BINDING OF ^{3}H-DIAZEPAM BY CEREBRAL CORTICAL SYNAPTIC MEMBRANES DURING CONDITIONING IN RATS

K. O. Korotkov, V. V. Zhulin, and R. I. Kruglikov

UDC 612.825.5:612.833

KEY WORDS: ^{3}H-diazepam; brain; conditioned reflex; specific binding.

The discovery of benzodiazepine receptors in the brain and the important role postulated for the benzodiazepine system of the brain, including an as yet unidentified endogenous ligand, in the regulation of its functional activity makes the study of the role of this system in learning and memory a problem for urgent consideration. The urgency of this problem is also determined by the fact that among the many pharmacological effects of benzodiazepine tranquilizers [1-3], memory disturbances of the anterograde amnesia type have been described [5, 7, 8, 11, 14-16].

The ability of benzodiazepine receptors of synaptic membranes to bind exogenous benzodiazepines, can serve as a parameter of the functional state of the benzodiazepine system of the brain, for it varies under the influence of functional loads [6, 12]. It has been suggested that changes in binding of exogenous benzodiazepines by synaptic membranes are due to changes in the number and affinity of the receptors [6] and competition between the endogenous ligand secreted during appropriate functional loads with exogenous benzodiazepines for the receptor [12].

The effect of conditioning on binding of ^{3}H-diazepam *in vitro* by synaptic membranes isolated from the cerebral cortex was investigated.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 130-150 g were used. Two-way defensive conditioned avoidance reflexes were formed in a shuttle box. The conditioned stimuli were flashes at the 6th second of action of which an electric shock was applied to the animals through the floor. Conditioning was continued until five out of six correct responses were obtained. Animals receiving the same number of photic and electrodermal stimulations as the corresponding experimental animals, but not as combinations, served as the active control (AC). Animals not exposed to any specific procedures served as the passive control. Each group consisted of 5-7 rats. The animals were decapitated immediately after the experiments, the brain removed, and the separated cortex was placed in liquid nitrogen. After thawing, the tissue was homogenized in a Potter's homogenizer in 25 volumes of Tris-HCl buffer solution consisting of 0.32 M sucrose, 0.001 EDTA, and 0.05 M Tris-HCl, pH 7.4, at 4°C. The suspension thus obtained was centrifuged for 15 min at 200g, the residue was discarded, and the supernatant was centrifuged for 20 min at 20,000g. The residue thus obtained (R_2) was suspended in 25 volumes (of the initial tissue) of 0.05 M Tris-HCl solution (pH 7.4) and the suspension was frozen at −20°C for keeping not more than 10 days. Binding of ^{3}H-diazepam (specific activity 87 Ci/mmole, Amersham, England) with synaptic membranes of R_2 was carried out in a total volume of 0.5 ml. The reaction mixture consisted of 50 µl of a solution of ^{3}H-diazepam of appropriate concentration (from 0.37 to 70 nM), 50 µl water or $2 \cdot 10^{-4}$ M diazepam (to determine nonspecific binding). The reaction was started by addition of 400 µl of membrane suspension from R_2. Incubation continued for 30 min at 0.5-1°C. The mixture was treated with 4 ml of 0.05 M Tris-HCl buffer, pH 7.4, and the suspension was filtered on a water-jet pump through GF/B filters (from Whatman, England), which were then washed twice with 4 ml of the same buffer. After drying overnight, the radioactivity of the filters was

Laboratory of Neurochemical Mechanisms of Conditioned Reflexes, Institute of Higher Nervous Activity and Neurophysiology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Rusinov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 695-696, December, 1981. Original article submitted July 15, 1981.

 0007-4888/81/9212-1668$07.50

TABLE 1. Specific Binding of ^{3}H-Diazepam by Cerebral Cortical Synaptic Membranes in pmoles/mg protein) in Control and Experimental Rats

Experimental conditions	^{3}H-diazepam concentration in reaction mixture, nM			
	0,37	1	10	30
1. Passive control	0,034±0,0028	0,145±0,0073	0,660±0,012	0,935±0,030
2. Active control	0,058±0,0081*	0,255±0,022*	1,130±0,081*	1,588±0,132*
3. Conditioning	0,042±0,0044	0,168±0,019†	0,710±0,045 †	1,035±0,103†

*$P_{2-1} < 0.05$.
†$P_{3-2} < 0.05$.

determined in Bray's scintillator on a Beckman-9000 counter. Specific binding of ^{3}H-diazepam was calculated as the difference between total binding (without cold diazepam in the reaction mixture) and nonspecific binding (with 10^{-5} M cold diazepam present in the mixture). The protein content was determined by the method in [10], and from each specimen of tissue two or three samples were prepared for each concentration of ^{3}H-diazepam. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Data on binding of ^{3}H-diazepam by the cerebral cortical synaptic membranes are given in Table 1. Conditioning led to a very small increase, but exposure to uncombined stimulation led to a significant increase in binding of ^{3}H-diazepam with synaptic membranes. This regular pattern was repeated over a wide range of ^{3}H-diazepam concentrations in the reaction mixture — from 0.37 to 30 nM (the saturating concentration is 50 nM [13]) and in its close to therapeutic concentrations [9]. The fact that binding of ^{3}H-diazepam was significantly increased with synaptic membranes obtained from the cerebral cortex of the active control animals can evidently be explained in the light of Simonov's information theory of emotions [4]. According to this theory, the degree of emotional excitation is proportional to the deficit of prognostically essential information to satisfy the corresponding need. In the course of defensive conditioning the animal receives information necessary to prevent (avoid) painful stimulation, whereas during uncombined presentation of the stimuli the animal does not receive any such information. As a result the degree of emotional excitation (in this case, fear) in animals of the active control was greater than during conditioning. The enhanced emotion of fear was matched by increased ability of the synaptic membranes to bind ^{3}H-diazepam. Structural changes arising during life in benzodiazepine receptors are extremely stable in character and are manifested *in vitro*. One mechanism of participation of the benzodiazepine system of the brain in learning and memory processes is probably modification of the benzodiazepine receptors, which may evidently arise on account of changes in the number of benzodiazepine binding sites and also in their affinity.

The authors are grateful to V. V. Rozhanets and M. V. Rodionova for help with the investigation and analysis of the results.

LITERATURE CITED

1. A. V. Bogatskii, S. A. Andronati, and N. Ya. Golovenko, Tranquilizers. 1,4,-Benzodiazepine and Related Structures [in Russian], Kiev (1980).
2. T. A. Voronina, in: Progress in Science and Technology. Series Pharmacology. Problems in Psychopharmacology [in Russian], Vol. 11, Moscow (1979), pp. 81-113.
3. P. V. Simonov, Theory of Reflection and the Psychophysiology of Emotions [in Russian], Moscow (1970).
4. E. O. Bixler, M. B. Scharf, C. R. Soldatas, et al., Life Sci., 25, 1379 (1979).
5. C. Braestrup, M. Nielsen, E. B. Nielsen, et al., Psychopharmacology, 65, 273 (1979).
6. P. R. Clarke, P. S. Eckersley, J. P. Frisby, et al., Br. J. Anaesth., 42, 690 (1970).
7. J. W. Dundee and D. A. George, Acta Anaesthesiol. Belg., 27, Suppl. 3-11 (1976).
8. S. Garrattini, E. Mussini, F. Mareucci, et al., in: The Benzodiazepines, (S. Garratini,, E. Mussini, and L. O. Randall, eds.), New York (1973), pp. 75-89.
9. M. Lees and S. Packman, Anal. Biochem., 47, 184 (1972).
10. R. Liljequist, M. Linnoila, and M. J. Mattila, Eur. J. Clin. Pharm., 13, 339 (1978).
11. A. S. Lippa, C. A. Klepner, L. Gunger, et al., Pharmacol. Biochem. Behav., 9, 853 (1978).

12. M. Möhler and T. Okada, Science, 198, 849 (1977).
13. S. K. Pandit, J. W. Dundee, and S. R. Keilty, Anaesthesia, 26, 421 (1971).
14. R. C. Petersen and M. M. Ghoneim, Prog. Neuropharmacol., 4, 81 (1980)
15. H. Roth, K. M. Hartse, P. G. Laab, et al., Psychopharmacology, 70, 231 (1980).

EFFECT OF ANTICONVULSANTS ON SEIZURES INDUCED BY KYNURENINE, QUINOLINIC ACID, STRYCHNINE, AND METRAZOL

I. V. Ryzhov and I. P. Lapin

UDC 616.8-009.24-092.9-085.213

KEY WORDS: kynurenine; quinolinic acid; anticonvulsants; seizures.

Experimental treatment of seizures induced by kynurenine (K) and quinolinic acid (QA), the most active convulsant metabolite of tryptophan, is particularly interesting because this model differs from others [6, 7, 11] in the fact that it is produced with the aid of endogenous brain metabolites. Consequently, if the efficacy of anticonvulsants on models and against various types of seizures in man is compared, a closer understanding can be obtained of seizures in whose genesis a role is played by K and QA. It was found previously that kynurenine-induced seizures are selectively weakened by the inhibitory amino acids taurine and glycine [8], whereas seizures induced by QA are weakened by GABA [9] and in agonist muscimol [8], which are also effective against K.

EXPERIMENTAL METHOD

Experiments were carried out on 1040 male SHR albino mice weighing 18-25 g in the fall. The anticonvulsants were dissolved in distilled water [all in powder form except diazepam (Seduxen solution in ampules); aqueous emulsions of primidone (hexamidine) and benzobarbital (benzonal) were prepared in 1% tragacanth solution] and were injected intraperitoneally. Animals of the control groups, which accompanied each experiment, received injections of distilled water. Before and after injection of the anticonvulsants the animals (10 mice in each group) were kept in metal boxes measuring 20 × 15 × 10 cm. By means of a semiautomatic apparatus [12] 50 μg of DL-K-sulfate (from Sigma, USA; 1% solution) or 5 μg QA (from Sigma, USA; 0.1 % solution) was injected 30 min later into the cerebral ventricles (through the right lateral ventricle). Two typical convulsants were used for comparison: strychnine sulfate (0.01% aqueous solution) and metrazol (0.8% aqueous solution), and these were injected subcutaneously as aqueous solutions 30 min after the anticonvulsants. All effects were recorded visually for 10 min after intraventricular and 30 min after subcutaneous injection of the convulsants. Four main parameters were determined: 1) the latent period of onset of seizures, 2) the frequency of clonic seizures in the group, 3) the frequency of tonic extensions in the group, 4) mortality.

The significance of differences in latent period was evaluated by Student's t test, and of the other parameters by the chi-square test.

EXPERIMENTAL RESULTS

The doses of convulsants (K, QA, strychnine, and metrazol) used were about equally effective (Table 1) as regards the frequency of clonic convulsions in the group (96, 95, 96, and 97% of animals respectively), although they differed in the frequency of tonic extension (14, 48, 89, and 60% of animals respectively) and in mortality (36, 65, 80, and 52%).

All the anticonvulsants tested had moderate anticonvulsant activity against seizures induced by K, which was manifested only as lengthening of the latent period of the seizures. Strengthening of this effect with an increase in dose was observed only in the case of primidone. Of all the drugs only benzobarbital reduced the frequency of clonic convulsions.

Laboratory of Psychopharmacology, V. M. Bekhterev Leningrad Psychoneurological Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 696-699, December 1981. Original article submitted June 19, 1981.

 0007-4888/81/9212-1670$07.50