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Binding of ^{3}H-Diazepam in Rat Brain Eleven Months After Termination of Corazole Kindling

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Binding of ^{3}H-diazepam in rat cerebellum decreases by 14% ($p<0.05$) 11 months after termination of kindling and one day after injection of a test dose of corazole (30 mg/kg), while it increases by 19.5% after a single injection of a convulsive dose of corazole (50-75 mg/kg). No changes are found in the cortex.

Key Words: *cerebellum; brain cortex; corazole kindling, diazepam; benzodiazepine receptors*

Kindling, produced by chronic electrical stimulation of brain structures [5,8] or chronic administration of convulsants [1,4,9,], induces a state of seizure predisposition (SP), due to which earlier subconvulsive agents evoke seizures. One of the features of this kindling-induced pathological state is that predisposition to seizures persists for a long time (weeks or months) after the termination of electrical stimulation or the discontinuation of con-

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vulsant [1,3,9]. The mechanism underlying this phenomenon remains unclear. Since seizures may appear due to impaired inhibitory control, disturbances of the $GABA_A$-receptor apparatus may be assumed to be one of the mechanisms of the kindling-induced pathological state of the brain.

The present study was aimed at the investigation of ^{3}H-diazepam binding with the benzodiazepine receptors forming part of the $GABA_A$-benzodiazepine-receptor complex and regulating the inhibitory mechanisms, in rat brain 11 months after the termination of corazole kindling.

MATERIALS AND METHODS

Experiments were carried out on 50 male Wistar rats. The animals were maintained under standard vivarium conditions and fed a standard ration. Two experimental series were performed. In series I (groups 1 and 2, 10 rats in each group) kindling was induced by daily intraperitoneal injections of 30 mg/kg corazole (a subconvulsive dose) during 28 days. The severity of the seizure reaction to the injections was evaluated daily using a six-point scale [1]. For evaluation of the duration of SP after termination of kindling, the animals of group 2 were periodically (after 6, 9, and 11 months) injected a test dose of corazole (30 mg/kg). In series II the experimental animals (10 rats) received a single injection of corazole in a convulsive dose (50-75 mg/kg), which produced a seizure response of 3-5 points, i.e., analogous to that observed in animals of the first series 11 months after the termination kindling. In both series the control animals (20 rats) were injected with the same volume of physiological saline under analogous conditions.

Twenty-four hours after the last injection of corazole to kindled animals (series I, group 1) and after the single injection of a convulsive dose of corazole (series II) the animals were decapitated. The cerebral cortex and cerebellum of each rat were homogenized in the cold using a Potter homogenizer in 10 ml isolation medium (0.32 M sucrose, 50 mM Tris-HCl, pH 7.4 at 4°C, and 1 mM EDTA). The homogenate was centrifuged at 1000 g for 10 min, the pellet was discharged, and the supernatant was recentrifuged at 20,000 g for 20 min. The sediment of coarse synaptic membranes (P_2) was frozen at -40°C. After thawing, the sediments were suspended in 10 ml of 50 mM Tris-HCl, pH 7.4, and centrifuged at 20,000 g for 20 min. The resultant sediment was resuspended in 4 ml of the same buffer solution. All isolation procedures were carried out at 4°C.

^{3}H-diazepam (68 Ci/mmol, Amersham, UK) in a volume of 0.1 ml aqueous solution was added to 0.4 ml of the suspension of synaptic membranes (final concentration in the sample 2 nM) and the mixture was incubated at 4°C for 40 min. Binding was terminated by passing the samples through CF/B filters (Whatman, UK) and followed by 4 washings with 2 ml of cold buffer saline. The filters were counted for radioactivity using ZhC-8 scintillator (Reakhim, Russia). Nonspecific binding was determined in parallel samples in the presence of 7-bromo-desmethyldiazepam as a competitor. Specific binding was calculated as the difference between the total and nonspecific binding. All samples were paired. The content of protein in the samples was measured after Lowry [6]. The reliability of differences was evaluated using the Student t test.

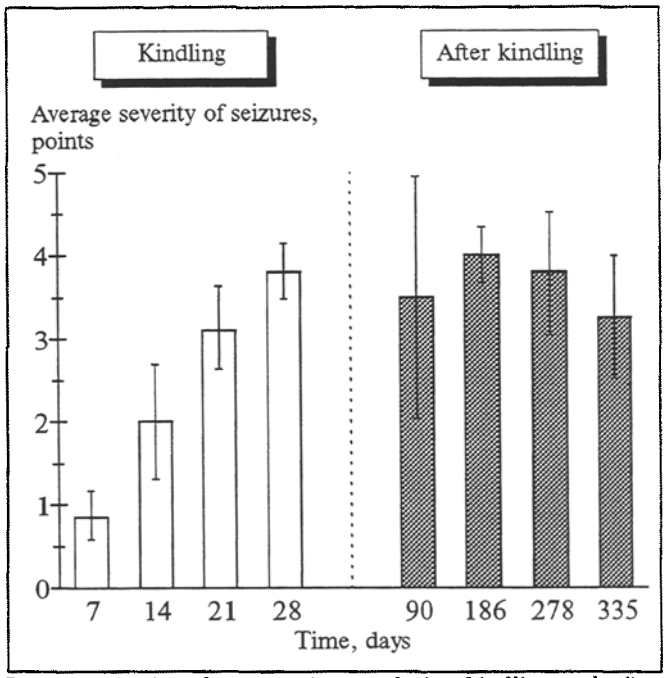

Fig. 1. Severity of seizures in rats during kindling and after its termination.

RESULTS

In series I (groups 1 and 2) daily administration of corazole resulted in the appearance of a 1-point seizures on the 3rd day in 20% of animals. The

TABLE 1. Binding of ^{3}H−Diazepam with Synaptic Membranes of Rat Brain 11 Months after Termination of Kindling ($M \pm m$)

Group and number (n) of animals	Specific binding, fmol/mg protein	
	neocortex	cerebellum
Control ($n=7$)	571.4±8.3	357.4±14.8
Experiment ($n=8$)	558.5±4.2	307.0±9.8*

Note. Here and in Table 2: * denotes $p<0.05$ in comparison with the control.

TABLE 2. Binding of ^{3}H−Diazepam with Synaptic Membranes of Rat Brain 24 Hours after a Single Injection of a Convulsive Dose of Corazole ($M\pm m$)

Group and number (n) of animals	Specific binding, fmol/mg protein	
	neocortex	cerebellum
Control ($n=8$)	505.4±12.0	265.5±8.0
Experiment ($n=8$)	527.8±5.0	317.3±82

number of animals with seizures and the severity of these gradually increased (Fig. 1).

The average severity of seizures was 3.29±0.19 immediately after the termination of kindling and 3.38±0.49 11 months later.

The level of ^{3}H-diazepam binding with synaptic membranes in the cerebellum measured 11 months after the termination of kindling in experimental animals (series I, group 1) was found to be 14% lower than in controls (Table 1), but no significant differences were observed in ^{3}H-diazepam binding in the cortex. A single injection of corazole increased the level of ^{3}H-diazepam binding in rat cerebellum by 19.5% (Table 2), but no reliable differences were found in the cortex.

Thus, these results, together with previous data [1,3,7] suggest that chronic administration of corazole in subconvulsive doses induces a gradual rise of SP, which manifests itself in the appearance of seizures and their aggravation in response to subsequent injections of the epileptogen. This SP syndrome persists for a long time (11 months) after the convulsant is discontinued.

The assay of ^{3}H-diazepam binding with synaptic membranes from rat brain revealed its reliable decrease 11 months after the termination of kindling in the cerebellum. This was not connected with the direct action of corazole, since the effect was not observed after a single injection of the agent even in doses 2-2.5 times higher than the subconvulsive doses. Moreover, acute administration of corazole resulted in an increase of ^{3}H-diazepam binding. These data imply that acute seizures induced by a single injection of corazole and seizures induced by corazole under conditions of kindling are different in nature and require different conditions for their realization. This is in conformity with our results and with data from other sources which showed that Ca- and NMDA-receptor antagonists inhibiting the development of SP in the brain do not greatly affect the realization of the seizure reaction itself [1,7]. In this connection, it is intriguing that in rats of the Krushinsky-Molodkina strain with a hereditary predisposition to audiogenic seizures, the binding of ^{3}H-diazepam with benzodiazepine receptors in the cerebellum was found to drop by 30-35% 24 hours after seizures [2]. The data presented here suggest that the mechanisms of chronic epileptogenesis (in our case in the form of kindling-induced corazole SP) and of the seizure reaction are not identical.

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