ROLE OF BENZODIAZEPINE RECEPTORS IN REALIZATION OF THE ANXIOLYTIC EFFECT OF COMPOUNDS IN ALCOHOL-DEPENDENT AND CONTROL RATS

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A study of the affinity of substances suggested for use as tranquilizers for benzodiazepine (BD) receptors in the brain is an essential step in the search for compounds with anxiolytic properties and helps to form some idea both of the possible mechanism of the new compound and the type of tranquilizers to which it belongs. Previously the writers discovered anxiolytic properties in a number of compounds with nonbenzodiazepine structure and showed that their activity is sharply reduced in animals with experimental alcoholism [3].

The aim of this investigation was to study the affinity of these compounds for brain BD-receptors of normal rats and rats physically dependent on ethanol.

EXPERIMENTAL METHOD

The following compounds were used: a derivative of the benzodiazepine series (diazepam), a bicyclic analog of urea (mebicar), delta-sleep-inducing peptide (DSIP); derivatives of a series of β-carbolines: 1-methyl-6-methoxy-tetrahydro-β-carboline (NK-424), 1-methyl-6-methoxy-dihydro-β-carboline (NIS-6), and β-carboline-3-carboxyethyl ester (NIS-26); derivatives of the aminoandrostane series: 17-β-acetylamino-4-androstene-3,16-dione (KLI-5), 17-β-acetylamino-5-androstene-3-β,16-β-diol (KLI-2), and 17-β-amino-5-androstene-3-β,16-β-diol hydrochloride (KLI-3); the GABA derivative sodium hydroxybutyrate.

A study of the anxiolytic, antineurotic, muscle-relaxing, and anticonvulsant activities of the compounds was carried out by methods described previously [1, 2, 5]. The compounds were injected intraperitoneally 30 min before the beginining of the experiment, because they exert their maximal pharmacologic effect during this time interval [7]). The results were analyzed by the Litchfield–Wilcoxon method with calculation of 50% effective doses (ED_{50}). Experiments to study the effect of the compounds on binding of ^{3}H-diazepam with rat brain synaptosomes followed the method in [10]. The protein was determined by a modified Lowry's method [8].

The significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

Of the compounds studied only diazepam possessed anticonvulsant and muscle-relaxing properties, within the spectrum of its pharmacologic action, as well as anxiolytic and antineurotic properties (Table 1). It had correspondingly high affinity for BD-receptors of the rat brain [11]. The combination of properties possessed by diazepam can be explained by its interaction with BD-receptors of both type II (realization of the anxiolytic action) and type I (muscle-relaxing, anticonvulsant action) [11]. In the carboline group, NIS-26 exhibited an antineurotic action besides its anxiolytic properties. Both effects of NIS-26 were produced in concentrations characteristic for diazepam. Meanwhile NIS-26 exhibits high affinity for BD-receptors [9], in agreement with data published previously. The absence of anticonvulsant and muscle-relaxing activity in its spectrum of pharmacological action suggests that it interacts selectively either with type II receptors or with specific β-carboline-binding proteins. The remaining compounds with non-benzodiazepine structure exhibited a sufficiently selective anxi-

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TABLE 1. Some Psychotropic Properties of Compounds with Different Chemical Structure

Compound	Anxiolytic effect	Antineurotic effect	Muscle-relaxing effect	Anticonvulsant action (antagonism with metrazol)
Diazepam	0,16 0,11÷0,24	1,0 0,76÷1,3	2,75 (1,7÷4,4)	0,51 0,39÷0,67
Sodium hydroxybutyrate	10,92 8,27—14,41	84,0 51,53—136,92	0 (30,0—100,0)	0 (5,0—20,0)
Mebicar	162,0 118,24—221,94	0 (500—2500)	0 (100—1000)	—
DSIP	0,017 0,011—0,024	0 (0,15—0,7)	0 (0,1—0,5)	0 (0,05—0,5)
KLI-2	15,0 12,5—18,0	0 (1—350)	0 (0,1—180)	0 (0,1—180)
KLI-3	20,0 15,3—26,0	0 (1—150)	0 (0,1—40)	0 (0,1—40)
KLI-5	5,0 3,8—6,5	0 (1—80)	0 (0,1—70)	0 (0,1—70)
NK-424	0,47 0,29—0,75	0 (1—15)	0 (0,1—25,0)	0 (0,1—25,0)
NIS	0,015 0,009—0,024	0 (0,05—0,3)	0 (0,001—5,0)	0 (0,001—5,0)
NIS-26	0,22 0,13±0,35	2,12 (1,51—2,96)	0 (0,01—15,0)	0 (0,001—15,0)

Legend. Values of ED_{50} (in mg/kg) given for each column; confidence intervals of means indicated at P = 0.05 level. 0) No effect. Dose range tested given in parentheses.

TABLE 2. Effect of Diazepam and of NIS-26 on Binding of ^{3}H-Diazepam with Brain Synaptosomes of Rats in a State of Alcoholic Abstinence (M ± m)

Compound	Control		Experiment	
	IC_{50}, mM	K_i, nm	IC_{50}, nm	K_i, nm
Diazepam	1,77	1,27±0,29 (3)	2,54	1,93±0,52(3)
NIS-26	1,1	0,78±0,13 (3)	3,3	2,39±0,45*(3)

Legend. Control: 8-month courses with no contact with ethanol, experiment: animals after contact with ethanol for 8 months, in a state of abstinence (deprived of ethanol for 24 h). *P < 0.05 compared with corresponding control. Number of experiments given in parentheses.

olytic effect, unaccompanied either by anticonvulsant or by muscle-relaxing effects, and in the radioligand investigations they did not compete with ^{3}H-diazepam even in millimolar concentrations. The effect of compounds with anxiolytic properties on binding of ^{3}H-diazepam with brain synaptosomes of intact rats (IC_{50}) was as follows: diazepam 8.8 ± 0.84 nM, sodium hydroxybutyrate > 0.1 mM, mebicar > 0.1 mM, DSIP > 0.1 mM, KLI-2 > 0.1 mM, KLI-3 > 0.1 mM, KLI-5 > 1 mM, NK-424 > 0.1 mM, NIS-6 > 1 mM, and NIS-26 3.13 ± 0.31 nM. Thus, among the compounds tested, which exhibit anxiolytic properties, three groups can now be distinguished: 1) compounds with benzodiazepine structure and possessing high affinity for BD-receptors (diazepam); 2) ligands of BD-receptors with nonbenzodiazepine structure (NIS-26); 3) compounds with a nonbenzodiazepine structure, which are not ligands of BD-receptors (mebicar, sodium hydroxybutyrate, DSIP, NK-424, NIS-6, KLI-2, KLI-3, KLI-5).

Solution of the problem of participation of BD-receptors in the phenomenon of the lowering of sensitivity to anxiolytic action of the compounds, observed during the development of experimental alcoholism, is very interesting. Naturally this problem is concerned only with compounds belonging to groups 1 and 2 (diazepam and NIS-26). On withholding of ethanol after enforced alcoholization, a decrease is observed in the binding constant of diazepam with the

corresponding brain receptors [6]. The study of binding of ^{3}H-diazepam with rat brain synaptosomes during a 24-hourly period of withdrawal after voluntary ethanol consumption for 8 months showed a decrease in stability of the ligand–receptor complex, which was expressed as an increase in the dissociation constant from 5.2 to 8.5 nM, whereas the number of binding sites remained unchanged [4]. The ability of unlabeled diazepam to compete with the ^{3}H-ligand for BD-receptors had a tendency to decrease correspondingly, and IC_{50} rose from 1.77 in the control animals to 2.54 nM in rats in a state of abstinence (Table 2).

More marked changes were observed in analogous investigations conducted with NIS-26. Effective concentrations of NIS-26 necessary to reduce binding of ^{3}H-diazepam by 50% were increased threefold in the experimental group, and the inhibition constant (K_i) rose from 0.78 to 2.39 nM (Table 2). These factors are in agreement with data obtained previously, according to which the decrease in the anxiolytic activity of NIS-26 was exhibited more strongly than that of diazepam on a model of experimental alcoholism [3]. It is possible that β-carboline-binding proteins undergo greater conformational changes in the period of abstinence than true BD-receptors. Nevertheless, the changes discovered in the affinity of BD-ligands for corresponding receptors in the state of abstinence cannot completely explain the 10-20-fold decrease of anxiolytic activity characteristic of diazepam and NIS-26. It is evident that an anxiolytic component, realized like compounds of group 3 without participation of the GABA-BD complex is present in the spectrum of action of the benzodiazepine tranquilizers.

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