Effect of Acoustic Stimulation on GABAergic Neurons in Limbic Structures of Krushinskii—Molodkina Rats

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Quantitative study of GABAergic and main cells in the hippocampus and piriform cortex of Krushinskii—Molodkina rats was performed 1 month after the incidence of seizure activity evoked by acoustic stimulation. The number of neurons significantly decreased in both regions and, particularly, in the hippocampus and central area of the piriform cortex.

Key Words: *Krushinskii—Molodkina rat; auditory epilepsy; hippocampus; piriform cortex; GABAergic cells*

Krushinskii—Molodkina (KM) rats are genetically predisposed to the development of seizure activity in response to acoustic stimulation. The GABAergic system plays the major role in the development of epileptiform activity. Deficiency of GABAergic transmission and increased excitation are also typical of auditory epilepsy [4,5,8]. This disorder is most pronounced in several brainstem structures (*e.g.*, the relay auditory nuclei that respond to acoustic stimulation) and limbic structures (first involved in epileptogenesis) [5,8]. Our previous studies showed that the number of GABAergic cells in the relay auditory nuclei of KM rats significantly decreases 1 month after acoustic stimulation [14].

Here we performed a quantitative study of GABAergic interneurons and main cells in the hippocampus and piriform cortex of KM rats.

MATERIALS AND METHODS

Experiments were performed on adult male KM rats. The animals were placed in individual cages (80×80×80 cm). Acoustic stimulus was presented for 30-40 sec using an electric bell. Further studies were performed on 5 rats that exhibited highest generalized seizures (limb clonus, total rigidity of vertebral muscles, lateral position, ataxia, and asphyxia) in response to stimulation. The behavior of animals was studied daily at 9:30-12.30 and 16:00-19.00 (5 h per day) over 1 month. Facial clonus and head tremor were observed in only 2 specimens. In control rats (n=5), stimulation was not performed.

After 30 days the animals were intraperitoneally anesthetized with sodium ethaminal (40 mg/kg) and perfused with 4% paraformaldehyde in phosphate buffered saline (pH 7.2-7.4) through the carotid artery. The brain was cryoprotected. Serial coronary sections were prepared on a freezing microtome. Adjacent sections were treated with cresyl violet to identify the portions and layers of the hippocampus and piriform cortex. Immunohistochemical study was performed to detect GABA-containing cells. GAD-67 served as the primary antibody in an immunohistochemical study and was detected with avidin-biotin complex (ABC). All reagents and antibodies were manufactured by Santa Cruz Biotechnology. Immunohistochemical treatment of preparations was performed according to manufacturer's recommendations.

Interneurons and main cells were studied in each third section stained with cresyl violet (10

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sections from the animal). Thirty fields of view were randomly selected. We examined radial, oriental, and pyramidal layers of CA1 and CA3, hilum, remaining part of the dentate gyrus, and upper, pyramidal, and lower layers of the anterior, central (middle), and posterior part of the piriform cortex. The study was performed with an ocular morphometric grid (size 0.00625 mm^2, magnification 40×40). The number of neurons and glial cells was calculated as follows: $N=Q^{-}\times 1/t$, where N is total cell number in the relative volume of dissected brain tissue; Q^{-} is cell number in the series of sections; and t is $^1/_5$ [13]. The results were analyzed by means of MINITAB software (Basic Study). The total number of GABAergic cells was estimated similarly.

RESULTS

One month after acoustic stimulation, we revealed a decrease in the number of cells in CA1: interneurons of the oriental layer/alveus, by 74% (1733±393 and 288±33 in the control and treatment groups, respectively, p=0.03); main cells (pyramidal cells) of the pyramidal layer, by 77% (11,860±555 and 2685±60 in the control and treatment groups, respectively, p=0.03); interneurons of the radial layer, by 79% (1203±53 and 250±70 in the control and treatment groups, respectively, p=0.03); and total number of GABAergic cells, by 62% (1900±115 and 538±23 in the control and treatment groups, respectively, p=0.002). The number of cells decreased in CA3: interneurons of the oriental layer/alveus, by 63% (1170±240 and 320±75 in the control and treatment groups, respectively, p=0.03); main cells of the pyramidal layer, by 68% (6575±73 and 2117±67 in the control and treatment groups, respectively, p=0.01); interneurons of the radial layer, by 75% (1028±23 and 253±93 in the control and treatment groups, respectively, p=0.005); and total number of GABAergic cells, by 70% (1909±40 and 574±70 in the control and treatment groups, respectively, p=0.04; Fig. 1). We revealed a decrease in the number of mossy cells in the hilum (1342±73 and 767±33 in the control and treatment groups, respectively, p=0.03) and remaining part of the dentate gyrus (70,338±204 and 46,095±100 in the control and treatment groups, respectively, p=0.04) by 43 and 35%, respectively. The number of cells decreased in the anterior part of the piriform cortex: interneurons of the upper layer, by 77% (282±58 and 183±48 in the control and treatment groups, respectively, p=0.02); pyramidal cells of the pyramidal layer, by 60% (2821±37 and 1123±153 in the control and treatment groups, respectively, p=0.003); interneurons of the lower layer, by 80% (997±71 and 200±30 in the control and treatment groups, respectively, p=0.02); semilunar (main) cells of the lower layer, by 59% (1680±60 and 658±148 in the control and treatment groups, respectively, p=0.008); and GABAergic cells, by 52% (1022±48 and 490±48 in the control and treatment groups, respectively, p=0.03). The number of cells decreased in the central part of the piriform cortex: interneurons of the upper layer, by 79% (889±37 and 191±21 in the control and treatment groups, respectively, p=0.04); pyramidal neurons, by 60% (2846±394 and 1146±105 in the control and treatment groups, respectively, p=0.01); interneurons of the lower layer, by 79% (1035±490 and 198±29 in the control and treatment groups, respectively, p=0.009); semilunar cells, by 58% (1918±321 and 805±252 in the control and treatment groups, respectively, p=0.001); and GABAergic cells, by 69% (1615±47 and 462±68 in the control and treatment groups, respectively, p=0.001). The number of cells decreased in the posterior part: interneurons of the upper layer, by 79% (194±43 and 1142±5391 in the control and treatment groups, respectively, p=0.008); pyramidal neurons, by 60% (1094±120 and 273±135 in the control and treatment groups, respectively, p=0.001); interneurons of the lower layer, by 79% (1077±499 and 228±52 in the control and treatment groups, respectively, p=0.009); semilunar cells, by 63% (1804±199 and 670±109 in the control and treatment groups, respectively, p=0.001); and GABAergic cells, by 70% (1503±43 and 460±15 in the control and treatment groups, respectively, p=0.001; Fig. 2).

Our results show that the number of interneurons and main cells in the hippocampus and piriform cortex of KM rats decreases on day 30 after

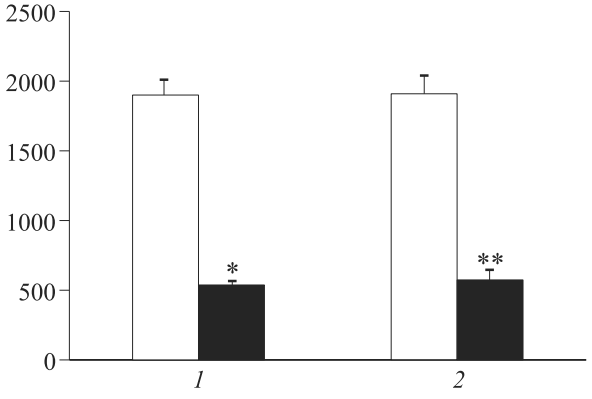

Fig. 1. Decrease in the number of GABAergic cells in the hippocampus 1 month after acoustic stimulation. GABAergic cells in the CA1 (*1*) and CA3 field (*2*). Here and in Fig. 2: light bars, control; dark bars, treatment. Ordinate: number of neurons in the relative volume. *p=0.02 and **p=0.04 compared to the control.

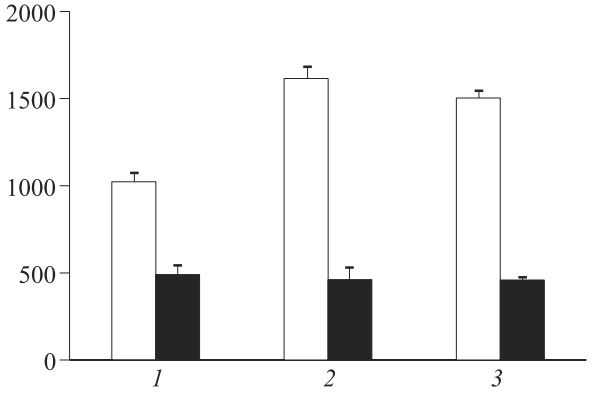

Fig. 2. Decrease in the number of GABAergic cells in the anterior (*1*), central (*2*), and posterior part (*3*) of the piriform cortex 1 month after acoustic stimulation.

the incidence of seizure activity in response to acoustic stimulation. These most "epileptogenic" structures are first involved in epileptogenesis. For example, hippocampal sclerosis develops even in the case of extrahippocampal epileptiform focus. The piriform cortex contributes to the involvement of motor structures in epileptogenesis and plays a role in the development of generalized seizures [3,11,12]. Our previous experiments showed that auditory seizure activity is accompanied by a significant decrease in the number of pyramidal cells and GABAergic cells in the subcortical auditory structure (inferior colliculi of the quadrigeminal plate) [14]. However, comparative study showed that this process is more pronounced in the hippocampus and piriform cortex. Auditory epilepsy in KM rats is accompanied by more pronounced changes in the limbic system, but not in structures directly responding to acoustic stimulation. The number of GABAergic cells in the hippocampus and piriform cortex also decreased in other types of epilepsy [2,3,11]. However, structural disorders of the limbic system in KM rats under conditions of acoustic stimulation are more severe than in other rats exposed to epileptogenic factors. These data confirm high importance of the genetic factor in epileptogenesis. Changes in activity of hippocampal GABA receptors in KM rats on day 30 after acoustic stimulation were previously reported [8]. Electrophysiologically, auditory epilepsy is accompanied by most severe disturbances in the brainstem region. The survived high-plasticity hippocampal neurons probably play a compensatory role, which partly recovers this structure. Therefore, electrophysiological changes were not revealed in the hippocampus. Previous studies showed that the GABAergic system of some brain regions, including the subcortical auditory area and hippocampus, has a specific structure in auditory epilepsy-predisposed animals. Multiple GABAergic interneurons in the inferior colliculi of the quadrigeminal plate can inhibit a considerable number of cells (*e.g.*, GABAergic cells), which leads to rapid development of the epileptiform focus [4,5]. KM rats differ from other animals by the structure of $GABA_A$ receptors in subcortical auditory structures and hippocampus [8,9]. Auditory epilepsy is accompanied by a significant decrease in the number of GABAergic cells in subcortical auditory structures [14] and, particularly, in the limbic system (*e.g.*, in the hippocampus). The hippocampus and piriform cortex contain multiform GABAergic neurons [1,7]. These heterogeneous cells are probably involved in various neuronal pathways of these structures [1,6,7,11]. Significant decrease in the number of a certain type (or types) cells due to epileptogenesis should affect the function of nerve pathways in these structures. The mechanisms of epileptogenesis in GABAergic cells require further investigations.

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