PERMEABILITY OF THE BLOOD–BRAIN BARRIER FOR [^{3}H]-GABA IN ALCOHOL POISONING

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Alcohol, especially chronic intake of alcohol, causes changes in the functional properties of the blood–brain barrier (BBB) and in the ability of various substances of exogenous and endogenous origin, such as lead, bismuth, acid dyes, albumin, mercury, phosphorus, sodium, iodine, etc., to penetrate into the brain (penetration is usually increased) [5]. In connection with the experimental study of the biological basis of alcoholism, the study of changes in the functional properties of the BBB and its permeability to endogenous substances which, in the modern view, may be involved in the mechanisms of formation of alcoholism, and in particular, for neurotransmitters, is particularly interesting. It has been shown that γ-aminobutyric acid (GABA), considered to be the inhibitory mediator of the CNS, participates in the mechanism of the depressive effects of ethanol, and that functional insufficiency of the GABA system may be one cause of hyperexcitation during withdrawal [6, 9].

For this reason, and also considering the fact that various GABA derivatives are used as drugs for the treatment of various manifestations of alcoholism, it was decided to study the ability of [^{3}H]-GABA to pass through the BBB and its distribution in brain structures in cases of acute and chronic alcohol poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 170-250 g. Alcohol was administered to the animals by the methods in [2,3]. Acute intoxication was induced by intraperitoneal injection of a 25% solution of ethanol in subnarcotic (3.7 g/kg) and narcotic (4.5 g/kg) doses, and also by injection of acetaldehyde in a dose of 450 mg/kg. Chronic alcoholization was induced by intraperitoneal injection of alcohol daily for 3 weeks in increasing doses: during the first week half the narcotic dose (2.25 g/kg), during the second week 2.5 g/kg, and during the third week 2.75 g/kg, and also after preliminary division of the animals into "heavy drinkers" and "light drinkers" with free choice between water and 15% ethanol solution, after which the animals were kept for the next 6 months under conditions of choice. H-GABA with specific activity of 4.4 mCi/ml and in a concentration in solution of 0.028 mg/ml was used as indicator. The indicator was injected into the caudal vein in a dose of 125 μCi/kg in accordance with the following scheme: 1) 1, 4, and 24 h after injection of a subnarcotic dose of ethanol; 2) at the time when the animal adopted the side position, recovered from it, and 24 h after injection of the narcotic dose of alcohol or acetaldehyde. The rats were decapitated 10 min after injection of the isotope and the brain was removed, stripped of its surface vessels, and frozen to −4°C. The content of indicator was determined in the cerebral cortex, hypothalamus, caudate nucleus, thalamus, and medulla. Pieces of tissue weighing 10-30 mg, taken from the corresponding part of the brain, were dissolved in 0.5 ml didodecyl-dimethylammonium hydrochloride (from Serva, West Germany). After all the material had dissolved 10 ml toluene scintillator was added to the sample. The level of radioactivity of the tissue was determined by the liquid scintillation method in a "Nuclear Chicago" spectrometer. The counting efficiency was checked against an external standard. For statistical analysis, the arithmetic mean and its confidence limits were calculated [1].

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TABLE 1. Effect of Acute Administration of Ethanol in a Subnarcotic Dose (3.7 g/kg) on Penetration of [^{3}H]-GABA from Blood into Brain Structures (in cpm/mg wet weight of brain tissue)

Experimental conditions	Time after administration of ethanol, h	Cortex	Hypothalamus	Thalamus	Caudate nucleus	Medulla
Control		82 (72—92)	135 (109—161)	70 (57—83)	86 (77—95)	104 (86—122)
Ethanol	1	73 (66—80)	101 (80—122)	69 (55—83)	72 (64—80)	61* (57—65)
	4	84 (75—93)	84* (75—93)	69 (56—82)	60* (55—65)	67* (60—74)
	24	28* (24—32)	24* (14—34)	21* (15—27)	19* (13—25)	18* (13—23)

Legend. Here and in Tables 2-4, *P < 0.05 compared with control.

TABLE 2. Effect of Injection of Ethanol in a Narcotic Dose (4.5 g/kg) and Acetaldehyde in a Narcotic Dose (450 mg/kg) on Penetration of [^{3}H]-GABA into Various Brain Structures (in cpm/mg wet weight of tissue)

Experimental conditions	Time of determination of parameter	Cortex	Hypothalamus	Thalamus	Caudate nucleus	Medulla
Control		83 (73—93)	113 (106—120)	83 (71—95)	87 (78—96)	84 (75—93)
Ethanol	Animal in side position	88 (71—105)	117 (87—137)	71 (67—75)	116* (101—131)	117* (100—134)
	Recovery from side position	83 (74—92)	96 (83—109)	74 (61—87)	88 (77—99)	96 (75—117)
	24 h after injection	58* (43—73)	113 (93—133)	82 (65—99)	64* (53—75)	95 (85—105)
Acetaldehyde	Animal in side position	52* (4—61)	152* (120—184)	62 (46—78)	129* (104—154)	84 (64—104)
	Recovery from side position	116* (101—131)	186* (166—206)	181* (148—214)	115* (92—138)	116* (97—135)
	24 h after injection	117* (101—133)	147* (116—178)	101 (78—124)	89 (71—107)	96 (80—112)

EXPERIMENTAL RESULTS

In a subnarcotic dose ethanol led to a progressive fall in [^{3}H]-GABA penetration starting from the time of its injection: in the medulla after 1 h, in the hypothalamus and caudate nucleus after 4 h, and in all the structures tested after 24 h (Table 1). When alcohol was injected in a narcotic dose (Table 2), at the time the animals assumed the side position, an increase in the accumulation of [^{3}H]-GABA was found in the caudate nucleus and medulla. On recovery from the side position, no changes in accumulation could be found, but after 24 h accumulation in the caudate nucleus and cortex was reduced.

According to the literature, acute alcohol intake differs in its effect on the GABA concentration in the brain of animals: In comparatively small doses (2-3 g/kg, intraperitoneally) it depresses it, but with an increase in the dose to 8 g/kg [5] it sharply increases it. To some degree this may be due to corresponding changes in the activity of enzymes which limit the brain GABA level: glutamate decarboxylase and aminobutyrate aminotransferase (GABA transferase, GABA-T). In particular it has been shown that in a dose of 2 g/kg, ethanol leads to an increase in GABA-T activity in the cerebral hemispheres and cerebellum, and with an increase in the dose up to 6 g/kg, activity of the enzyme falls, and this is accompanied by a rise in the GABA level [4]. With these facts in mind, and also modern views on the BBB, according to which penetration of substances into the brain is determined by the functional metabolic state of the brain [7], it can be tentatively suggested

TABLE 3. Effect of Chronic Administration of Ethanol, Intraperitoneally for 3 Weeks, on [^{3}H]-GABA Content in Various Brain Structures (in cpm/mg wet weight of tissue)

Experimental conditions	Time of determination of parameter	Cortex	Hypothalamus	Thalamus	Caudate nucleus	Medulla
Control	—	82 (76—88)	137 (128—146)	70 (63—77)	73 (69—77)	90 (79—101)
Ethanol:						
2,75 g/kg	1st week	96 (84—108)	118 (102—134)	76 (62—90)	66 (57—75)	103 (87—119)
3,0 g/kg	2nd week	80 (69—91)	91* (81—101)	74 (63—75)	74 (61—87)	72 (51—93)
3,25 g/kg	3rd week	88 (74—92)	124 (106—142)	71 (63—79)	74 (67—81)	79 (70—88)

TABLE 4. Effect of Voluntary Consumption of Alcohol for 6 Months under Conditions of Choice between a 15% Solution and Water on [^{3}H]-GABA Content in Various Brain Structures (in cpm/mg wet weight of tissue)

Group of animals	Experimental conditions	Cortex	Hypothalamus	Thalamus	Caudate nucleus	Medulla
Control	Without alcoholization	82 (76—88)	137 (128—146)	70 (63—77)	73 (69—77)	90 (79—101)
Light drinkers	Consumption of alcohol for 6 months	65 (51—79)	108* (96—120)	62 (57—69)	68 (64—72)	84 (53—115)
Big drinkers	The same	110* (90—130)	72* (61—83)	66 (57—75)	68 (60—76)	73 (62—84)

that the change in permeability of the BBB for GABA discovered in the present experiments under the influence of subnarcotic and narcotic doses of alcohol was evidently due to a change in the content of endogenous GABA, with a consequent change in the ability of [^{3}H]-GABA to pass through the BBB when injected by the systemic route.

The increased entry of [^{3}H]-GABA into the brain structures at the time when the animal adopted the side position after administration of a narcotic dose of alcohol and the decrease in its entry 24 h later are evidence of phasic changes in BBB permeability, evidently connected with changes in the functional state of the CNS due to the arrival or disappearance (after 24 h) of alcohol from the body. The effect of acetaldehyde in these experiments was manifested chiefly as increased accumulation of [^{3}H]-GABA in different brain structures (Table 2) and it was not connected with any change in the functional state of the CNS. This was reflected most clearly in the increased accumulation of [^{3}H]-GABA in all the brain structures tested when the animals recovered from the side position. This state of affairs may indicate that the increased accumulation of exogenous GABA in the brain after administration of acetaldehyde was connected with its toxic membrane-directed (nonenzymic) effects, which ultimately led to more marked structural changes in the BBB, which are characterized by greater inertia than the metabolically determined functional changes in the BBB induced by alcohol.

Alcoholization of the animals for 3 weeks led to a fall in [^{3}H]-GABA accumulation in the brain and hypothalamus followed by restoration of the normal level of accumulation of the label in the hypothalamus in the 3rd week without any change in permeability of the BBB in other structures (Table 3). This state of affairs evidently reflects the compensatory powers of the BBB in chronic alcoholization, aimed at maintaining the metabolic processes in the brain at a definite level. After contact with alcohol for 6 months the "light drinkers" showed a significant decrease of permeability in the hypothalamus compared with rats not exposed to alcohol (Table 4). In the "big drinkers" a significant decrease in permeability in the hypothalamus and an increase in the cortex was observed compared both with the non-alcoholization control and with the group of "light drinkers." These data may be evidence of significant changes in functional relations between individual elements of the GABA system in animals consuming alcohol in large quantities.

The results on accumulation of [^{3}H]-GABA in the various brain structures under the influence of alcohol obtained in these experiments are difficult to compare with data in the literature on the content of endogenous GABA in the brain of animals receiving alcohol, chiefly because in investigations of this kind the GABA content was determined not in individual brain structures, but mainly in the cerebral hemispheres and cerebellum. Furthermore, data on the GABA content in the brain during alcoholization are extremely contradictory in character. They range from an increase in its level [8] to a decrease, or to no change [5].

At the same time it will be evident that significant factors affecting the GABA content in brain structures are the method of administration of the alcohol (compulsory or voluntary consumption under free choice conditions) and the duration of chronic exposure.

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EFFECT OF PANTOGAM, NICOTINAMIDE, AND PHENAZEPAM ON SEIZURE ACTIVITY

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Pantogam, the calcium salt of D-homopantothenic acid (synthesized in the "Vitaminy" Scientific Production Department by V. M. Kopelevich, T. D. Marieva, and V. I. Gunar), is known to possess antiepileptic activity [1, 4]. It has also been shown that nicotinamide (the hypothetical endogenous ligand of benzodiazepine receptors [12]) can inhibit some forms of epileptic activity [5, 6]. Phenazepam is one of the most effective drugs with an anticonvulsant action [2].

In the investigation described below a comparative study was made of the effects of pantogam, nicotinamide, phenazepam, and combinations of them on generalized seizure activity.

EXPERIMENTAL METHOD

Noninbred albino mice weighing 18-24 g were used. Clonic seizures were induced by subcutaneous injection of metrazol in a dose of 60-70 mg/kg body weight, and clonico-tonic convulsions were induced by intraperitoneal injection of metrazol in a dose of 80-100 mg/kg

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