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EFFECT OF A SINGLE INJECTION OF ETHANOL ON PERMEABILITY OF THE BLOOD-BRAIN BARRIER FOR ^{14}C-TYROSINE, ^{14}C-DOPA, AND HORSERADISH PEROXIDASE

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When administered peripherally ethanol easily passes through the blood-brain barrier (BBB) and enters the brain [10]. It has also been shown that ethanol disturbs the entry of products taking part in metabolism into the brain and, in particular, amino acids [6, 12], which pass through the BBB with the participation of specific mechanisms of active transport [13]. It can accordingly be concluded that the activity of at least some of the eight known [13] systems for the transport of low-molecular-weight metabolites to the brain is modified by ethanol. The mechanism of nonspecific transport of high-molecular weight compounds of protein type into the brain (the discovery of interendothelial tight junctions, vesicular transport, endothelial tubules, diffusion through the endothelial cytoplasm), reflecting the state of the barrier function of the BBB, are normally at a low functional level, but they may be activated under the influence of various stress-inducing and injurious factors [4, 8-14], which may include the intake of ethanol.

The aim of this investigation was to make a comparative study of the effect of a single intake of ethanol on some mechanisms of specific and nonspecific transport of materials across the BBB.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-350 g. Ethanol was injected intraperitoneally in doses of 1, 2, and 4 g/kg body weight. After 60 min, under ether anesthesia, ^{14}C-L-tyrosine or ^{14}C-L-dopa (specific radioactivity 492 and 5.4 kCi/mmole respectively) was injected into the femoral vein in a dose of 5 μCi, dissolved in 0.5 ml of physiological saline. In the experiments with ^{14}C-dopa, to prevent its peripheral metabolism, carbidops (α-methyldopa hydrazine, an inhibitor of dopa-decarboxylase [7]) was injected intraperitoneally in a dose of 80 mg/kg 75 min before the indicator. Immediately

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TABLE 1. Effect of a Single Dose of Ethanol on Permeability of the BBB for ^{14}C-Tyrosine

Experimental conditions	Radioactivity in brain structures (number of counts per minute recorded in supernatant per 100 mg tissue)			
	cortex	hypothalamus	medulla	cerebellum
Control (physiological saline, intraperitoneally)	591±33 (6)	515±19 (6)	510±29 (6)	562±34 (6)
Ethanol, intraperitoneally)				
1 g/kg	622±26 (6)	544±32 (6)	493±27 (6)	540±41 (6)
2 g/kg	724±30* (6)	666±38*** (6)	650±29* (6)	744±37** (6)
4 g/kg	926±50*** (6)	862±71*4 (6)	875±74** (6)	880±59*** (6)

Legend. Ethanol was injected as a 10% (1 and 2 g/kg) or 25% (4 g/kg) solution 60 min before ^{14}C-L-tyrosine (5 Ci, intravenously). *P < 0.02, **P < 0.01, ***P < 0.002, ****P < 0.001. Here and in Table 2, number of specimens shown in parentheses.

TABLE 2. Effect of a Single Dose of Ethanol on Permeability of the BBB for ^{14}C-dopa

Experimental conditions	Radioactivity in brain structures (number of counts per minute recorded in supernatant per 100 mg tissue)			
	cortex	hypothalamus	medulla	cerebellum
Control (physiological saline, intraperitoneally)	128±6 (4)	88±8 (4)	94±10 (4)	140±5 (4)
	538±23 (6)	380±21 (6)	438±36 (6)	625±58 (6)
Ethanol, intraperitoneally:				
1 g/kg	490±43 (5)	351±29 (5)	389±36 (5)	551±52 (5)
2 g/kg	698±18*** (6)	454±35 (6)	533±25* (6)	746±33* (6)
4 g/kg	674±20** (6)	517±25** (6)	531±24* (6)	803±45** (6)

Legend. Carbidopa was injected 75 min before ^{14}C-L-dopa. *P < 0.05, **P < 0.01, ***P < 0.001 compared with effect of carbidopa.

after injection of the indicators, the blood vessels of the brain were rinsed with physiological saline, heated to 36°C, in a volume of 60 ml through the left ventricle, after preliminary division of the right atrium, to remove blood. Pieces of brain tissue weighing 100 mg, from the region of the cortex, cerebellum, hypothalamus, and medulla, were homogenized in 0.3 ml of 0.6 M $HClO_4$ solution. The homogenate was centrifuged for 20 min at 10,000 g and the supernatant treated with 0.3 ml of 1.5 M $KHCO_3$. After repeated centrifugation 0.3 ml of the supernatant was added to 10 ml of "Aquasol" universal mixtures and the number of counts recorded on an LKB (Sweden) liquid scintillation counter. The numerical results were subjected to statistical analysis by Student's t test. Horseradish peroxidases in a dose of 50 mg/kg dissolved in 0.5 ml of physiological saline, was injected into the animals' femoral vein under ether anesthesia 60 min after injection of ethanol. The animals were decapitated 10-15 min after injection of the peroxidase, the brain was removed, and slices 2 mm thick were fixed in a 2% solution of glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C. Sections 40 μ thick, after preliminary treatment in 0.01% hydrogen peroxide solution and repeated rinsing in phosphate buffer, were examined under the light microscope.

EXPERIMENTAL RESULTS

The control experiments showed that permeability of BBB for ^{14}C-tyrosine was four times greater in all structures than permeability for ^{14}C-dopa (Tables 1 and 2). Carbidopa increased its penetration into the brain up to a level comparable with the degree of permeability for ^{14}C-tyrosine. In view of the ability of carbidopa to inhibit the highly active dopa-decarboxylase of the cerebral capillaries [7], which, in accordance with modern views on the BBB [2], is the principal morphological and functional component of the barrier, it can be concluded that the increase in permeability of the brain structures for ^{14}C-dopa observed in the present experiments, was not only the result of preservation of ^{14}C-dopa in the blood, but it was largely due to facilitation of its passage through the actual mechanisms of the BBB. This state of affairs suggests that a change in activity of the enzymes contained in the microcapillaries of the brain, and responsible for metabolism of physicologically active substances, is one of the effective mechanisms of the change in their ability to pass through the BBB.

Ethanol, in a dose of 1 g/kg, did not affect permeability of the BBB for ^{14}C-tyrosine and ^{14}C-dopa. With doses of ethanol of 2 and 4 g/kg a dose-dependent increase in permeability of the BBB was observed for ^{14}C-tyrosine in all structures studied, whereas the permeability for ^{14}C-dopa was increased by ethanol in a dose of 2 g/kg, and it increased by virtually the same degree when the dose of ethanol was increased to 4 g/kg. This last result may indicate a comparatively stronger effect of ethanol with respect to the greater transport of ^{14}C-tyrosine than of ^{14}C-dopa into the brain. Horseradish peroxidase is a high-molecular-weight compound which, under normal conditions, does not pass through the BBB, and which is therefore used to investigate the barrier function of the BBB [8, 14], for it reflects the level of activity of the mechanisms of nonspecific transport. In the present experiments ethanol, in any of the doses used, did not enable horseradish peroxidase to pass from the lumen of the microcapillaries into the brain parenchyma. This may be evidence of the relative integrity of the barrier function of the BBB after a single intake of ethanol. It can be concluded from these experimental results that a single intake of ethanol into animals in doses comparable with those causing moderately severe drunkenness in man causes a change in activity of the system transporting tyrosine and dopa into the brain. The barrier function of the BBB is unchanged under these circumstances. Increased penetration of peripherally injected tyrosine and dopa into the brain after administration of ethanol is in agreement with the results of investigations [1, 3, 5] which demonstrated increased turnover of noradrenalin and dopamine, of which tyrosine and dopa are precursors, after a single dose of ethanol, chronic ethanol intake, and free consumption of ethanol by animals. It can accordingly be postulated that the change in activity of the system transporting neutral amino acids through the BBB under the influence of ethanol is an adaptive mechanism, aimed at making good the deficiency of neurotransmitters caused by ethanol. Considering data in the literature [3] on the role of central catecholaminergic systems in the development of dependence on alcohol, it can be concluded that the change in activity of the transport system of the BBB is one component in the complex series of mechanisms of formation of alcohol motivation. The presence of competitive relations between substrates (amino acids) for the transport system [9,11] and the increased passage of ^{14}C-tyrosine and ^{14}C-dopa through the BBB, observed in the present experiments under the influence of ethanol may indicate the possibility of a change (decrease) in the transport of other amino acids into the brain, and in turn, this may be accompanied by disturbance of metabolic processes in the brain.

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