Changes of Acetylcholinesterase Activity in Brain Areas and Liver of Sucrose- and Ethanol-Fed Rats

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The effects of chronic ethanol or sucrose administration to rats on acetylcholinesterase from brain and liver were investigated. Membrane-bound and soluble acetylcholinesterase activities were determined in fractions prepared by centrifugation. The thermal stability and the effects of temperature and different types of alcohols on acetylcholinesterase activity were also studied. Membrane-bound acetylcholinesterase activity increased (p < 0.01) in the liver after chronic ethanol administration, whereas no differences among groups in the encephalic areas, except in the brain stem soluble form, were found. Membrane-bound acetylcholinesterase from the ethanol- and sucrose-treated groups was more stable at the different temperatures assayed between 10 and 50°C than that corresponding to the control group. Non-linear Arrhenius plots were obtained with preparations of membrane-bound acetylcholinesterase from rat liver, with discontinuities at 30°C (control or sucrose groups) or 34–35°C (alcohol group). Assays made with membrane-bound or soluble enzyme from brain showed linear Arrhenius plots in all groups studied. The inhibitory effects of increasing concentrations of ethanol, n-propanol and n-butanol on acetylcholinesterase preparations from forebrain, cerebellum, brain stem and liver of the three experimental groups (control, sucrose-fed and ethanol-fed) were very similar. However, n-butanol displayed a biphasic action on particulate or soluble preparations of rat forebrain. n-Butanol inhibited (competitive inhibition) at higher concentrations (250–500 mM), while at lower concentrations (10–25 mM), the alcohol inhibited at low substrate concentrations but activated at high substrate concentration. These results suggest that the liver is more affected by ethanol than the brain. Moreover, the lipid composition of membranes is probably modified by ethanol or sucrose ingestion and this would affect membrane fluidity and consequently the behaviour of acetylcholinesterase.

KEY WORDS: Acetylcholinesterase; brain; ethanol-fed rats; liver; sucrose-fed rats.

INTRODUCTION

The medical aspects of alcoholism, including studies of ethanol intoxication and dependence, have gained great interest in recent years. Neurological disorders, liver damage, peripheral neuropathies and impaired fetal development in the offspring of alcoholic mothers have been described as consequences of alcohol abuse.
There is increasing evidence that many of the pathological changes associated with chronic ethanol abuse arise as a result of disorders in membrane function that follow changes in the lipid composition of neural and non-neural membranes (1). It has been demonstrated that chronic ethanol administration affects the activity of certain membrane-bound enzymes (Na⁺/K⁺-ATPase, Ca²⁺-ATPase, 5'-nucleotidase, acetylcholinesterase and adenylate cyclase) (1–4). Ethanol interacts with membrane lipid bilayer molecules and thereby expands the membrane and increases lipid fluidity. However, prolonged exposure to alcohol seems to result in compensatory changes in the membrane aimed at restoring the original state of fluidity (5). Changes in lipids and in fluidity are also known to influence such physiologically important membrane enzymes as Na⁺/K⁺-ATPase and hormone-responsive adenylate cyclase (4).

Ethanol may also affect a variety of neurotransmitter systems in different brain regions. For example, it has been reported that after long-term ethanol ingestion brain cholinergic function is persistently altered (6,7). Acetylcholinesterase (AChE; EC 3.1.1.7) plays a key physiological role in cholinergic synapses by rapidly hydrolyzing the neurotransmitter acetylcholine, hence terminating synaptic transmission. This enzyme is mainly found in nervous tissue and neuromuscular junctions. However, AChE has also been localized in tissues where no cholinergic functions are known to occur (8).

AChE is found in a range of molecular forms which are classified as collagen-tailed asymmetric species (A) and globular (G) components (9). Globular AChE forms exist in tissues and fluids as amphiphilic (detergent-soluble, G₄) and hydrophilic (salt-soluble, G¹) molecules. The mammalian brain contains abundant amphiphilic AChE tetramers (G₄) in vivo and few monomers (G¹). The amphiphilic AChE tetramers of the brain consist of two pairs of subunits, one pair being disulfide-bonded and the other two subunits being bonded by disulfide bridges to a hydrophobic 20-kDa polypeptide anchor.

In rat liver, AChE is associated with the plasma membrane and Golgi apparatus (10–11). Most of the liver enzyme corresponds to detergent-soluble tetramers and dimers. The dimers are sensitive to glycosylphosphatidylinositol residues for membrane attachment. In addition, a small fraction of AChE tetramers is released from the liver by detergent-free buffers, this pool probably representing the enzyme destined for secretion.

The purpose of this study was to investigate the effects produced by chronic ethanol administration to rats on AChE activity from several brain areas and the liver. We also focused our attention on whether the administration of a carbohydrate-enriched diet and the ingestion of ethanol have similar effects on brain and liver AChE activity. Finally, the thermal stability of AChE, the dependence of the enzyme activity on temperature, and the actions of some alcohols on the kinetic properties of AChE were also investigated.

**EXPERIMENTAL PROCEDURE**

**Materials.** Acetylthiocholine chloride, bovine serum albumin, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), eserine (physostigmine), ethopropazine and Tris(hydroxymethyl)aminomethane were from Sigma Chemical Co. (St Louis, MO, U.S.A.). The ethanol and sucrose given to rats were from Panreac Química S.A. (Barcelona, Spain). The alcohols used in AChE assays were from Merck (Darmstadt, Germany). All other products were from Probus (Barcelona, Spain). Chemicals were of analytical grade or of the highest purity commercially available.

**Animals.** All animal experiments were carried out in accordance to European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Male Wistar rats (Criffa S.A., Barcelona, Spain), 6 weeks old and weighing 140–160 g, were used. The animals were divided into three groups. One group (called "ethanol group") was given a 20% (v/v) ethanol solution as the sole source of liquid for 10 months. A second group ("sucrose group") receiving water ad libitum was used. Ethanol was introduced in the diet gradually, the final concentration of the alcohol (20%) being reached after three weeks. Administration of isocaloric sucrose to the rats of the ethanol group the day before.

All rats were maintained on a laboratory rat-chow basal diet (Panlab A-04, Barcelona, Spain) containing 3.8% fat, 17.0% crude protein and 56.5% carbohydrate ad libitum. Animals were fasted overnight and killed by cervical dislocation. The abdominal cavity was immediately opened and blood was drawn from the vein cava with heparinized needles and syringes. Livers and brains were removed (immediately after death), exhaustively washed in saline, and the forebrain, cerebellum and brain stem dissected out. Samples were stored at −80°C until use and thawed only once.

For estimation of alcohol in rat serum, ice-cold perchloric acid was added to blood and, after separation of the pellet, the concentration of alcohol was determined by a spectrophotometric assay and an appropriate diagnostic kit (Boehringer-Mannheim test combination).

**Preparation of the Membrane-Bound and Soluble Acetylcholinesterase.** Liver and selected brain areas from rats of the three experimental groups were individually minced and then homogenized in a Potter-Elvehjem homogenizer (five strokes) with 10 volumes of 0.25 M sucrose dissolved in 10 mM Tris/HCl buffer, pH 7.4. The homogenate from neural tissues was centrifuged at 1,500 g for 15 minutes to remove cell debris. After centrifugation of the supernatant at 105,000 g for 1 hour, the new supernatant was saved. This fraction was referred to as “soluble” AChE. The remaining pellet was resuspended in 2 ml of the above sucrose-Tris buffer, the suspension