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Effects of Millimeter Wavelength Electromagnetic Radiation on Neurons: Electrophysiological Study*

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Effects of millimeter wavelength (MM) radiation (61.22 and 75.00 GHz) on spontaneous firing frequency of *Lymnae stagnalis* pacemaker neurons has been studied. MM exposure has been found to produce biphasic changes in the firing frequency. When initiated, exposure produced short-term deep inhibition, followed by a slower increase in the firing frequency. The rate of the temperature increase plays an important part in the development of the response. The model based on membrane potential regulation by two different systems functioning at different rates and at opposite directions agrees well with experimental data on changes in the firing under exposure. The results obtained suggest that MM radiation exposure at the power used in treatment can activate thermoreceptors and other temperature-sensitive nerve endings located in superficial skin layers.

INTRODUCTION

Millimeter wavelength (MM) electromagnetic radiation has been used to treat various diseases [2]. In most cases, the optimal parameters of exposure are selected by the physician empirically, since primary mechanisms of MM radiation effects on living organisms remain poorly explored.

Due to the small depth of penetration (less than 1 mm) MM radiation can only directly affect the structures located in superficial skin layers such as receptors, nerve endings, immunocompetent cells, and so on. The increase in the immune state of the organism may be considered as a conceivable mechanism for the treatment effects of MM radiation [3]. It is known that the immune and nervous systems are intimately related. MM radiation can affect immunocompetent cells

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directly as well as through the nervous system. The aim of this work was to clarify if MM radiation can affect the spontaneous firing frequency of neurons. As a model to study the effect of MM radiation on receptors and nervous endings, the nerve cells of *Lymnae stagnalis* were used. Previously, the identified neurons of *Lymnae stagnalis* have been used to investigate effects of decimeter wavelength electromagnetic radiation [4–5].

METHOD

The prepared nerve ring of *Lymnae stagnalis* was placed in an acrylic resin cell and was attached to the wax foundation of the cell with cactaceous thorns. The cell contained about 2 ml of physiological solution with pH 7.5 and with the NaCl concentration of 80 mM, KCl — 1.6 mM, $MgCl_2$ — 4 mM, $CaCl_2$ — 2 mM, Tris — 2 mM. The experiments were performed on the pacemaker BP-4 neuron [6] from the great parietal ganglion. The electrical activity of neurons was recorded with glass intracellular microelectrodes. The microelectrode was filled with 2 M of KCl and had the resistance of 5–10 MΩ. To amplify the action potential, a TEV-200 system (Dagan. Corp., the USA) was used. After amplifying, the signal was tape recorded for the following computer analysis.

The cells were exposed to continuous MM radiation at frequencies of 61.22 and 75.00 GHz. As a source of radiation, a G4-142 device was used. Cells were exposed with a 3.6 x 1.8 mm rectangle waveguide, of which the end was isolated by teflon tape to prevent the ingress of solution into the waveguide. Power flux density at the output of the waveguide varied from 0 to 130 mW/cm^2. Accepted power was also estimated by the rate of temperature increase in the solution layer thick about 0.1 mm adjacent to the teflon gasket. Specific absorption rate (SAR) was calculated with the formula SAR = 4200 dT/dt [W/kg], where dT/dt is the rate of the increase in temperature (C°/s). SAR was 0–4200 W/kg with the temperature increase being 0–2.2 C° per 20 seconds of exposure.

RESULTS AND DISCUSSION

On normal physiological conditions, the BP-4 neuron fires with a steady frequency. On starting exposure, the firing frequency of this neuron decreased (Fig. 1). With increasing SAR, this effect also increased. At SAR = 4200 W/kg, complete discontinuance of the firing was observed at several neurons. In Fig. 2, the typical changes are shown in the firing frequency under prolonged exposure of the neuron. After starting the exposure, at first, the firing frequency decreased in proportion to SAR (by 69 ± 22%, 23 experiments, with SAR = 4200 W/kg) and then gradually increased to the level exceeding the initial one (by 68 ± 21%, 9 experiments, with SAR = 4200 Wt/kg). After the completion of exposure, the opposite picture was observed: at first, the firing frequency increased and then gradually decreased

towards the new level approximate to the initial one. The increase in the firing frequency agreed well with the exponent with time constant 3.7 ± 1.9 min. The effects observed at the same SAR did not depend on the frequency of exposure (62.22 or 75.00 GHz).

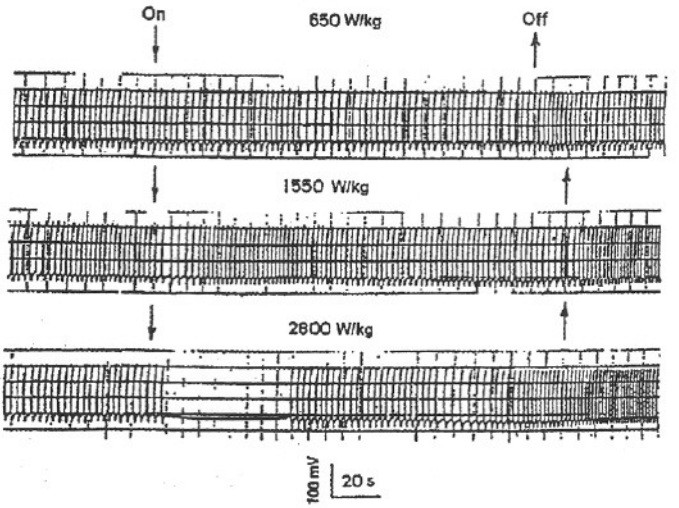

FIGURE 1. Effect of MM radiation (75 GHz) on the firing frequency of a BP-4 neuron at various SARs. The arrows indicate the beginning and termination of exposure.

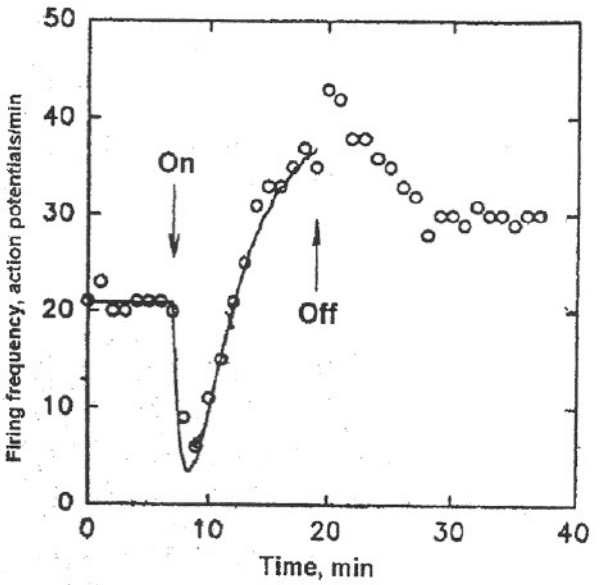

FIGURE 2. Changes in the firing frequency of a BP-4 neuron on exposure with SAR = 3150 W/kg. The arrows indicate switching on and off exposure and solid line indicate the values predicted with the model.

The neuron's firing frequency is proportional to membrane potential [7] and is controlled mainly by the Na-pump and passive transport system. To identify the systems producing the effects observed, 0.05 mM of ouabain, a specific inhibitor of the Na-pump was used [8]. This experiment suggests that the inhibition observed is a result of the increase in the Na-pump activity resulting in membrane hyperpolarization and the firing frequency increase is caused by the increase in sodium and potassium penetrability ratio producing membrane hyperpolarization [9].

In some cases, the rate of temperature increase may contribute to the excitable cell responses to heating [7, 10]. Therefore, on modeling thermal effects of MM radiation it is necessary to take into account not only the temperature increase in itself, but the rate of the temperature increase as well. In experiments with the heating, physiological solution was heated by 2°C with a rate of 0.96 °C/s, which was corresponded to irradiation with SAR of 4030 W/kg. For the heating, changes were similar to those on exposure. The dynamical inhibition was 72 ± 15%, and increase in the firing frequency was approximately 60 ± 14%, which is close to changes in the firing frequency on exposure with SAR = 4030 W/kg. Thus, the effect of MM radiation is identical to that of heating and, most likely, is due to the thermal action of MM radiation.

Previously, threshold rates of the temperature increase for the BP-4 neuron were determined in the experiment on heating solution at a different rate. The minimum rate on the heating required to record the response was 0.0025°C/s [10].

In the model, the firing frequency regulation was attributed to two systems of the membrane transport regulation, the sodium pump and ionic component, which modify membrane potential at opposite directions and with different rates. Time constants of the potential change for the abrupt increase in temperature were assumed to be equal 6 s for hyperpolarization [8] and 3.7 min for depolarization of the cell. The temperature increase was described by the single-exponential function:

$$\Delta T = \Delta T(\infty)[1 - \exp(-t/\tau)], \quad (1)$$

where $\Delta T(\infty)$ is a stationary temperature increase and τ is the time constant calculated with the formula [11]:

$$\tau = \mu L^2, \quad (2)$$

where L is the depth of microwave penetration and $\mu = 1000$ s/cm^2. Values of L for various frequencies were taken from Polk's article [12]. The model agreed well the changes in electrical activity on exposure and heating (Fig. 2).

With this model, we studied the dependence of the neuron's firing inhibition on the frequency of irradiation. With choosing SAR for each frequency, the stationary temperature increases were made identical (2 °C) for all frequencies.

In Fig. 3, an obvious frequency-dependent nature from the effect of a neuron's firing dynamical inhibition can be seen. The curve of this dependence has an S-shaped form with the mean point at a frequency of 15 GHz. The effect peaks at frequencies above 30 GHz. As can be seen in Fig. 3, the frequencies used in the experiments were in the range of maximal effect.

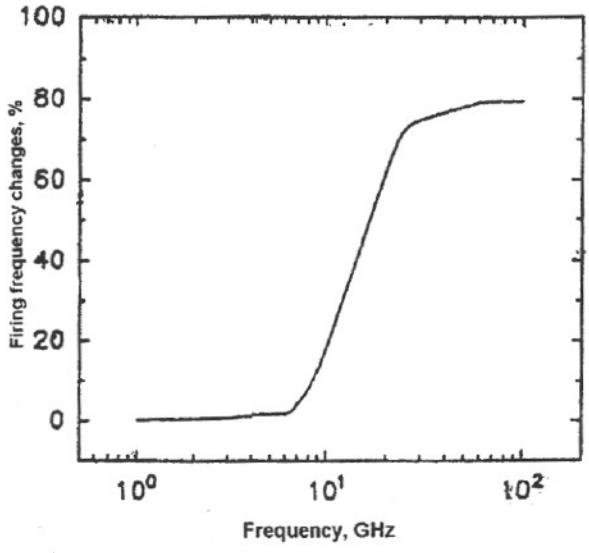

FIGURE 3. Calculated dependence of the firing frequency inhibition on the exposure frequency. For each frequency, SAR was choused so that stationary temperature increase was 2°C.

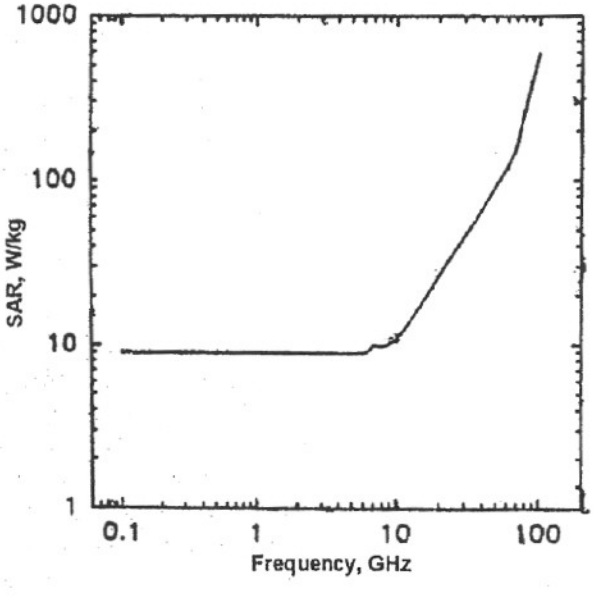

FIGURE 4. Frequency dependence of calculated threshold SAR values producing the firing frequency inhibition at a level of 3%.

In Fig. 4, the dependence of the calculated threshold SAR values produced 3% inhibition of a neuron's firing frequency on the exposure frequency. It has been shown that the minimum statistically significant change in a neuron's firing frequency may be observed at the 3% level. At frequencies of 1 and 75 GHz, threshold SAR values were 9 and 110 Wt/kg. The corresponding power flux densities were 30 and 4 mW/cm^2, i.e., high frequency values of power flux density was substantially lower than for low frequency ones.

An irradiation pulse slowly decaying during several minutes may produce the inhibition alone, without the following increase in the firing frequency. In Fig. 5, the inhibition in response to abrupt increase in temperature following its slow decrease is shown. On the contrary, the irradiation at slowly increasing power produced the increase in the firing frequency alone, without the initial inhibition. If the basis for treatment effects of MM radiation is thermal activation of various different receptors and free nerve endings, then, selecting regimes of exposure, it is possible to observe changing phases in the firing frequency and test the treatment efficacy of these phases.

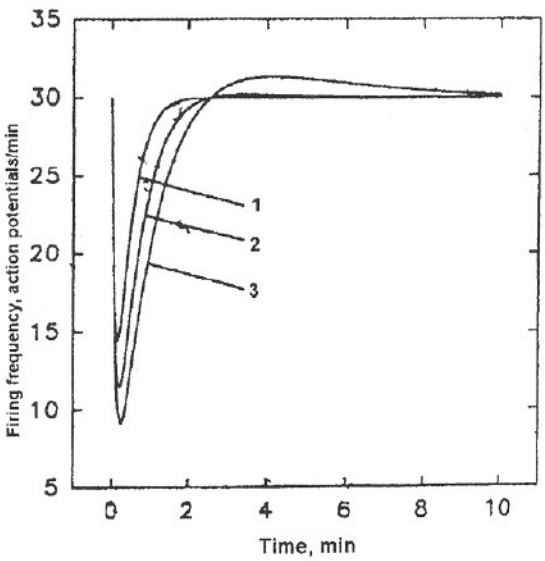

FIGURE 5. Firing frequency changes in response to abrupt temperature increase by 2°C with the following decrease by the exponential law with a time constant equal to 0.5 (1), 1 (2), and 2 min (3).

Threshold stimulus for the human cold receptors is a temperature decrease at a rate of 0.001 °C/s with the stimulus duration no less than 3 s [13]. The thermal sensitivity of *Lymnae stagnalis* neurons is in the range of thermal sensitivity of

human skin receptors. The experiments have shown that the increase in temperature by a fewer degrees can produce the recorded changes in electrical activity. The increase of temperature by several degrees in response to some medications [14], can undoubtedly affect thermoreceptors and other thermosensitive nerve endings, including mechanoreceptors. Mechanoreceptors have found to have high termosensitivity due to the Na-pump [15] and, along with thermoreceptors and other thermosensitive cells, appear to play a significant part in the biological effects of MM radiation.

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