Effect Hypokinesia on the Ultrastructure of Rat Neocortex

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The effect of 40, 90, and 120-day hypokinesia and nonstressful hypokinesia on the ultrastructure of cingular convolution, motor and frontal-parietal areas of rat cortex were investigated. Differences were found only in the ultrastructure of cingular convolution after hypokinesic stress. In each area both procedures led to a decrease in the number and variety of spike and axospike synapses.

Key Words: *ultrastructure; hypokinesia; stress; neocortex; rat*

Investigation of reorganization of different parts of brain under different functional conditions is of practical and theoretical significance. The mechanisms underlying the effects of hypokinesia and corresponding morphological changes in the brain have been extensively investigated. There is only superficial knowledge on the effects of hypokinesia on structural alterations in motor and associative areas of the neocrtex, which are directly related to generation of higher motor programs. Here we describe the influence of hypokinesic stress and nonstressful hypokinesia on the ultrastructure of cingular convolution, frontal-parietal (PA^s) and motor (FP^a/FP^p) [5] areas of rat cortex.

MATERIALS AND METHODS

Experiments were performed on 25 mature male random-bred rats. They were divided into control group and experimental groups 5 animals each. Control animals were maintained under normal laboratory conditions. To induce hypokinesic stress, the rats were placed into individual boxes, where the distance from rat body to the box wall did not exceed 5 cm for 40, 90, and 120 days (groups 1, 2, 3 respectively). Nonstressful hypokinesia was modeled by placing of 2-2.5-month-old rats in a larger chamber (20 cm^3) for 90 days. In the beginning of this period small animals could perform numeral motor acts; as the animals were growing, the extent of hypokinesia increased. As a result, the rats could perform single motor acts. Under these conditions stress did not develop due to high adaptational ability of immature organism and gradual restriction of motor activity. The absense of stress was confirmed by normal serum levels of cortisone and adrenocorticotropic hormone and by behavior of rats in the maze and open field tests [6].

The rats were perfused with phosphate buffer containing 2.5% glutaraldehyde (pH 7.2-7.4) through the thoracic aorta under intraperitoneally Nembutal anesthesia (40 mg/kg). Pieces of the cortex were divided into two parts corresponding to the upper and bottom levels of transverse section, postfixed with osmium tetroxide, dehydrated through alcohol and acetone solutions of ascending concentrations, and embedded in Araldite. The sections (thickness 60 nm) were contrasted with lead citrate [10] and examined in an EM-100C electron microscope (JEOL).

RESULTS

Nonstressful and stressful hypokinesia caused small ultrastructural changes in the neocortex. The differ-

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ences were found only in the ultrastructure of cingular convolution, specifically, in some middle-size round cells with spikes and in the axospike synapses. For instance, after 40-day hypokinesic stress and nonstressful hypokinesia weak changes were observed in some mitochondria and cytoplasmic reticulum in neurons. A decrease in the number of microtubules or their shift to the periphery and appearance in the clarified areas of pathologic inclusions were observed in thick dendrites. Considerable regions with normal and altered neurons were isolated by astrosyte processes. Partly due to this isolation the number of active axosomal synapses in some cells decreased from 5-7 to 2-3. However, this decrease was also observed in the nonisolated neurons, which can be due to small number of synaptic terminals arranged on a neuron and the absence of active zone on some of them. In changed axospike synapses the number of synaptic vesicles decreased, they were located far from active zone, synaptic gap was extended, synaptic membranes and contacting surfaces were convoluted, and the structure of synaptic contact was almost symmetrical. The spikes were less numerous than in the control: they were represented by small spike-like processes with underdeveloped or reduced spike apparatus. As the duration of hypokinetic stress increased, the number of spikes and their variety increased, there appeared single neurons with different extent of chromatolysis and synapses at different stages of degeneration by dark type.

Only a small number of the upper-layer small round cells and axospike synapses was changed. These changes were similar to the mentioned above with the exception of neuronal chromatolysis, degeneration of synapses and pathologic insertions in cingular convolution. At the same time, for all periods of hypokinesia (particularly after 120 days) the number of the spikes resembling short spike-like processes decreased considerably. Nonstressful hypokinesia also induced a decrease in the number and variety of spikes in the upper layer of motor cortex. Other changes were not observed.

The structure of the frontal-parietal area remained practically normal after both procedures. Only in the upper layers single neurons and axospike synapses with insignificant changes of mainly functional character were observed. At the same time, the number and variety of spikes was considerably lower after both procedures, which was more obvious in the upper layer.

Thus, in the investigated areas of rat neocortex hypokinesia combined with stress induced greater structural changes than hypokinesia alone. Both influences led to structural alterations only in a relatively deep layers of limbic zone, namely, cingular convolution, whereas frontoparietal and motor areas respond weakly. It was shown that hypokinesia induces pronounced structural disorders in the amygdala and hyppocampus [4,13]. This may be due to the fact that these structures are more sensitive to external stimuli, while neocortex areas are characterized by high compensatory abilities, which allows them to retain normal organization as long as possible [1,3]. Glial cells may play a certain role in protection of these areas. For instance, after different periods of hypokinesic stress the number of neurons with satellites and individual satellite forms in motor areas increases considerably [3].

In all studied brain regions, including practically native frontal parietal cortex, we revealed a common feature for both types of hypokinesia: a decrease in the number and variety of spikes, and, accordingly, of axospike synapses. It is known that these dendritic structures are involved in intimate reactions of neurons. Their large number and variety are the characteristic features in highly organized associative brain regions where convergence of polymodal signals is realized. The number, size, and shape of spikes as well as the degree of development of the spike apparatus depend on the activity of nervous system: its stimulation increases and suppression decreases these parameters [8,11]. Therefore it cannot be excluded that the reaction of spikes to hypokinesia is associated to a certain extent with reorganization in convergence due to sensory deprivation of proprioceptor and other sensory receptors. This suggestion is supported by the findings that structural changes occur in the upper layers of motor and frontal-parietal areas, which are most significant for the convergence processes [1,2,7].

Interestingly, in each area after both procedures alterations were observed predominantly in neurons. Since this is also true for other parts of the end brain [11], it can be suggested that the investigated neocortex areas contain cells which are highly sensitive to hypokinesia and stress and other secondary factors. It is difficult to identify these cells only on the basis of electron microscopic data; nevertheless, these data show that even prolonged and essential restriction of motor activity does not lead to changes in the structure of large pyramid cells in the lower layers of motor area, and are similar to the projectional cells of the main pyramidal way in rodents. It was shown that reaction of limbic area to hypokinesia is the same as that of other investigated areas of the neocortex and significantly differ from that in the limbic areas: amygdala and hippocampus [13]. This argues in favour of its high associative properties, owing to which the synthesis of neocortical and limbic information can occur there. It was suggested that this

area is part of associative (but not of limbic) zone of the neocortex [9,12].

Our results show that hypokinesia by itself does not lead to significant structural changes in associative areas of the neocortex; combined action of hypokinesia and stress factors leads to alterations only in the limbic area, which may be linked to its function: as an "emotional" region it directly responds to stress. However, the structure even in this region is retained practically native, structural changes are less numerous and superficial, pointing to the possibility of their restoration.

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