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EFFECT OF COMPOUNDS WITH ANXIOLYTIC PROPERTIES ON VOLUNTARY ETHANOL CONSUMPTION BY RATS

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KEY WORDS: ethanol; benzodiazepine tranquilizers; anxiolytic system

Although tranquilizers differing in chemical structure, and mainly of the benzodiazepine series, are actively used in clinical practice for the treatment of characteristic disorders such as anxiety, fear, and emotional stress, it is still not clear whether these drugs affect addiction to ethanol. In view of the latest data on the role of emotionally negative conflict situations in the development of pathological craving for ethanol [1, 8, 11, 13], the possibility that the treatment of alcoholism with tranquilizers may be pathogenetic in character cannot be ruled out [1, 6, 12, 14]. However, there is no direct proof that tranquilizers act on the formation of addiction to ethanol, or on the level of ethanol consumption at times other than during stress.

The aim of this investigation was to study the role of the effects of various chemical compounds with anxiolytic properties on voluntary consumption of 15% ethanol solution by rats at different stages of experimental alcoholism.

EXPERIMENTAL METHOD

The following compounds were used: diazepam, sodium hydroxybutyrate, mebicar,* derivatives of the aminoandrostane series: 17-β-acetylamino-5-androstene-3β,16β-diol (KLI-2), 17-β-amino-5-androstene-3β,16β-diol hydrochloride (KLI-3), 17-β-acetylamino-4-androstene-3,16-dione (KLI-5), and a derivative of the β-carboline series: 1-methyl-6-methoxytetrahydrocarboline (NK-424).

To study the effect of the compounds on alcohol motivation, intact rats weighing 200-220 g were placed in individual cages with free access to water and to 15% ethanol, and the drugs were administered simultaneously for 10 days. To analyze the effect of the compounds on the ethanol consumption of rats with established physical dependence on ethanol, animals which had been in contact with ethanol for 8 months (weighing 450-500 g) were used. Before the experiments the voluntary ethanol consumption of these animals was recorded for 10 days and rats whose daily consumption was not less than 40-50 ml/kg of 15% ethanol solution were used in the experiments [3]. The test compounds were injected intraperitoneally into these animals for 2 weeks in aqueous solution and in the form of a suspension with Tween-80 (aminoandrostanes, β-carbolines, diazepam).

The consumption of water and 15% ethanol solution was recorded daily. The experimental data were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Data reflecting the effects of compounds with anxiolytic properties in doses corresponding to ED_{50} of their effect in intact animals on the formation of alcohol motivation and on

*2,4,6,8-tetramethyl-2,4,6,8-tetra-azobicyclo-(3,3,o)-octadione-3.7.

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TABLE 1. Effect of Compounds in Doses Equivalent to ED_{50} for Anxiolytic Effect in Intact Animals on Voluntary Consumption of Ethanol by Rats (M ± m)

Compound	Dose, mg/kg/day	Average daily consumption of 15% ethanol by rats, ml/kg					
		first stage			third stage		
		control	experiment	reduction of consumption, % of control	background	experiment	reduction of consumption, % of background
Diazepam	0,2	26,4±4,2	17,3±4,8*	34,5	51,1±7,8	37,0±6,5	27,6
Sodium hydroxybutyrate	10,0	30,2±3,8	11,4±1,6*	62,3	47,4±3,9	42,9±5,2	9,5
Mebicar	200,0	30,2±3,8	20,6±4,3*	31,8	47,4±3,9	40,6±2,7	14,4
KLI-2	15,0	21,7±2,3	18,0±3,6	17,1	42,6±5,1	29,1±3,8*	31,7
KLI-3	20,0	21,7±2,3	14,9±2,8*	31,4	42,6±5,1	36,2±3,0	15,1
KLI-5	5,0	21,7±2,3	20,1±0,9	7,4	42,6±5,1	41,4±2,3	2,9
NK-424	0,5	27,3±4,4	12,5±2,3*	54,3	48,7±4,3	39,8±2,9	18,3

Legend. Here and in Table 2: *P < 0.05 compared with control.

TABLE 2. Effect of Compounds in Doses Corresponding to ED_{50} for Anxiolytic Effect in Rats with Established Physical Dependence on Ethanol on Its Voluntary Consumption (M ± m)

Compound	Dose, mg/kg/day	Average daily consumption of 15% ethanol by rats, ml/kg					
		first stage			third stage		
		control	experiment	Reduction of consumption, % of control	background	experiment	Reduction of consumption, % of background
Diazepam	4,0	28,3±5,98	10,82±4,03*	61,8	35,08±4,93	19,95±2,44*	43,2
Sodium hydroxybutyrate	60,0	27,2±4,7	14,1±2,8*	48,4	41,7±6,24	23,81±4,53*	43,0
Mebicar	1000,0	27,2±4,7	23,2±4,33	14,4	41,7±6,24	18,84±5,7*	54,9
KLI-2	80,0	29,6±2,1	15,02±1,84*	49,3	45,52±6,54	20,6±5,2*	54,8
KLI-3	100,0	20,4±0,41	8,57±0,84*	57,3	36,23±5,31	17,71±3,8*	51,8
KLI-5	30,0	29,6±2,1	18,31±2,36*	38,2	45,52±6,54	20,46±2,9*	55,1
NK-424	3,0	31,54±6,03	16,41±2,29*	48,9	44,34±4,47	29,53±3,75*	33,5

the level of ethanol consumption in animals physically dependent on ethanol are given in Table 1. It was found that administration of the classical benzodiazepine tranquilizer diazepam to the animals in doses that are anxiolytic for intact animals did not affect the rate of formation of alcohol motivation. Other compounds, with anxiolytic properties unconnected with their action on benzodiazepine receptors (mebicar, aminoandrostanes), also behaved similarly. The exceptions were sodium hydroxybutyrate and NK-424, which lowered the level of ethanol consumption by the rats statistically significantly at the stage of formation of alcohol motivation. Meanwhile, at the stage of physical dependence on ethanol, none of the drugs, in the doses tested, had any effect on the level of ethanol consumption by the animals. Since of all the compounds studied, the mechanism of the anxiolytic action has been established only for diazepam (complex formation with type I benzodiazepine receptors), it can be concluded that these receptors do not participate in the control of alcohol motivation. This hypothesis is confirmed by the absence of changes in the affinity constant and in the number of benzodiazepine binding sites in the animals' brain after single and chronic administration of ethanol [5, 9]. Meanwhile disappearance of the inhibitory effect of NK-424 and sodium hydroxybutyrate on ethanol consumption at the stage of physical dependence is evidence of the development of specific pathogenetic changes in the brain under the influence of chronic ethanol consumption, preventing the manifestation of this activity. These results are in agreement with those obtained by the writers previously, which were evidence of a sharp decline in the anxiolytic activity of the compounds, as revealed by the method of competition between the rats for an area of the floor safe from electric shocks, after chronic contact of the animals with ethanol [4].

Since under the conditions of the model indicated above, unlike the conflict situation method [7], a wide spectrum of compounds of different chemical classes was effective [2], it can be postulated that in this case a different mechanism of anxiolytic action, unconnected with benzodiazepine receptors, was revealed. Possibly benzodiazepines themselves are equally endowed with two types of activity: that mediated through benzodiazepine receptors, and that

realized through a different anxiolytic system. The latter is evidently most sensitive to the action of ethanol. If the hypothetical "type 2 anxiolytic system" participates in the formation of alcohol motivation, these compounds will evidently reduce alcohol consumption in doses corresponding to ED_{50} for their anxiolytic effect. To test this hypothesis similar experiments were carried out using compounds in doses equivalent to ED_{50} for anxiolytic effect in animals after contact with ethanol for 8 months (by the test of competition for an area safe from electric shocks). It will be clear from Table 2 that these doses were an order of magnitude or more greater than the corresponding doses for intact animals. All the compounds, in these doses, effectively reduced voluntary consumption of ethanol by the animals at both the first and the third stages of experimental alcoholism. These data are evidence in support of the concept that a nonbenzodiazepine anxiolytic system also exists [10]. It is evidently this system which is highly sensitive to the action of ethanol and which participates in the control of its voluntary consumption.

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IMMUNOHISTOCHEMICAL DEMONSTRATION OF PRO-OPIOMELANOCORTIN PEPTIDE FRAGMENTS (β-ENDORPHIN AND ACTH) IN RAT AND MOUSE ADRENALS

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It has been discovered in recent years that many neuropeptides, including opioid peptides, are formed not only in structures of the brain and hypothalamohypophyseal system, but also in many peripheral organs and tissues [7, 9]. For example, the mammalian adrenals have been shown to be the principal site of synthesis and secretion of endogenous opioid neuropeptides of the enkephalin group, formed from proenkephalin and prodynorphin [3, 4, 8]. Meanwhile data showing that the adrenal tissues contain neuropeptides formed from proopiomelanocortin (POMC), a high-molecular-weight polypeptide, and the precursor of several pituitary hormones and opioid peptides of the endorphin group (β-, γ-lipotrophins, α-, β-, and γ-melanocyte-stimulating hormone, ACTH, α-, β-, and γ-endorphins), are contradictory [2, 5, 6].

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