EXPERIMENTAL BIOLOGY

Experimental Study of Relationship between Biological Hazards of Low-Dose Radiofrequency Exposure and Energy Flow Density in *Spirostomum Ambiguum* Infusoria Exposed at a Mobile Connection Frequency (1 GHz)

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Radiofrequency exposure at the mobile connection frequency (1 GHz) at different energy flow densities, 5 μW/cm^2 (2-fold below the maximum permissible level) and 50 μW/cm^2 (5-fold surpassing this level), caused a reduction of motor activity in unicellular hydrobionts *Spirostomum ambiguum* Ehrbg infusoria. In all cases, the effect was similar by the intensity and developed in a jump-wise manner after exposure of a certain duration, after which did not increase with prolongation of the exposure. The duration of radiofrequency exposure safe for the object varied significantly: 8-9 h and 10 min at 5 and 50 μW, respectively. These innovation data on harmful biological effects of very low radiofrequency exposure (5 μW/cm^2), the threshold form of biological reaction, presence of "safe" periods of exposure, and the data demonstrating a clear-cut relationship between these periods and energy flow density are interesting from theoretical viewpoint and in connection with the problem of evaluating permissible levels of radiofrequency exposure of biological objects.

Key Words: *radiofrequency exposure; energy flow density; disorders in motor activity of Protozoa*

Evaluation of health hazards of electromagnetic field radiofrequencies (RF) at which mobile connection, so popular today, is realized attracts much recent attention.

Exposure to RF radiation (300 MHz-300 GHz) is standardized by energy flow density (State Standard 12.1.006-84) in Sanitary Standards and Regulations of work with electromagnetic field sources, protection of the population from exposure to electromagnetic fields, and other norm-setting documents. In 2003 sanitary epidemiological regulations and standards SanPiN 2.1.8/2.2.4.1190-03 have been introduced in Russia, establishing for RF exposure the most stringent in the world maximum permissible mean value for energy flow density (EFD) at the level of 10 μW/cm^2. These standards are based on observations of few volunteers and in model experiments on warm-blooded animals, cell cultures, various lower animals and plants [4,5].

On the other hand, previous studies on unicellular hydrobionts *Spirostomum ambiguum* Ehrbg infusoria often used as test objects in ecotoxicology and pharmaceutics [1,12] showed that the negative effects of

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RF exposure could be observed at the permissible EFD values. Conditions corresponding to the mobile connection frequency (1 GHz) were used in those studies. The effect was evaluated by reduction of spontaneous motor activity (SMA) in infusoria [10]. SMA reduced significantly after 30-40 min exposure of spirostomas in RF field in comparison with the control. The degree of those disorders did not change with prolongation of the exposure to 10 h. The efficiency of exposure at lower and higher electromagnetic energy flow densities was not studied in those experiments.

These findings, significant for the common problem of biological effects of weak electromagnetic field and the problem of hazards of electromagnetic radiation at the mobile connection frequency prompted us carry out experiments on a wider scale.

We studied the reaction of spirostomas to RF exposure at the same frequency (1 GHz) at a lower (5 μW/cm^2) and higher (50 μW/cm^2) EFD. The former value corresponded to a 2-fold reduction of the maximum permissible level, the latter to a 5-fold elevation of this parameter. RF exposure with an EFD of 5 μW/cm^2 is highly prevalent in areas adjacent to the mobile connection basic stations of standards CDMA IS-95, CDMA IMT-MC-450, while the EFD of 50 μW/cm^2 is present close to the mobile connection basic stations of the GSM 900/1800 standard. It has been also shown that the exposure levels of about 50 μW/cm^2 were associated with about 80% calls from mobile phones [7]. Comparison of the findings of those and previous experiments and their analysis are presented in this paper.

MATERIALS AND METHODS

Laboratory culture of unicellular hydrobionts *Sp. ambiguum* infusoria served as the test object. These infusoria are rather large: 1-3 mm long and 0.3-0.5 mm in diameter, due to which they can be observed at just a 2-fold magnification. The spirostomas were kept in mass cultures in biological tubes at 20±1°C in tap water, precipitated during at least 3 days before culturing and filtered through a decalcified filter. In order to maintain the culture, the spirostomas in the log-phase were put into a fresh tube and were fed with yeast once a week.

The device intended for ultrahigh frequency (UHF) exposure of biological objects and water was a narrow-range antenna to which electromagnetic field energy was delivered through a coaxial cable. A P2-52 device for measurements of the standing wave coefficient and voltage served as the UHF source. This device consisted of an indicator block and a block with replaced generators, working in the continuous generation mode. EFD was measured by placing the platform with the test object closer or further from the antenna in accordance with experimentally measured relationship between EFD and the distance from the antenna. The error of EFD measurement at the site of the sample location varied from 15 to 20%.

Exposure of spirostomas to UHF radiation was carried out in water suspension in open plastic Petri dishes 4 cm in diameter. The thickness of water was no more than 0.5 cm (depth of electromagnetic radiation penetration at a frequency of 1 GHz being about 2 cm [8]). Control culture was placed under similar conditions without exposure to electromagnetic field. The length of UHF exposure varied from 1 min to 10 h, depending on EFD.

The changes in the spirostoma SMA were evaluated directly after exposure. In accordance with methodological recommendations [6], individual infusoria were collected from exposed and control suspensions and placed individually into special boxes (8×2.5×0.5 cm) with 1-2 mm deep cells 5-mm in diameter for scintillation. Changes in SMA were evaluated by the number of crossings of 8 (but not 4, as described previously [10]) lines perpendicular to each other, superimposed on the MBS-10 microscope ocular. Due to this method, the results were expressed in absolute values, this improving their reliability. Twenty spirostomas were taken for each period of the study from experimental and control groups in each experimental series (3-5 series were carried out). Hence, changes in SMA were studied in more than 1800 spirostomas. The results were processed using Microsoft®Excel'2003 SR-2 Origin® software by Fisher's test.

RESULTS

SMA of spirostomas exposed to electromagnetic field of the mobile connection parameters (1 GHz) at EFD of 10 μW/cm^2 (maximum allowable level in Russia [3]) during a short period of just 30-40 min dropped by 40% in comparison with the control [10]. The effect developed in a jump-wise manner and did not depend on the length of exposure after reaching the threshold level.

Electromagnetic radiation with EFP of 5 μW/cm^2 (2-fold less than the allowable level) also caused a pronounced, approximately the same as at 10 μW/cm^2, reduction of SMA (Fig. 1), but this effect was observed after a much longer exposure (8-9 h; Fig. 1). The effect also did not depend on the duration of exposure after reaching the threshold level.

The general regularity and degree of SMA changes in response to exposure of spirostomas at the same frequency at a higher EFD (50 μW/cm^2) were the same. No effect was observed during some period, after which the experimental infusoria "jumped" to

a new functional level, which was characterized by SMA reduction by about the same value as in previous experiments (by an average of 40%). On the other hand, the disorders developed within no more than 10 min of exposure to EFD of 50 μW/cm². SMA during 10-min threshold exposure in 3 series of these experiments differed significantly from the controls for each series, which confirmed our conclusions (Table 1).

Hence, hydrobionts exposed to continuously generated low intense electromagnetic radiation at the mobile connection frequency developed pronounced disorders in motor activity. An exposure duration threshold was detected. At EFD of 10 μW/cm² the duration of "safe" exposure was rather low: the threshold was detected after 30-40 min of irradiation [10]. At EFG of 5 μW/cm² the duration of "safe" exposure was much longer and the threshold was observed after 8-9 h. At EFD of 50 μW/cm² the safe period of exposure to electromagnetic field was no more than 10 min. The degree of changes after the threshold length was much similar in exposures of all kinds. At EFD used in our study it did not depend on the falling energy value.

The practical significance of these data is associated with the problem of standardizing the hazards of low intense RF exposure of the biota. The innovation data on the negative biological effect at EFD level 2-fold lower than the maximally allowable level in Russia, on the existence of safe periods of this exposure, and on the relationship between the duration of safe exposure and EFD are particularly important.

From the theoretical viewpoint, the uncommon features of the reaction (significant efficiency of very low dose exposure, threshold pattern of the effect, and saturation during superthreshold exposure) have been found previously in a cycle of priority studies [2,9,11] of the effects of low dose (for the object) X-ray and γ-radiation on various test objects, including infusoria. This does not preclude the existence of some universal mechanism of formation of a response of biological systems to weak exposure to various electromagnetic radiations, which deserves special studies.

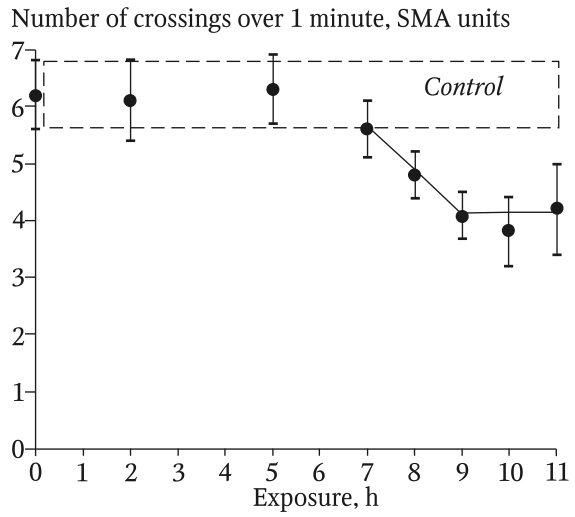

Fig. 1. Changes in SMA of spirostomas (in abs. units with quadratic error) exposed to electromagnetic field with EFD of 5 μW/cm² at a frequency of 1 GHz during up to 11 h.

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TABLE 1. Mean SMA Values in Spirostomas in the Control and after Electromagnetic Exposure at a Frequency of 1 GHz at EFD of 50 μW/cm² during 10 min (*M*±*m*)

Series	Control (*n*=20	Experiment (*n*=20)	Experimental to control SMA proportion, %
1	5.9±0.4	3.4±0.4	57.6±11.1*
2	6.4±0.3	4.1±0.4	64.1±10.7*
3	6.0±0.5	3.8±0.6	63.3±10.8*
Mean (*n*=60)	6.1±0.4	3.8±0.5	62.3±6.3*

Note. *$p<0.05$.

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