ETHANOL OXIDATION IN THE LIVING BRAIN

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INTRODUCTION

It is postulated that the first metabolite of ethanol, acetaldehyde (AA), mediates many effects of ethanol in a brain, including behavioural, neurochemical and neurotoxic actions, and plays a crucial role in the development of alcoholism (Hunt, 1996; Smith et al., 1997; Deitrich, 2004; Quertermont et al., 2005). AA derived from the peripheral metabolism of ethanol penetrates from blood to brain with difficulty, because of the metabolic barrier represented by aldehyde dehydrogenase in capillary endothelium and surrounding astrocytes (Sippel, 1974; Westcott et al., 1980; Zimatkin, 1991). Therefore, for its central action, AA should be produced inside the brain. It seems possible because all of the main ethanol metabolizing enzymes (alcohol dehydrogenase, cytochrome P450 2E1 and catalase) are available in the brain tissue (Zimatkin et al., 1998). The slow, but measurable oxidation of ethanol, and the formation of AA in the naive adult rat brain homogenates was demonstrated, and the crucial role of catalase in this process was established (Aragon et al., 1992; Gill et al., 1992; Hamby-Mason et al., 1997; Zimatkin et al., 1998). The significant oxidation of ethanol to AA was found in fetal and neonatal brain homogenates (Hamby-Mason et al., 1997; Person et al., 2000), as well as in the primary culture of astrocytes (Eysseric et al., 1997). Recently, in addition to catalase, the important role of CYP450 2E1 in ethanol oxidation in the rodent brains has been demonstrated using specific enzyme inhibitors, mice with genetic deficiency in catalase and plays a crucial role in the development of alcoholism including behavioural, neurochemical and neurotoxic actions. All chemicals were obtained from Sigma–Aldrich. For the in vivo examination of ethanol oxidation in the brain under general anesthesia (calepsol, 100 mg/kg, i.p.), the rats were placed into the stereotaxic apparatus, and through the opening in the skull a solution (85 and 90 mM) of ethanol (in 0.9% NaCl) was injected into the lateral brain ventricle using a syringe and micro pump (the stereotaxic coordinates: P = −0.9; L = 1.5; D = 3.5 mm (Paxinos and Watson, 1986). The speed of perfusion varied from 6 to 43 µl/min. To study the effect of the catalase inhibitor 3-amino-1,2,4-triazole (aminotriazole) the initial ethanol solution (perfused for 30 min) was substituted by the same ethanol solution containing 10 mM aminotriazole (for 4.5 h). The samples of perfusate released from the large cistern (Cisterna magna) of the brain stem were collected through a needle and plastic capillary and were obtained every 5 or 10 min during 1–5 h. The samples were collected into Eppendorf tubes containing the pre-cooled acetaldehyde-binding solution (0.1 M phosphate buffer pH 7.25; 7 mM semicarbazide; 0.15% trilon B; 0.25 mM Na azide). The exact volume of every sample was calculated by weighing the Eppendorf tubes before and after sampling.

Animals, chemicals and experimental design

Male Wistar rats (N = 20; 200–240 g) were obtained from the breeding colony of the Grodno State Medical University. All chemicals were obtained from Sigma–Aldrich. For the in vivo examination of ethanol oxidation in the brain under general anesthesia (calepsol, 100 mg/kg, i.p.), the rats were placed into the stereotaxic apparatus, and through the opening in the skull a solution (85 and 90 mM) of ethanol (in 0.9% NaCl) was injected into the lateral brain ventricle using a syringe and micro pump (the stereotaxic coordinates: P = −0.9; L = 1.5; D = 3.5 mm (Paxinos and Watson, 1986). The speed of perfusion varied from 6 to 43 µl/min. To study the effect of the catalase inhibitor 3-amino-1,2,4-triazole (aminotriazole) the initial ethanol solution (perfused for 30 min) was substituted by the same ethanol solution containing 10 mM aminotriazole (for 4.5 h). The samples of perfusate released from the large cistern (Cisterna magna) of the brain stem were collected through a needle and plastic capillary and were obtained every 5 or 10 min during 1–5 h. The samples were collected into Eppendorf tubes containing the pre-cooled acetaldehyde-binding solution (0.1 M phosphate buffer pH 7.25; 7 mM semicarbazide; 0.15% trilon B; 0.25 mM Na azide). The exact volume of every sample was calculated by weighing the Eppendorf tubes before and after sampling.

Ethanol and AA determination

The samples obtained were stored for 30 min at 4°C followed by the addition of 50 µl of 4 M perchloric acid and 19 mM thiourea (for the reduction of the artificial AA formation from ethanol) and 1 mM 1-propanol as internal standard and stored again for 30 min at 4°C. Two hundred microlitres of the mixture were transferred quickly to the 15 ml glass vials containing 100 mg K2CO3. Then those vials were incubated for 20 min at 65°C, and 2 ml of head-space gas was injected into HP 6890 gas chromatograph with flame ionization detector and column (250 × 2 mm, filled with chromatone N-AW-DMCS, treated with Carbowax 5% 20 M), inlet

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Fig. 1. Ethanol and acetaldehyde levels in perfusate at the course of ventricular perfusion of rat brain by ethanol solution. (a) Levels of ethanol; (b) Levels of acetaldehyde. Ventricular perfusion of brain in anesthetized rats by 85 mM solution of ethanol in 0.9% NaCl with the increasing speed of perfusion (6–43 µl/min). Each data point is mean ± SEM; N = 5.

170°C, oven 70°C, detector 220°C. Under these conditions, the retention time for acetaldehyde was 0.47 min, for ethanol 0.9 min, for propanol 1.43 min. Relative peak heights were determined by comparison with the standards prepared by the addition of the known amounts of acetaldehyde or ethanol to the perfusing fluid. To exclude artificial AA, the calibration of its formation was carried out by the addition of various ethanol concentrations to the perfusate. The levels of artificial AA obtained were subtracted from the data obtained.
Statistics
The data are represented as mean ± SEM. Statistical analysis used repeated measures ANOVA; post-hoc comparisons were performed using Dunnet’s test; differences were considered significant at \( P < 0.05 \). Computer program STATISTICA 6.0 was used in this study.

RESULTS
It was found that the passage of ethanol through the ventricular system of the brain results in the significant decrease of ethanol concentration (from 85 to 2 mM) (Fig. 1(a)). In addition, we found the appearance of AA in the perfusate, obtained from the cisterna magna (Fig. 1(b)). AA was not detected in the cerebrospinal fluid and perfusate following the perfusion by 0.9% NaCl without ethanol.

Increasing the perfusion speed of 85 mM ethanol solution from 6 to 43 μl/min results in the increasing of ethanol concentration in the perfusate from 2 to 35 mM (Fig. 1(a)) and AA from 10 to 50 μM (Fig. 1(b)). The repeated measures ANOVA revealed the significant effect of the perfusion speed for ethanol elimination (\( F = 33.8; \ P < 1 \times 10^{-15} \)) and AA accumulation (\( F = 85.3; \ P < 1 \times 10^{-20} \)).

The addition of 10 mM aminotriazole to the perfusing fluid significantly increased the ethanol level in the perfusate (\( P < 0.01 \)–0.001) (Fig. 2(a)). This effect appeared in the first 30 min period and still slowly increased during the next 2 h. A one-way ANOVA revealed the significant effect of time for that effect of aminotriazole (\( F = 2.1; \ P < 0.05 \)). No significant effect of 10 mM aminotriazole on AA level in the perfusate was found (Fig. 2(b)).

DISCUSSION
It may be suggested that during the ventricular perfusion of the rat brain, ethanol is being diluted in the cerebrospinal fluid, diffusing into the brain tissues according to the concentration gradient, partly diffusing into the blood and is removed by the blood-flow. However, the blood ethanol concentration in those animals did not exceed the endogenous levels, probably because of its dilution in the peripheral blood (data not shown). This may be one of the reasons for ethanol elimination from the perfusing fluid (non-enzymatic).

In addition, ethanol was partly oxidized in the brain tissues and the AA accumulation in the perfusate is the important confirmation of that. That catalase participates in this process, is shown by the inhibition of this enzyme by aminotriazole, since this resulted in decreased ethanol elimination in the brain. Most of the AA formed from ethanol should be oxidized rapidly by the brain aldehyde dehydrogenase to acetate or removed by binding with the brain tissues (Zimatkin et al., 1998). The remaining AA is diffusing in the brain ventricle fluid and is detected in the perfusate. No AA was found in the blood of the experimental animals, probably due to the blood-brain metabolic barrier represented by aldehyde dehydrogenase, working in both directions (Zimatkin, 1991) and quick elimination of AA at the periphery.

The blood-brain barrier (the barrier between the blood and brain nerve cells) contains a much more powerful metabolic barrier for AA as compared to the other brain barriers, including the barrier between brain tissue and cerebrospinal fluid. The AA formed from ethanol metabolism in the brain neurons and glial cells can diffuse via the intercellular clefts into the perfusing fluid.

The concentration of AA in the perfusate was about \( 1/1000 – 1/10000 \) of ethanol elimination. In brain homogenates, where there is no non-enzymatic removal of ethanol and brain barriers, the ratio of ethanol elimination/AA accumulation was \( 1/40 – 1/100 \) (Zimatkin et al., 1998). The lower level of AA in the perfusate may also be explained by its quick oxidation in the ependymal cells lining the brain ventricles and containing high aldehyde dehydrogenase activity (Zimatkin, 1991).

Accelerating the speed of perfusion results in the increase of ethanol and AA concentration in the perfusate. At higher speeds of perfusion the smaller fractions of ethanol and AA are eliminated by diffusion, by the blood flow or oxidized by brain tissue. In the latter case, there is a limit to the capacity of ethanol and aldehyde oxidizing enzymes.

The whole volume of rat brain ventricles is 200–300 μl. Therefore, the speed of ventricular perfusion of the brain used
in our experiments (6–43 µl/min) was higher as compared to microdialysis techniques which use the perfusion of the much smaller intercellular space of the brain tissue. The optimal speed of perfusion chosen for the further experiments was 12 µl/min. At lower speeds of perfusion all the ethanol from the perfusing fluid was eliminated. Therefore, the further inhibition of the process could not be detected.

The addition of 10 mM aminotriazole to the perfusing fluid significantly increases the ethanol levels in the perfusate. This indicates the decrease of the enzymatic portion of ethanol elimination rate because of the inhibition of ethanol oxidation by catalase. It is known that catalase is the main enzyme of ethanol oxidation in the brain and aminotriazole at concentrations 5–10 mM (20 min pre-incubation period) is a potent inhibitor of brain catalase and ethanol oxidation in brain homogenates (Aragon et al., 1992; Gill et al., 1992; Zimatin et al., 1998; Zimatin et al., 2006). In vivo experiments on mice, was showed the maximal inhibition of brain catalase occurs within 5–10 h following the intraperitoneal aminotriazole administration in the dose of 0.5 g/kg (Escarabajal et al., 2000). We demonstrated the maximal inhibition of ethanol elimination from the perfusing fluid following 2.5 h of perfusion with aminotriazole. This inhibitory effect of aminotriazole supports the enzymatic character of ethanol elimination in the brain during the ventricular perfusion (Fig. 2(a)). The absence of a significant decrease of AA in this experiment can be explained by the inhibitory effect of aminotriazole on brain aldehyde dehydrogenase, which we found earlier (unpublished data).

In conclusion, for the first time, the possibility of ethanol oxidation and acetaldehyde production in the living brain was demonstrated. The enzyme catalase is involved in this process.

Acknowledgements — The authors are grateful to Dr Richard Deitrich for the revision of the manuscript and his valuable comments.

REFERENCES


