ACTION OF AN ELECTROMAGNETIC FIELD ON CONDITIONED REFLEX RETENTION AND NUCLEIC ACID CONCENTRATION IN BRAIN TISSUES AFTER CAROTID ARTERY COMPRESSION

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UDC 616.831-008.1-02:616.133-089.814-092.9]-085.849.11-07:[616.831-008.939.633.2+616.831.31-092:612.833.81.

KEY WORDS: brain; conditioned reflex; compression of the carotid arteries; RNA, DNA, irradiation with decimeter waves.

Reports have recently been published on a new method of treatment of cerebrovascular diseases by electromagnetic fields [7, 8].

In this connection it is interesting to study the action of an electromagnetic field in the decimeter waveband (DMW irradiation) on restoration of brain function when the circulation in the carotid arterial system is disturbed. The aim of the present investigation was to study the action of DMW irradiation of the brain in rats after compression of the carotid arteries on reproduction of a defensive conditioned reflex and on the DNA and RNA levels in brain structures.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 54 noninbred male rats weighing 150-200 g. The cerebral circulation was disturbed by bilateral compression of the common carotid arteries under ether anesthesia for 10-12 min. The depth of disturbance of brain function and the pattern of its recovery in all the rats were assessed quantitatively on the basis of the ability of the animals to reproduce a defensive conditioned passive avoidance reflex formed before compression. The reflex was formed in a modified setup [11] consisting of an illuminated (safe) compartment and a darkened (dangerous) compartment, connected by an opening. An electric current could be passed through the wire mesh floor of the darkened compartment. Under the conditions used the rats received electrical stimulation with a voltage of about 40 V. To form the reflex the animals were placed in the illuminated compartment of the apparatus facing away from the opening. The rat quickly ran into the dark compartment where it received an electric shock, which lasted until the rat returned into the safe compartment. After this the rat was immediately removed from the apparatus and this concluded the procedure of reflex formation. To test reproduction of the reflex thus formed the animal was replaced in the illuminated compartment and the length of time it remained there before entering the darkened compartment was recorded. If for 600 sec the rat did not move into the darkened compartment, it was removed from the apparatus and the reflex was considered conventionally to be completely retained.

The experiment began with formation of the conditioned reflex in the rats. The stability of its formation was tested after 2 h. Operations were then performed on all the rats and they were divided into two groups; control and experimental. Retention of the reflex was again tested 2 h after compression and on the 2nd day. In the experimental rats testing retention of the reflex was preceded by DMW irradiation.

DMW irradiation was given by means of a G4-37A high-frequency signal generator, generating electromagnetic waves in the decimeter band with a frequency of 460 MHz. During irradiation the cylindrical BYa2 093 292 source pointing along the axial line of the body was firmly applied to the rat's head. Irradiation lasted 3 min. The energy flux density was

Laboratory of Physiology, Experimental Division, Research Institute of Health Resorts and Physiotherapy, Ministry of Health of the USSR, Moscow. [Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh (deceased).] Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 62-64, May, 1983. Original article submitted September 14, 1982.

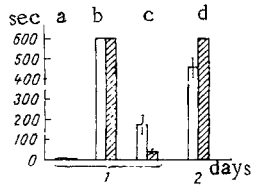

Fig. 1. Action of DMW irradiation of rat's head after bilateral compression of the common carotid arteries on reproduction of defensive conditioned reflex. Abscissa, time after compression of carotid arteries (in days); ordinate, duration of rat's stay in illuminated compartment before moving into darkened compartment where it received an electric shock (in sec). Unshaded columns — control rats not subjected to DMW irradiation after compression of carotid arteries; shaded columns — experimental rats subjected to the action of DMW irradiation after compression of carotid arteries. a, b) Conditioned reflex formation, c) reproduction of reflex after compression of carotid arteries.

80 mW/cm^2. The choice of dose was determined by the activating action of similar doses of DMW irradiation on the genetic apparatus of brain neurons [5].

Animals which tolerated DMW irradiation on the 2nd day after the operation and survived were decapitated by guillotine after retention of the reflex had been tested. The RNA and DNA concentrations were determined [9] in tissues of the cerebral cortex and brain stem by means of an SF-4A spectrophotometer in animals of three groups (five rats in each group): intact, experimental, and control. Rats with compressed carotid arteries, subjected to the same manipulations except DMW irradiation, served as the control. The experimental results were analyzed by Student's t test and the Wilcoxon–Mann–Whitney U test.

EXPERIMENTAL RESULTS

The duration of the rats' stay in the illuminated compartment 2 h after reflex formation was 600 sec, i.e., complete retention of the reflex was observed (Fig. 1a). After compression of the carotid arteries, reproduction of the reflex was worsened in both control and irradiated rats (Fig. 1b). DMW irradiation of the rat's head worsened reproduction of the reflex by a much greater degree than in the control ($P < 0.01$).

On the 2nd day after compression, immediately after repeated DMW irradiation the state of the rats worsened sharply in 63.6% of cases, as shown by rapid and haphazard running of the animals, sudden jumps, and convulsions which terminated in death of the rats. In the control group on the 2nd day after the operation some rats also died, but far fewer (21.2%) than in the experimental group. Comparison of mortality among rats in the experimental and control groups and determination of the significance of differences in alternative variation ($n = 43$; $P < 0.01$) showed that the lethal action of DMW irradiation on the rats was significant. Investigations on eight intact rats showed that DMW irradiation of the rat's head in the same dose for 3 min daily for 6 days did not give rise to such effects.

Meanwhile in the experimental group, in rats which survived (36.4%) on the 2nd day after irradiation, complete reproduction of the reflex was observed (Fig. 1). Recovery of the reflex on the 2nd day after the operation in the control rats was appreciably better than on the 1st day but had not reached the initial value. Restoration of the reflex in the experimental rats was thus more pronounced than in the controls ($P < 0.01$). Analysis of the time to form the reflex in animals of the experimental group revealed that in the rats which survived the duration of stay in the illuminated compartment was longer (5.2 ± 1.6 sec) than in the case of rats which died (3.2 ± 0.9 sec).

The nucleic acid concentrations in brain structures were determined in rats which survived on the 2nd day after DMW irradiation and testing reproduction of the reflex. As Table 1 shows, compression of the carotid arteries itself significantly reduced the RNA concentration in the cerebral cortex. Irradiation of the head of a rat with compressed arteries significantly increased the RNA concentration in the cerebral cortex compared with that in rats with compressed arteries, but not irradiated; however, this procedure did not restore the ini-

TABLE 1. Nucleic Acid Concentrations (in mg% phosphorus per dry weight of tissue) in Brain Tissues of Rats of Different Groups (M ± m)

Tissue	Nucleic acid	Normal (group 1)	Compression of carotid arteries (group 2)	Compression of carotid arteries + DMW irradiation (group 3)
Cerebral cortex	RNA	137,4±0,7	120,8±1,5*	126,0±1,2*
	DNA	45,9±2,3	36,1±5,2	46,9±1,9
Brain stem	RNA	102,9±4,9	105,8±6,1	111,0±7,5
	DNA	34,3±1,6	48,1±10,1	50,4±4,3

*P < 0.05.

tial RNA concentration. Changes in the RNA concentration in the brain stem and DNA concentration in all parts of the brain studied were not significant.

DMW irradiation of the rat's head in the early period of disturbance of the cerebral circulation thus in some cases worsened the animals' state and caused convulsions, followed by death, whereas in other cases it improved recovery of brain function as reflected in the parameters of conditioned-reflex activity. This dissimilarity in the action of DMW irradiation is probably attributable to individual differences between rats, for, as reflex formation showed, the animals differed in the speed of their response. Negative effects of DMW irradiation were observed in rats which responded faster. In less reactive rats irradiation improved the restoration of brain function.

Comparison of normalization of conditioned-reflex activity with an increase in RNA concentration, mainly in the cerebral cortex, under the influence of DMW irradiation suggests one possible compensatory mechanism of the action of irradiation in the early stage of cerebrovascular disturbance due to compression of the carotid arteries. Decimeter waves of an electromagnetic field irradiate primarily structures of the cerebral cortex that are most affected by circulatory insufficiency resulting from compression of the carotid arteries [2], activate RNA synthesis in them, and in turn this promotes intensification of protein synthesis, i.e., provision of structural materials for brain function. This hypothesis is supported by data on activation of RNA synthesis in other forms of hypoxia (adaptation to high altitude hypoxia) [6], and also the results of investigations which showed the role of nucleic acids in mechanisms of fixation of adaptive memory [4] and an increase in RNA concentration in brain structures during the formation of positive and negative conditioned reflexes [1].

It is also important to discuss the results of the present experiments from the standpoint of the possible use of DMW irradiation of the head under clinical conditions. Correlation has been demonstrated between the appearance of paroxysms and vascular diseases of the brain, including those due to looping of the internal carotid artery [10]. If the blood supply in the vertebrobasilar system and the carotid arterial system is disturbed, pathological manifestations appear, but they are not always similar in their course. In particular, epileptic manifestations occurred in 21% of cases [3]. In the present experiments, on compression of the carotid arteries various different pathological manifestations were found, and in some experiments convulsions terminating in death were observed in the animals. Under these circumstances DMW irradiation after compression of the carotid arteries not only had a beneficial action, but in the group of more reactive rats it caused convulsions and death of the animals. Comparison of clinical observations with the results of the present experiments suggests that in cases when animals are predisposed to epileptic fits, DMW irradiation aggravates the development of pathological changes.

To conclude, DMW irradiation of the head may have an unfavorable action in circulatory disturbances in the territory supplied by the carotid arteries and this must be taken into account in clinical practice.

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RHEOLOGIC PROPERTIES OF RED CELL SUSPENSIONS FROM PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITHIN THE PHYSIOLOGICAL TEMPERATURE RANGE

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UDC 616.1-008.9-056.43-092.9-07:616.153.915-074

KEY WORDS: rheology; viscosity; phase transition; myocardial infarction.

Investigations of structural changes in erythrocyte membranes under the influence of temperature within the range 34-43°C have shown that a structural phase transition takes place in them [4, 11, 14, 15]. The viscosity of suspensions of red cells and their "ghosts" varies as a nonlinear (stepwise) function of temperature between 34 and 42°C and pH 5.5 to 8.5 under normal conditions in the region of phase transition. Inflections lie between temperatures of 37 and 40°C and pH between 7.3 and 7.5 [12]. Consequently, the nonlinear character of the change in membrane viscosity depending on temperature or pH means that phase transitions in membranes, just as in liquid crystal polymers [9], can be identified sufficiently definitely.

We used viscosity as the parameter with which to investigate red cell membranes from patients with acute myocardial infarction, during its course. Special attention was paid to the presence of inflections on the ηT curves, for they can be recorded by a technique developed by one of us (V.G.K.).

EXPERIMENTAL METHOD

Altogether 75 patients with acute myocardial infarction (40 during treatment and 35 in the acute period) and also 20 healthy subjects aged 20-80 years were investigated. Blood (0.3-0.5 ml) was taken from a vein or finger three or four times during treatment (1st, 9th, and 30th days). The red cells were washed free from plasma with phosphate buffer containing 0.103 M Na_2HPO_4 and 0.155 M NaH_2PO_4, pH 7.4 [10], without the addition of complex-forming compounds, by centrifuging three times at 2-3°C. The speed of centrifugation was 1500-2000 rev/15 min. A viscosimeter with capillary tube 0.54 mm in section, 100 mm long, and with the height of the column of liquid Δh = 20 mm above the capillary, was inserted into a centrifuge tube containing the suspension in a volume of V = 0.2-0.3 ml (Fig. 1). The shear velocity was 2.5 sec^{-1}. The tube was immersed in a type UT-15 ultrathermostat or a waterbath to the extent of three-quarters of its volume. The viscosity of the red cell suspension was measured between 34 and 42°C at intervals of 1°C from below upward. The incubation time of the suspen-

Department of Medical Chemistry and Department of Internal Medicine, Faculty of Clinical Medicine, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 64-67, May, 1983. Original article submitted June 17, 1982.

 0007-4888/83/9505-0642$07.50