EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS ON THE STATE OF MOUSE BRAIN BENZODIAZEPINE RECEPTORS

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Antidepressants of various types are known to exhibit their clinical therapeutic effect only during chronic administration, and not before 2-3 weeks. To explain the mechanism of this action it is evidently necessary to study neurochemical changes induced by prolonged administration of antidepressants. The tricyclic antidepressants have shown to be capable of inducing changes in α- and β-adrenoreceptors [2], and also in histamine H_2-receptors and imipramine receptors [3, 6]. Meanwhile in animals undergoing chronic antidepressant therapy activity in an open field test is reduced; this can be interpreted as a sedative effect induced by the antidepressants. Investigations have shown definite interconnection between monoaminergic systems and the GABA-benzodiazepine system [7].

The object of this investigation was to study the effect of chronic administration of antidepressants of various groups on the state of the benzodiazepine receptors of the brain.

EXPERIMENTAL METHOD

Male CBWA mice weighing 20-23 g were kept in the animal house in cages holding 40-50 animals, and received food and water ad lib. Chronic administration of aqueous solutions of drugs (or of water in the control) was carried out perorally for 2 weeks twice a day using an atraumatic tube, in a volume of 0.3 ml. The animals were decapitated 24 h after the last dose of the drugs and the brain was quickly removed, washed in physiological saline, and homogenized in a glass homogenizer with Teflon pestle (300 rpm, 40 grindings). All operations were carried out in the cold. Isolation medium contained 0.32 M sucrose, 0.05 M Tris-HCl buffer, pH 7.4 (at 25°C), and 1 mM EDTA. The homogenate (10%) was centrifuged for 10 min at 2500g and the residue was washed in half the original volume of isolation medium and centrifuged again. The pooled supernatants were centrifuged for 20 min at 15,000g and the residue of the unpurified fraction of synaptosomes (P_2) was suspended in 0.05 M Tris-HCl buffer, pH 7.4. Further processing of the material was carried out differently in the two series of experiments. In series I (July 1982) the suspension was frozen overnight at −20°C, then thawed, and washed fives times in 0.05 M Tris-HCl buffer, pH 7.4 (5 ml to 1 g of original tissue), poured into polyethylene flasks in a volume of 10 ml each, and kept at −20°C up to 1 month. In the experiments of series II (November 1982) the membranes were washed five times on the day of isolation (the conditions of washing were the same) and kept at the temperature of liquid nitrogen. In both series membranes of all four groups of animals were used in each experiment. Binding of [^{3}H]flunitrazepam with washed synaptic membranes was carried out by the method in [9] with modifications. The incubation mixture (0.5 ml) contained 40 mM Tris-HCl buffer, pH 7.4 (at 25°C), [^{3}H]flunitrazepam (Amersham Corporation, England) in concentrations of 11.6, 9.4, 7.2, 5.1, 2.6, and 0.65 nM, displacing agents, and 0.3-0.4 mg protein of synaptic membranes. Incubation was carried out at 0.5°C for 30 min, the reaction was stopped by the addition of 4 ml of cold buffer to the samples, and the material was then quickly filtered under a vacuum through GFB glass fiber filters (Whatman, England). The filters were washed twice with 4 ml of the same buffer (filtration and washing took not more than 15 sec). The dried filters were

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TABLE 1. Effect of Antidepressants on Binding of [^{3}H]Flunitrazepam with Synaptic Membranes of Mouse Brain

Drug	K_i, nM
Diazepam	10
B-1	2 000
Pyrazidol	>2 000
Bufuralin	≥20 000
Chlorimipramine	>20 000
Zimelidine	>200 000
Maclobamide	500 000
M-1	500 000

Legend. Mean values for data from 2-3 experiments given. K_g for inhibitor (K_i) calculated by equation $K_i = IC_{50}(1 + C/K_g)$, where IC_{50} is the concentration of inhibitor required to give 50% inhibition of binding; C the concentration of [^{3}H]flunitrazepam (5.8 mM); K_g the association constant of [^{3}H]flunitrazepam (1.45 nM).

TABLE 2. Effect of Chronic Administration of Antidepressants on Binding Sites of [^{3}H]Flunitrazepam with Synaptic Membranes of Mouse Brain (M ± m)

Series of experiments	Measured value	Substance administered			
		H_2O	chlorimipramine	M-1	zimelidine
I (two experiments)	K_g, nM	1,78±0,29	1,35±0,03	1,34±0,07	1,52±0,11
	B_{max}, fmoles/mg protein	482±63	662±188	780±75	752±46
II (three experiments)	K_g, nM	2,40±0,08	2,06±0,12	1,55±0,06	1,67±0,11
	B_{max}, fmoles/mg protein	1080±61	1318±82	1224±58	1378±109
Mean relative values, % (five experiments)	K_g, nM	100 (60—95)	81* (60—95)	72* (66—89)	80 (63—122)
	B_{max}, fmoles/mg protein	100	135* (120—167)	152* (113—232)	161* (120—214)

Legend. *P ≤ 0.05 by Wilcoxon's T test; limits of variations shown in parentheses.

placed in flasks containing 5 ml of Bray's scintillator, vigorously shaken, and after incubation for 12 h at room temperature their radioactivity was counted on an SL-4000 (Contron, France) counter. Nonspecific binding was determined in samples containing 5×10^{-6} M diazepam. In the present experiments it was equal to binding on the filters and did not exceed 5% with 9.4 nM [^{3}H]flunitrazepam. Adsorption isotherms were analyzed by Scatchard's method. The protein concentration in the samples was determined by Peterson's method [5]. Behavioral tests were set up by the methods described in [1], with six animals in each group. Doses of the drugs in the acute experiments were the same as in chronic experiments. Metrazol was injected subcutaneously in a dose of 180 mg/kg.

EXPERIMENTAL RESULTS

The antidepressants of the different groups themselves had no significant affinity for benzodiazepine receptors of the mouse brain (Table 1). The only exception was compound B-1, synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR, a benzfuran derivative, but its affinity also was two orders of magnitude lower than that of diazepam. It can be concluded from these data, like the results obtained by other workers, that these antidepressants, at least in therapeutic doses, do not interact with benzodiazepine receptors [4].

To study the effect of chronic antidepressant administration on brain benzodiazepine receptors chlorimipramine was chosen as a representative of the typical tricyclic antidepressants, as well as zimelidine and the morpholine derivative M-1, synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR, as representatives of atypical antidepressants.

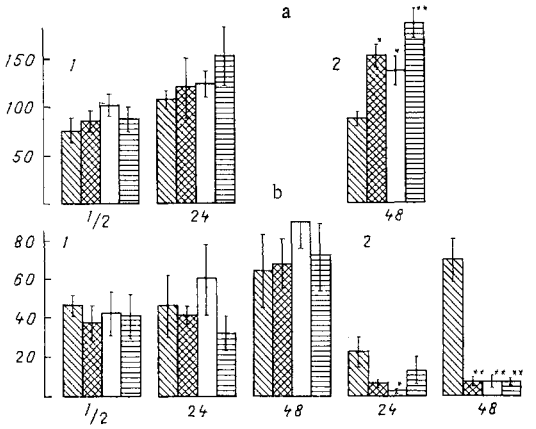

Fig. 1. Effect of antidepressants on behavioral responses of mice. a) Latent period of onset of tonic convulsions in mice after injection of metrazol. Abscissa, interval (in h) after last injection of drug; ordinate, time (in min); b) evoked aggressiveness in mice. Abscissa, interval (in h) after last injection of drug; ordinate, number of fights during electrical stimulation by a current of 0.45 mA for 2 min. 1) Single injection; 2) chronic administration. First columns – control, second – chlorimipramine, third and fourth – M-1 and zimelidine. $*P \leq 0.05$, $**P \leq 0.01$.

It will be clear from Table 2 that rather different values were obtained in the two series of experiments for the concentration of binding sites (B_{max}) for membranes isolated from control animals. These differences were evidently due to the additional cycle of freezing and thawing and the lower keeping temperature of the membranes in the experiments of series I. Despite these differences the tendency for the values of B_{max} in all five experiments of the two series remained constant: membranes isolated from animals receiving antidepressants had a higher concentration of benzodiazepine receptors than membranes of the control animals. The affinity of binding states for [^{3}H]flunitrazepam in animals receiving zimelidine was unchanged under these circumstances. A slight but significant reduction in the dissociation constant (K_g) was observed only for animals receiving M-1 and chlorimipramine.

To verify the functional importance of the changes discovered a preliminary study was made of the behavior of different groups of animals in the evoked aggression test, and the latent period of onset of tonic convulsions and of death after injection of metrazol also were investigated. In mice receiving antidepressants, 24 h after withholding them a tendency was observed for aggressiveness to diminish compared with the control (Fig. 1). When the same animals were tested another 24 h later, the differences between the control and experimental groups became significant (Fig. 1a, 1). Chronic administration of the antidepressants significantly increased the latent period of onset of tonic convulsions and of death after administration of metrazol; this effect was still present even 48 h after withholding of the drugs. In behavioral tests in animals receiving antidepressants, a "benzodiazepine-like" result of chronic administration of these drugs was found. This result was not due evidently to residual drug remaining in the body or its active metabolites, for a single dose of these antidepressants did not cause similar changes in behavior of the mice (Fig. 1b, 1). A common property of antidepressants is their ability to affect monoamine metabolism or transport directly, potentiating or facilitating serotoninergic and (or) noradrenergic transmission in the brain. However, the long latency of manifestation of the therapeutic action of antidepressants is convincing evidence that in practice it is not the direct but the indirect effects of these drugs that are more important. It has been shown, for instance, that besides changes in adrenoreceptors and in histamine and imipramine receptors, for each of which the antidepressants studied have appreciable affinity, during chronic administration of these drugs [8] opiate receptors, with which the antidepressants do not interact, also are changed. The functionally important change in the number of benzodiazepine receptors which we found supplements the picture of stable neurochemical changes induced by these drugs.

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EFFECT OF ETHANOL ON BRAIN ENKEPHALIN CONCENTRATION IN RATS WITH DIFFERENT LEVELS OF ALCOHOL MOTIVATION

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Current hypotheses put forward to explain interaction between alcohol and opiates in the body accept both the possibility that substances with opiate activity may be formed in response to administration of ethanol and the direct action of alcohol on functional activity of the enkephalinergic system [5]. To investigate the presence of common mechanisms of action of ethanol and opiates it is necessary to study the effect of ethanol on the concentrations of met- and leu-enkephalin during chronic alcohol intake. The aim of the present investigation was therefore to measure concentrations of enkephalins at different stages of development of chronic experimental alcoholism and in rats with different alcohol motivation.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing initially 200-250 g. The duration of alcohol narcosis was determined by the time in the side position after intraperitoneal injection of 25% ethanol solution in a dose of 4.5 g/kg. The peptide concentrations were measured 15 days after determination of the duration of ethanol narcosis. Chronic alcoholization of the animals was carried out by allowing them free choice between water and 15% ethanol solution. During the period of daily determination of alcohol intake the animals were kept in individual cages. Rats alcoholized for 10 days and 3 and 10 months were used in the experiments. A state of abstinence was simulated by depriving rats of ethanol for 24 h after 10 months of alcoholization. At each stage of chronic experimental alcoholism two groups of animals were selected: one group consisted of rats consuming ethanol in a volume of not more than 10% of the total fluid intake (light drinkers), the other of animals consuming not less than 70% of ethanol solution (heavy drinkers). The animals were decapitated and the brain divided into parts by the method in [8]. Brain tissue was quickly frozen in liquid nitrogen, weighed, and placed in 0.1 M acetic acid, previously warmed, treated on a water bath for 5-7 min, cooled on ice, and homogenized in a homogenizer with Teflon pestle. The samples were then centrifuged at 10,000g and the residue was again homogenized in acetic acid and centrifuged. The two supernatants were pooled and lyophilized. The residue was dissolved in 50 mM Tris-HCl buffer, pH 8.0, containing 0.05% bovine serum albumin and 0.05% sodium azide (RIA buffer). The samples were centrifuged for 5 min at 8000g, divided into aliquots, and frozen. Concentrations of enkephalins were determined radioimmunologically. Antibodies and iodinated peptides were

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