EFFECT OF ETHANOL AND PROBENECID ON EXCRETORY FUNCTION OF THE CHOROID PLEXUS OF THE RAT BRAIN

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KEY WORDS: probenecid; ethanol; choroid plexus; tyrosine; tryptophan; dopa.

Acute and chronic administration of ethanol to animals has been shown to increase the permeability of the blood-brain barrier (BBB) for certain amino acids which are precursors of neurotransmitters and, in particular, or tyrosine, tryptophan, and dopa [5, 11]. This may be due both to its direct effect on the transport mechanisms of the BBB and also other indirect effects of ethanol, linked with its ability to cause cerebral hypoxia [6], to induce a positive emotional state [2], to modify activity of neurotransmitter systems [1] and also, perhaps, certain other factors. The importance of indirect effects of ethanol in the mechanism of increased permeability of BBB is confirmed by data showing that ethanol has no effect on activity of the enzyme γ-glutamyl transpeptidase [11], contained in microcapillaries of the brain and regarded as a possible carrier of amino acids through the BBB [4]. It can be tentatively suggested that among the indirect factors causing increased accumulation of certain amino acids in the brain, besides those mentioned above, may be added an inhibitory effect on the excretory mechanisms of the choroid plexus.

The aim of the present investigation was to test this hypothesis experimentally.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-350 g by ventriculocisternal perfusion of the CSF spaces of the brain [10] with artificial CSF containing (in mM): Na^+ 127.6, N^+ 2.5, Ca^{2+} 1.3, Mg^{2+} 1.0, Cl^- 134.5, at pH 7.4. The afferent and efferent cannulas (internal diameter 0.4 and 0.8 mm, respectively) were implanted stereotaxically under pentobarbital anesthesia (30-60 mg/kg, intraperitoneally), in accordance with an atlas of the brain [9], into the right lateral ventricle (A = 8.0 mm; L = 0.95 mm H = 4.5 mm from the surface of the skull) and into the cisterna magna (in the midline between the occipital tuberosity and the first cervical vertebra, H = 1.5 mm). Probenecid (100 or 200 mg/kg, intraperitoneally) was injected 15 min before the ethanol (in the form of a 25% solution in doses of 2 or 4 g/kg, intraperitoneally, 60 min before the isotopes). ^{14}C-L-tyrosine, ^{14}C-L-tryptophan, and ^{14}C-L-dopa (specific radioactivity 492, 0.5, and 5.4 mCi/mmole, respectively) in a dose of 5 μCi, dissolved in 0.5 ml physiological saline, were injected into the animals' femoral vein. In the experiments with ^{14}C-dopa, its peripheral metabolism was prevented by injecting carbidopa (alpha-methyldopa hydrazine), an effective inhibitor of dopa-decarboxylase, 75 min before the indicator in a dose of 80 mg/kg, intraperitoneally. Perfusion was carried out 1.5, 15, and 30 min after injection of the isotope at the rate of 25 μl/min until 10 μl of perfusate was obtained, and this was mixed with 10 ml of "Aquasol" universal cocktail and the number of disintegrations was recorded on an LKB liquid scintillation counter (Sweden). The results were subjected to statistical anaysis by Student's t test.

EXPERIMENTAL RESULTS

The maximal initial level of radioactivity (without preliminary injection of probenecid and ethanol), determined in the present experiments after 15-30 min, was 2-2.5 times lower (Table 1) than the level of radioactivity observed in the previous investigation [3, 5] in brain tissue following injection of similar doses of labeled tyrosine and dopa. A marked degree of radioactivity (10-15 times higher than the natural background level) was observed under

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TABLE 1. Effect of Probenecid and Ethanol on Radioactivty (in cpm/100 μl CSF) of the CSF after Intravenous Injection of ^{14}C-Tyrosine (Tyr), ^{14}C-Tryptophan (Trp), and ^{14}C-Dopa (M ± m)

Experimental conditions	Isotope	Time after injection			
		1	15	30	60
Control (physiological saline, intraperitoneally)	^{14}C-Tyr	108±14 (12)	225±18 (12)	316±26 (12)	283±23 (10)
	^{14}C-Trp	92±11 (11)	196±21 (11)	248±22 (11)	210±14 (9)
	^{14}C-Dopa	84±6 (12)	162±15 (12)	254±31 (12)	231±17 (10)
Probenecid intraperitoneally: 100 mg/kg	^{14}C-Tyr	65±9** (11)	157±10*** (11)	208±19*** (9)	162±18** (9)
	^{14}C-Trp	51±7* (10)	120±8*** (10)	144±11*# (10)	135±10*** (8)
	^{14}C-Dopa	58±6** (10)	134±12* (20)	172±16*** (9)	150±14** (8)
200 mg/kg	^{14}C-Tyr	42±8*** (9)	121±14*** (9)	93±7*# (9)	139±12*** (7)
	^{14}C-Trp	30±4*# (10)	94±11*** (10)	86±5*# (8)	108±9*# (7)
	^{14}C- Dopa	54±8* (9)	116±12** (7)	74±8*# (7)	144±21** (6)
Ethanol intraperitoneally: 2 g/kg	^{14}C-Tyr	116±42 (9)	198±21 (9)	331±23 (9)	304±30 (7)
	^{14}C-Trp	103±9 (8)	204±19 (8)	259±25 (8)	226±26 (6)
	^{14}C-Dopa	92±11 (8)	159±17 (8)	241±18 (7)	214±22 (6)
4 g/kg	^{14}C-Tyr	102±8 (10)	192±14 (10)	305±34 (9)	272±24 (7)
	^{14}C-Trp	98±12 (10)	183±18 (9)	226±31 (8)	197±18 (6)
	^{14}C-Dopa	92±9 (10)	174±16 (10)	238±20 (7)	224±19 (6)

Legend. *p < 0.02, **p < 0.01, ***p < 0.002, ****p < 0.001 compared with control. Number of specimens in parentheses.

these cirucmstances in the CSF 15-30 min after intravenous injection of the isotopes, i.e., later than in brain structures in which the degree of radioactivity was close to the maximal level 60 sec after their intravenous injection [3, 5]. This state of affairs evidently indicates different kinetic parameters of distribution of substances passing through the BBB from the blood, in the brain structures and CSF, and also emphasizes the fact that the concepts of blood-brain barrier and blood-CSF barrier are not identical. This is an important distinction, not only theoretically but also practically, for it is connected with the ability of parameters of central bioavailability of physiologically active substances to be assessed clinically in accordance with the degree of their accumulation in the CSF.

Probenecid, which can inhibit mechanisms of excretion of acidic and weakly acidic substances in the body [8], including the excretory mechanisms of the choroid plexus [7], led to dose-dependent lowering of the level of radioactivity of the CSF after injection of each of the indicators (Table 1). It can accordingly be concluded that probenecid is able to inhibit the excretory function of the choroid plexus, relative to tyrosine, tryptophan, and dopa also. The effect of probenecid was preserved when it was given jointly with ethanol. Unlike probenecid, ethanol had no significant effect, in either of the doses used, on the level of radioactivity of the CSF (Table 1), a possible indication of preservation of the excretory function of the choroid plexus against the background of alcohol intoxication.

It can be concluded from these results that blockade of the excretory function of the choroid plexus by ethanol is not a possible cause of the increase in the concentrations of the amino acids tyrosine, tryptophan, and dopa in the brain, which is observed after their intravenous injection under conditions of alcohol intoxication [3, 5, 11]. Meanwhile probenecid, which has an inhibitory effect on the excretory mechanisms of the choroid plexus, can evidently delay elimination not only of penicillin [12], and barbiturates [7], from the brain and CSF, but also of certain other substances including amino acids.

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EFFECT OF A 3-HYDROXYPYRIDINE ANTIOXIDANT ON LEARNING IMPAIRMENT AND LIPOFUSCIN ACCUMULATION INDUCED BY ETHANOL

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In the modern view the cell membrane is an important component of the primary process of aging [3], and activation of lipid peroxidation (LPO), observed during aging when antioxidant protection is weakend, is a universal mechanism of biomembrane modification and damage. Disturbance of structural and functional regulation of neuron membranes may lead to irreversible brain damage and to intracellular lipofuscin formation. Most investigators consider that the principal role in the genesis of lipofuscin is played by LPO processes, followed (or accompanied) by polymerization of low-molecular-weight aldehydes with primary amino groups of proteins. As a result of polymerization, compounds of the Schiff base type are formed, thanks to the presence of which lipofuscin possesses the property of autofluorescence [6]. On the other hand we know that under the influence of chronic ethanol consumption by rats and mice the content of saturated fatty acids is increased in their brain phospholipids; the cholesterol concentration also is increased, leading to a change in the structural organization of the membranes [7]. It has also been shown that prolonged alcoholization leads to persistent impairment of learning ability in mice [10]. The parameters of behavior under these circumstances approximate to those in old animals, and long-term alcoholization can thus be regarded as a model of rapid aging [11].

The ability of natural and synthetic antioxidants (tocopherol, ionol, etc.) to delay the development of aging processes is well known [4]. The aim of this investigation was to assess the protective action of the antioxidant 2-ethyl-6-methyl-3-hydroxypyridine (3-HP) on impairment of learning ability and lipofuscin accumulation in the brain tissues of animals induced by ethanol. It was shown previously that 3-HP can lengthen the survival of animals [4] and improve learning capacity in old rats [1].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female albino mice aged initially 3 months, and exposed to ethanol for 5 months by Freund's method [10] in our own modification. The animals were divided into three groups: 1) mice receiving 15% ethanol solution instead of drinking water for 5 months; 2) mice receiving 15% ethanol solution together with 3-HP instead of drinking water; 3) intact mice receiving water. Mice initially aged 15 months also were used. All the animals were kept on a standard diet. In the course of the experiments it was found that both control and experimental animals, weighing 32-37 g, consumed on average 4-5 ml per mouse per day throughout the experiment, but the volume of alcohol consumed by one mouse daily, calculated as absolute ethanol, was 0.56-0.75 ml, and the amount of 3-HP consumed simultaneously with ethanol was 0.60-0.75 mg/day per mouse (or 20-25 mg/kg body weight/day).

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