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ACETALDEHYDE OCCURRENCE IN CEREBROSPINAL FLUID DURING ETHANOL OXIDATION IN RATS AND ITS DEPENDENCE ON THE BLOOD LEVEL AND ON DIETARY FACTORS

HANS PETTERSSON and KARL-HEINZ KIESSLING

The Alcohol Research Group of the Swedish Medical Research Council and Department of Animal Nutrition, The Swedish University of Agriculture, Forestry and Veterinary Medicine, S-750 07 Uppsala, Sweden

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Abstract—After administration of ethanol to rats fed either an normal (I) or a cyanamide contaminated (II) standard laboratory diet, acetaldehyde and ethanol were determined in cerebrospinal fluid and blood. The acetaldehyde level in the cerebrospinal fluid from group I showed a plateau at approximately 30 μM while the blood concentration ranged from 20 to 60 μM. In group II the acetaldehyde in the cerebrospinal fluid varied according to the blood level. When the acetaldehyde in the blood of group I exceeded 50 μM after infusion of acetaldehyde, the concentration in cerebrospinal fluid started to rise. The ethanol concentration in the cerebrospinal fluid was correlated directly to the concentration in the blood independent of the type of feed. The difference in the occurrence of acetaldehyde in cerebrospinal fluid from the two groups may be explained by an inhibition of the brain aldehyde dehydrogenase with a low K_m towards acetaldehyde.

It has been proposed that many of the observable effects on the central nervous system after alcohol consumption are produced by acetaldehyde [1, 2]. However, there are few reports on the presence and level of acetaldehyde in the brain after alcohol consumption [3–6]. During the last years controversial reports have appeared using head-space gas chromatography for the detection and estimation of acetaldehyde concentrations in brain tissue [7–11].

After inhibition of non-enzymatic formation of acetaldehyde and correction for the blood content in the brain tissue and by using the freeze stop technique, no acetaldehyde could be detected in the brain unless the acetaldehyde level in cerebral blood exceeded 200 μM after injection of acetaldehyde [2, 8]. An enzymatic blood–brain barrier has, therefore, been postulated [7]. By measuring the acetaldehyde level in cerebrospinal fluid, the problems with anoxia, blood contamination and non-enzymatic acetaldehyde formation [7, 8, 12, 13] are excluded or minimized.

A dietary factor, later shown to be cyanamide [17], in a standard commercial diet obtained from Astra-Ewos AB, Södertälje, Sweden causes an elevation in the blood level of acetaldehyde [14]. This diet has been used in the present work as an aid in the study of acetaldehyde occurrence in cerebrospinal fluid.

MATERIALS AND METHODS

Female Sprague–Dawley rats weighing about 200 g were fed Astra-Ewos R-3 or Anticimex 210, (Sollentuna, Sweden) laboratory rat diet until the start of the experiment.

Ethanol alone. Ethanol (0.5; 1.0; 1.5; 2.0 g/kg body wt) was injected intraperitoneally (i.p.). Sampling was performed 90 min after ethanol injection. Ten min before sampling the rats were anaesthetized with 60 mg/kg body wt of mebumal (Apoteksbolaget).

Ethanol and acetaldehyde. Ethanol 1.5 g/kg (40% sol. in saline) was injected i.p. and 15 min later the rats were anaesthetized. An i.p. infusion (10 or 20 μl/min) of acetaldehyde (0.75 or 1.0 M in saline) was started 20–25 min after ethanol injection. After 30 min infusion sampling was performed.

Sampling. The fluid samples were collected with heparinized blood pipettes, mixed directly in 1 ml of ice cold 6% perchloric acid and kept cold in capped tubes until the final determinations. The first sample taken was 100 μl blood from the tip of the tail. Then cerebrospinal fluid was sampled. A cut through the skin was made and a hypodermic needle 23 × 1 in. was inserted through the cartilage between the skull and the first vertebrae into the cisterna magna and the fourth ventricle. With the help of the pressure of the cerebrospinal fluid and the capillary effect of the pipettes, 50–100 μl cerebrospinal fluid could be sampled. If there was any visible contamination with blood, the sample was excluded. Blood (100 μl) from the left jugular vein and carotid artery was also sampled.

The whole sampling procedure was completed within 5 min. The acid-precipitated proteins were removed by centrifugation at 1.200 g for 10 min in cold, the supernatants neutralized immediately with 300 μl 2 M K_2CO_3 and the precipitated potassium-perchlorate removed by centrifugation. The final supernatants were taken for acetaldehyde and ethanol determinations.

Acetaldehyde determination. The acetaldehyde concentration was determined with an enzymatic method according to Tottmar and Marchner [15]. Aldehyde dehydrogenase (EC 1.2.1.3; ALDH) was obtained as

a partially purified enzyme from rat liver mitochondria. The preparation was rich in a multiple form of the enzyme, which has a low K_m towards acetaldehyde. The high concentration of this multiple form makes the method rather specific for acetaldehyde but a reaction with any endogenic aldehydes with a comparably low K_m and high concentrations in the sample cannot be excluded. However, no ALDH-reaction could be detected in sample mixtures with extracts from blood and cerebrospinal fluid of normal, untreated rats.

The reaction mixture contained 100–500 μl of the final supernatants, 2.4 ml 50 mM pyrophosphate buffer pH 8.8, 0–400 μl aqua dest., 10 μl ALDH and 5 μl 50 mM NAD. The reaction was initiated with NAD, and the formation of $NADH_2$ was measured in an Aminco-Bowman spectrofluorometer. The reaction was completed within 2–3 min.

There was no disappearance of acetaldehyde from the reaction mixture at the experimental conditions used. No spontaneous formation of acetaldehyde from ethanol was observed [12]. Minimum detectability was 0.20 nmol, corresponding to a concentration of 6 μM in the sample.

Ethanol determination. Enzymatic conversion of ethanol to acetaldehyde and spectrophotometric determination of $NADH_2$ produced was performed according to Dickinson and Dalziel [16].

RESULTS

Acetaldehyde in peripheral blood. A maximum in the acetaldehyde concentration in peripheral blood from the tail after ethanol injection was reached between 45 and 90 min followed by a slow decline. The time course was similar for both diets. Depending on the ethanol dose, the Astra-Ewos diet caused an acetaldehyde level of 9–166 μM and Anticimex 10–63 μM, 90 min after ethanol administration.

Intraperitoneal injection of 50–100 mg/kg acetaldehyde (1.0 M in saline) 30 min after an ethanol dose of 1.5 g/kg caused an acetaldehyde peak with a maximum 5 min after injection. This peak declined rapidly and the normal level was reached within 10 min. To obtain a high and stable acetaldehyde level in the blood, the rats were infused i.p. with acetaldehyde, 20 min after ethanol administration. After 10–15 min the acetaldehyde reached a stable level and this level persisted with small fluctuations during the infusion period. The acetaldehyde level differed among rats and depended upon the infusion rate and the amount given.

Acetaldehyde in blood and cerebrospinal fluid. Administration of 0.5 g/kg of ethanol resulted in an ethanol level below 1 mM in blood 90 min later, independent of the diet consumed by the animal. The acetaldehyde level detected in blood and cerebrospinal fluid at that time ranged from 10 to 30 μM irrespective of the type of feed given to the animals.

Differences in acetaldehyde levels were only observed between rats fed the two diets when higher doses of ethanol were administered.

Astra-Ewos R-3-rats. Higher ethanol concentrations gave higher acetaldehyde levels in the blood in rats fed the R-3 diet. A good correlation (0.83) between the ethanol and acetaldehyde concentrations is found

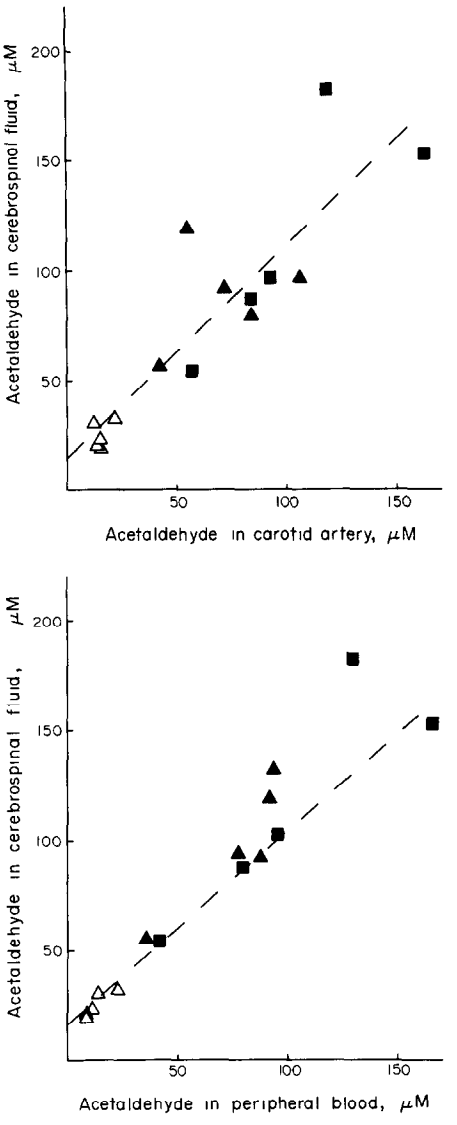

Fig. 1. Acetaldehyde concentration in cerebrospinal fluid from rats fed Astra-Ewos R-3 diet, and its dependence on the blood level.The marks represent acetaldehyde concentrations 90 min after administration of 0.5 (△), 1.0 (▲) and 1.5 (■) g ethanol per kg body wt. Carotid artery: regression coefficient 0.99 and correlation coefficient 0.85. Peripheral blood: regression coefficient 0.88 and correlation coefficient 0.99.

in the blood from the carotid artery, (regression coefficient 3.99). The acetaldehyde in cerebrospinal fluid varied according to the acetaldehyde concentration in the blood (see Fig. 1). A very good correlation (0.997) between the ethanol levels in blood from carotid artery and cerebrospinal fluid was also found (regression coefficient 1.13).

Infusion of acetaldehyde raises the acetaldehyde concentrations in both blood and cerebrospinal fluid.

Anticimex 210-rats. With this diet higher ethanol concentrations gave only slightly higher acetaldehyde levels in the blood (correlation 0.71, regression 1.20). No correlation was found between acetaldehyde in blood and cerebrospinal fluid, but there was a correlation (0.73) between the levels found in the carotid

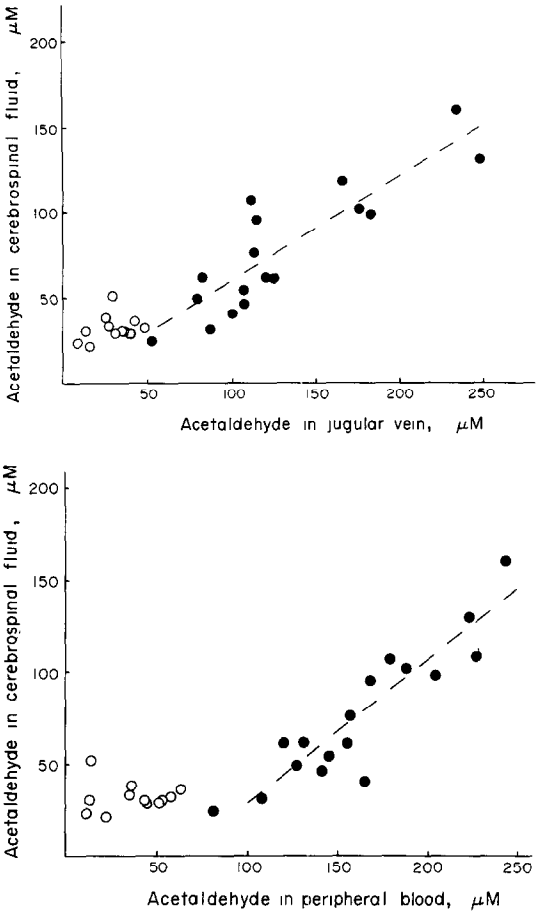

Fig. 2. Acetaldehyde concentration in cerebrospinal fluid from rats fed Anticimex 210 diet and its dependence on the blood level. Open circles (○)—90 min after administration of 0.5, 1.0, 1.5 or 2.0 g ethanol per kg body wt. Filled circles (●)—after administration of 1.5 g ethanol per kg body wt and acetaldehyde infusion.

artery and the jugular vein, although the range was small (9–57 μM). The acetaldehyde concentration in cerebrospinal fluid appeared to be constant around 30 μM, whereas it ranged between 20 and 60 μM in blood. A very good correlation (0.998) between the ethanol levels in blood from carotid artery and in cerebrospinal fluid was also found (regression coefficient 1.20).

Infusion of acetaldehyde raises the acetaldehyde concentration in the blood. When the level reached approximately 50 μM in the jugular vein or the carotid artery and approximately 100 μM in the tail, the acetaldehyde level in cerebrospinal fluid began to rise. The slope of these increases were 0.61 for the jugular vein (correlation 0.87) and 0.79 for the tail, (correlation 0.92) (see Fig. 2). In the carotid artery the acetaldehyde concentrations were more scattered. For eighteen values between 48 and 357 μM a slope of 0.36 and a correlation of 0.70 were found.

Effect of cyanamide on the acetaldehyde level in cerebrospinal fluid. Cyanamide (5 or 10 mg/kg body wt) administered 3 hr before ethanol caused an elevation of acetaldehyde in the blood of rats fed the Anticimex 210 diet. The concentration of acetaldehyde in blood, 90 min after injection of (1.5 g/kg body wt) ethanol, was around 200 μM. Approximately the same level was detected in cerebrospinal fluid.

DISCUSSION

While the present experiment was in progress, it was shown by Marchner and Tottmar [17], that the dietary factor in the Astra-Ewos R-3 diet was cyanamide, a known inhibitor of aldehyde dehydrogenase. The effects of this diet may, therefore, be looked upon as a pre-treatment with low doses of cyanamide. The Astra-Ewos R-3 diet was also shown to decrease the activity of the aldehyde dehydrogenase with a low K_m towards acetaldehyde in the liver [18]. Thus, the difference of acetaldehyde occurrence in cerebrospinal fluid after feeding the two diets may be explained by an inhibition of the low K_m aldehyde dehydrogenase in the brain similar to what occurred in the liver. In the rats fed the R-3 diet acetaldehyde in the cerebrospinal fluid varied according to the blood level with a slope of 1 (see Fig. 1). The same is shown for ethanol, another compound with a high lipid solubility, the metabolism of which is insignificant in the brain [19].

The plateau at approximately 30 μM in cerebrospinal fluid of rats fed the 210 diet when the blood level ranges from 20 to 50 μM (20–100 μM in peripheral blood) (see Fig. 2) is probably an effect of a steady state between diffusion into and out of the cerebrospinal fluid and a metabolism by the low K_m aldehyde dehydrogenase. The higher values in cerebrospinal fluid compared with the blood at low blood levels is probably caused by a decreasing level in the blood at the sampling time. The slope after the plateau is less than 1, indicating that another aldehyde dehydrogenase, the high K_m enzyme, metabolizes part of the acetaldehyde when the low K_m enzyme is saturated.

The diffusion in and out of the blood capillaries in the brain may also affect the slope and is probably partly responsible for the difference in the slope between carotid artery and the jugular vein.

Sippel [7] and Sippel and Eriksson [8] could not find any significant acetaldehyde level in brain tissue at blood levels lower than 200 μM although they fed the rats the Astra-Ewos laboratory diet. The blood acetaldehyde concentrations reported by them indicate a certain inhibition of the liver aldehyde dehydrogenase but to a lesser degree than in our experiments. They have also injected acetaldehyde to elevate the blood level but the low doses might have been metabolized before their sampling.

A special enzymatic blood-brain barrier to protect the brain against acetaldehyde is not probable. Acetaldehyde can be detected in cerebrospinal fluid inside the blood-brain barrier and no barrier between cerebrospinal fluid and brain tissue is recognized [20]. Nevertheless, it cannot be excluded that a small part of the acetaldehyde-metabolizing capacity can be located in the choroid plexus and the barrier. The presence of an active aldehyde dehydrogenase in brain tissue would result in a lower level of acetaldehyde in the tissue than in the cerebrospinal fluid. Our results compared with Sippel and Erikssons [8] indicate this.

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