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BINDING OF ^{3}H-DIAZEPAM WITH BRAIN SYNAPTIC MEMBRANES DURING THE DEVELOPMENT OF GENERALIZED EPILEPTIC ACTIVITY

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UDC 616.853-092.9-07:616.831.31-008.949.4:615.214.22]-073,916

KEY WORDS: ^{3}H-diazepam, epileptic activity, synaptic membranes.

The importance of injury to GABA-ergic mechanisms in the pathogenesis of epilepsy has been discussed for quite a long time [13, 16]. It is also known that the distinct antiepileptic properties of the benzodiazepines (BD) are due to their potentiating effect on the inhibitory action of GABA [8, 12]. Under normal conditions complex mutual modulating relations exist between the ligand–receptor systems (GABA–GABA receptor, BD–BD receptor) [7, 9, 15]. It has been suggested that both receptors constitute a single structural-functional complex [10]. It can accordingly be postulated that the development of epileptic activity (EA) in the CNS is connected to a certain degree with changes in the properties of BD receptors, leading to disturbance of the possible regulatory effect of their endogenous ligands on GABA-ergic mechanisms.

The aim of the present investigation was to study the effect of the development of generalized EA in the rat cerebral cortex on binding of ^{3}H-diazepam with synaptic membranes.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. A 0.50% solution of bemegride was injected intramuscularly into the animals in a dose of 24 mg/kg. As a result, 5-7 min after the injection of bemegride the rats developed a characteristic fit of clonicotonic convulsions, falling on to their side with a well-marked phase of tonic extension. Animals of the control group were given an intramuscular injection of the same volume of physiological saline. The animals were decapitated 20 min after the injection of bemegride or physiological saline and the brain was removed and washed in cold physiological saline three times to remove all the blood. The cerebral cortex was carefully separated from the white matter and homogenized in a glass homogenizer with Teflon pestle, in the ratio of 10 ml of buffer solution to 1 g of cortex. A buffer solution of the following composition (in mM) was used: sucrose 320, EDTA-Na_2 1, Tris-HCl 5.0; pH 7.4 (20°C). The homogenate was centrifuged on a K-24 centrifuge (East Germany) at 1500g for 10 min. The supernatant was carefully collected in a separate tube, and the residue rehomogenized in the original volume of isolation medium, after which it was centrifuged for 10 min at 1500g. The pooled supernatant was centrifuged for 20 min at 9000g. The supernatant was removed and the residue, resuspended in 10 mM Tris-HCl, pH 7.4 (20°C) at the rate of 10 ml of buffer to 1 g of original cortex, and centrifuged for 20 min at 9000g. The residue was again resuspended in 20 ml of 6 mM Tris-HCl, pH 7.4 (20°C) and allowed to stand for 2 h at 0-4°C. The suspension was then centrifuged at 13,000g for 20 min. The supernatant was discarded and the solid residue, consisting of the coarse fraction of synaptic membranes, was kept at 0°C for not more than 12 h. The whole procedure of isolation of the synaptic membrane fraction was carried out at 0-4°C.

The residue of membranes was resuspended in 5 ml of buffer solution containing 50 mM Tris-HCl, pH 7.2 (20°C). The protein concentration in the suspension was determined by the biuret

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Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 686-688, December, 1985. Original article submitted April 17, 1985.

 0007-4888/85/0012-1664$09.50

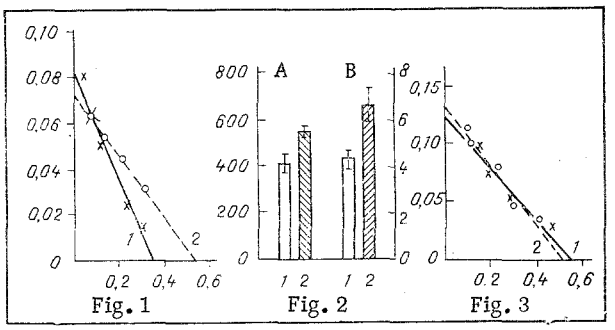

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Graph showing dependence of quantity of ^{3}H-diazepam bound on its concentration added to incubation medium (Scatchard plot). Abscissa, number of binding sites (in pmoles/mg protein); ordinate, ratio of number of binding sites of protein to concentration of added ^{3}H-diazepam (in ml/mg protein). 1) Control, 2) bemegride.

Fig. 2. Effect of development of EA on binding of ^{3}H-diazepam with synaptic membranes of rat cerebral cortex. Ordinate: on left — B_{max} (in fmoles/mg protein), on right — K_d (in nM). A) B_{max}, b) K_d. 1) Control, 2) bemegride. Arithmetic mean values of three independent measurements are given.

Fig. 3. Effect of bemegride on binding of ^{3}H-diazepam with BD receptors of synaptic membranes (Scatchard plot). Legend as to Fig. 1.

reaction. Radioligand analysis of binding was carried out as described previously [3], using as the label ^{3}H-N-methyldiazepam (England) with activity of 71 Ci/mmole. Radioactivity of the preparation was measured on a counter of "Beckman" type (USA). The maximal number of binding sites (B_{max}) and the dissociation constant (K_d) of the ligand—receptor complex were determined by the method in [14].

EXPERIMENTAL RESULTS

The experiments showed that dependence of the quantity of ^{3}H-diazepam bound with synaptic membranes on the concentration of label in the incubation medium is linear in character on a Scatchard plot (Fig. 1). This is evidence of the existence of only one type of binding site with K_d = 4.40 ± 0.15 nM and B_{max} = 410 ± 35 fmoles/mg protein.

The development of a fit of generalized convulsions by the rats in response to injection of bemegride did not change the linearity of this relationship (Fig. 1, 2). However, 20 min after injection of the convulsant, i.e., 10-15 min after the fit, the maximal number of binding sites for ^{3}H-diazepam on the synaptic membranes increased to 550 ± 12 fmoles/mg protein. Meanwhile an increase in K_d of the H-diazepam—receptor complex to 6.7 ± 0.75 nM was observed (Fig. 2).

The character of binding of $_3$H-diazepam with receptors of the synaptic membranes did not change after incubation of the synaptic membranes with bemegride (final bemegride concentration 10^{-7} M) *in vitro* for 20 min at 37°C. For ^{3}H-diazepam B_{max} on synaptic membranes and K_d of the ^{3}H-diazepam—receptor complex remained at the control level (Fig. 3).

These investigations showed that changes in K_d and B_{max} are caused by the development of convulsions in the rats and not by the direct effect of bemegride on the synaptic membranes.

When the possible causes of the change in properties of BD receptors during generalized EA are examined, the writers' previous results, showing that the development of various forms of EA [2, 4], including generalized [4], is connected with activation of lipid peroxidation (LPO) in the brain membranes, might well be recalled.

As a result of activation of LPO changes take place in the properties of membranes and, in particular, the flowability of their lipid bilayer is reduced [3, 6]; this may lead to changes in the affinity of the membrane receptors, and also to interference with interaction between the receptors [11]. In fact, an increase in the viscosity of the synaptic membranes of the brain has been found during induction of ascorbate-induced LPO in them [3, 6], accompanied by a decrease in the number and affinity of the β-adreno-receptors [6]. Specific binding of serotonin with brain microsomal membranes also was reduced during induction of LPO [3]. Changes in the properties of the brain membrane receptors were observed not only during activation of LPO in systems *in vitro*, but also during the development of pathological processes connected with intensification of LPO, such as stress [5] and the development of convulsions during hyperbaric oxygenation[1], in the CNS.

On the basis of these data it was logical to suggest that the change in properties of the BD receptors during development of experimental EA in rats was due to activation of LPO. Evidence in support of this hypothesis is given by results obtained during activation of ascorbate-dependent LPO *in vitro* in the microsomal fraction of rat cerebral cortical membranes [3]. Under these circumstances it was found that activation of LPO leads to an increase of 30% in specific binding of ^{3}H-diazepam [3]. Analysis by the Scatchard plot method showed that this increase is due to an increase in the value of B_{max} for ^{3}H-diazepam from 630 to 820 fmoles/mg protein.

This means that activation of LPO *in vitro* causes changes in specific binding of ^{3}H-diazepam with rat brain membranes, changes that are very similar to those taking place during the development of generalized EA.

The results are thus evidence of substantial changes in the affinity and number of BD receptors of brain synaptic membranes during the development of generalized EA. These changes are evidently due to peroxidation in the lipid matrix of the membranes. In this case, to make the antiepileptic action of BD more effective, these drugs should best be used in a combination with anti-oxidants.

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