METHODS

A Novel Method for Evaluation of Ethanol Oxidation in Living Brain

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We propose a method for intravital examination of ethanol metabolism in rat brain. Calipsol-anesthetized rats were fixed in stereotaxis and ethanol solution was infused into the lateral cerebral ventricle via an orifice in the skull at a constant rate with a syringe or micropump. Elimination of ethanol and production of acetaldehyde (ethanol metabolite) were measured in perfusate samples from the cisterna magna by gas chromatography. The method is highly sensitive, reliable, and reproducible and allows to study the kinetics and enzyme mechanisms of ethanol oxidation in the brain and regulation of this process.

Key Words: *ethanol oxidation; acetaldehyde; living brain*

Acetaldehyde (AA) is a primary metabolite of ethanol, which mediates various psychopharmacological, behavioral, and neurochemical effects of alcohol in the brain and plays a key role in the pathogenesis of alcoholism [3,8,9]. However, AA produced during intensive oxidation of ethanol at the periphery cannot cross the blood-brain barrier. Therefore, the neurotropic effects are caused by AA produced in the brain. This hypothesis was proved *in vitro* [1,2,4,5,10]. In these studies, AA was detected in brain homogenates or brain cells incubated with ethanol. However, it is still unknown whether oxidation of exogenous ethanol could occur in living brain. For solving this problem, the formation of ethanol metabolites in the brain after direct intracerebral injection of ethanol should be demonstrated *in vivo*.

Our aim was to develop a method for intravital study of ethanol metabolism in the rat brain.

MATERIALS AND METHODS

For evaluation of ethanol metabolism in living brain, we perfused the cerebral ventricular system in rats with ethanol solution. The solution was infused into the lateral cerebral ventricle, while the examined samples of perfusate were taken from the cisterna magna. The rats anesthetized with calipsol (100 mg/kg intraperitoneally) were placed into a stereotactic apparatus, soft tissues were cut, and the skull was drilled. A needle was introduced into the lateral ventricle via the orifice according to stereotactic coordinates of rat brain atlas (P -0.9; L 1.5; D 3.5 mm) [6]. The needle was connected to a syringe and micropump via a fluoroplastic tube. The ethanol dissolved to a certain concentration in 0.9% NaCl saline was infused at a constant rate (Fig. 1). The second outflow needle was inserted into the center of the cisterna magna via a puncture in the dura matter at a point located 1.5 mm caudally to the locus of its maximal mobility determined by dura matter pressing with a bulbous-end probe (Fig. 2).

The outflow needle was connected to a fluoroplastic tube, whose open end was placed into a collection tube containing AA-binding solution (0.9% NaCl, 0.1 M phosphate buffer, pH 7.2, 7 mM semicarbazide, 0.15% EDTA, 0.25 mM sodium azide, 4°C). The volume of binding solution 2-fold sur-

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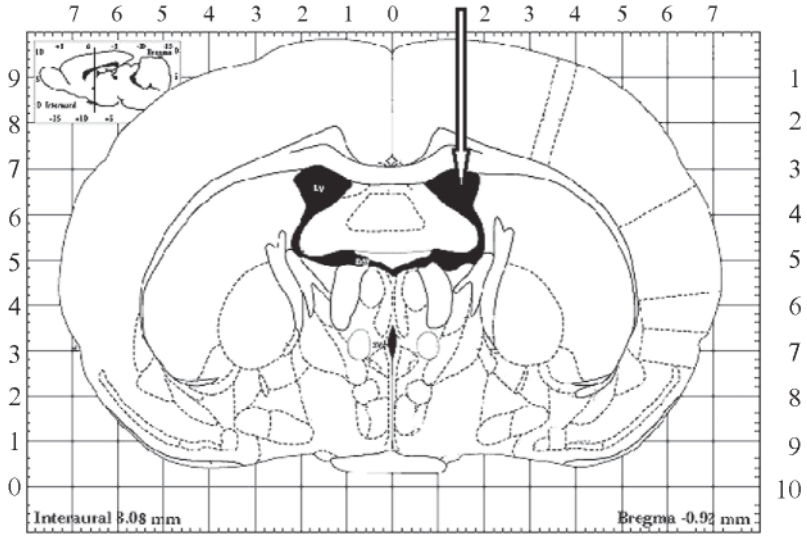

Fig. 1. Frontal section of the rat brain. The arrow points to the place of inflow of the perfusion solution into the lateral cerebral ventricle.

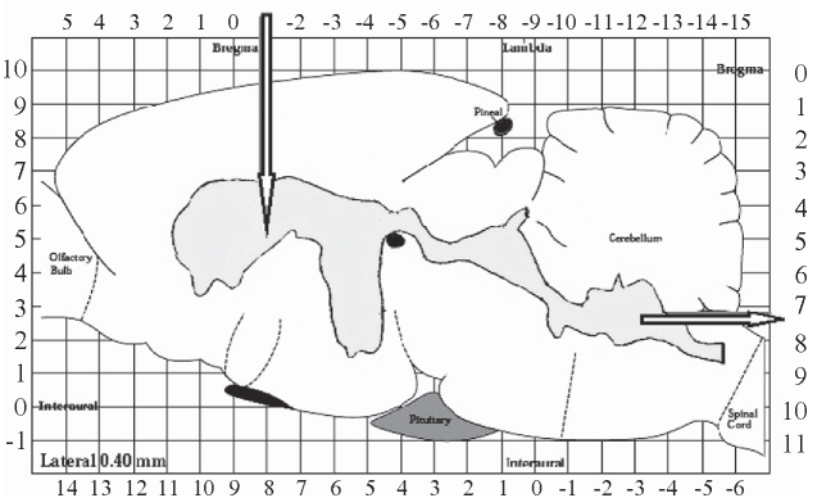

Fig. 2. Sagittal section of the rat brain. Reconstruction of the ventricular system. The arrows point to the places of inflow of the perfusion solution and outflow of the perfusate.

passed the expected amount of the perfusate. The amount of perfusate in each probe was calculated as the difference of test tube weight before and after sampling.

The perfusion solution with various concentrations of ethanol (1-400 mM) was infused into the lateral cerebral ventricle at a constant rate of 6-48 μl/min using a syringe and a micropump. The perfusate flowed out under a pressure created by infused solution. Each sample was collected for 5 or 10 min. The experiment lasted for 0.5-4.0 h.

The content of ethanol and AA in the perfusate was measured by gas chromatography [1]. The mixture of perfusate with AA-binding solution was kept at 4°C for 30 min, thereafter it was supplemented with 50 μl 4 M chloric acid with the internal standard (propranolol=1) and thiourea (19 mM) needed to decrease the oxidizing potency of chloric acid towards ethanol and to diminish the artifact production of aldehyde. After 30 min, the mixture was heated to 65°C over 20 min in a water bath, 2 ml gas-vapor phase was introduced into a HP-6890 gas chromatograph supplied with flame ionization detector and a 2.5 m×2 mm column filled with chromaton N-AW DMCS impregnated with Carbowax 5% 20 M (injector 170°C, furnace 70°C, detector 220°C).

To correct the data for artifact AA, its production was determined in samples with various ethanol concentrations and subtracted from the corresponding values. In each sample, the differences between AA and ethanol levels in the inflow and outflow perfusion solutions were determined.

RESULTS

Depending on ethanol concentration in the perfusion solution and perfusion rate, 5-90% of infused ethanol oxidized during passage via the cerebral ventricular system and perfusate accumulated 15-80 μM AA. For example, when the perfusion solution with 180 mM ethanol passed through the brain at a rate of 12 μl/min over 4 h, 60-90% injected ethanol was oxidized. The perfusate contained AA in a concentration of 10-60 μM (Fig. 3).

Therefore, the proposed method can measure the intensity of intravital oxidation of ethanol in the brain. It produces no significant damage to the brain and prevents entry of artifact substances affecting the results of metabolic study. Repeated sampling of the perfusate during long-term period makes it possible to study the kinetics of ethanol oxidation in the brain for more than 2 h.

Probably, the entire brain was involved in ethanol oxidation during intraventricular perfusion in our experiments. While routing via the ventricles and channels in the brain carried by the perfusion fluid, ethanol can diffuse freely into the cerebral tissue. The product of its oxidation (AA) returns partially to the perfusate, where it can be detected.

The proposed novel method to study ethanol metabolism in the living brain is sensitive, reliable, and reproducible. It makes it possible to examine the kinetics of ethanol metabolism, its enzymatic mechanisms, and the regulation pathways. This method can be used to study the mechanisms of alcohol effects on the brain and the pathogenesis of alcoholism as well as to search for a novel approaches to treat this disease. The original method of intraventricular perfusion can also be used in the investigations of metabolism of various substances in the brain.

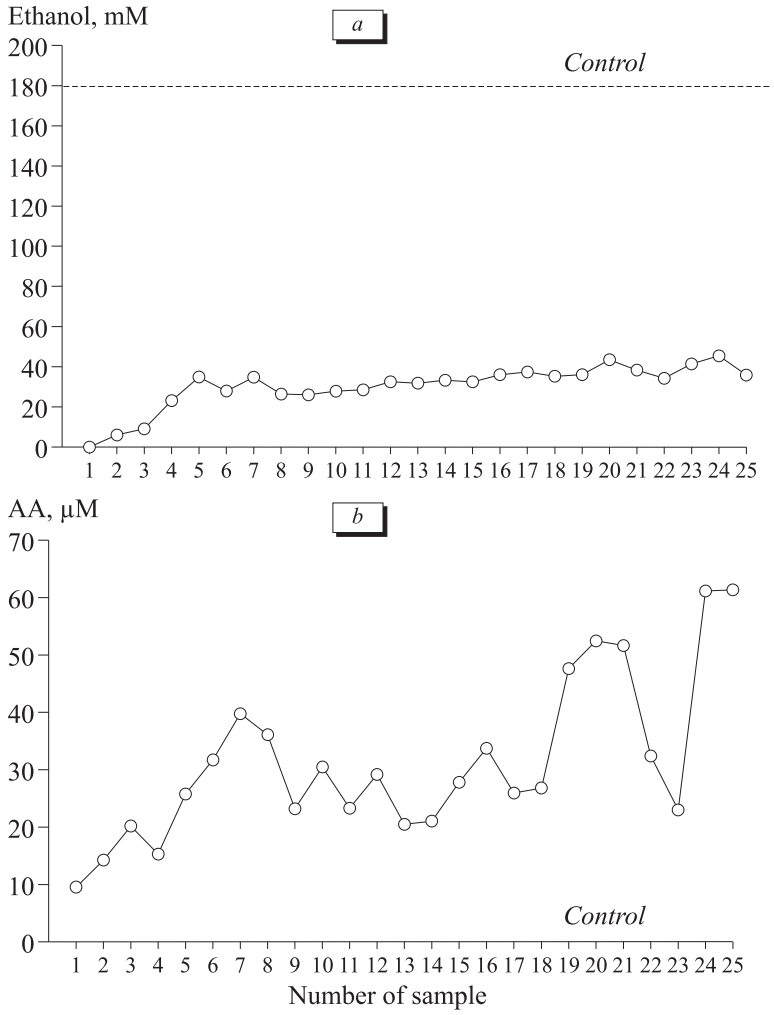

Fig. 3. The contents of ethanol (*a*) and AA (*b*) in rat cerebral perfusate during 4-h intraventricular perfusion of the brain with ethanol solution (180 mM). The perfusate samples were taken every 10 min and studied by gas chromatography.

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