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ULTRACYTOCHEMICAL CHANGES IN THE BRAIN AND LIVER FOLLOWING EXPOSURE TO LOW-INTENSITY NONIONIZING MICROWAVE RADIATION

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Data in the literature indicate that nonionizing microwave radiations (NMR) may have opposite biological actions on the body. They may depress or stimulate excitation and inhibition in the brain [1, 2, 4, 9], they may induce pathomorphological changes in elements of the nervous system [1, 3, 7], yet at the same time they are used therapeutically [5]. Nevertheless, changes in ultrastructure and biochemical parameters arising in various tissues following exposure of the body to NMR of low intensity have not been adequately studied, so that no solid conclusions can be drawn regarding the harmful and useful action of this type of energy.

The object of this investigation was a combined study of the fine changes in structure and metabolism of brain and liver cells following repeated exposure of animals to low-intensity NMR.

EXPERIMENTAL METHOD

Experiments were carried out on 478 albino rats by morphological and biochemical methods. The morphological methods included electron microscopy, histochemical determination of the content of glycogen, RNA, and DNA in the cells, and of activity of succinate, malate, glucose-6-phosphate, and lactate dehydrogenases (SDH, MDH, G6PDH, LDH), and measurement of the area of the cell nuclei in sections stained with hematoxylin and eosin. The biochemical methods included determination of the oxidative and phosphorylating activity of mitochondria isolated in a substrate containing Tris-sucrose, and determination of activity of the enzymes SDH, G6PDH, and phosphorylase, and of the glycogen content. The results were subjected to statistical analysis.

Animals were exposed to NMR by means of the Luch-58 therapeutic apparatus (wavelength 12.6 cm) under dosimetric control; the animals were kept in an anechoic chamber in special cages. Animals exposed to NMR with an intensity of 50 $\mu W/cm^2$ for 3, 6, or 7 h followed by repetition of this dose on 10 consecutive days or for 2 months, and also animals exposed to NMR with an intensity of 10 $\mu W/cm^2$ for 2 months (40 min per session 3 times a day with intervals of 3-4 h) were used in the experiments. The parameters were determined immediately after the end of irradiation and after 20 and 60 days of the recovery period. Additional loads were used (hypoxic hypoxia in a pressure chamber — raising the animals to an altitude of 8 km,

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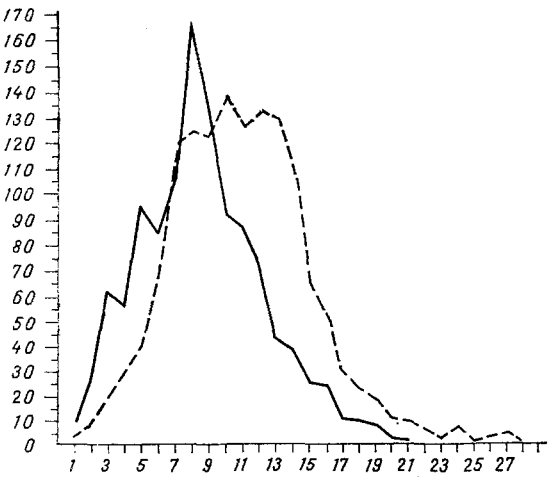

Fig. 1. Distribution of mean areas of nuclei of control rats (continuous line) and animals exposed to NMR with an intensity of 50 μW/cm² (broken line). Abscissa, class of area of nuclei; ordinate, number of nuclei in the given class.

where they remained for 20 min, and exposure to NMR of higher intensity — 500 μW/cm² — in a single session of 6 h). These two groups of animals served as the control, together with a group of intact animals.

Histochemical reactions for the enzymes were carried out on sections obtained in a cryostat from native brain and liver tissue, followed by incubation in a special medium containing a particular substrate (succinate, lactate, malate, G6P) and the dye nitro-BT. The results of the reactions were assessed from the character of the topographic distribution of formazan grains and were described by numbers on a five-point system: 5 points — sharp increase in activity, 4 points — high activity, 3 points — moderate, 2 points — low, 0 (1 point) — very low [10, 11]. For electron microscopy the brain was perfused by the method adopted in the Laboratory of Electron Microscopy, Brain Institute, Academy of Medical Sciences of the USSR.

EXPERIMENTAL RESULTS

A study of the histochemical parameters in the intact animals showed high G6PDH activity in the liver, as reflected in a dense distribution of formazan granules in the hepatocytes. Reactions for SDH revealed a moderate and uniform distribution of formazan granules in all cells in the lobules of the liver. The reactions for MDH and LDH showed lower activity of these enzymes than of SDH and G6PDH in the hepatocytes. Strongly positive reactions were given for glycogen and nucleic acids in the hepatocytes of intact animals, but no free neutral lipids could be detected. Changes in these histochemical parameters in the brain tissues were rather less marked than those in the liver. However, even in the brain also reactions for SDH and G6PDH were clearly positive, rather weaker for MDH, and weak for LDH. Activity of the same enzyme differed in cells in different brain formations. For instance, G6PDH activity was higher in the cortical cells than in the subcortical formations, MDH activity was higher in cells of the vascular plexuses of the cerebral ventricles, and so on. The amounts of glycogen and tigroid substances also differed in nerve cells in different brain formations. These high-energy chemical compounds were demonstrated more clearly in large neurons, and granules of RNA and DNA also were more clearly visible in these cells.

In animals exposed to low-intensity NMR (50 or 10 μW/cm²) the histochemical parameters were distinctly different from those in the brain and liver of intact animals. These changes were expressed as an increase and redistribution of the activity of the various enzymes studied, of glycogen utilization, of dispersion of basal substance, an increase in the nucleic acid concentrations, and the appearance of small foci of infiltration with neutral lipids (in the liver). Changes were noted as early as on the 1st day after the end of exposure to NMR with an intensity of 50 μW/cm², irrespective of the duration of the session. The changes in the parameters studied under these circumstances showed certain special features, which were dependent neither on the duration nor the number of repetitions of the session (dose), but also on the specificity of the cells of different formations of the brain

TABLE 1. Oxidative Phosphorylation in Brain and Liver Mitochondria of Albino Rats Following Exposure to NMR with an Intensity of 50 μW/cm²

Exposure	Time of investigation	Phosphorylating activity of mitochondria (P)	Significance of differences, P	Oxidation activity of mitochondria (O)	Significance of difference (P)	Ratio of phosphorylation to oxidation (P/O)
		Brain				
Control		0,185±0,074		0,995±0,076		0,180
NMR, 50 μW/cm², 10 days, 6 h/session	Immediately after exposure	0,105±0,87	>0,05	0,461±0,100	<0,02	0,230
The same	1 month later	0,178±0,110	>0,05	0,871±0,220	>0,05	0,200
The same + hypoxia	" " "	0,80±0,019	<0,05	0,961±0,140	>0,05	0,083
The same + NMR 500 μW/cm², 6 h	" " "	0,257±0,047	>0,05	0,876±0,160	>0,05	0,290
		Liver				
Control		0,326±0,018		1,165±0,160		0,280
NMR, 500 μW/cm², 10 days, 6 h/session	Immediately after exposure	0,029±0,018	<0,001	0,292±0,086	<0,01	0,100
The same	1 month later	0,181±0,057	>0,05	0,794±0,160	>0,05	0,220
The same + hypoxia	" " "	0,103±0,015	>0,05	1,393±0,076	<0,05	0,074
The same + NMR 500 μW/cm², 6 h	" " "	0,131±0,085	>0,05	0,757±0,170	>0,05	0,170

and liver, and also on the time of the investigations. (Individual parameters also were changed after 30-60 days of the recovery period.) The character of the changes also was repeated in animals exposed to NMR with an intensity of 10 μW/cm² for 2 months, when the duration of the session (a single dose) was 40 min and the sessions were repeated 3 times a day at intervals of 3-4 h.

Changes in ultrastructure of the cells were found in animals exposed to NMR with intensities of 50 and 10 μW/cm². They consisted of changes in shape, number, electron density, and size of the organelles located in the cytoplasm and karyoplasm, determined both electronmicroscopically and morphometrically.

Karyometric investigations of the nuclei of the intact and irradiated animals revealed individual differences under normal conditions and after exposure to NMR. For instance, the mean area of the nuclei in intact animals varied from 114 to 167 μ² and in irradiated animals from 133 to 187 μ².

Analysis of the preparations showed that the mean area of the nuclei in the control was 139 ± 6.4 μ², with a range of variation from 60 to 260 μ². In the irradiated animals the mean area of the nuclei was 162.7 ± 7.3 μ², with a wider range of variations — from 60 to 340 μ².

The karyometric data were divided for analysis into classes with an interval of 10 μ², after which curves showing the distribution of mean areas of the nuclei in the control and experimental animals were plotted (Fig. 1).

It will be clear from the graph that the curve of distribution of classes of areas of the nuclei from intact animals had three peaks, corresponding to 90, 110, and 140 μ², and that the largest number of nuclei occurred in class 8 (166 nuclei), the smallest number in class 21 (one nucleus). The distribution curve for the irradiated animals had five peaks. The first peak occurred at 140 μ², the second at 160 μ², the third at 180 μ², the fourth at 300 μ², and the fifth at 330 μ². The largest number of nuclei occurred in class 10 (139 nuclei), the smallest in class 28 (one nucleus).

The fact that the curve had several peaks can be regarded as evidence of polyploidization of the cells. The appearance of "intermediate" classes was perhaps due to aneuploidy, resulting from activation of endomitosis, or to a simultaneous change in the DNA and protein content in the heterochromatin.

The results of the morphological and histochemical investigations of the state of metabolism of individual chemical substances (the content of glycogen, tigroid substance, RNA, DNA, etc., including key enzymes of the aerobic, anaerobic, and pentose cycles), and

TABLE 2. Glycogen Content and Enzyme Activity in Brain and Liver Tissues of Albino Rats Following Exposure to NMR (50 μW/cm², 10 days, 6 h per session)

Parameter		Control	NMR		NMR + hypoxia	NMR + NMR 500 μW/cm², 6 h
			1 day later	30 days later		
Glycogen, mg%						
in brain		264,0±13,8	—	268,0±22,7	—	273,0±52
	P			>0,05		>0,05
in liver		960,0±157	577,0±33,4	1090,0±213,4	726,0±79,7	2570,0±319,4
	P		>0,05	>0,05	>0,05	<0,05
G6PDH:						
in brain		35,90±3,18	42,33±2,14	39,17±4,59	22,33±3,18	35,58±3,00
	P		<0,01	<0,05	<0,05	>0,05
in liver		48,50±3,18	59,17±1,76	49,00±3,18	33,00±3,72	45,00±3,35
	P		<0,05	>0,05	<0,01	>0,05
Phosphorylase						
in brain		63,8±8,5	84,9±11,5	57,57±13,5	89,7±9,5	57,47±0,03
	P		>0,05	>0,05	<0,05	>0,05
in liver		99,4±8,2	155,9±9,9	109,0±13,5	157,0±15,9	82,28±12,66
	P		<0,05	>0,05	<0,05	>0,05

also of the state of the brain and liver cell ultrastructure following exposure to NMR were supplemented by the results of biochemical investigations, which agreed with them (Tables 1 and 2).

It will be clear from Tables 1 and 2 that the oxidative and phosphorylating activity of the brain and liver mitochondria of the irradiated animals was considerably reduced ($P < 0.01$), in agreement with the increased activity of the various enzymes shown by the histochemical tests and changes in the ultrastructure of the mitochondria. It was not restored 1 month after the end of exposure to NMR, as was shown also by the high level of activity of the enzymes in the histological tests.

Exposure of animals to low doses of NMR applied repeatedly thus changes the structural and functional basis of the mechanisms regulating metabolic processes in the cell and causes conformational changes in biologically active chemical compounds, thereby exerting an injurious action on the fine structures of the cell. NMR in the above doses caused a redistribution of the load on the known pathways of energy formation, uncouples oxidative phosphorylation in the mitochondria, and intensifies glycolysis on account of the compensatory synthesis and increased activity of the key enzymes, all of which have a beneficial action on the formation of the necessary amount of energy both for the specific function of the organelles and for the reconstruction of their fine structure. Normalization of the cell structure after dystrophic changes takes place as a result of intracellular regenerative processes [6, 8]. Exposure to NMR in these doses increases the resistance of the organism to hypoxia and also to the action of higher doses of NMR.

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AUTORADIOGRAPHIC AND ELECTRON-MICROSCOPIC INVESTIGATION OF THE EFFECT OF SOME SOVIET DRESSING MATERIALS ON EXPERIMENTAL WOUND HEALING

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KEY WORDS: dressings; wound; autoradiography; scanning electron microscopy.

Interest in the study of different aspects of wound healing has increased appreciably in recent years. To some extent this is due to the fact that many new techniques are now available for the research worker [3, 5-7]. However, in a field such as the development of new dressing materials, modern methods of morphological control have so far hardly been used at all. It is evident that the use of electron microscopy and autoradiography would help both to reveal structural differences in the course of wound healing taking place under different dressings, and also to verify the functional properties of dressings.

In the investigation described below some Soviet dressing materials were evaluated by autoradiography and scanning electron microscopy during use in experiments on animals.

EXPERIMENTAL METHOD

Experiments were carried out on 20 noninbred albino rats weighing 150-170 g, on which full-thickness excised wounds measuring 1.5 × 1.5 cm were inflicted in the dorsal region under ether anesthesia. The animals were divided into four groups. In group 1 (control) traditional cotton and gauze dressings were used, in group 2 the wounds were dressed with a double layer of atraumatic nontissue material, in group 3 cotton and gauze dressings also were used but Kapron gauze modified with a surfactant was chosen, and in group 4 dressings made from Kombutek-II were used. The dressings were changed every 2 days. During the dressing procedure the area of the wounds was determined by planimetry and squash preparations were taken from their surfaces. Biopsy material taken from the wounds was investigated once only, on the 8th day after the operation. To obtain autoradiographs, 5-[^{3}H]uridine (specific activity 18 Ci/mmole) was used as RNA precursor; fragments of tissue measuring 2 × 2 × 2 mm were incubated in medium with the precursor for 3.5 h at 37°C. After incubation the material was fixed with 15% formalin solution. Autoradiographs were obtained on paraffin sections 2-5 μ thick by the ordinary method using type M photographic emulsion. The number of tracks above the nuclei and cytoplasm of 100 fibroblasts was counted in sections stained with hematoxylin and eosin. The numerical results were subjected to statistical analysis by Wilcoxon's test.

Samples of dressing materials before and after application to the wounds were investigated with the scanning electron microscopy. The dressings were fixed with 3% glutaraldehyde solution in phosphate buffer, dehydrated in alcohols of increasing strength, and dried. The contact surfaces of the materials were sprayed with silver and examined in the ISM-2 scanning electron microscope.

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