CHARACTERISTICS OF BENZODIAZEPINE RECEPTORS IN RATS DIFFERING IN PREDISPOSITION TO EXPERIMENTAL ALCOHOLISM

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Clarification of the role of benzodiazepines as compounds increasing the affinity of GABA for the GABA-benzodiazepine complex [9], and the discovery that ethanol has a similar action [8] suggested that the state of these systems may be an important factor in the elucidation of the neurochemical mechanisms of development of dependence on ethanol.

In the present investigation the number and affinity of benzodiazepine receptors for diazepam in the cerebral cortex and hippocampus of rats differently predisposed to the development of experimental alcoholism were studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 200-250 g. Predisposition to ethanol consumption was estimated from the duration of ethanol narcosis [1]. Chronic alcoholization of the animals was carried out under conditions of a free choice between water and a 15% solution of ethanol [2]. Ethanol was injected once intraperitoneally, in a dose of 2.5 g/kg. Control animals received the same volume of physiological saline. The animals were decapitated 1 h after the injection. Characteristics of benzodiazepine receptors were studied in brain fraction P_2 [9]. Samples were incubated at 0°C. Bound and free N-methyl-^{3}H-diazepam were separated by means of GF/B filters. Protein was determined by Lowry's method [6]. The dissociation constant (K_d) and the number of binding sites were analyzed by the method of least squares. To construct each straight line 6 or 7 points were used. The significance of differences between groups was determined by Student's test.

EXPERIMENTAL RESULTS

The study of the number of benzodiazepine receptors and of their affinity for diazepam revealed no differences in animals predisposed and not predisposed to the development of experimental alcoholism, and divided into corresponding groups depending on the duration of ethanol narcosis (Table 1). A single injection of ethanol was followed by the appearance of statistically significant differences in the number of benzodiazepine binding sites between animals predisposed and not predisposed to the development of experimental alcoholism. Considering that the tranquilizing effect is produced with the aid of benzodiazepine receptors, this suggests that the larger number of these receptors is responsible for the manifestation of the tranquilizing action of ethanol in rats predisposed to alcoholism, whereas in animals not predisposed to alcoholism ethanol has instead an anxiogenic action, reducing the number of diazepam binding sites.

Voluntary consumption of ethanol solution for 3.5 months was not accompanied by any considerable changes in the characteristics of the benzodiazepine receptors (Table 2). Even a longer consumption of ethanol (10 months), when physical dependence on ethanol had already been formed in the animals, did not lead to any changes in the number of receptors or in their affinity for diazepam in the parts of the brain studied.

In the period of maximal severity of abstinence symptoms [3], 24 h after withdrawal of ethanol, a sharp decrease was found in the number of benzodiazepine receptors and in their af-

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TABLE 1. Characteristics of Benzodiazepine Receptors in Rats Differing in Predisposition to Development of Experimental Alcoholism (M ± m)

Test object	Predisposition of rats to alcoholism	Experimental conditions			
		injection of physiological saline		injection of ethanol (2.5 g/kg)	
		K_d, nM	number of binding sites (B), femtomoles/mg protein	K_d, nM	number of binding sites (B), femtomoles/mg protein
Cerebral cortex	Predisposed	6,9±2,8 (4)	1535±348 (4)	8,40±1,23 (4)	1575±165 (4)
	Not predisposed	7,15±2,0 (4)	1472±232 (4)	5,88±1,77 (4)	903±197 * (4)
Hippocampus	Predisposed	4,45±0,67	1504±173 (4)	4,45±0,56 (4)	1233±140 (3)
	Not predisposed	4,55±0,70 (4)	1289±153 (4)	3,80±0,51 (4)	1073±103 (3)

Legend. Number of animals shown in parentheses. *P < 0.05 compared with predisposed rats.

TABLE 2. Characteristics of Benzodiazepine Receptors in Rats During Voluntary Chronic Alcoholization (M ± m)

Experimental conditions	Cerebral cortex		Hippocampus	
	K_d, nM	number of diazepam binding sites (B_{max}), femtomoles/mg prot.	K_d, nM	number of diazepam binding sites (B_{max}), femtomoles/mg prot.
Control	4,28±0,84	1998±120	4,20±0,23	1677±115
Alcoholization for 3,5 months	4,60±0,53	2102±102	3,65±0,56	1853±250
Control	5,20±0,81	1823±236	5,65±1,20	1548±174
Alcoholization for 10 months	5,01±0,59	1662±119	5,21±0,80	1561±163
24 h after withdrawal of ethanol	8,50±0,68*	1129±74*	5,23±0,67	1472±169

Legend. Animals of the same age as the experimental rats, but with no contact with ethanol, were used as the control. Data from three independent experiments given. *P < 0.05 compared with control.

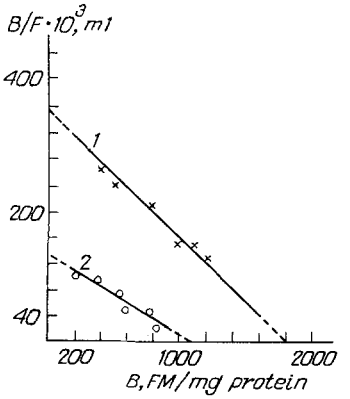

Fig. 1. Scatchard plot for binding of diazepam with benzodiazepine receptors in cerebral cortex rats (1) and of experimental animals (2) after 10 months of voluntary alcoholization, in a state of abstinence. 1) K_d = 5.2, B_{max} = 1823; 2) K_d = 8.5, B_{max} = 1129. Legend: FM — femtomoles.

finity in the cerebral cortex of the rats. These changes were quite specific, for no such changes in benzodiazepine receptors were found in the hippocampus (Fig. 1; Table 2). Similar results were obtained on mice, in which as a result of withdrawal of ethanol after chronic administration, the concentration of binding sites and the affinity of the benzodiazepine receptors decreased [5]. Arising from data showing that diazepam reduces aggressiveness, restlessness, and tremor arising on withdrawal of ethanol [4], it can be postulated that these characteristic manifestations of the abstinence syndrome are in fact due to an increase in the number of benzodiazepine receptors. The possibility likewise cannot be ruled out that the

reduction we found in the number of benzodiazepine receptors determines the animals' response to stress, which arises in rats with formed physical dependence on alcohol when deprived of ethanol. In particular, we know that stress, induced by various physical factors, is accompanied by a considerable decrease in the number of benzodiazepine receptors [7]. Without discussing the question of specificity of the patterns discovered in relation to the realization of mechanisms of formation of ethanol dependence, it can be tentatively suggested that weakening of functional activity of the GABA-benzodiazepine complex in animals predisposed to the development of experimental alcoholism is one of the neurochemical mechanisms of development of the abstinence syndrome.

LITERATURE CITED

1. Yu. V. Burov, T. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
2. Yu. V. Burov, R. Yu. Ukhananov, and A. I.Maiskii, Byull. Éksp. Biol. Med., No. 9, 67 (1982).
3. I. V. Viglinskaya, in: Experimental and Clinical Psychopharmacology [in Russian], Moscow (1980), p. 107.
4. L. M. Aaronson, D. D. Himma, and M. Okamoto, J. Pharmacol. Exp. Ther., 221, 319 (1982).
5. G. Freund, Life Sci., 27, 987 (1980).
6. M. B. Lies and J. Bachman, Anal. Biochem., 47, 184 (1972).
7. J. Millan and T. Duka, Mod. Probl. Pharmacopsychiat., 17, 123 (1982).
8. L. Nestoros, Science, 209, 708 (1980).
9. S. M. Paul, P. J. Marangos, and P. Skolnick, Biol. Psychiat., 16, 213 (1981).

POTENTIATING ACTION OF SALTS OF BIVALENT AND TRIVALENT METALS ON THE ANALGESIC EFFECT OF MORPHINE

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Evidence that La^{+++} cations potentiate morphine-induced analgesia has been published in [3, 5, 6]. The present writers showed previously [2] in experimens *in vitro* that La^{+++} cations, and also Mn^{++}, and Ni^{++} cations and lanthanides, interacting with specific binding sites of morphine and D-Ala^2-D-Leu^5-enkephalin (DADL) on rat brain membrane preparations, increase the affinity of opiate ligands for the corresponding receptors.

The aim of this investigation was to study the effect of salts of various metals ($MnCl_2$, $NiCl_2$, $GdCl_3$, and $LaCl_3$) on the analgesic effect of morphine. These salts were chosen because in experiments *in vitro* they were found to have the strongest activating action on affinity of opioid ligands for opiate receptors of rat brain membranes.

EXPERIMENTAL METHOD

The analgesic activity of the compounds in experiments on mice weighing 21-27 g was investigated after intracisternal [7] injection by the hot plate method (52°C). After preliminary assessment of the animals' response to painful contact stimulation 3 times, with an interval of 5 min, solutions of salts of the metals in isotonic NaCl solution were injected intracisternally in a dose of 10 µl. Morphine was again injected intracisternally in a dose of 2 mg per mouse 45 min after injection of the salts of the metals. In a parallel investigation

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