Cell Biology Research Progress

Cell Response to Electromagnetic Field

Nuclear and Membrane Mechanisms

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Preface

Interaction of humans with a new ecological factor – artificial electromagnetic fields, causes public concerns. The artificial electromagnetic fields are denominated as an electromagnetic noise [1]. Factors of electromagnetic nature may have a significant regulatory effect on cell as they can penetrate into the cell and interact directly with the subcellular structures. Microwave irradiation of low intensity has been widely used in medicine [2], but on the other hand, there are evidences of the negative impact of low-energy microwave radiation on human health [3]. All this makes the study of biological mechanisms of microwave radiation influence of practical importance. Due to the fact that the energy of a quantum of electromagnetic radiation of centimeter and millimeter wavelength is not enough to break covalent chemical bond, the possibility of the influence of the radiation on biological objects is denied by some scientists. According to A.C.L. Basset, such scientist called studies of the effects of non-ionizing electromagnetic radiation on biological objects as "Pathological Science" and "the Emperor's Clothes." [4]. We believe that such statementsare simply unreasonable. Biological effects of EMF are discussed in the reviews [5-7], but till now a theory that explains all biological effects caused by electromagnetic fields (EMF) has notbeen developed.

Biological cell is the elementary unit in which the main biological processes – birth, death, heredity occur. These elementary biological processes occurring on the cell level are called in specific terms: cell proliferation, cell differentiation, apoptosis, and cell mutagenesis. The effects of EMF on these processes will be discussed in this book. The study of the influence of electromagnetic factors on the cell nucleus is of particular interest, because of the critical importance of cell nucleus as a storage of genetic information and its governing role in cell methabolism.

There is an extensive scientific literature devoted to the study of the influence of low energy EMF on biological cell. There is a wealth of experimental data concerning EMF effects on the physical properties of the water, the oxidative potential of the cell and free radical production, but these problems will not be discussed in this book. In our opinion, the important fact is that the effects of EMF on the cell induced by exposure to irradiation may be detected in hours and days after exposure. This fact indicates that the receiving system has a memory. Only two actively functioning cell compartments – cell nucleus and cell membrane have a 'long live' memory that enables the cell to remember the external non-specific signals and to respond to them in the proper way. Therefore, in this book we will pay closer attention to these cell compartments. Several subsystems in the nucleus are responding to external non-specific factors on different levels – the level of gene expression by means of regulation of DNA-protein interactions via their conformational changes; level of chemical modifications of DNA; level of chemical modifications of regulatory proteins; level of conformational changes in chromatin; level of regulation of nuclear membrane properties, and so on. Only some of these regulatory systems were studied in relation to EMF effect on the cell. On the other hand, cell membrane is an active cell structure that takes part in regulation of all cellular processes and has a 'memory'. This memory is related to the changes of membrane properties – fluidity and permeability. Therefore, in this book we will also consider changes in cell membranes in connection to the EMF influence on the cell.

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Contents

Chapter I

Brief Overview of the Studies Performed During the 20th Century on the Cell Effects Caused with Low Intensity Electromagnetic Fields (EMF)

In this Chapter we will briefly discuss some effects of EMF on the molecular and cellular levels, studied in the 20th century. The investigation of biological effects of electricity and magnetism began as soon as the experimental investigations of these physical phenomena started in 18-19 centuries. Prominent physicists - Benjamin Franklin in the USA, Luigi Galvani in Italy were interested in biological effects of magnets and electricity. They used biological objects as indicators of electric current. In the 20th century the physical mechanisms of the interaction of electromagnetic radiation with biologic tissues were suggested. It was believed that via these mechanisms the energy of electromagnetic waves is transformed to heat. Main features of this interaction were described in [1-4].

The processes of transition of high frequency electromagnetic radiation to cell may be described as follows. The main basic mechanisms are polarization of bound charges, orientation of permanent dipoles and displacement of free charge carriers (electronic as well as ionic). Orientation polarization is the main cause of energy absorption in biological aqueous substances for frequencies between 1 and 20 GHz. The orientation movements do not lead to molecular changes. Another mechanism occurs mainly in the frequency range between 100 kHz and 100 MHz and can be described as a bounding layer polarization, frequently occurring in structures with different electric properties. Due to the poor electric conductivity of the cell membrane, in comparison to interior and exterior cell space, the electric high-frequency current produced by the electromagnetic field in tissue essentially flows around the cell for lower frequencies (e.g. 10 kHz). Due to charge displacements, the cell acts here like a large dipole. At high frequencies on account of the capacity of the cell membrane a large part of high-frequency current then flows through the cell membrane and cytoplasm. Electric charge displacement around and within a biological cell induces the electric potential which imposes to the existing potential of the membrane [3].

The modern systems of communication use EMF of range 0.3 - 300 GHz, so-called microwave radiation. In the Russian and Ukrainian literature this diapason is divided in two: high frequency radiation - 3 - 30 GHz, and extremely high frequency radiation - 30 - 300 GHz. Because the frequencies of alternating current in electric power grids are 50 or 60 Hz, the special interest induces extremely low frequency (ELF) radiation – 3 – 300 Hz (0 – 300 Hz).

An important characteristic of the interaction of electromagnetic radiation with biological objects is specific absorption rate (SAR). Tissue heating effect can result from elevations of tissue temperature induced by RF energy deposited or absorbed in biological systems through local, partial-body or whole-body exposures. The bulk properties of complex permittivity and electrical conductivity cause the electric fields and currents induced to be absorbed and dissipated in cells and tissues of the human body. For a single pulse or brief application of RF energy, the exposure duration may not be long enough for significant conductive or convective heat transfer to contribute to tissue temperature rise. In this case, the time rate of rise in temperature is proportional to SAR. For longer exposure durations, RF energy-induced temperature rise depends on the animal or tissue target and their thermal regulatory behavior and active compensation process. For local or partial body exposures, if the amount of RF energy absorbed is excessive, rapid temperature rise and local tissue damage can occur. Under moderate conditions, a temperature rise on the order of 1°C in humans and laboratory animals can result from an SAR input of 4 $W \cdot kg^{-1}$. However, this temperature rise falls within the normal range of human thermoregulatory capacity [5].

It is believed that excess of energy of microwave irradiation absorption above 4 W / kg causes heating and serious damage of biological tissues. The maximum undamaging exposure level for human organism is 0.4 W/kg [6].

For microwave radiation the level of surface power density of less than 10 mW/cm^2 is assumed as not inducing the significant thermal effects in the biological objects. Therefore it was assumed to regard the effects of microwaves with surface power density of more than 10 mW/cm^2 as 'thermal' and with power less than 10 mW/cm^2 as 'non-thermal' [7].

In many cases the biological effects of electromagnetic fields associated with their thermal effect [7-9]. In this book we will consider only so-called non-thermal effects, the effects of low-intensity electromagnetic fields (less than 10 mW/cm^2).

Below we will describe some essential experimental results dealing with EMF effects on macromolecules, cell membranes and cell nucleus, obtained in 20^{th} century, in chronological order.

Microwave radiation may cause an increase in the intensity of infrared absorption of DNA preparations in the form of wet and dry films of DNA [10], as well as monomers of amino acids and nucleotides [11]. The DNA in solution has several resonant frequencies of absorption in the microwave region [12]. The frequency spectrum of DNA absorption changes during the transition from cyclic, super coiled DNA, to linear DNA molecules [13], and depends on the length of the DNA molecule [14]. But these results were criticized [15, 16]. In recent publication the old idea of resonant absorption spectrum of DNA molecules is revived: DNA of human colon carcinoma in aqueous solution shows an absorption peak in the vicinity of 2,8 kHz [17].

It was supposed that electromagnetic waves could cause changes in enzyme activity due to their resonance with the oscillations of electrically charged protein molecules. The effects of microwave radiation on enzyme activity *in vitro* in a frequency-dependent manner are known. For example, irradiation of the enzyme-substrate system of lipoxygenase-polyunsaturated fatty acids by microwaves with a frequency 21 - 30 and 31 - 40 GHz led to a significant decrease in activity of the enzyme, while the EMF irradiation with a frequency of 41 - 50 GHz did not alter the rate of enzymatic reaction [18]. The early observations of non thermal effects of microwaves on enzyme activity were supported in some later works. Exposure to microwaves (2.45 GHz, 50°C) of a hydrated lipase enzyme, suspended in organic media, enhanced the reaction rate by 2–3 folds in comparison with exposure to classical heating. The observed apparent non-thermal effects depended on the hydration state of the enzyme in the organic medium [19]. The parameters of α-amylase and protease activity and kinematic viscosity of the enzyme solutions were determined in enzyme *in vitro* systems exposed to microwaves. The microwave radiation was applied in the range from 54 to 69 GHz. The stimulating and inhibiting enzyme activity frequencies were determined. The obtained results show that conformational transitions appear under the action of the EHF electromagnetic radiation. Under the influence of the inhibiting enzyme activity EHF frequency the inside molecular hydrophobic physical interactions occur more often, while under the influence of stimulating frequency the degree of enzyme hydration increases [20]. But in other investigations the possibility of the non-thermal change of enzymatic activity by microwaves was not confirmed. For instance, in a set of experiments it was demonstrated that unspecific cuts, caused by trypsin digestion of the three model proteins in enzyme systems, stimulated by microwave irradiation, were absent. In author's opinion it appears evident that incorrect temperature monitoring in the past led to erroneous conclusions about the involvement of the non-thermal microwave effects in microwave-assisted protein digestion experiments [21]. The results of resent theoretic work (non-equilibrium molecular dynamics simulations) envisage the possibility of conformational rearrangements of protein structure under the influence of external magnetic field (frequency 2.45 GHz and an r.m.s. electric field intensity range of 0.01–0.05 V/Å). The peptide displays a natural tendency to form stable elements of secondary structure which are stabilized by tertiary interactions with proximate regions of the peptide. The presence of external EMF suppresses the formation of the secondary structure, involving a mechanism of localized dipolar alignment, which serves to enhance intra-protein perturbations in hydrogen bonds. The presence of a electric field perturbs the conformational states, due to the local residue-specific dipolar orientation with the field altering not only hydrogen bonding arrangement dynamics, but also secondary and tertiary structure motifs, and, thus, enhancing sampling of peptide energy landscape. The more rapid structural changes corresponding to shorter-lived secondary and tertiary contacts increase the backbone fluctuations, rendering the more globular state of the peptide entropically and energetically favored [22]. In our opinion, the results of this work seems not applicable to real conditions of biologic experiment, because the lowest energy level 0,01 V/cm^2 corresponds to energy power density approximately of 50 W/cm^2.

Microwave radiation causes significant changes in the ion transport via cell membranes. Exposure of human erythrocytes to a field with a frequency of 1.009 GHz leads to exit of potassium ions from the cells and to entrance of sodium ions to the cell by the gradient of concentration [23-25]. Changes in the transport of ions H + and Cl-across the membrane of red blood cells were observed in cells exposed to microwaves [26]. The effects of microwaves on the transport of these ions were observed in rat spleen cells [24], *E. coli* and *B.*

subtilis cells [27]. Microwaves induce the increase of the availability of hydrogen atoms in cell membrane-incorporated peptides by changing the packing density of lipid membranes [28]. Microwave irradiation (0.9 GHz) of lecithin artificial membranes caused a significant increase in the conductivity of the membranes. It was accompanied by increasing of diffusion of Tetraphenylboron sodium ions through the membranes and layers of electrolyte [29]. In experiments in vitro low-energy microwave irradiation (200 $\mu W/cm^2$) caused a significant increase in the permeability of cell membranes to human buccal epithelial to dye indigocarmin in response of cells to radiation with a wavelength of 1.6 cm and 7.1 mm [30] and in the response of cells to radiation with a wavelength of 1.6 and 8 mm [31].

One of the possible mechanisms of increasing passive permeability of membranes for ions may be associated with changes in the density of packing of membrane lipids and a change in lipid-protein interactions under the influence of microwave irradiation [32].

Exposure to microwaves of wavelength 8 mm leads to decrease in transmembrane potential of smooth muscle cells. The mechanism of such effect is connected mainly with increasing of membrane permeability to sodium ions and inhibition of active transport of these ions [33]. A similar effect of the reducing of the transmembrane potential is observed under the microwave irradiation in isolated ganglia of mollusks *Planorbis corneus* [34].

Microwave radiation (2450 MHz) inhibits Na-K ATPase in human erythrocytes. Exposure of membrane suspensions to electromagnetic radiation at a dose rate of 6 W/kg and at five temperatures between 23 and 27 degrees C resulted in an activity change only for the Na+/K+ ATPase at 25 degrees C. The activity decreased by approximately 35% compared to sham-irradiated samples. [35]. By means of patch-clamp technique it was shown that the stimulating effect of microwave irradiation on ion transport through ion channels [36]. In [37] was found that the EMF (42.5 MHz, 0.1 mW/cm^2) affects the activity of Ca^{2+}-activated K^+ channels with low activity and reduced activity of channels with high-output activity. The solution previously exposed to EMF (42.2 GHz, 2 mW/cm^2) also induces increase in activity of ion channels [38].

It was shown experimentally that that the energy of the electromagnetic field can be used for active transport of ions across the membrane [39, 40]. In authors' opinion, this occurs if the cyclic electron transport in transmembrane enzyme coincides in phase with the external oscillating field. In this case, the energy of electromagnetic field is absorbed by membrane-incorporated enzyme transport complex and is converted into chemical energy by ion transport against the gradient of concentration.

Charge transfer processes induced by EMF affect an electric potential across the cell membrane. Induced transmembrane potential is involved in regulating the of cell activity [41]. The effect of microwave irradiation on the chloride current through the cell membrane algae *Nitellopsis obtusa* depended on the frequency EMF. The frequencies of 49, 70 and 76 GHz caused the increase of chloride current by 200 - 400%, at frequencies of 41.5 and 71 GHz the chloride conductivity decreased. The authors believe that microwaves affect certain links in the chain of control of chloride channels, and do not affect directly the same channels [42]. EMF (53 - 78 GHz) effects on currents through the membrane and transports Tetraphenylboron sodium through artificial membrane. The authors attribute the effect observed to thermal effect of microwave radiation [43]. It was proposed that microwaves energy absorption is associated with the so-called "migratory" or "spans" effect of the transfer of charge carriers through the membrane. Coincidence of times of flight (hopping) of ions via membrane with the period of the external field leads to an acceleration of charge carriers transport and with additional active conductivity [44].

The frequency-dependent increase in the concentration of intracellular calcium on exposure to modulated electromagnetic fields can lead to changes in the frequency-dependent changes in the functional activity of cells as a result of the changes in the activity of Ca^{2+}-dependent enzymes [45].

Microwave irradiation causes a number of morphological changes, increasing of the volume of nuclei and cells [46]. Microwave irradiation of frequency 3 GHz and 5 mW/cm^2 intensity induces stimulation of cell metabolism in culture as may be detected by changes in nucleolar and nuclear volume [47].

Effects of microwave radiation on electrokinetic properties of nuclei and chromatin were studied in living cells. The method of assessment of electrocinetic properties of cell nuclei was performed as follows. Cells were placed in electrophoretic cell in 3,03 mM phosphate buffer solution with addition of 2,89 mM calcium chloride. At electric field strength 15 V/cm, and electric current 0,1 mA the displacement of cell nuclei in living cell was determined. In some cells the nuclei are displaced to anode, in other cells – they are not (remained unmovable). The percentage of negatively charged nuclei was determined as electronegativity of cell nuclei (ENN). In one measurement ENN was determined among 100 cells in one preparation; the measurement was repeated in 8-10 preparations for every variant of experiment. Microwave exposure of cells (wavelength of 7.1 mm, E = 0,2 mW/cm^2, exposure time 1-60 seconds) caused a decrease in the electronegativity of the nuclei of human buccal epithelium cells and condensation of chromatin in the nuclei. Chromatin condensation was determined by quantity of granules of heterochromatin located near nuclear envelope. The observed effects depend on irradiation dose and individual peculiarities of donors [48, 49].

In the study of the influence of microwave irradiation on ENN of cells of human buccal epithelium it was found that the effect of microwave irradiation depends on the initial level of ENN: at a high ENN level in control, the exposure results in the decrease of ENN; if ENN in control is low the exposure results in ENN increase. Therefore, the fact that the same influence induces different answer in cells of different donors seems very strange. But in complex biological systems the character of response depends on the initial state of the system. In physiology this regularity is known as "The law of initial value" or Wilder's law [50, 51].

Cell membrane permeability of vital stain indigocarmin increases after cell exposure to microwaves [52, 53].

Electric field (field strength 20-80 V/cm, a.c. frequency 50 Hz, exposure time 5 min) applied to cells of human buccal epithelium induced increase of ENN, increase of cell membrane permeability to vital stains and induced chromatin decondensation [54].

Microwave radiation increases transcription activity and reduces replication of tumor cells of rats [55]. The incorporation of ^{3}H-thymidine into DNA was stimulated by microwave irradiation of human lymphocytes [56]. Microwave radiation can cause the increase in transcription in result of increase in intracellular calcium concentration [57]. Stimulation of proliferation of human fibroblasts in vitro was observed when exposed to microwave radiation at a frequency of 53 - 78 GHz, at very low intensity (5x10-18 W/cm^2) [58]. The mutagenic effect of electromagnetic radiation was studied in many works. Noconsensus exists in assessment of the mutagenic potential of EMF. No mutagenic effect of low-level microwave radiation was revealed in experiments on *Drosophila* [59,60], *Salmonella typhimurium*, yeast cells [61, 62], mouse cells [63] and human lymphocytes [64, 65]. However, there are works, demonstrating mutagenic action of microwave radiation. Possible increase in the frequency of mutations after microwave irradiation of *Drosophila* larvae (7.2 mm wavelength, intensity 5 mW/cm^2) observed in [66]. Mutagenic effect of microwaves was observed in a series of works of V.Garaj-Vrhovac. The number of chromosomal aberrations increased in cultured cells of Chinese hamster after exposure to microwaves (7.7 GHz, 30 mW/cm^2) (15, 30, 60 min) [67]. The power density 30 mW/cm^2 is relatively high and microwaves can produce a considerable thermal effect in the biological sample. In the further works of this scientific group the non-thermal intensities of microwave irradiation were used. One of the most efficient tests to assess genotoxic effect of environmental factors is the micronucleus test. Micronucleus assay (MN) was widely applied in assessment of EMF-induced genotoxic effects. The number of chromosomal aberrations and the number of micronuclei increased after irradiation in cultured V79 Chinese hamster fibroblasts (frequency 7.7 GHz, power density 0.5 mW/cm^2, exposure time 15, 30 and 60 min) [68]. The frequency of chromosomal aberration increases after the human whole-blood samples exposure to continuous microwave radiation (frequency 7.7 GHz, power density 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min) [69]. The number of chromosomal aberrations increased in human lymphocytes among persons exposed to microwave radiation [70]. The MN and chromosomal aberrations rate increased in human peripheral blood lymphocytes exposed *in vitro* to electromagnetic fields with frequencies 2.45 and 7.7 GHz at power density of 30 mW/cm^2, after cell exposure of 30 and 60 min [71].

The specific (non-thermal) mutagenic effect of EMF was not revealed in some studies. The exposure of human peripheral blood lymphocytes to 2450 MHz microwaves during 30 and 120 min at a constant temperature of 36.1 degrees C did not influence the cell kinetics nor the sister chromatid exchange (SCE) frequency [73]. No genotoxic effects were found in human peripheral blood lymphocytes cultured *in vitro* and exposed to 50 Hz sinusoidal magnetic fields for 72 h at different intensities (1.0, 0.75, 0.5, 0.25, and 0.05 mT rms), as assessed by the micronucleus assay [74], also no genotoxic effects were revealed after cell exposure to microwaves [75-77].

In the pioneer works of H. Lai and N.P. Singh it was found that single and double DNA strand breaks accumulate in rat brain cells after microwave irradiation [78, 79]. Pulse radiation exposure (500 pulses per second, duration 2 s) and field 2.45 GHz (exposure time - 2 hours) induced the single and double strand DNA breaks in rat brain cells [79, 80]. Melatonin (1 mg / kg) or N-tert-butlylfenilnitron (100 mg / kg) injected into animals immediately before or immediately after radiation exposure prevented the appearance of DNA alterations [80]. However, these results were not confirmed in [81].

Many investigations of EMF-induced DNA strand breaks were conducted in the first decade of the 21st century. The DNA strand breaks are shown in animals [82] and cultured cells [83, 84] after exposure to microwaves and to