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Cellular Composition of the Piriform Cortex of the Rat Brain in Experimental Epilepsy

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The cellular composition of all layers of the anterior, central, and posterior parts of the piriform cortex of the rat brain was studied two weeks and one month after specific electrical stimulation (kindling) of the ventral hippocampus. Stereomicroscopic analysis at both two weeks and one month after kindling showed significant decreases in the numbers of pyramidal cells and interneurons in all layers of all parts of the piriform cortex. At two weeks, the numbers of pyramidal cells and interneurons in the central part of the piriform cortex also decreased in rats in which electrodes were inserted into the ventral hippocampus but without stimulation. These results, along with published data, led to a series of suggestions regarding the involvement of the piriform cortex in epileptogenesis.

KEY WORDS: experimental epilepsy, piriform cortex, cellular composition, rat.

The main characteristic of epilepsy is the ability of focal convulsions affecting different brain structures to develop into generalized convulsions. The key question for understanding this process is the identification and investigation of these structures. One such structure is the piriform cortex (PC), which is the primary olfactory area. Apart from its involvement in the perception of olfactory information, this area, because of its unique system of associative connections and multiple contacts with other limbic nuclei, is involved in the process of memory and propagation of excitatory waves [1, 2, 6, 15]. This structure is therefore under active investigation in a variety of brain diseases, including temporal epilepsy, which is the most common form of this disease [3]. There are grounds for believing that the neural networks of the PC are critical for the development of epilepsy [15].

Kindling, a specific electrical stimulation of epileptogenic brain formations leading to the development of epilepsy-like states, is one of the most widely used experimental models of epilepsy. The involvement of the PC in the process of epileptogenesis in kindling is demonstrated by various data, including the appearance in this structure of early "epileptic" and interictal discharges in response to electrical stimulation of the "epileptogenic" parts of the brain, a significant increase in glucose utilization in this zone during kindling of the amygdaloid body, induction in the PC of an early gene (c-fos) at the earliest stages of kindling of the amygdaloid body, and more [3, 7, 9].

Nonetheless, our understanding of the importance of the PC in epileptogenesis is far from complete. Many questions require detailed study, these including morphological rearrangements occurring in this area at different stages of epileptogenesis. With the exception of descriptions of structural changes occurring in the PC in kindling of the amygdaloid body [8, 10, 12], this question has received little study.

The aim of the present work was to perform a quantitative analysis of different types of cells in the anterior, posterior, and central parts of the PC after kindling of the ventral hippocampus.

MATERIALS AND METHODS

Studies were performed on 25 male mongrel rats consisting of two experimental groups and one control group. All conditions for studies with experimental animals were observed. Animals of group 1 were anesthetized with sodi-

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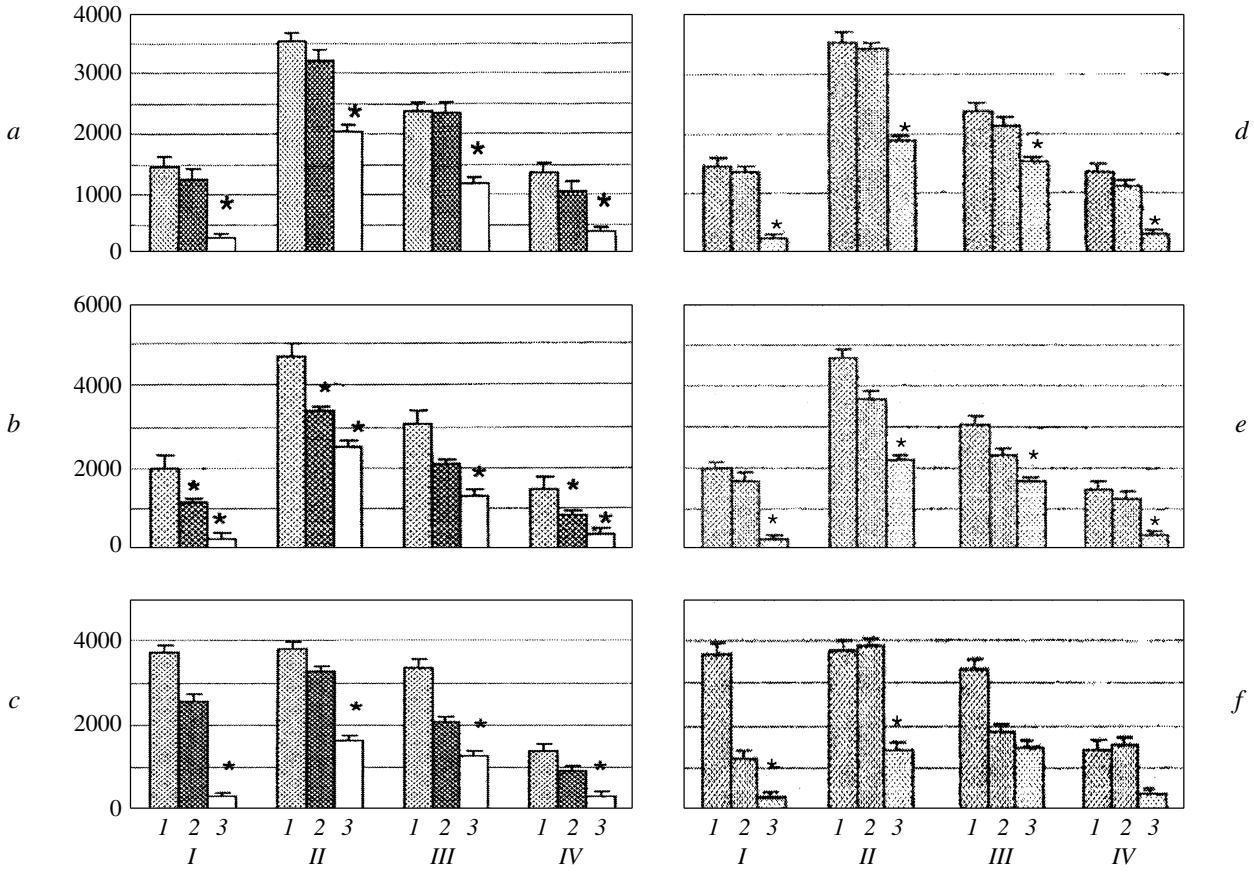

Fig. 1. Numbers of neurons in the rat piriform cortex (PC) two weeks (*a–c*) and one month (*d–f*) after kindling of the ventral hippocampus. *a*, *d*) Anterior part of the PC; *b*, *e*) central part of the PC; *c*, *f*) posterior part of the PC. The horizontal axes show: *1*) controls (intact animals); *2*) implantation of electrodes without stimulation; *3*) kindling; *I* is the number of interneurons in the upper layer; *II* is the number of pyramidal neurons in the middle layer; *III* is the number of neurons in the lower layer; *IV* is the number of interneurons in the lower layer (per standard volume of tissue). Asterisks show significant differences compared with controls. Vertical bars show standard errors.

um ethaminal (40 mg/kg, i.p.) and electrodes were inserted into the ventral hippocampus. This structure was stimulated using a rapid kindling protocol (stimulation parameters for the ventral hippocampus were: duration 10 sec, intensity 450 μA, frequency 40 Hz) on post-operative day 7 [11]; 24 h after stimulation, animals were presented with five test stimuli with 5-min intervals. Further studies were performed using animals showing generalized convulsions (degree IV–V). Electrodes were implanted in animals of experimental group 2 using the same protocol, though stimulation was not applied. Control animals were kept in standard animal-house conditions.

Brains were studied in animals of group 1 at two weeks and one month after completion of test stimulation and in animals of group 2 at the same times after implantation of electrodes. Each study involved the brains from five animals. Control and experimental animals anesthetized with sodium ethaminal (40 mg/kg) were perfused with 4% paraformaldehyde in phosphate buffer pH 7.2–7.4 via the carotid artery. Brains were additionally fixed in the same solution, cryoprotected in special fluids, and serial sections of thickness 4 μm were cut on a cryomicrotome and stained with cresyl violet by the Nissl method. Neuron numbers were analyzed by stereoscopic methods in all layers of the anterior, posterior, and central parts of the PC. Neurons were counted on each fifth section (10 sections for each animal) using an ocular morphometric grid (objective ×40, ocular ×20). The numbers of cells in the PC were calculated as $N = Q \times 1/t$, where N is the total number of cells per unit volume of the brain tissue from which the section was taken, Q is the number of cells in the series of sections being analyzed, and t is 1/5 [14]. Statistical significance was assessed using the Basic Statistic program (Minitab).

RESULTS

After kindling of the ventral hippocampus, a significant decrease in the number of cells was noted at all levels and in all parts of the PC (Fig. 1).

Two weeks after kindling, the proportion of interneurons in the upper layer of the anterior part decreased by 70%, the numbers of pyramidal neurons in the middle and lower layers decreasing by 42% and 50%, and the number of interneurons in the lower layer decreased by 73% (see Fig. 1*a*). In the ventral part, the proportion of interneurons in the upper layer decreased by 80%, the numbers of pyramidal neurons in the middle and lower layers decreased by 47% and 57%, and the number of interneurons in the lower layer decreased by 73% (see Fig. 1*b*). In the posterior part, the proportion of interneurons in the upper layer decreased by 82%, the numbers of neurons in the middle and lower layers decreased by 49% and 60%, and the number of interneurons in the lower layer decreased by 76% (see Fig. 1*c*). In the central part, significant changes were also seen in animals with implanted electrodes but without stimulation (group 2). Thus, there was 43% reduction in the proportion of interneurons in the upper layer, a 28% reduction in pyramidal neurons in the middle layer, and a 43% reduction in interneurons in the lower layer (see Fig. 1*b*).

At one month after kindling, the anterior part showed a 77% decrease in the proportion of interneurons in the upper layer, 44% and 77% decreases in pyramidal neurons in the middle and lower layers, and a 36% decrease in interneurons in the lower layer (see Fig. 1*d*). In the central part, the proportion of interneurons in the upper layer decreased by 86%, pyramidal neurons in the middle and lower layers decreased by 53% and 45%, and interneurons in the lower layer decreased by 74% (see Fig. 1*e*). In the posterior part, the proportion of interneurons in the upper layer decreased by 82%, pyramidal cells in the middle and lower layers decreased by 58% and 55%, and interneurons in the lower layer decreased by 74%. No significant changes were seen in the numbers of cells in any part of the PC one month after electrode implantation without stimulation.

DISCUSSION

Thus, two weeks and one month after kindling of the hippocampus, the various layers of the anterior, central and posterior parts of the PC showed significant decreases in the numbers of pyramidal cells and interneurons. These data again demonstrate the involvement of this structure in the kindling of epileptogenesis. A number of studies have provided evidence for the different levels of readiness of the anterior, posterior, and central parts of the PC for induction of convulsions. In particular, a number of functional rearrangements have been described in response to convulsions evoked in other limbic areas [4, 8, 9]; in kindling of the amygdaloid body, a significant decrease in the number of cells (predominantly interneurons) has been described only in the central part of the PC [12], etc. In contrast to these data, our investigations demonstrated no significant differences in the extent of kindling of different zones of the PC: both time points after electrical stimulation of the hippocampus were sufficient for significant rearrangements to be induced in the cellular composition of its different layers and areas. According to classical data on the structure of the PC [5, 15], the superficial layer is its major input – the axons of the mitral cells making up the lateral olfactory tract terminate on the apical dendrites of the main cells (Ia) and the bodies of superficial (layer II) and deep (layer III) pyramidal cells. The bodies of class 3 pyramidal-like neurons, semilunar cells, are located in the superficial part of layer II and their numerous apical dendrites extend in layer Ia. Associative and commissural inputs terminate in layer Ib and on basal dendrites of layer III. Various interneurons concentrated mainly in layers I and III mediate the coordinated functioning of these structural elements. This highly organized combined activity undoubtedly changes as a result of the kindling-mediated decrease in the numbers of interneurons and pyramidal neurons in the various layers. Thus, the rearrangements in the cytoarchitectonics of the PC occurring as a result of kindling of the hippocampus must be reflected in the receipt and processing of both specific and multimodal information. Although changes applied to the whole cellular composition, interneurons of the upper layers of the central area were of particular note, as the decrease in the relative content of these cells was relatively more marked. One view is that this zone is involved in the temporal transition of kindling from stage III to stage IV–V [8], which suggests its access to structures able to maintain expression of generalized convulsions.

The disappearance of interneurons, the absolute majority of which are gamma-aminobutyric acid (GABA)-containing inhibitory cells, must mediate increased excitation of pyramidal neurons in the PC in kindling. There are two hypotheses explaining how the convulsion-evoked decrease in the number of interneurons (mainly inhibitory cells) initiates epileptogenesis. According to the first, normal inhibition and excitation are maintained by "non-main" cells exposed to treatment, i.e., interneurons; the death of these cells thus deactivates inhibitory influences, with the result that main cells are disinhibited and become hyperexcited. According to the second hypothesis, the initial loss of interneurons stimulates previously non-contacting main cells to form aberrant connections [13].

As regards the decrease in the number of main (pyramidal) neurons (a process often described in the "epileptic" hippocampus), this must mediate increased GABAergic innervation of the remaining pyramidal neurons and, thus, a greater level of inhibition of these cells. Evidently, the sprouting of GABAergic fibers characteristic of this process, as well as the increased hyperinnervation of the surviving projection cells, induces synchronization of their membrane potentials, resulting in subsequent excitation of the inputs which must facilitate initiation of convulsive activity in most hippocampal neurons and its propagation in the surviving parts of the hippocampal formation.

In the transitional zone, the number of interneurons decreased significantly not only after kindling, but also two weeks after electrode implantation. This fact may support known biochemical data showing that electrode implantation into the amygdaloid body without stimulation affects GABA levels in a number of limbic structures [10]. We can thus conclude that kindling of the ventral hippocampus produces significant impairment of all subsections of the PC. This must undoubtedly be reflected in the process of epileptogenesis, at different stages of which these subsections are, in all probability, involved to different extents.

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