ULTRASTRUCTURAL CHANGES IN THE CEREBRAL CORTEX AFTER TRANSCRANIAL MICROPOLARIZATION

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An electron-microscopic investigation of the cerebral cortex of cats and monkeys after transcranial micropolarization (TCMP) revealed ultrastructural changes the severity of which depended on the intensity of the current and the duration of its action. In the focus of exposure the current acted directly on the brain tissue, but the cerebral cortical cells differed in their sensitivity to different conditions of TCMP. The most reactive tissue was the glia, followed by the bodies of neurons and synaptic structures. In areas of brain remote from the focus of TCMP the ultrastructural organization of the synapses was principally altered. The morphological changes discovered were not pathological.

KEY WORDS: transcranial micropolarization; ultrastructural changes; glia; neuron; synapse.

Exposure of various brain structures to an electric field is an effective method of treating certain diseases of the brain [4]. It has also been shown that micropolarization (MP) of neocortical and deep brain structures can be used to exert a controlled influence on conditioning and on learning and memory processes [7, 6]. Meanwhile, most neurophysiological and morphological investigations have been undertaken by the use of electrodes implanted directly into the brain tissue [2, 7, 6]. The method of transcranial micropolarization (TCMP), suggested by G. V. Gal'dinov and S. P. Shklyaruk working in the writers' laboratory, is a more sparing method and offers wide opportunities for acting on the brain not only for experimental purposes, but also, under certain conditions and within certain limitations, in clinical practice.

The object of the present investigation was to study the ultrastructure of the cerebral cortex after TCMP.

EXPERIMENTAL METHOD

The brains of cats and monkeys exposed to various regimes of TCMP in acute experiments were used (the physiological investigations were carried out by G. V. Gal'dinov and S. P. Shklyaruk). The following intensities of TCMP were used: 1) 0.5 mA for 5-7 min; 2) 0.5-1 mA for 30 min; 3) 1-2 mA for 2-3 h. Percutaneous agar electrodes 1.5 cm^2 in area were used to produce TCMP. TCMP was applied to the temporal, occipital, prefrontal, and frontal regions of the cortex. The middle layers of zones of the cortex beneath the electrodes and of distant zones were studied with the electron microscope. The brains of intact animals served as the control.

Material was fixed by the immersion method successively in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and in 1% OsO_4 solution in the same buffer, then dehydrated in alcohols of increasing concentrations and embedded in Araldite. Ultrathin sections were cut on the LKB Ultrotome, stained with uranyl acetate solution and lead citrate, and studied and photographed in the IEM-7A and IEM-100B electron microscopes. Attention was concentrated on morphological changes in neurons, synapses, and glia.

EXPERIMENTAL RESULTS

Ultrastructural analysis showed that different components of the cortical nerve tissue of the brain differed in their sensitivity to different regimes of TCMP.

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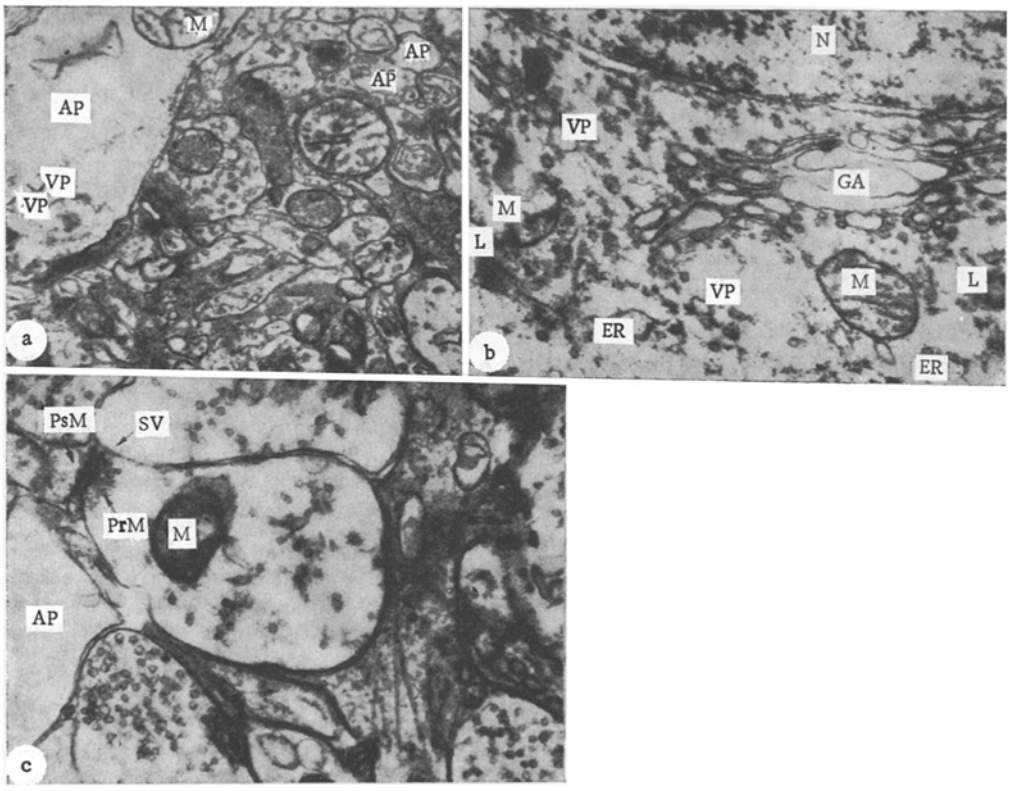

Fig. 1. Cerebral cortex of monkey after exposure to TCMP: a) swollen astrocytic processes in middle layers of motor cortex (current 0.5 mA, 5-7 min, 15,000 ×); b) nucleus and cytoplasm of neuron in layer III of visual cortex (current 0.5-1 mA, 30 min, 15,000 ×); c) axodendritic synapse with a few synaptic vesicles grouped near presynaptic membrane (current 1-2 mA, 2-3 h, 25,000 ×); GA) Golgi apparatus; AP) astrocytic processes; VP) vacuole-like profiles; L) lysosomes; M) mitochondria; PrM) presynaptic membrane; PsM) postsynaptic membrane; SV) synaptic vesicles; ER) endoplasmic reticulum; N) nucleus.

In areas of the cortex where very weak polarization was applied with a current of not more than 0.5 mA for 5-7 min, a marked glial response was primarily observed (Fig. 1a): an increase in the number and swelling of the processes of the astrocytes, a decrease in the electron density of their matrix, vacuolation, and fragmentation of the cristae in the mitochondria. Among the oligodendrocytes, fewer modified cells were observed than among the astrocytes. Structural changes in components of the nerve cells were not significant.

Glial cells respond to different procedures sooner than neurons. For instance, the glia has increased sensitivity to neuropharmacological agents, to the action of a magnetic field, to x rays, and to the development of cerebral edema [1, 12, 15]. In other words, with its protective and barrier functions, the glia apparently takes the first "shocks" of any procedure and responds by various structural changes.

Under the influence of a current of 0.5-1 mA for 30 min, morphological changes were found not only in the glial cells and their processes, but also in the perikaryon of a certain proportion of neurons (Fig. 1b). In such neurons the nuclear chromatin was distributed irregularly and there were many deep invaginations of the nuclear membrane, and the perinuclear space was widened. An increased number of vesicular structures, lysosomes, multivesicular bodies of varied electron density, widening of the cisterns of the Golgi apparatus, swelling of the mitochondria with localized areas of translucency of the matrix and with partly destroyed cristae were observed in the cytoplasm of some cells. In other neurons, widening of the cisterns of the endoplasmic reticulum, disappearance of a sharp reduction in the number of ribosomes on the membranes of the tubules, vacuole-like profiles, and absence of organelles from some segments of the cytoplasm were observed. Under these circumstances synapses whose structural components showed signs of submicroscopic changes were found in the neuropil: swelling of many of the mitochondria and a decrease in the number of cristae, the appearance of polymorphic synaptic vesicles, changes in the number of vesicles, and changes in the electron density of the synaptic membranes.

The most marked morphological changes in the nerve and glial cells were observed in areas of the cortex where stronger polarization with a current of 1-2 mA and an exposure of 2-3 h was used. The number of synapses with structural changes also was appreciably increased (Fig. 1c). These changes consisted of a marked decrease in the number of synaptic vesicles, their concentration in a single complex close to the presynaptic membrane, the appearance of vacuole-like profiles or laminated membranous structures, and swelling and translucency of the matrix of the axon terminals. At the same time, synapses with agglutination of the synaptic vesicles located in the center of the terminal or filling it entirely, and with increased electron density of the synaptic membranes, were found. In unpolarized areas of the cortex remote from the focus of exposure, various changes also were found in some synapses.

Under acute experimental conditions, TCMP thus causes ultrastructural changes in the cerebral cortex, the severity of which depends on the intensity of the current and the duration of its action.

In the focus of exposure the current acts directly on the brain tissue, but different structures of the cortex differ in their sensitivity to different TCMP regimes. Most sensitive is the glia, followed by the bodies of neurons and synapses. In areas of the brain remote from the focus of TCMP the ultrastructural organization of the synapses was most affected, evidence of the trans-synaptic influences of foci of MP on distant brain structures.

It is difficult at present to identify the morphological changes which are purely functional in character from changes thay may subsequently become irreversible and pathological. Changes in the functional state, with an increase or decrease in functional activity of nerve tissue are of necessity accompanied by fine ultrastructural changes. Such ultrastructural changes have been found in the CNS under the influence of amphetamine, chlorpromazine, photic stimulation, motor overloads, and electrical stimulation [5, 8, 9, 14]. The authors cited regard these changes as a reflection of the intensification of metabolism in the event of their "activation," i.e., they place these ultrastructural changes in the category of functional changes.

In the present experiments the varied intensity of the structural changes in the nerve tissue point to a high degree of polymorphism and heterogeneity of the response to TCMP. The polymorphism was perhaps due to differences in the functional background of activity of the cells and, correspondingly, to differences in their sensitivity to the action of the current, as in the case of responses to various pharmacological and other agents [9]. Similar submicroscopic changes in the synapses during exposure to various factors have led some workers to regard the structural changes as different "degrees" of functional stress [9, 14]. Eccles [13], in particular, considers that changes in presynaptic structures are evidence of activation of synapses.

Consequently, the direct and indirect evidence obtained suggests that the ultrastructural changes do not go beyond the limit of functional changes taking place in the cerebral cortex after TCMP.

The process of storage and retrieval of information is linked by many workers with particular changes in nerve tissue; information processing is based both on active interaction between neurons and glial cells and, on increased conductance of the synapses. The more strongly the synapses and the whole of the neuronal pathway are activated, the more effective the memory [3, 10, 11, 13].

The present experiments showed that TCMP causes submicroscopic changes in the glia, neurons, and synapses, proof of the effectiveness of this type of procedure on brain tissue. Further investigations will probably help to reveal the connection between these changes and trace formation – a most important property of nerve tissue.

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SPECIFIC SECRETORY INCLUSIONS IN THE PYLORIC PART OF THE MOUSE STOMACH

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Specific granules were found in the epithelium of the pyloric part of the mouse stomach on staining with aldehyde-fuchsin. They were PAS-negative and did not stain with lead hematoxylin. Their production in intact mice is limited to a small zone of the pylorus, with the appearance of a circumscribed spot. α-Adrenergic stimulation caused widening of the zone in which these granules were found. Combined treatment with Inderal and general cooling led to a sharp increase in the zone of distribution of the cells containing these granules.

KEY WORDS: epithelium of pylorus; secretory granules; adrenergic stimulation; exposure to low temperatures.

Inclusions of mucoid and mucin and inclusions containing enterohormones are found in the epithelial cells of the gastrointestinal tract.

This paper gives the results of experiments showing the presence of special and hitherto undescribed inclusions in the stomach, revealed by staining with aldehyde-fuchsin. In intact animals these inclusions are found only in a narrow zone of the pylorus. In other parts of the stomach and in the intestine, no such inclusions are found and, for that reason, they are called specific inclusions.

EXPERIMENTAL METHOD

The pyloric part of the stomach of albino mice was investigated. Experiments were carried out on 15 mice, of which six were intact. Seven mice received injections of adrenoblockers for 1 week: Two animals received Inderal (propranolol), 3 received dihydroergotoxin, and 2 received both adrenoblockers simultaneously. The other two mice received Inderal and exposed to general cooling at 5°C. Material from the experimental animals was fixed by Bouin's method and that from the control animals in Bouin's or Carnoy's fluid or formalin and embedded in paraffin wax. Serial sections were cut transversely and longitudinally to the pylorus and stained with aldehyde-fuchsin (preliminary oxidation with permanganate, counterstaining with hematoxylin-orange), and lead hematoxylin and by the PAS reaction.

EXPERIMENTAL RESULTS

On staining the epithelial cells with aldehyde-fuchsin, the apices of the cells containing mucoid stained diffusely. In addition, in a small proportion of cells bright dark violet granules could be seen. They had distinct outlines and were distributed throughout the cytoplasm. The concentration of these granules was not predominantly high in the apical or basal parts of the cells (Fig. 1). Sometimes the granules were concentrated around the nucleus (Fig. 2). In intact mice cells with granules of this type were found in a small zone of the pylorus, close to its junction with the duodenum. The zone was oval in shape and contained 20 to 30 pits. The

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