MONOACYLGLYCEROPHOSPHATIDE ACCUMULATION AND CHANGES IN PROPERTIES OF BENZODIAZEPINE RECEPTORS IN BRAIN SYNAPTOSOMES

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On the basis of recent data it is possible to postulate the participation of membrane phospholipids in processes of ligand–receptor interaction [1, 3]. In particular, it has been shown [4] that specific binding of ^{3}H-diazepam with synaptosomal membranes of the rat cerebral cortex is increased as a result of hydrolysis of the phopholipids by phospholipase A_2, and this served as the basis for the hypothesis of a change in the properties of benzodiazepine receptors because of modification of the phospholipid matrix of neuronal membranes. At the same time, in view of the accumulation of monoacylglycerophosphatides (MAGP) and of free fatty acids, products of phospholipid hydrolysis by phospholipase A_2, in membranes [5], it can be tentatively suggested that the effect of phospholipase A_2 on the properties of receptors must be mediated either through the combined action of these compounds or the action of only one of them.

Solution of this problem would reveal the possible mechanism of regulation of the properties of receptors for diazepam, an effective psychotropic drug, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Plasma membranes of synaptosomes were obtained from the cerebral cortex of Wistar rats weighing 160–180 g. The animals were decaptitated in the cold and the gray matter of the cortex was isolated. The material was homogenized (Teflon – glass) in 10 volumes of medium containing 0.32 M sucrose, 1 mM EDTA, 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged for 10 min at 1000g, the supernatant was collected, the residue was homogenized under the same conditons, and the second portion of supernatant was selected. The collected supernatant was centrifuged at 10,000g for 20 min and the residue resuspended in 50 mM Tris-HCl and subjected to hypo-osmotic shock for 2 h in a medium of 6 mM Tris-HCl, pH 7.4. After sedimentation for 20 min at 20,000g the synaptosomal membrane fraction was obtained. The protein concentration was determined by the biuret method. Analysis of ligand–receptor interaction was carried out with the aid of ^{3}H-diazepam (from Amersham Corporation, England) in accordance with the technique described in [2]. Treatment with phospholipase A_2 (from Boehringer, West Germany) was given at 37°C. Preparation of arachidonic acid and lysophosphatidylcholine used were from Serva (West Germany). The membrane were treated with these preparations for 30 min at 37°C, and their excess, not incorporated into the membranes, was removed by centrifugation.

EXPERIMENTAL RESULTS

In the experiments of series I the action of phospholipase A_2 on interaction of ^{3}H-diazepam with benzodiazepine receptors in plasma membranes of brain synaptosomes was studied. Incubation of phospholipase A_2 with the membrane was shown to lead to an increase in specific binding of the labeled ligand by 30%; the magnitude of the effect, moreover, depends on the duration of incubation with the enzyme (Fig. 1). The degree of hydrolysis of membrane phospholipids during incubation of synaptosomal membranes in the presence of 2 μg of phospholipase A_2/mg protein for 40 min was 20%.

A similar effect was produced by the action of lysophosphatidylcholine on the membranes

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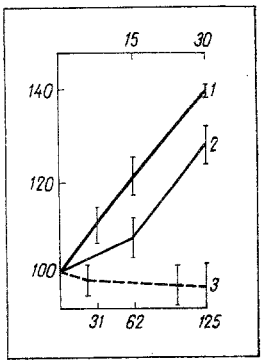

Fig. 1. Changes in specific binding of ^{3}H-diazepam with synaptosomal membranes under the influence of lysophosphatidylcholine (1), phospholipase A_2 (2), and arachidonic acid (3). Abscissa: bottom — concentration of lysophosphatidylcholine and arachidonic acid (in μg/mg protein, 37°C, duration of treatment 30 min), top — incubation time (in min; 37°C, 2 μg/mg protein); ordinate, changes in specific binding of ^{3}H-diazepam (in % of control).

(Fig. 1). In this case the degree of specific binding of ^{3}H-diazepam with the membranes rose steadily up to an MAGP concentration in the incubation medium of 125 μg/mg protein. By contrast, addition of arachidonic acid to the membrane suspension had no appreciable effect on the ability of the benzodiazepine receptors to bind ^{3}H-diazepam specifically within a concentration range of the free fatty acid of 0 to 400 μg/mg protein. The increase in binding of ^{3}H-diazepam with the membranes that was found could be explained either by a change in the properties of the benzodiazepine receptors themselves or by the appearance of additional binding sites of the ligand in the membranes, for example, on account of loosening of the phospholipid matrix due to the detergent action of MAGP. Accordingly, in the experiments of series II the properties of the receptors for diazepam, altered by modification of the phospholipid matrix of the synaptosomal membranes, due to the action of phospholipase A_2 and lysophosphatidylcholine, were analyzed by Scatchard's method. Under these circumstances it was shown that both phospholipase A_2 and lysophosphatidylcholine cause a significant decrease in the dissociation constant for ^{3}H-diazepam but do not change the value reflecting the maximal number of specific binding sites for this ligand, which is evidence of an increase in the affinity of the benzodiazepine receptors, i.e., of a change in their properties.

It must be pointed out that solubilization of benzodiazepine receptors from synaptosomal membranes led to total disappearance of the action of lysophosphatidylcholine on specific binding of ^{3}H-diazepam (preparations of the solubilized receptors were generously by A. Ya. Korneev, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR).

The results of this investigation thus lead to two basic conclusions: 1) the effect of phospholipase A_2 on the properties of benzodiazepine receptors of synaptosomal membranes of cerebral cortical cells is due to accumulation of MAGP in the membranes; 2) modification of the phospholipid matrix of the membranes, accompanied by MAGP accumulation, causes changes in the properties of benzodiazepine receptors, manifested as an increase in their affinity. Taken as a whole these results indicate that membrane phospholipids play a regulatory role in ligand—receptor interaction, and they suggest ways for modifying the properties of benzodiazepine receptors in a particular manner in order to enhance the effectiveness of psychotropic drugs of the benzodiazepine series.

LITERATURE CITED

1. E. M. Kreps, V. A. Tyurin, N. V. Gorbunov, et al., Dokl. Akad. Nauk SSSR, 273, No. 3, 753 (1983).
2. M. L. Libe, E. D. Bogdanova, A. E. Rozenberg, et al., Byull. Éksp. Biol. Med., No. 11,

552 (1981).
3. F. Hirata and J. Axelrod, Science, 209, 1082 (1980).
4. E. Ueno and K. Kurijama, Neuropharmacology, 20, 1169 (1981).
5. H. van den Bosch, Biochim. Biophys. Acta, 604, 191 (1980).

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

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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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