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EFFECT OF CHRONIC ETHANOL INTAKE ON PERMEABILITY OF THE BLOOD–BRAIN BARRIER FOR ^{14}C-TYROSINE AND HORSERADISH PEROXIDASE IN RATS

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Administration of a single dose of ethanol has been shown to increase the permeability of the blood–brain barrier (BBB) for precursors of certain neurotransmitters and, in particular, for tryptophan [8] and dopa [3], possibly as a result of mobilization of adaptive processes aimed at making good the neurotransmitter deficiency [1] arising under the influence of ethanol. At the same time, there is evidence to show that chronic ethanol consumption by animals leads to inhibition of transport of the serotonin precursor, tryptophan, into the brain and this is accompanied by a fall in the neurotransmitter level in the brain [2]. This phenomenon may lie at the basis of changes in activity of the transport function of BBB under the influence of chronic ethanol intake. Morphological investigations [5] have shown that transendothelial pinocytosis probably arises during chronic ethanol intake by animals, possible evidence of changes in the barrier function of BBB.

The aim of this investigation was to study the effect of chronic alcohol administration to animals on the transport and barrier functions of BBB with respect to peripherally injected ^{14}C-tyrosine.

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar rats weighing 380-450 g, divided depending on the quantity of alcohol consumed during 3 weeks of free choice between 10% ethanol solution and water, into heavy drinkers (over 3.5 g ethanol/kg body weight daily) and light drinkers (under 2 g ethanol/kg body weight daily). After division of the animals in this way, a 25% solution of ethanol was administered by the intragastric route 3 times a day for 10 days in doses which increased daily (8-11 g/kg). Control animals received equivalent volumes of physiological saline by gastric tube. The experimental heavy and light drinking animals were divided into four groups: in the animals of group 1, the last (30th) dose of

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TABLE 1. Effect of Chronic Administration of Ethanol on Peremability of BBB for ^{14}C-Tyrosine (radioactivity in superantant from 100 mg tissue)

Brain region	Control	Ethanol (4 g/kg, intraperitoneally, instead of last dose given by gastric tube)	Physical dependence	Abstinence	
				without administration of ethanol	ethanol (4 g/kg, intraperitoneally)
Heavy drinking rats					
Cortex	580±54 (6)	1271±120[d,f] (5)	778±82[b] (6)	732±64[b,f] (4)	1312±116[a] (6)
Hypothalamus	527±46[a] (6)	964±64[b,e] (5)	589±64[b] (6)	659±52[b,e] (4)	924±88[c,i] (6)
Medulla	468±31 (6)	1084±95[c,e] (5)	568±43[b] (6)	625±68[c,e] (4)	978[a]±104[d,i] (6)
Cerebellum	538±35 (6)	1158±92[c,g] (5)	647±58[b] (6)	700±72[c,e] (4)	1090±72[d] (6)
Light drinking rats					
Cortex	519±25 (6)	1572±114[d,f,k]	751±51[c,i] (6)	926±102[d,f,i,] (5)	1279±131[d,k] (6)
Hypothalamus	458±39[a] (6)	1228±61[d,e] (5)	637±50[b,i] (6)	799±86[c,e,i] (5)	1154±96[d,f,i] (6)
Medulla	474±29 (6)	1342±108[d,e,j]	640±39[b,h] (6)	759±78[d,e,h]	1193±112[d,e,j,l]
Cerebellum	530±24 (6)	1515±118[d,g,i]	727±65[b,h] (6)	842±64[b,d,h]	1205±108[g,k] (6)

Legend. a) $P < 0.05$ compared with other control; b) $P < 0.02$; c) $P < 0.01$; d) $P < 0.001$ compared with internal control; e) $P < 0.02$; f) $P < 0.01$; g) $P < 0.001$ compared with corresponding opposite (light and heavy drinking) group; h) $P < 0.005$; i) $P < 0.02$ for light drinkers in a state of physical dependence compared with animals in a state of abstinence; j) $P < 0.05$, k) $P < 0.01$ for light drinkers receiving intraperitoneal injection of ethanol instead of last dose of gastric tube, compared with animals in a state of abstinence; l) $P < 0.05$ for light drinkers compared with heavy drinkers in a state of abstinence, after intraperitoneal injection of ethanol.

ethanol was given, not by gastric tube, but by intraperitoneal injection in a dose of 4 g/kg, and the permeability of BBB for ^{14}C-tyrosine and horseradish peroxidase (HRP) was determined in these animals 60 min later. In the remaining animals (groups 2-4) the permeability of BBB was determined 4-6 h (state of physical dependence) and 24 h (state of abstinence) after the last intubation, and also in the state of abstinence after intraperitoneal injeciton of ethanol in a dose of 4 g/kg. Under ether anesthesia ^{14}C-tyrosine (specific radioactivity 492 mCi/mmole) was injected into the animals' femoral vein in a dose of 5 μCi, dissolved in 0.5 ml of physiological saline. Immediately after injection of the indicator, and after division of the right atrium, the cerebral vessels were washed free from blood by injection of 60 ml of physiological saline, heated to 36°C, through the left ventricle of the heart. Pieces of brain tissue weighing 100 mg from the cerebral 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 0.3 ml of 1.5 M $KHCO_3$ was added to the supernatant. After recentrifugation 0.3 ml of supernatant was added to 10 ml of "Aquasol" universal cocktail and the number of counts recorded on a liquid scintillation counter (LKB, Sweden). The numerical results were subjected to statistical analysis by Student's t test. HRP was injected into the femoral vein of the animals under ether anesthesia in a dose of 50 mg, dissolved in 0.5 ml physiological saline, 60 min after administration of ethanol. The animals were decapitated 10-15 min after the injection of HRP, the brain was removed, and slices 2 mm thick were fixed in 2% glutaraldehyde solution 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 frequent rinsing in phosphate buffer, were examined under a light microscope.

EXPERIMENTAL RESULTS

After intragastric administration of ethanol the animals showed a progressive loss of body weight on average by 90 g. Three of the 24 heavy-drinking rats and five of the 27 light drinkers died after losing on average 120 and 140 g weight respectively, possible evidence of a high degree of intoxication of the animals receiving ethanol. Permeability of the BBB for ^{14}C-tyrosine was virtually identical in the control rats, both light and heavy drinkers (Table 1). Ethanol, injected intraperitoneally instead of the last dose given by gastric tube, increased the permeability of BBB of the labeled indicator in all structures studied

on average by 2-3 times. This effect of ethanol was significantly greater in the light than in the heavy drinkers. Permeability of rats of both groups in a state of physical dependence was increased about equally, but in a state of abstinence, without injection of ethanol, it was significantly greater in the light drinkers. Ethanol injected intraperitoneally during abstinence increased the permeability of BBB in all structures studied, but by a greater degree in the light drinkers. In the experiments with HRP, which is a high-molecular-weight compound which normally does not pass through the BBB, and which is used to study the barrier function of BBB [4, 9], chronic alcoholization of the animals did not lead to passage of HRP from the microcapillary lumen into the brain parenchyma. This fact may be evidence that chronic alcoholization of the animals for 10 days in these experiments did not affect the mechanisms of nonspecific transport of materials through BBB and did not change its barrier function.

It can be concluded from these results that during chronic alcoholization it is the transport function of BBB which is the first to change. Transport of peripheral tyrosine is increased under these circumstances, which could indicate the activation of adaptive mechanisms aimed at making good the noradrenalin deficiency in the brain, which is observed in animals during chronic administration of ethanol [1]. Data on the smaller quantity of ^{14}C-tyrosine reaching the brain of heavy-drinking animals are confirmed by investigations which revealed a lower noradrenalin concentration in certain brain structures and, in particular, in the hypothalamus of rats predisposed (heavy-drinking) to the formation of experimental alcoholism [1].

Allowing for data on competitive relations between substrates for the system transporting neutral amino acids into the brain [6, 7], the greater increase in permeability of BBB for ^{14}C-tyrosine observed in the present experiments in light-drinking rats suggests a more severe disturbance of transport of other neutral amino acids into the brain in the animals of this group also, and this may ultimately lead to severe disturbances of brain metabolism. On this basis it can be concluded that processes of adaptation of the transport systems of BBB to the action of ethanol are more balanced in character in heavy than in light-drinking animals.

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