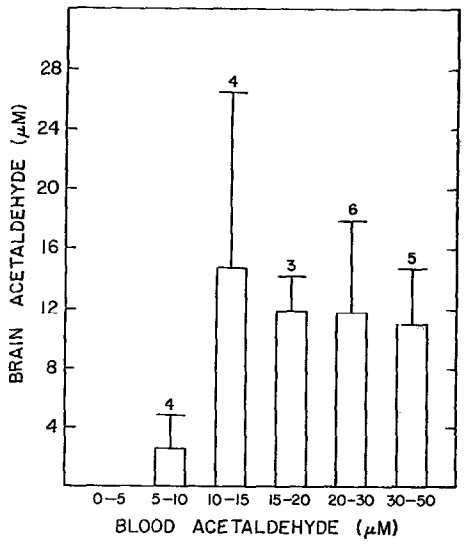

Fig. 2. Relationship between acetaldehyde concentrations in interstitial fluid collected from the caudate nucleus and from blood. Acetaldehyde was measured in rat brain perfusate and blood by use of gas chromatography. The acetaldehyde concentration in brain fluid was estimated by correcting for perfusion exchange efficiency. The bar indicates one standard deviation and the numerals indicate the number of individual measurements. Animals were given a 4.5 g/kg dose of ethanol intragastrically and samples were removed at time intervals ranging from 30 to 120 min.

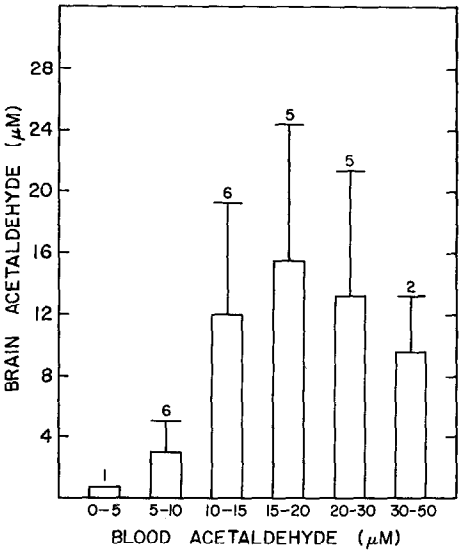

Fig. 3. Relationship between acetaldehyde concentration in interstitial brain fluid collected from the diencephalon and from blood. Details as in the legend for Fig. 2.

between blood and brain [19], and (2) is uniformly distributed in the brain [20, 21].* Thus, assuming that the concentrations of ethanol are equal in both brain fluid and blood, the calculated efficiency of exchange was found in all experiments to range between 1.5 and 8 per cent. This is in agreement with values reported by others using the push–pull perfusion technique [12]. Even though the degree of efficacy varied, the actual percent of ethanol exchange in push–pull perfusates was calculated for each animal under each experimental condition. By assuming that the acetaldehyde exchange efficiency was equal to ethanol exchange efficiency, the actual brain fluid concentration of acetaldehyde could be calculated.

Acetaldehyde levels in the brain. The concentration of acetaldehyde in brain fluid was determined in both the caudate nucleus and in diencephalic regions (thalamus and hypothalamus); the data are presented in Figs. 2 and 3. Most significant is the fact that, even with low concentrations of blood acetaldehyde (5–20 μM), acetaldehyde can be detected in the brain. As the level of blood acetaldehyde increases, brain interstitial fluid acetaldehyde rises to a plateau of 10–15 μM. This occurred in both brain regions that were sampled.

Effects of disulfiram on blood and brain acetaldehyde levels. Disulfiram drastically inhibits the aldehyde dehydrogenase catalyzed oxidation of acetaldehyde [23], resulting in higher blood acetaldehyde levels after a dose of ethanol. In the disulfiram-treated animal, the level of acetaldehyde in a given region of the brain rose as the level of acetaldehyde in the blood increased. As shown in Fig. 4, the slope of the brain acetaldehyde vs blood acetaldehyde line is 0.34, with a correlation coefficient of 0.83. The elevated concentration of brain acetaldehyde could be due to two factors: (1) more acetaldehyde crossing the blood–brain barrier causing brain aldehyde dehydrogenase to become saturated, thus allowing acetaldehyde to accumulate; or (2) brain aldehyde dehydrogenase being inhibited by disulfiram.

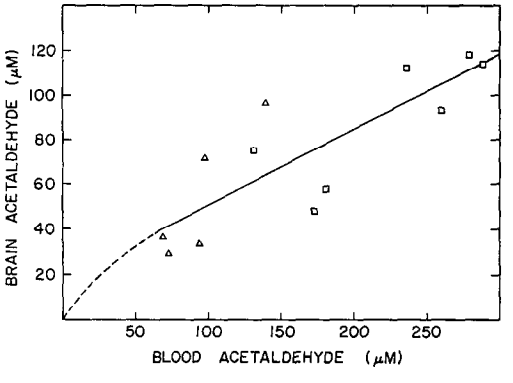

Fig. 4. Brain and blood acetaldehyde concentrations in rats pretreated with disulfiram (600 mg/kg) 20 hr prior to the administration of ethanol (4.5 g/kg intragastrically). Acetaldehyde concentrations in brain perfusate samples and blood collected simultaneously were measured by gas chromatography. Brain fluid acetaldehyde was estimated by correcting for perfusion exchange efficiency. Samples were collected between 30 and 120 min after ethanol treatment. Key: (△) time points from rats perfused in the caudate nucleus and (□) time points from rats perfused in the diencephalon.

* Recently, experiments using a different perfusion technique have provided some evidence to suggest that ethanol may not be distributed uniformly throughout the brain and that it may not even be distributed equally between blood and brain [22]. The *in vivo* exchange efficiency for individual perfusions was not calculated. This may be the cause for the different conclusions reached in this study as compared to the others [20, 21].

Table 1. Acetaldehyde concentration in whole brain and blood of rats with and without prior disulfiram treatment*

Rat No.	Disulfiram treatment	Blood ethanol (mg/100 ml)	Blood acetaldehyde (μM)	Brain acetaldehyde (nmoles/g)
1	–	279	31	0–5†
2	–	450	18	0–5†
3	+	160	92	20
4	+	299	120	28
5	+	175	95	18
6	+	166	78	15
7	+	186	185	53
8	+	135	95	35

* Disulfiram-pretreated (600 mg/kg) and untreated rats were given a 4.5 g/kg dose of ethanol and 1 hr later were killed, their brains removed, and ethanol and acetaldehyde levels in them determined by methods described in the text. Just prior to killing the animals, peripheral tail blood samples were obtained and blood levels of ethanol and acetaldehyde determined.

† In measuring these acetaldehyde concentrations, no acetaldehyde could be detected. Since our limit of accurate detectability of whole brain acetaldehyde was 5 nmoles/g, this can be considered to be the maximum concentration of brain acetaldehyde.

Concentration of acetaldehyde in whole brain. From selected animals administered ethanol, the whole brain was rapidly removed to determine the concentration of both ethanol and acetaldehyde. The results are presented in Table 1 for rats with or without disulfiram pretreatment. The level of brain ethanol in nmoles/g was assumed to be essentially equal to the blood concentration in μmoles/l [21] so that a comparison could be made between the levels of acetaldehyde in the brain and the blood. The brain acetaldehyde concentration was corrected for blood contamination, which averaged 23 μl/g in the samples. The results indicated that whole brain acetaldehyde could be detected only in the presence of disulfiram where blood acetaldehyde levels greater than 50 μM were achieved.

DISCUSSION

Although it has been reported that the concentration of acetaldehyde in brain is equal to or greater than that found in blood [2], recent studies show that the acetaldehyde remains at zero or a low level when blood acetaldehyde is below 100–200 μM. Eriksson and Sippel [9] reported that acetaldehyde cannot be found in rat brain until the blood level increases above 200 μM. However, Tabakoff *et al.* [10] found low levels of brain acetaldehyde (6 nmoles/g) in mice during the course of normal ethanol metabolism. Both groups removed the brain from the animal to measure acetaldehyde; we have employed the push–pull perfusion technique to sample acetaldehyde in brain fluid. The advantages of this technique for collecting brain samples are that samples are obtained from the interstitial fluid of a live animal and that blood does not contaminate the sample as is the case when a whole brain is used. Because acetaldehyde levels can be high in blood, such contamination could constitute an artifact with respect to the concentration of acetaldehyde in brain. A disadvantage of the push–pull technique is the relatively low efficiency of exchange (1.5–8 per cent) of the perfusion solution with interstitial fluid. Thus, many samples of brain perfusate contained acetaldehyde levels which approached the lower limits of detectability.

Analysis of samples of whole brain revealed that, when the concentration of acetaldehyde in the blood ranged from 17 to 30 μM, no acetaldehyde could be detected. In contrast, the amount of acetaldehyde in brain fluid was typically between 5 and 25 μM when blood acetaldehyde was in the same 17–30 μM range. Thus, not only can acetaldehyde cross the blood–brain barrier, but a higher concentration is apparently contained in the interstitial fluid of the brain than in the brain tissue (neurophil) itself.

It appears that an effective aldehyde-metabolizing system exists in the brain which keeps acetaldehyde at a low level during ethanol metabolism. This is most likely due to the presence of aldehyde dehydrogenase, although, conceivably, aldehyde oxidase could serve in some capacity in the metabolism of brain acetaldehyde. The K_m for the enzyme involved must be relatively low since the metabolism occurs rapidly at these micromolar levels. Tabakoff *et al.* [24], as well as Erwin and Deitrich [25], have shown that a low K_m aldehyde dehydrogenase is located in brain. When the concentration of acetaldehyde becomes so high that the activity of the aldehyde dehydrogenase system cannot remove it as rapidly as new acetaldehyde enters the brain, a build-up of the metabolite would occur. Pettersson and Kiessling [11] suggested that a high K_m brain enzyme exists, as is found in liver [7], which could then prevent the concentration of acetaldehyde from reaching the level found in blood.

Although acetaldehyde is found in ventricular CSF [11], it is possible that it penetrates the blood–CSF barrier rather then entering by way of the brain–CSF barrier, thus never coming into contact with the

Table 2. Calculated concentration of acetaldehyde in brain cells*

[Acetaldehyde]				
Blood (μM)	Interstitial fluid (μM)	Whole brain (nmoles/g)	Calculated brain cellular (μM)	Cell/interstitial fluid
0–30	10	< 5	4 (maximum)†	—
70–120	44	25	23	0.52
> 120	89	53	49	0.55

* Cellular brain levels of acetaldehyde were calculated using data in Figs. 2–4, Table 1, and equation 1. Equation 1 assumes that interstitial fluids comprise 10 per cent of the brain volume, while cells comprise the other 90 per cent.

† Maximum calculations were made assuming that whole brain acetaldehyde was 5 nmoles/g, the limit of accurate detectability. Thus, the calculated brain cellular acetaldehyde concentration is somewhere between 0 and 4 μM.

brain cells. Our findings that acetaldehyde does indeed exist in brain interstitial fluid, however, indicate that acetaldehyde could impinge upon the environment of the neuron and could thereby exert some physiological effects on neuronal tissue.

Treatment of the animal with disulfiram causes a marked increase in blood acetaldehyde after the administration of ethanol, with brain fluid acetaldehyde now increasing porportionately with blood acetaldehyde. This is similar to the results found by Kiianmaa and Virtanen [26], who reported that blood and CSF levels of acetaldehyde were significantly correlated ($r = 0.681$ to 0.976) after rats that had been fed a diet containing an aldehyde dehydrogenase inhibitor, cyanamide, were given ethanol. The increased amount of acetaldehyde found either in brain perfusates or in whole brain could result from the *in vivo* inhibition of brain aldehyde dehydrogenase. Recently, it was shown that approximately 50 per cent of the conversion of 3,4-dihydroxyphenylacetaldehyde, the aldehyde derived from the monoamine oxidase reaction on dopamine, was inhibited in animals given disulfiram [15]. In addition, the aldehyde dehydrogenase activity is differentially inhibited *in vivo* in different regions of the brain when the same substrate is assayed [27]. If this form of aldehyde dehydrogenase is the same isozyme that metabolizes acetaldehyde, it could be expected that the inhibition of acetaldehyde would occur in brain as it does in liver after disulfiram treatment.

The finding that acetaldehyde is detectable in the extracellular fluid shows that acetaldehyde can cross the blood–brain barrier and is not totally metabolized in the capillaries as had been sugggested [9]. The brain itself seems to have the capacity to metabolize the bulk of the acetaldehyde that can diffuse into it. It has been demonstrated in mice that the activity of aldehyde dehydrogenase is sufficient to metabolize all acetaldehyde that enters the brain when the blood levels are below 70 μM [10]. A similar situation must exist in the rat. We observed that, even though acetaldehyde was present in interstitial fluid, essentially none was found in the whole brain. This implies that very little acetaldehyde is actually in the cell, perhaps because of the rapid metabolism occurring there. The difference in acetaldehyde in brain interstitial fluid and brain cells can be explained by diffusion rates of acetaldehyde from the blood to the interstitial fluid and then to the cells, as well as the metabolism rate in the cells.

From the data obtained, the concentration of acetaldehyde, [AcH], in the brain of the rat can be estimated by the following equation, assuming a theoretical interstitial fluid content of 10 per cent, a value in the range often quoted [28]:

$$[\mathrm{AcH}]_{\mathrm{Total}} = 0.1\,[\mathrm{Ach}]_{\mathrm{Fluid}} + 0.9\,[\mathrm{AcH}]_{\mathrm{Cells}} \quad (1)$$

Table 2 presents calculated cellular acetaldehyde levels using the above equation and data from Figs. 2–4 and Table 1. At best, with normal metabolism of ethanol, the level of acetaldehyde found in the cell is between 0 and 40 per cent of that found in the interstitial fluid. Possibly, the level of acetaldehyde may be at the K_m for aldehyde dehydrogenase (ca. μM). In the presence of disulfiram, when the total acetaldehyde level in the brain is augmented, the intracellular content becomes elevated, equaling 50 per cent of that found in the interstitial fluid. During the normal metabolism of ethanol, the aldehyde dehydrogenases in the cell could be capable of maintaining the acetaldehyde level at an extremely low concentration. The physiological effect of acetaldehyde on the cell would have to be exerted at a low micromolar level, if such an effect does exist.

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