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EFFECTS OF DRUGS ON BLOOD-BRAIN BARRIER PERMEABILITY IN RATS CHRONICALLY INTOXICATED BY ETHANOL

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Summary. - *Male Wistar rats were divided in free choice conditions into heavy-drinkers consuming > 3.5 g/kg of ethanol daily (HD), and light-drinkers consuming < 2.0 g/kg/day (LD). Subsequent 30 day intragastric administration of 25% ethanol (8-11 g/kg/day) caused an increase in permeability of the BBB to ^{14}C-tyrosine, ^{14}C-tryptophan and ^{14} C-DOPA at all the stages of alcoholization. At late stages of intoxication (20-30 days) the penetration of horseradish peroxidase (HRP) indicating pinocytosis was present. All the changes were more pronounced in LD than in HD rats. Disulfiram, and to a lesser extent phenazepam and diazepam, when repeatedly injected (for 16-30 days) together with ethanol aggravated its effects. Clonidine and haloperidol antagonized ethanol-induced increase of the BBB permeability to labelled compounds and HRP in the two groups of rats. Picamilon, lithium oxybutyrate, chlorpromazine and alpha-tocopherol had little effect on the penetration of radioactive tracers but effectively antagonized the penetration of HRP. The other drugs were without effects on the BBB regulatory and barrier functions altered by chronic alcoholization. In view of the BBB dysfunction in chronic alcoholics the adequacy of the above-mentioned drugs for their treatment is discussed.*

Riassunto (Effetti dei farmaci sulla permeabilità della barriera ematoencefalica nei ratti intossicati da etanolo). - *La somministrazione intragastrica di alte dosi di etanolo (nell'ordine di 8-11 g/kg/die per 30 giorni) provoca, nel ratto, l'incremento della permeabilità della barriera ematoencefalica (BBE) alla ^{14}C-tirosina, al ^{14}C-triptofano, al ^{14}C-DOPA ed alla perossidasi di rafano (HRP). L'aumento della permeabilità è significativamente più marcato nei ratti «modesti bevitori» (ovvero animali che posti in condizione di libera scelta tra l'acqua ed una soluzione alcolica al 15% assumono giornalmente meno di 2,0 g/kg di etanolo) rispetto ai ratti «forti bevitori» (che consumano invece più di 3,5 g/kg di etanolo al giorno). Inoltre sono stati studiati gli effetti combinati dell'etanolo e di alcuni farmaci psicotropi, solitamente impiegati nel trattamento dell'alcolismo. La somministrazione cronica di disulfiram, fenazepam o diazepam, associata a quella di etanolo, aumenta l'effetto di quest'ultimo sulla permeabilità della BEE. Al contrario, la clonidina e l'aloperidolo riducono l'incremento della permeabilità indotto dall'etanolo, svolgendo un'azione protettrice sulla BEE. Tale protezione, soprattutto in termini di una riduzione della pinocitosi indotta dall'etanolo, è svolta anche da altri farmaci quali il picamilon, l'idrossibutirrato di litio, la cloropromazina e l'alfa-tocoferolo.*

Introduction

According to modern concepts of neurophysiology the blood-brain barrier (BBB) may be considered a polyfunctional membrane under control of the nervous and endocrine systems. Its functioning is aimed at the maintenance of a stable homeostasis of the brain [1]. Ethanol is known to exert a pronunced membrane effect [2] and also to affect the BBB functions when injected into animals [3-5]. Psychotropic drugs, including those used for the treatment of alcoholism and related disorders, have more or less evident membrane effects [6-8]. This suggests that psychotropic drugs can interact with ethanol in affecting the BBB functions, particularly in cases of barrier dysfunctions induced by preceding ethanol intoxication. Theoretically, the effect of drugs on the BBB function altered by ethanol can reveal itself in increasing, decreasing or having no effect on the permeability of the barrier. The knowledge of a combined action of ethanol and drugs at the level of the BBB may be useful for working out some general principles of an adequate pathogenetic treatment of alcoholism and concomitant disorders, which also include the mechanisms of correction and regulation of the BBB functions.

The present work was devoted to study the chronic effect of ethanol itself and in combination with some psychotropic drugs (used for alcoholism treatment) on regulatory and protective functions of BBB.

Materials and methods

Male Wistar rats, weighing 300-400 g, were used in all experiments. They were divided under free choice conditions into heavy-drinkers, consuming more than 3.5 g/kg/day of ethanol in 15% solution (HD), and light-drinkers consuming less than 2 g/kg/day (LD). After preliminary selection, animals were subjected to the procedure of three 10-day periods of intragastric intubation with 25% ethanol solution in gradually increasing doses from 8 to 11 g/kg/day during each 10-day period. A daily dose of ethanol was divided into 3 equal parts and intubated at 8 h intervals. The BBB functions were tested on 10th, 20th, 25th and 30th days of chronic alcoholization, 60 min after the last intubation, as well as in the state of physical dependence (4-6 h after the last intubation), and in abstinence (20-24 h later). Moreover, the BBB functions were also tested after an i.p. injection of 4 g/kg of ethanol into chronically alcoholized rats. Drugs of different groups, which are used for the treatment of alcoholism like disulfiram, apomorphine, clonidine, mebicar, phenazepam, diazepam, inmecarb (derivate of 1-benzyl-2-3-dimethylindolcarbonic acid), lithium oxybutyrate, haloperidol, picamilon (isonicotinoil-gamma-oxybutyric acid), were injected i.p. every day during the 2nd half of alcoholization (16-30 days).

The BBB regulatory functions were tested according to the method described previously [9]. ^{14}C-tyrosine, ^{14}C-tryptophan and ^{14}C-DOPA (specific activites 492, 0.5 and 5.4 mCi/mmol, respectively) were used as tracers. To prevent ^{14}C-DOPA peripheral degradation, carbidopa (alpha-ethyldopa hydrazine) 80 mg/kg i.p. was injected 75 min before the tracer. Statistical analysis of the data was done according to Student's t-test. Horseradish peroxidase (HRP, type II, Sigma or Boehringer) was used to evaluate the BBB protective functions. HRP (50 mg/kg) was injected into the femoral vein under light ether anaesthesia. The fixative perfusion of brain tissues, preparation and treatment of microscopic slices were done by the method of Lehtosalo *et al.* [10]. About 50 brain slices, 40 microns thick, were examined under light and electronic microscope in order to look for pinocytosis in microcapillary walls, which normally does not exist but can be induced by different factors damaging BBB.

Results and discussion

The chronic alcoholization of animals was followed by progressive loss of body weights which by the 30th day of intubation were 96 ± 5 g in HD and 115 ± 11 g in LD. This indicates a marked degree of intoxication of both groups of animals, as well as a higher sensitivity of LD to the toxic effect of ethanol in comparison to HD. The only initial difference (before systemic intubation of ethanol) of BBB permeability in two groups of rats was an increase in penetration of labelled tyrosine into the hypothalamus of HD as compared to LD (Table 1). This suggests a relationship between the selective increase of BBB permeability in hypothalamus (one of the main emotiogenic structures of the brain) and a high level of alcohol motivation. Therefore this can be considered a psychophysiological background of alcohol motivation and dependence. This suggestion is supported by the data indicating higher tyrosine hydroxylase activity in the hypothalamus of HD as compared to LD [11]. An increased turnover of brain dopamine and norepinephrine produced by ethanol have been also reported [12, 13]. A significant involvement of catecholaminergic structures of the brain, including those at hypothalamic level in the mechanisms of positive reinforcement should be also mentioned [14]. The overall data suggest a relationship between an increase in penetration through the BBB of the precursor (tyrosine) and an increase in neurotransmitters turnover (dopamine, norepinephrine) in emotiogenic structures of the brain such as hypothalamus. Relatively slight initial differences of tyrosine penetration through the hypothalamic BBB in HD and LD rats supports, rather than contradicts, the above-mentioned statements since only less than 2% of the total amount of tyrosine entering the brain is involved in the catecholamine synthesis [15]. The chronic alcoholization of animals also produced a progressive deterioration of regulatory and protective functions of the BBB (Table 2). At all inter-

Table 1. - *Permeability of blood-brain barrier to ^{14}C-tyrosine in heavy- and light-drinkers*

Rats	Cortex	Hypothalamus	Medulla	Cerebellum
Heavy-drinkers	572 ± 58 (9)	546 ± 52 (10)	473 ± 29 (10)	526 ± 31 (9)
Light-drinkers	529 ± 26 (8)	452 ± 30* (10)	459 ± 33 (9)	541 ± 27 (10)

The data are expressed as counts/min in supernatant from 100 mg of wet weight. Means $\pm$ SEM. In parentheses number of rats. * $p < 0.02$ as compared to heavy drinkers.

Table 2. - *Effects of 30-days chronic alcoholization on blood-brain barrier permeability to ^{14}C-tyrosine, ^{14}C-tryptophan, ^{14}C-dopa and horseradish peroxidase (HRP) in heavy- and light-drinkers (HD and LD)*

Tracer	Control		10 days		20 days		25 days		30 days	
	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD
^{14}C-tyrosine	530±48 (22)	495±32 (18)	645±54 (*a*) (14)	688±62 (*b*) (12)	824±67 (*c*) (14)	1720±146 (*c*) (12)	—	—	1688±152 (*c*) (10)	2547±216 (*c*) (8)
^{14}C-tryptophan	504±36 (18)	422±23 (14)	579±44 (*a*) (12)	543±38 (*a*) (10)	730±52 (*b*) (12)	970±84 (*c*) (12)	—	—	1461±108 (*c*) (12)	2124±168 (*c*) (10)
^{14}C-dopa	488±31 (20)	396±22 (18)	502±21 (10)	472±30 (*a*) (9)	683±49 (*b*) (13)	1128±74 (*c*) (10)	—	—	1520±132 (*c*) (10)	2034±152 (*c*) (6)
HRP	0	0	0	0	0	LP	LP	DP	DP	DP

The data are expressed as counts/min in supernatant from 100 mg of wet tissue (cerebral cortex + hypothalamus + medulla oblongata + cerebellum). Means ± SEM. In parentheses number of rats. LP: local pinocytosis (HRP found in less than 20% of microcapillaries). DP: diffuse pinocytosis (HRP found in more than 80% of microcapillaries). (*a*) $p < 0.05$, (*b*) $p < 0.01$, (*c*) $p < 0.001$ as compared to corresponding controls.

vals alcoholization induced more pronounced deterioration of the BBB functions in LD than in HD rats.

By the 20th day in LD, together with a greater BBB permeability to tracers, a penetration of HRP into the microcapillary walls was also observed (local pinocytosis, present in less than 20% of slices). HRP was found mainly in the walls of the microcapillary endothelium and in some cases close to the basal membrane surrounding the capillaries. The HRP is a large compound (MW - 43,000) which normally does not penetrate from blood to brain [16]. Its appearance in the wall of endothelium of LD probably represents the first sign of the structural damage of the BBB. The penetration of HRP into the microcapillaries of HD was found on the 25th day when in LD a diffuse pinocytosis was present (HRP in more than 80% of slices). On the 30th day a diffuse pinocytosis was found in the microcapillaries of the two groups of animals. Its intensity (the number of pinocytotic vesicles in one capillary cut) in LD was about 35-55% greater than that observed in HD rats. This also speaks in favour of higher sensitivity to toxic effect of ethanol displayed by LD rats. It must be also pointed out that the BBB failed to develop any tolerance to a damaging effect of ethanol. This is in contrast with the data indicating that tolerance to ethanol due to its fluidizing effect may develop in some biological membranes [17, 18]. This implies that BBB presents a complex morphological and functional organization that makes it different from relatively simple membranes to which the BBB is sometimes compared. In the two groups of rats more pronounced disturbances of the BBB were observed in abstinence than in physical dependence. Moreover, i.p. injection of ethanol to the abstinent rats produced even greater increase of BBB permeability to tracers than its intragastric administration. The development of a diffuse pinocytosis in microcapillaries of HD and LD rats obviously resulted in irreversible changes of the BBB. In fact, on the 30th day of alcoholization the survival of HD and LD was 72 and 56%, respectively.

As regards the drugs, disulfiram (100-200 mg/kg) exerted the most damaging action on BBB. In fact, on the 20th day of alcoholization HD and LD rats displayed a significant increase of the BBB permeability to tracers, as well as to HRP. Moreover, by the 25th day disulfiram caused the death of all rats in both groups. Diazepam (4-8 mg/kg) and phenazepam (2-4 mg/kg) produced considerably smaller increase of BBB permeability to tracers, statistically significant for hypothalamus and medulla. They also induced a local and diffuse pinocytosis in the capillaries of HD rats on 20th and 25th day of alcoholization, respectively. On the contrary, a protective effect both on regulatory and barrier functions of the BBB was ensured by the presynaptic adrenergic agonist clonidine (0.025-0.1 mg/kg), and the dopaminergic blocker haloperidol (1.0-2.0 mg/kg).

The protective effects, particularly in terms of reduction of pinocytosis, were also induced by picamilon (10-40 mg/kg), lithium oxybutyrate (50-100 mg/kg), chlorpromazine (1.0-2.0 mg/kg) and alpha-tocopherol (50 mg/kg). As regards dopaminergic agonists, apomorphine (0.05-0.2 mg/kg), mebicar (1000-1500 mg/kg) and inmecarb (40-80 mg/kg) failed to produce any tangible effects upon the BBB functions altered by chronic alcoholization.

In conclusion, from the point of view of the safety and integrity of BBB, closely related to the regulation of homeostasis of the brain, the use of disulfiram for the treatment of alcoholism appears to be unacceptable. With regards to benzodiazepine tranquillizers, their capacity to induce dependence and abstinence has to be taken into account. On the other hand, clonidine and haloperidol can effectively counteract the damaging effect of ethanol at any stage. Picamilon, lithium oxybutyrate, chlorpromazine and alpha-tocopherol are supposed to reduce the damage of BBB at the late stage of alcoholism when cerebral functional and morphological changes appear.

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