EFFECT OF GABA ANTAGONIST-INDUCED SEIZURES ON ^{3}H-MUSCIMOL AND ^{3}H-DIAZEPAM BINDING IN THE RAT STRIATUM

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Disturbances in the neuronal receptor system are known to develop under the influence of various convulsants [1, 8]. A special role in the development of seizures is played by modulation of the functional state of receptors for GABA, the principal inhibitory neurotransmitter in the mammalian brain [6, 11]. Changes in the GABA-benzodiazepine receptor complex during seizures induced by GABA antagonists, namely picrotoxin and bicuculline, have not been adequately studied. The investigation described below was aimed at elucidating this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 170-220 g. Bicuculline (Sigma, USA) and picrotoxin (Serva, Germany) were dissolved in dimethylsulfoxide and injected intraperitoneally in a dose of 8 mg/kg (1.42 and 1.23 LD_{50} respectively) calculated on the basis of 0.1 ml per 100 g body weight. Control animals received an injection of the equivalent volume of solvent. The rats were decapitated 10 min after injection of bicuculline (duration of seizures 7.3 ± 0.2 min) and 20 min after injection of picrotoxin (duration of seizures 9.1 ± 0.3 min). In the experiments with ^{3}H-muscimol the synaptic membranes of the striatum were treated with Triton X-100 [2]. The incubation medium, in a total volume of 1 ml, contained the membrane preparation (200-300 μg protein), 10 mM Tris-HCl, pH 7.4, and ^{3}H-muscimol (NEN, Germany, 20 Ci/mmole) in concentration of 1-15 nM. Nonspecific binding was estimated in the presence of 1 mM GABA (Reanal, Hungary). The samples were incubated for 30 min at 0-3°C. The contents of the tubes were transferred to GF/C filters (Whatman, England) and washed with 10 ml of ice-cold buffer. Coarse synaptic membranes of the striatum were prepared for the study of ^{3}H-diazepam binding in a similar way but the procedure did not include treatment with Triton X-100, and 50 mM K-phosphate buffer, pH 7.4, was used for washing the membranes. The incubation mixture (1 ml) contained 150-220 μg membranes, 50 mM K-phosphate buffer, pH 7.4, and ^{3}H-diazepam (Amersham, England, 77 Ci/mmole) in concentrations of 0.5-5 nM. Nonspecific binding was assessed in the presence of 30 μM diazepam. The samples were incubated for 1 h at 0-3°C. Unbound label was separated on GF/C filters. Radioactivity was measured on a RackBeta 1217-802 counter. Parameters of specific binding of the ligands were determined from the results of five separate experiments, using double parallel tests. The calculation was done on a Scatchard plot using linear paired regression analysis by the method of least squares, on an EC-1841 computer. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

Data on specific binding of ^{3}H-muscimol with synaptic membranes of the rat striatum after exposure to the convulsive action of the GABA antagonists are given in Table 1. No significant changes were found in the parameters of ligand binding during seizures induced by picrotoxin, a blocker of the chloride ion channels of $GABA_A$-receptors. During bicuculline poisoning an increase was observed in affinity of the receptors for muscimol. This was reflected in a fall of 27%

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TABLE 1. Characteristics of Specific Binding of ^{3}H-Muscimol with Synaptic Membranes of Rat Striatum after Seizures Induced by GABA Antagonists (M ± m)

Substance	k_d, nM	B_{max}, fmoles/mg protein
Dimethyl sulfoxide	11,0±0,7	312,0±48,0
Picrotoxin	9,8±0,9	332,8±35,0
Bicuculline	8,0±1,0*	342,3±61,6

Legend. Here and in Table 2: *p < 0.05.

TABLE 2. Kinetic Characteristics of ^{3}H-Diazepam Binding with Membranes of Rat Striatum after Administration of GABA Antagonists (M ± m)

Substance	K_d, nM	B_{max}, fmoles/mg protein
Dimethyl sulfoxide	5,7±0,7	165,8±12,0
Picrotoxin	6,7±1,3	215,6±15,7*
Bicuculline	10,6±0,6*	349,2±27,9*

in the value of K_d. Changes in density of the receptors were not significant in either case. It can be tentatively suggested that the increase in affinity of the receptors for the GABA agonist muscimol was due to compensatory modifications of the GABA-ergic systems of the striatum. It was shown previously in our laboratory that similar changes are also observed with ^{3}H-GABA binding (in course of publication). This suggests that specific binding sites for ^{3}H-GABA and ^{3}H-muscimol react more strongly on the convulsive effect of bicuculline than on that of picrotoxin. This may possibly account for the different character of the seizure syndrome under the influence of these particular GABA antagonists [9].

Table 2 gives the results of experiments to study specific binding of ^{3}H-diazepam. Seizures induced by picrotoxin and bicuculline were accompanied by an increase in density of binding sites of the ligand by 30 and 111% respectively. Meanwhile the affinity of the receptors for diazepam was reduced after exposure to bicuculline (by 86%). The changes thus discovered may be linked with compensatory reactions of the GABA-benzodiazepine receptor complex in the course of seizures. Meanwhile many convulsants possess membranotropic effects [3]. It has been suggested that activation of lipid peroxidation in neuronal membranes by chemical convulsants may be the basis of disturbances of the functional state of the GABA-benzodiazepine receptor complex [1]. Phosphatidylserine, an important component of membranes, was shown to have an appreciable influence on specific binding of ^{3}H-flunitrazepam with receptors in the rat cerebellum. In this case the modulating effect of GABA agonists on benzodiazepine binding was altered [5]. The appearance of hypoxia during development of the seizure syndrome may also lead to the formation of disturbances of benzodiazepine and $GABA_A$-receptors. Prolonged hypoxia, for instance, led to increased binding of ^{3}H-muscimol and ^{3}H-flunitrazepam with rat brain membranes [10].

The state of $GABA_A$-receptors under the influence of chemical convulsants has been studied by a number of workers. It has been shown, for instance, that characteristic changes in ^{3}H-diazepam binding are observed in the cerebral cortex of rats subjected to the convulsant action of bemegride: the affinity of the receptors for the ligand is reduced but the density of the binding sites is increased [1]. Our own data show that similar changes were observed also under the influence of GABA antagonists: picrotoxin and bicuculline. Evidence has been obtained that changes in the functional state of the chloride ionophore, which is sensitive to GABA, take place in the course of seizures induced by the benzodiazepine receptor antagonist Ro15-1788 and the β-carboline FG 7142 [8]. Disturbances of specific binding of ^{3}H-diazepam develop in the rat brain during the convulsant action of urea [4].

The results of this investigation thus indicate that an increase in the concentration of binding sites for ^{3}H-diazepam is observed in the rat striatum during seizures induced by the GABA antagonists picrotoxin and bicuculline. Under the influence of bicuculline, the affinity of benzodiazepine receptors for the ligand also was reduced. Seizures induced by bicuculline were accompanied by a significant increase in affinity of $GABA_A$-receptors for ^{3}H-muscimol. The changes

discovered may reflect compensatory changes in the GABA-ergic systems of the rat striatum during poisoning by GABA antagonists.

The possibility of membranotoxic effects of picrotoxin and bicuculline likewise cannot be ruled out.

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EFFECT OF THE DIABETOGENIC AGENT DITHISONE ON ZINC CONCENTRATION IN PANCREATIC ISLETS OF ANIMALS OF DIFFERENT SPECIES

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The high selectivity of action of dithisone on the insulin-producing cells is one of its advantages over other substances (alloxan etc.) used as a model of diabetes mellitus [1, 2, 4, 6, 8-11]. In view of the high affinity of dithisone for zinc ions, the study of changes in the content of this metal in the pancreatic islets of animals of different species receiving dithisone must be interesting from the point of view of the study of the role of disturbances of zinc metabolism in the endocrine part of the pancreas in the mechanism of development of diabetes. Highly sensitive and selective methods of cytochemical and biochemical determination of zinc in the insular tissue of the pancreas have been developed in order to carry out such investigations.

In this paper the zinc concentration in the pancreatic islets in animals of different species was studied after injection of dithisone.

EXPERIMENTAL METHOD

The experiments were carried out on 42 cats, 293 rabbits, 49 golden hamsters, 68 mice, and 25 pigeons. Dithisone was injected intravenously in doses of 50-100 mg/kg. The blood sugar level was determined by the Hagedorn—Jensen

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