FORMATION OF AND EFFECT OF DIAZEPAM ON A CORTICAL EPILEPTIC COMPLEX AFTER BRAIN SECTION AT DIFFERENT LEVELS

G. N. Kryzhanovskii,* R. F. Makul'kin, A. A. Shandra, B. A. Lobasyuk, and M. N. Lebedyuk

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A set of epileptic foci created in the cerebral cortex by application of convulsants has been shown to be a unique system in which the role of determinant structure, determining the behavior of each focus and of the system as a whole, is played by the most powerful focus [1-6].

Since diazepam is a highly effective antiepileptic agent, it was important to investigate the effect of this drug on an epileptic complex. The effects of diazepam were studied on the whole brain and also on cerveau isolé, cortex isolé, and hemisphère isolé preparations. By using these brain preparations it was possible to obtain additional information on the mechanism of formation of the complex and of the action of diazepam.

EXPERIMENTAL METHOD

Acute and chronic experiments were conducted on 22 cats. Under ether anesthesia the skin and subcutaneous fascia was divided by a midline incision running from the nasal bones to the occiput. The eye was drained. Wide access to the various parts of the frontal and parieto-occipital regions of the neocortex of one hemisphere was obtained through burr-holes drilled in the vault of the skull and the orbit. Scattered foci of paroxysmal activity were created by application of a piece of filter paper (2 mm²) soaked in a 0.1-0.5% solution of strychnine. Foci of this type were created in different parts of the coronary, posterior sigmoid, and lateral gyri. A focus of powerful epileptic activity was formed in the orbital or ectosylvian gyrus by application of a 1-3% solution or of a small crystal of strychnine. The midbrain was divided by Villablanca's method [13] and neuronal isolation of the cortex and hemisphere was performed by Khananashvili's method [9]. Biopotentials were derived by a monopolar technique, the reference electrode was secured in the nasal bones, and cotton threads soaked in Ringer's solution served as active electrodes. Cortical electrical activity was recorded on the 4-ÉEG-3 ink-writing electroencephalograph. Diazepam (Seduxen, from Gedeon Richter, Hungary, in ampul form) was injected intravenously or intraperitoneally in a dose of 0.5-5 mg/kg.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of diazepam on epileptic complexes created in the intact cortex was investigated. After application of a 0.1% solution of strychnine to the coronary (zone 2) and posterior sigmoid gyrus (zone 3) spike potentials of different amplitude, and not synchronized with each other, appeared in these areas of the cortex (Fig. 1A). After the appearance of activity in these foci the pieces of filter paper with strychnine were removed and a 3% solution of strychnine was applied to the anterior ectosylvian gyrus (zone 1) in order to create a more powerful, hyperactive focus. With the formation of this focus and an increase in the intensity of its activity, an increase in the amplitude and frequency of the discharges was observed in the foci in the coronary and sigmoid gyri, and

*Corresponding Member, Academy of Medical Sciences of the USSR.

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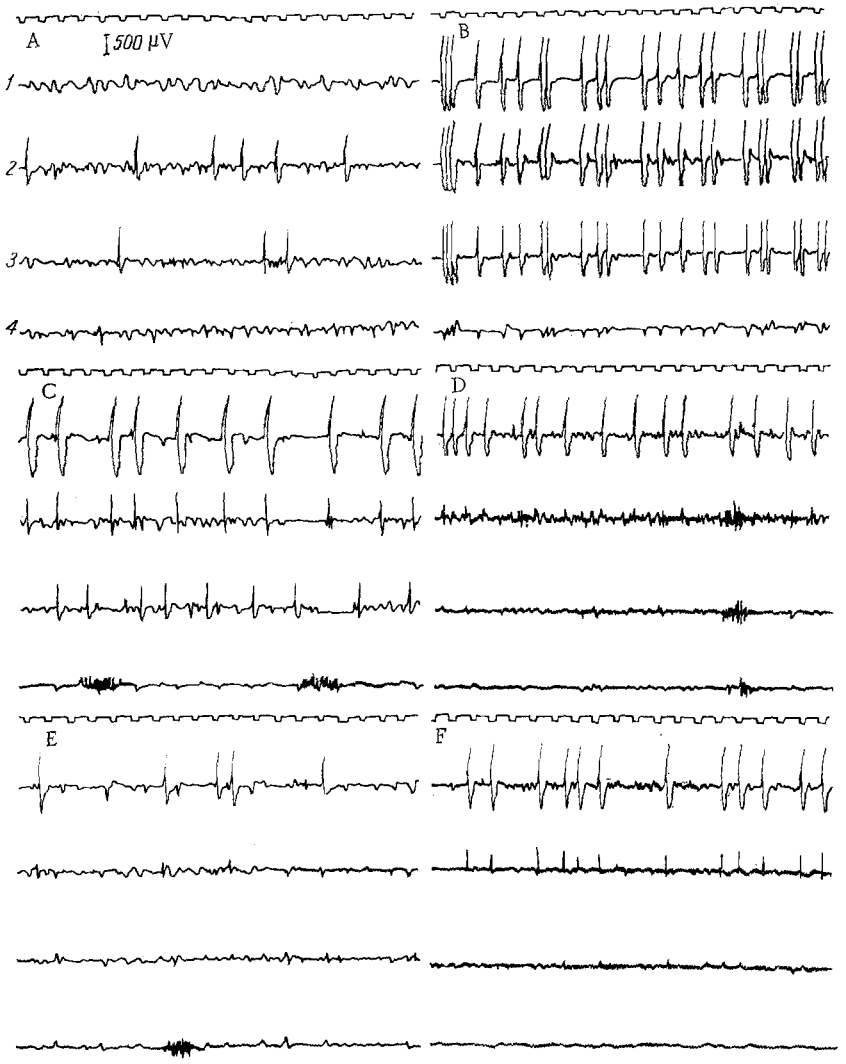

Fig. 1. Effect of diazepam on activity of epileptic complex created in the intact cortex. A) Formation of foci of enhanced excitability in zones 2 and 3 by application of 0.1% strychnine solution (application of substances ceased after the appearance of epileptic activity); B) 14 min after formation of determinant focus in zone 1 by application of 3% strychnine solution: synchronization of epileptic activity in all zones of complex; C) 2 min, D) 12 min, E) 24 min after injection of 3 mg/kg diazepam; F) 8 min after reapplication of 3% strychnine solution to zone 1 to restore activity of complex. 1) Anterior ectosylvian gyrus, 2) coronary, 3) posterior and 4) anterior sigmoid gyrus. Calibration: 500 μV, time marker 1 sec.

activity in these foci became synchronized with discharges in the hyperactive focus. In this way a single functional epileptic complex consisting of three foci was produced, in which the role of determinant structure, determining the behavior of the whole complex, was played by the hyperactive focus in the ectosylvian cortex (Fig. 1B).

A few minutes after intraperitoneal or a few seconds after intravenous injection of diazepam in a dose of 3 mg/kg (at the stage when all foci of the complex were generating paroxysmal discharges) a fall in the level of paroxysmal activity was observed, as reflected in a decrease in the amplitude and frequency of spike potentials in the dependent foci (Fig. 1C). The amplitude of the epileptic discharges in the determinant focus showed no significant changes in this period. Depression of activity was initially more marked in areas of the neocortex not affected by strychnine, where paroxysmal activity induced from the determinant focus was recorded. Discharges were then suppressed in the foci farthest from the determinant

focus (zone 3) and to a lesser degree in the focus nearest to the determinant focus (zone 2). Besides lowering of the level of paroxysmal activity in these foci, synchronization of the discharges in them was disturbed and the foci began to generate spike potentials independently of one another. At the stage when paroxysmal activity was suppressed in all the dependent foci, it still continued in the determinant focus (Fig. 1D). Later (10-30 min after injection of diazepam) this activity was reduced (Fig. 1E), and in some experiments paroxysmal activity was suppressed in the determinant focus also. Simultaneously with abolition of the epileptic complex, bursts of spindle-like activity were formed under the influence of diazepam in all zones of the neocortex (Fig. 1, C-D). The intensity and rate of appearance of the effects of suppression of epileptic discharges varied depending on the power of the epileptic foci, the distances between them, their localization in different cortical zones, the dose of diazepam, and the individual sensitivity of the animal to the drug. If in the stage of total suppression of the epileptic foci a further application of the concentrated strychnine solution was made to the focus to restore the activity of the complex, spike potentials of relatively high amplitude appeared as a rule only in that focus, synchronized potentials of low amplitude were recorded in the nearest focus to it, and in other zones no activity was recorded (Fig. 1F).

The ability of diazepam to suppress epileptic activity was manifested clearly in animals with mesencephalic section. In cerveau isolé preparations foci of epileptic activity were formed after application of weaker solutions of strychnine (0.05%). These foci appeared more quickly (40-60 sec after strychnine application) than in the cortex of the intact brain, and the more distant foci were more easily united into complexes under the influence of the determinant focus. Marked generalization of the process also took place, as shown by the spread of paroxysmal activity to areas of the cortex not affected by strychnine (Fig. 2A). Under the influence of diazepam generalization ceased and epileptic discharges were preserved: They were grouped together more frequently than in the intact brain (Fig. 2B). Spike potentials disappeared in all foci 15-30 min after injection of diazepam; the disappearance occurred in the same order as in the cortex of the intact brain (Fig. 2, C-F). Simultaneously with suppression of the discharges, a marked increase was observed in the amplitude and frequency of bursts of "spindles" in the neocortex.

The formation of a complex of epileptic foci under the influence of a determinant focus also was observed in the cortex of the hemisphère isolé and cortex isolé preparations (Fig. 3). Under these conditions the level of paroxysmal activity also fell under the influence of diazepam and synchronization of the discharges was disturbed (Fig. 3, B-D).

In cortex isolé preparations epileptic activity formed under the influence of strychnine had certain special features: In many cases it resembled paroxysmal discharges of the spike and after-discharge type (Fig. 3E). These potentials resembled those that are characteristically found after acetylcholine [6]. After administration of diazepam the after-discharge was first reduced and then completely disappeared, after which the negative—positive spike discharges followed the same course, initially in the dependent foci and later in the determinant focus (Fig. 3F).

Diazepam thus depresses epileptic foci and their complexes in the cerebral cortex; the order of depression of these foci, moreover, is the same as that after the use of other agents, such as the so-called cortical narcotics [3]. These effects of diazepam also were observed on cortex isolé preparations and after mesencephalic section, evidence that diazepam acts directly on the cortex. Diazepam is known to stimulate the GABA-ergic system and to potentiate GABA inhibition in the cortex [7, 8, 10-12].

It was shown previously that an increase in excitability and decrease in inhibitory control in certain areas of the neocortex is a condition that enables involvement of these structures in epileptic activity [3]. The decrease in sensitivity of the neocortex to direct epileptic stimulation (in the present experiments, by repeated application of strychnine solutions), and also to impulses arising from the determinant focus, under the influence of diazepam thus contributes to the limitation of secondary epileptogenesis.

The appearance of clearly defined spindle-like activity in the cortex simultaneously with suppression of epileptic activity under the influence of diazepam suggests activation of the thalamocortical mechanism responsible for the formation of spindle-like activity in the cortex. It can be tentatively suggested that generalized epileptic activity, i.e., hyper-

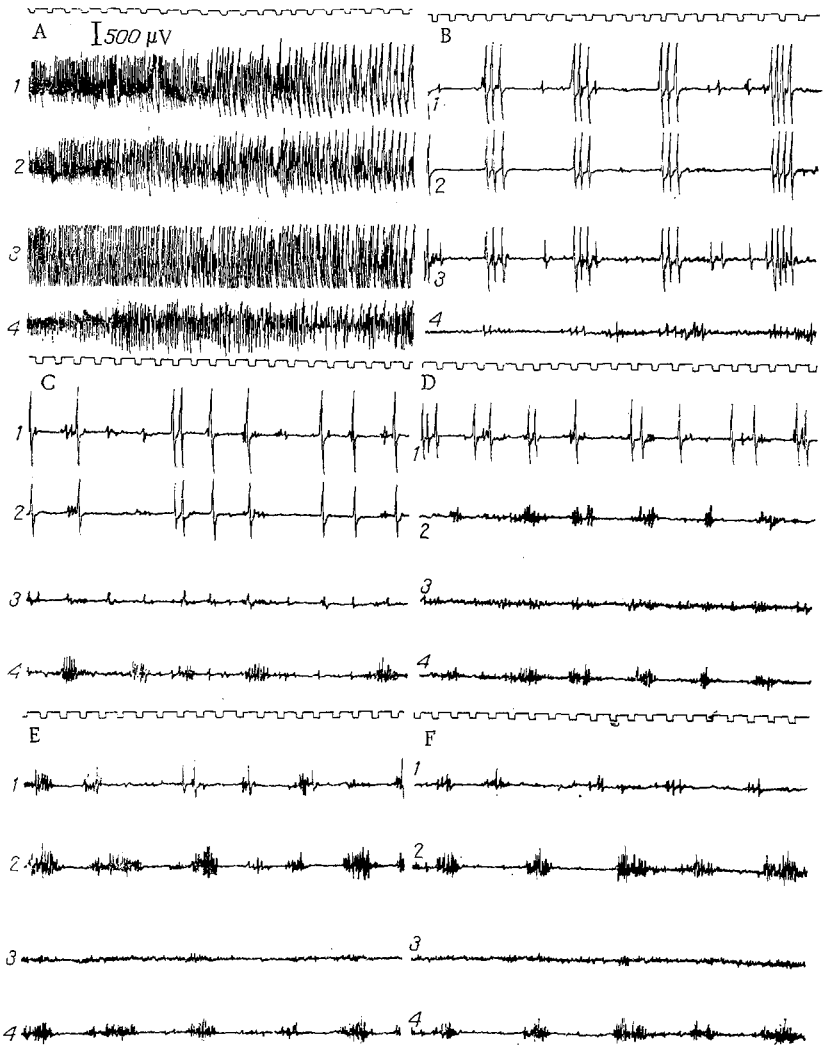

Fig. 2. Effect of diazepam on activity of epileptic complex created in cortex of cerveau isolé. A) Epileptic complex, exhibiting generalized activity with involvement of area 4, unaffected by strychnine, in the epileptic process, created by application of 0.1% solution of strychnine to zones 2 and 3 and a crystal of strychnine to zone 1 (determinant focus); B) 1 min, C) 3 min, D) 12 min, E) 30 min, F) 40 min after injection of 1 mg/kg diazepam. Remainder of legend as in Fig. 1.

synchronization, depresses the natural apparatus of thalamocortical synchronization or modulates it significantly. When epileptic activity is suppressed, on the other hand, this apparatus exhibits its own activity. The physiological thalamocortical synchronization and pathological generalized hypersynchronization in the brain during epileptogenesis may perhaps function by different mechanisms. The fact that complexes of synchronized activity appeared in the cortex isolé suggests that the cortex contains built-in synchronizing mechanisms.

The results of this investigation are also important for the clinical diagnosis of determinant and dependent foci on the basis of the functional heterogeneity of these foci and differences in their resistance to diazepam revealed in these experiments.

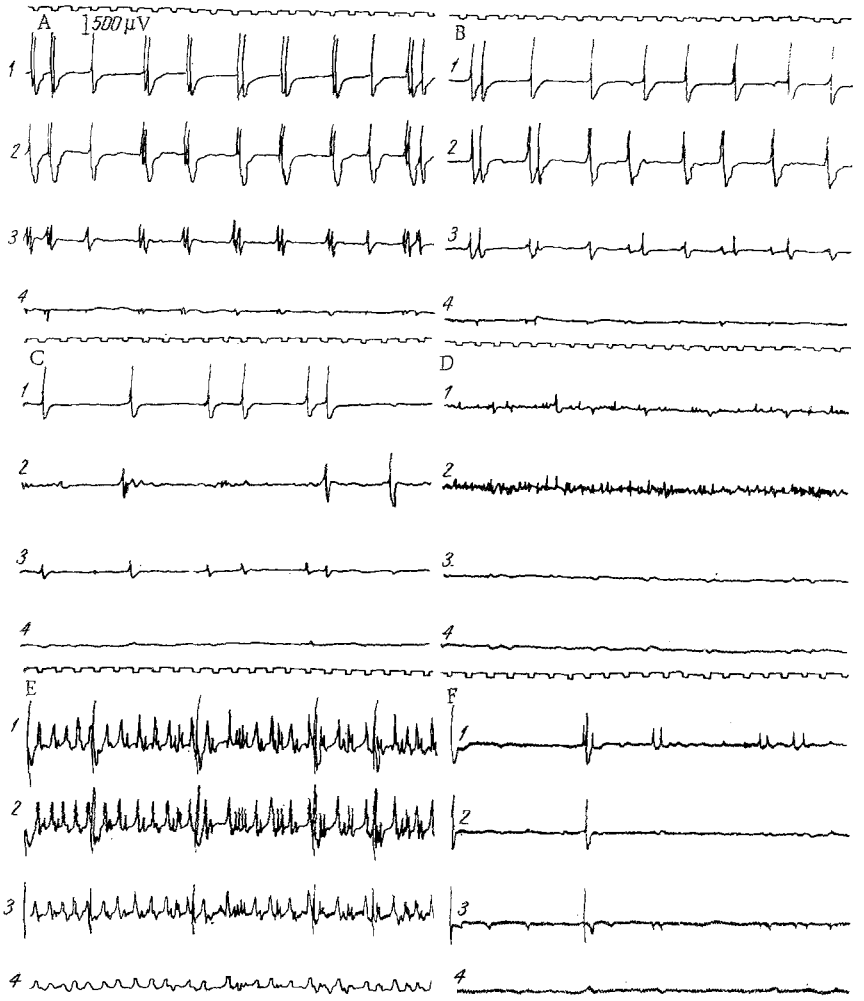

Fig. 3. Effect of diazepam on activity of epileptic complex created in cortex of hemisphère isolé and in cortex isolé preparation. Experiment No. 1: A) epileptic complex created in neocortex of hemisphère isolé by application of 0.1% strychnine solution to zones 2 and 3 and of a crystal of strychnine to zone 1 (determinant focus); B) 4 min, C) 6 min, D) 32 min after injection of 2 mg/kg diazepam. Experiment No. 2: E) epileptic complex created in cortex isolé by application of 0.1% strychnine solution to zones 2 and 3 and of a crystal of strychnine to zone 1 (determinant focus); F) 3 min after injection of 3 mg/kg diazepam. Remainder of legend as in Fig. 1.

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EFFECT OF IMMUNIZATION WITH SMALL DOSES OF ANTIGEN ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

Yu. N. Zubzhitskii and E. G. Alksnis

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Some authorities consider that sensitization of man and animals under the influence of foreign information potentiates the development of atherosclerosis [9, 14]. Investigations [12, 13] have shown, for instance, that keeping animals on a high-cholesterol diet, accompanied by immunization with various antigens, leads to more severe changes in the aorta than the high-cholesterol diet alone for the same duration. However, it should be pointed out that other workers [8, 10] have found that injection of heterologous proteins have a protective effect against the development of atherosclerosis.

In the experiments described above antigens of different types were injected in different doses before the beginning of cholesterol feeding or during feeding, so that the results were not at all comparable. Nevertheless the study of the character of the antigen, its dose, and the times of its injection is an essential factor for the understanding of the role of immunization in the development of experimental atherosclerosis.

The object of this investigation was to study the effect of immunization on the development of atherosclerosis in rabbits with established experimental hyperlipidemia, on the grounds that in atherosclerosis the affected individual is frequently exposed to antigen stimulation, and its role in the course of the disease has not yet been explained.

EXPERIMENTAL METHOD

Experiments were carred out on male rabbits weighing 2.5 kg. Atherosclerosis was induced by feeding the animals on a diet containing 500 mg cholesterol daily [2] for 13-15 weeks.

Both autologous γ-globulin obtained by the salting out method [7] and heterologous (human) therapeutic standard γ-globulin were used for immunization. Heterologous γ-globulin was injected subcutaneously during the 6th or 9th week of cholesterol feeding on three successive days (20, 25, and 30 mg protein, respectively). The course of injections was repeated after an interval of 7 days (total dose of antigen injected 150 mg). Since autologous protein is much less antigenic than heterologous, the course of immunization was longer and was not interrupted [4]: Autologous γ-globulin (total dose 163 mg) was injected in the 11th week of cholesterol feeding on ten consecutive days, intravenously in increasing concentrations (from 5 to 30 mg protein), together with mechanically disintegrated yeast cells (*Candida albicans;* from 1.2 to 12 mg protein, total dose of yeast protein 62 mg). The last injection in both series of experiments contained γ-globulin labeled with ^{125}I [11], and it was given intravenously. A culture of *C. albicans* was used because it has common antigens with vascular tissue structures [6], so that cross-reacting antibodies injuring the vessel

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