MORPHOLOGICAL BASES FOR EVOLUTION OF FUNCTIONS

Histochemical Study of Effects of Weak Electromagnetic Field on Structures of the Rat Midbrain

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Abstract—Histochemical activity of the mitochondrial enzyme cytochrome oxidase (CO) in auditory and non-auditory structures of rat midbrain was studied after exposure to low electromagnetic field (EMF). An increase of the enzyme activity was shown in several midbrain structures of the experimental animals.

INTRODUCTION

Susceptibility of CNS to action of ultrahigh-frequency electromagnetic fields (UHF EMF) of low intensity is well known. Bioeffects of this action have been studied at various levels: from molecular to behavioral [1]. Studies on the EMF effects on the nervous system of the higher vertebrates have been performed for the last 15 years at the Laboratory of Physiology of Sensory-Motor Systems of Ukhtomskii Research Institute of Physiology. Results of these studies have shown the impulse-modulated electromagnetic irradiation of low power to change parameters of bioelectric activity of the cerebral cortex of animals [2]. Mapping of the brain by histochemical method of detection of cytochrome oxidase (CO) allowed suggesting that EMF stimulated auditory and somatosensory peripheral receptors, while this effect as mediated by CNS ascending tracts affected certain brain centers [3]. Analysis of behavioral responses also indicated a possibility of perception of weak electromagnetic signals. Their effects on animal organism produced behavioral responses that are similar with orientation reactions to species-specific auditory signals [4]. Analysis of structural changes occurring in Corti organ after the EMF irradiation of animals

provided direct evidence of the EMF action on auditory receptors [5]. Stimulation of sensory receptors implies changes of neuronal activity of certain CNS structures. In turn, the neuronal activity is inexorably connected with certain metabolic needs of the nervous tissue and can be revealed histochemically, including Wong-Riley method of detection of cytochrome oxidase (CO) [6].

The goal of the present study was histochemical study by the CO-method of EMF effects on metabolic activity of the auditory (inferior colliculus, IC) and non-auditory (superior colliculus, SC, and the periaqueductal central gray matter, CGM) centers of the rat midbrain.

MATERIALS AND METHODS

The study was carried out in 12 breedless adult white male rats. The animals were divided into 4 groups, in each of which 2 animals were EMF irradiated, while one animal remained control. The animals were irradiated for 5 days at the same day time, for 2 h/day. The animals during the experiment were placed in the transparent plastic boxes that were inside a shielded sound-proof room measuring $3 \times 2.5 \times 2$ m. Irradiation with impulse-modulated UHF electromagnetic field was produced

Fig. 1. Distribution of CO activity in binary converted images of IC sections of intact (b) and irradiated (a) rats. Magnification $\times 20$. Arrows indicate mediolateral orientation of the structure; dots indicate lateral boundary of the structure.

using a GTs-31 generator and a megaphone-like antenna in the end of the echo-free chamber. The carrying frequency was 980 mHz, the frequency of modulating signal was 1 kHz with duration of 0.5 ms. The energy flow density of the EMF of the UHF was 0.5 mW/cm^2 . The level of the applied power was determined using a Ya6P-110 measuring device. Control animals were placed in the same chamber for the same time period, but with the switched-off generator. To avoid artifacts due to possible circadian fluctuations of the brain metabolic activity, the alternation of the stay of control and experimental animals in the chamber was changed during the experiment. After the last irradiation the brain of the animals was treated by the Wong-Riley original method [6]. To identify cytoarchitectonic structures, the obtained material was compared with Nissl-stained preparations.

The computer analysis of midbrain sections was performed using an installation with a video camera and software Mathcad and Gimp for video image processing. The processing procedures developed in the Laboratory [7] were as follows: binary image conversion whose essence is to reduce the number of gray colors to two—black and white, which allows demonstrating illustratively changes of the spatially arranged distribution of CO activity; construction of the optical density graphs that

allow demonstrating graphically the enzyme activity dynamics.

RESULTS

In the inferior colliculus (IC) of intact animals, in the frontal sections treated by the CO method, the same nuclei are differentiated by their enzyme activity, which are identified cytoarchitectonically. The highest level of CO activity is found in the central nucleus; its surrounding pericentral and external nuclei are characterized by a lower level of the enzyme activity. A small increase of staining is observed in the ventromedial part of the central nucleus. After the EMF irradiation two areas with increased CO activity appear in the IC: one of them is located in the central nucleus medioventrally, while the other, laterally, at the boundary of the IC central and pericentral nuclei. Boundaries of this area are determined after a binominal image transformation of the IC frontal sections (Figs. 1a, 1b).

In frontal sections of the intact rat midbrain on treatment with the CO method, a lamination is revealed at the level of the superior colliculus (SC) due to the heterogeneous distribution of the enzyme. The superficial complex of the layers is characterized by the highest CO activity. The optic tract layer with a low reaction is present between the su-

Fig. 2. Distribution of CO activity in binary converted images of SC sections and optical density graphs of the middle SC layer complex of intact ((a), (c)) and irradiated ((b), (d)) rats. Magnification $\times 10$. *Short arrows* designate columns with high CO activity; *long arrow* on the right indicates rostrocaudal (r, c) direction of sections.

perforial and middle layer complexes. Middle layer complex has a moderate CO activity. In intact animals the enzyme distribution along the layers is homogenous (Fig. 2a).

In frontal sections of SC after the EMF action, the three-layer complexes are seen as clearly as in control, but the enzyme distribution character changes. In the middle complex a heterogeneous

Fig. 3. Distribution of CO activity in the rat CGM. Magnification $\times 20$. Columns: M—medial, VLd—ventrolateral dorsal, VLv—ventrolateral ventral, D—dorsal, DL—dorsolateral; AQ—Sylvian aqueduct, nIII—the nerve nucleus.

CO distribution is observed in the form of alternating columns of the moderately and weakly stained neuropil (Fig. 2b). The columns are clearly seen at the rostral and middle SC levels and became smoothed at the caudal levels. Graphs of optical density confirm these observations and demonstrate regular alternation of zones with the more and less high CO activity (Figs. 2c, 2d).

The central gray matter (CGM) of the rat mid-brain is shown by our previous study to be divided to 5 columns differing by the enzyme activity [8]. The medial column (M) surrounds the Sylvian aqueduct and has a low level of the CO activity. Ven-

trally, the highly active ventrolateral dorsal (VLd) and the moderately active ventrolateral ventral (VLv) columns are located, the latter bordering the highly active III nerve nucleus adjacent to CGM. Another highly active column of CGM, the dorsal one (D), is located dorsally to M. Between the columns VLd and D with high CO activity there is present an area with the moderate level of the enzyme activity—the dorsolateral (DL) column of the CGM (Fig. 3). Study of CGM after the EMF irradiation of the animals shows non-uniform change of the enzyme activity within this structure (estimation was performed by measuring optical densi-

ty of individual columns). Since the histochemical CO detection was performed in cryostat sections whose thickness can somewhat vary, dynamics of the enzyme activity was judged by difference of optical density of the studied structure and the mean optical density of the whole section. A preliminary estimation of the mean optical density of the whole preparation indicates that this value varies depending on the section thickness, but does not differ in intact and experimental animals. Thereby, we eliminated errors related to different thickness of sections. The obtained value was designated as the relative optical density ($\Delta\tau$). Results of the performed study showed that the relative optical density of the CGM columns after the EMF action changes in comparison with these parameters in intact animals as follows: this density in the columns D and VLd increases, in the column M it remains constant (Fig. 4). The statistical significance of differences of the relative optical density of the CGM columns in experimental and control animals was evaluated according to Fisher's criterion. According to this evaluation, the optical density of the columns D, VLv, VLd, and DL changes statistically significantly, whereas that of M—insignificantly.

DISCUSSION

Our previous study performed by photometry method [3] showed the CO activity in rats after UHF EMF action to rise in the dorsal cochlear nucleus, lateral segment of olive, and central nucleus of the IC. The present work has established the appearance of 2 areas with increased CO activity in the rat inferior colliculus after the EMF action, one of them located in the medioventral portion of the central nucleus, the other located laterally, at the boundary of the IC central and pericentral nuclei, in the zone involving their border areas.

The IC central nucleus is arranged tonotopically, its medioventral portion corresponding to the site of projection of the high-frequency part of the inner ear cochlea [9]. The lateral area of the IC central nucleus is described in rats and other animals on the basis of other connections than other parts of the structure, of increased CO activity, special functional characteristics of its neurons, and high density of GABAergic receptors [10–13]. An opinion is put forward that this area participates in pro-

Fig. 4. Relative optical density of CGM columns in intact animals and in animals irradiated with UHF EMF. *Abscissa:* CGM columns, *ordinate:* their relative optical density. (1) Norm, (2) EMF.

cesses providing location of the sound source and is activated by presentation of a stimulus of any frequency [11].

Thus, the change of CO activity of the inferior colliculus after the EMF action occurs in the same way as after exposure to high-frequency acoustic stimuli.

As shown in the present study, after action of EMF, in the middle layer complex of the rat superior colliculus there appear alternating columns with moderate and high CO activity. The appearance of columns with high CO activity in the middle layer complex and deep gray layer of the rat SC was found after a unilateral cochlea destruction, which seems to be due to the spatially arranged organization of afferents coming here from auditory structures [14].

This suggestion is confirmed by the data of morphological and electrophysiological studies. The study of evoked potentials in SC neurons in mouse [15], cat [16], and guinea pig [17] indicates that the neurons located in the middle and deep layer complexes and responding selectively to auditory and somatosensory stimuli form clusters oriented perpendicularly to the SC surface.

In the light of the above data, the non-uniform change of CO activity after EMF irradiation can be accounted for (as shown in our earlier study [5]) by susceptibility of auditory receptors to this action and by a subsequent change of activity of auditory of ascending tracts that have discrete terminal areas in the SC; this results in changes of metabolic activity of certain pools [5].

According to results of the performed study, the

CO activity of CGM columns after EMF action changes as follows: in D and VLd it increases, in DL and VLv it decreased, and in M it does not change.

The midbrain CGM is a polyfunctional structure participating in organization of species-specific vocalization, endogenous analgesia, control of some behavior forms and visceral functions [18]. The complex character of connections, transmitter heterogeneity, and incomplete information about functional role of CGM substructures makes it difficult to interpret the obtained data. A suggestion about their functional role can be made based on peculiarities of connections of individual CGM columns [19–24]. Some nuclei of the brainstem reticular formation, zona incerta, and somatosensory cortex project to all CGM substructures. However, other connections are related to certain CGM columns. Thus, analysis of connections of the VLd and D columns allows suggesting that interaction of motor, somatosensory, and limbic systems occurs on neurons of these columns, while efferents are sent to motoneurons innervating muscles of tongue and larynx as well as facial and masticatory muscles, i.e., the structures that participate in sound generation. Besides, the column D has an output to neurons of plates 6–8 of the spinal cord C₄–T₈ segments and auditory relay structures, i.e., its neurons seem to interact in regulation of the auditory–motor coordination. The DL-column, apart from the afferents common to the whole CGM, also has reciprocal connections with hypothalamus, while VLv and M form internal connections of the CGM.

Thus, the increase of CO activity after EMF action occurs in the CGM columns that receive polysensory inputs including the auditory ones and project to sensory and motor CNS structures.

In summary, it can be concluded that the studied midbrain structures respond differently to the EMF action. The character of this response allows suggesting that it is related to EMF effects on auditory receptor cells and subsequent activation of ascending tracts that mediate effects of electromagnetic fields not only on auditory, but also on integrative brain centers. Probably, the known behavioral responses to EMF effects are a consequence of activation of these structures.

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