ELECTROMAGNETIC FIELDS in BIOLOGY and MEDICINE

Marko S. Markov

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
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CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Version Date: 20150107

International Standard Book Number-13: 978-1-4822-4851-7 (eBook - PDF)

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5 Biophysical Mechanisms for Nonthermal Microwave Effects

Igor Belyaev

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5.1 THERMAL AND NT BIOLOGICAL EFFECTS OF MWs

Exposures to radio-frequency (RF, 3 kHz–300 GHz) electromagnetic radiation or microwaves (MWs, 300 MHz–300 GHz) vary in many parameters: intensity (incident flux power density [PD], specific absorption rate [SAR]), wavelength/frequency, near field/far field, polarization (linear, circular), continuous wave (CW) and pulsed fields (which include variables such as pulse repetition rate, pulse width or duty cycle, pulse shape, and pulse to average power), modulation (amplitude, frequency, phase, complex), static magnetic field (SMF) and electromagnetic stray fields at the place of exposure, overall duration and intermittence of exposure (continuous, interrupted), and acute and chronic exposures. With increased absorption of energy, the so-called thermal effects of MWs are usually observed that deal with MW-induced heating. SAR is a main determinate for thermal MW effects. Several other physical parameters of exposure including carrier frequency,

modulation, polarization, and coherence time of exposure have been reported to be of importance for the so-called nonthermal (NT) biological effects that are induced by MW at intensities well below measurable heating. The literature on the NT MW effects is very broad. There are four lines of evidence for the NT MW effects: (1) altered cellular responses in laboratory in vitro studies and results of chronic exposures in vivo studies (Cook et al., 2006; Grigoriev et al., 2003; Huss et al., 2007; Lai, 2005), (2) results of medical application of NT MW in the former Soviet Union countries (Betskii et al., 2000; Devyatkov et al., 1994; Pakhomov and Murphy, 2000; Sit'ko, 1989), (3) hypersensitivity to electromagnetic fields (EMFs) (Hagstrom et al., 2013; Havas and Marrongelle, 2013), and (4) epidemiological studies suggesting increased cancer risks for mobile phone users (Hardell et al., 2013a,b). It should be noted that along with detrimental effects, beneficial effects of NT MW have also been reported (Arendash et al., 2010; Dragicevic et al., 2011) supporting long-standing notion that interplay of physical MW parameters and biological parameters of the exposed object defines the value of the NT MW effect (Sit'ko, 1989). Majority of scientific community in this field is moving to better characterize and discover mechanisms for NT MW effects. International IARC expert panel has concluded: "Although it has been argued that RF radiation cannot induce physiological effects at exposure intensities that do not cause an increase in tissue temperature, it is likely that not all mechanisms of interaction between weak RF-EMF (with the various signal modulations used in wireless communications) and biological structures have been discovered or fully characterized." This paper is not intended to be a comprehensive review of the literature on the NT MW effects. In this review, we will describe recent developments in physical mechanisms.

5.2 MOST IMPORTANT PHYSICAL VARIABLES FOR THE NT MW EFFECTS

It is widely accepted that the NT MW effects depend on a variety of physical parameters. Most representative so far IARC expert review panel on RF carcinogenicity has stated that "the reproducibility of reported effects may be influenced by exposure characteristics (including SAR or power density (PD), duration of exposure, carrier frequency, type of modulation, polarization, continuous versus intermittent exposures, pulsed-field variables, and background electromagnetic environment" (IARC, 2013). These exposure characteristics were recently described in detail (Belyaev, 2010a) and are briefly summarized in the following.

5.2.1 Carrier Frequency

Resonance response of leaving cells to NT MW was reported. Slight changes in carrier frequency about 2–4 MHz resulted in disappearance of NT MW effects because of high quality of resonance-like responses (Belyaev et al., 1992a, 1996; Shcheglov et al., 1997a). Significant narrowing in resonance response with decreasing PD has been found when studying the growth rate in yeast cells (Grundler, 1992) and chromatin conformation in thymocytes of rats (Belyaev and Kravchenko, 1994). In Grundler's study, the half-with of resonance decreased from 16 MHz to 4 MHz as PD decreased from 10^{-2} W/cm^2 to 5 pW/cm^2 (Grundler, 1992). Small change in carrier frequency by 10 MHz has reproducibly resulted in cell-type-dependent appearance (915 MHz) or disappearance (905 MHz) in effects of GSM mobile phone on DNA repair foci in human cells (Belyaev et al., 2009; Markova et al., 2005, 2010).

5.2.2 Nonlinearity: Sigmoid Dependencies and Power Windows

One of the earliest observations of a threshold in response to NT MW was published by Frey (1967). In this study, a threshold of 30 μW/cm^2 was found by Frey to evoke brain stem responses to RF in cats (Frey, 1967). This value was four orders of magnitude lower than intensities needed to cause

internal body temperature increase. Devyatkov and colleagues have found that a wide variety of the NT MW effects in vitro and in vivo display sigmoid dependence on intensity above certain intensity thresholds (Devyatkov, 1973).

In their pioneering study on blood–brain barrier (BBB) permeability, Oscar and Hawkins exposed rats to MW at 1.3 GHz and analyzed BBB permeability by measuring uptake of several neutral polar substances in certain areas of the brain (Oscar and Hawkins, 1977). A single, 20 min exposure to CW MW increased the uptake of D-mannitol at average PD of less than 3 mW/cm^2. Increased permeability was observed both immediately and 4 h after exposure, but not 24 h after exposure. After an initial rise at 0.01 mW/cm^2, the permeability of cerebral vessels to saccharides decreased with increasing MW power at 1 mW/cm^2. Thus, the effects of MW were observed within the power window of 0.01–0.4 mW/cm^2. The findings on *power windows* for BBB permeability have been subsequently corroborated by the group of Persson and Salford (Persson et al., 1997; Salford et al., 1994). In their recent study, the effects of GSM MW on the permeability of the BBB and signs of neuronal damage in rats were investigated using a GSM programmable mobile phone in the 900 MHz band (Eberhardt et al., 2008). The rats were exposed for 2 h at an SAR of 0.12, 1.2, 12, or 120 mW/kg. Albumin extravagation and also its uptake into neurons increased after 14 days. The occurrence of dark neurons in the rat brains increased later, after 28 days. Both effects were already seen at 0.12 mW/kg with only a slight increase, if any, at higher SAR values.

The data obtained in experiments with *Escherichia coli* cells and rat thymocytes provided new evidence for sigmoid PD dependence and suggested that, similar to the effects of extremely low frequency (ELF) EMF, MW effects may be observed within specific *intensity windows* (Belyaev et al., 1992c, 1996; Belyaev and Kravchenko, 1994; Shcheglov et al., 1997b). The most striking example of the sigmoid PD dependence was found at the resonance frequency of 51.755 GHz (Belyaev et al., 1996). When exposing *E. coli* cells at the cell density of 4×10^8 cell/mL, the effect reached saturation at the PD of 10^{-18}–10^{-17} W/cm^2 and did not change up to PD of 10^{-3} W/cm^2. This suggested that the PD dependence of MW effects at specific resonance frequencies might have intensity threshold just above the background level.

The dependence of the effect on the exposure level is usually not linear, and the reduction in the effect is much slower than expected according to the decreasing SAR (Suhhova et al., 2013).

5.2.3 Coherence Time

MW exposure of L929 fibroblasts was performed by the Litovitz's group (Litovitz et al., 1993). MW at 915 MHz modulated at 55, 60, or 65 Hz approximately doubled ornithine decarboxylase (ODC) activity after 8 h. Switching the modulation frequency from 55 to 65 Hz at coherence times of 1.0 s or less abolished enhancement, while times of 10 s or longer provided full enhancement. These results suggested that the MW coherence effects are remarkably similar to those of ELF magnetic fields observed by the same authors (Litovitz et al., 1997b).

5.2.4 Modulation

Significant numbers of in vitro and in vivo studies from diverse research groups demonstrate that the NT RF effects depend upon modulation (Blackman, 1984; Blackman et al., 1980; Byus et al., 1984, 1988; d'Ambrosio et al., 2002; Dutta et al., 1984, 1989; Gapeev et al., 1997; Huber et al., 2002, 2005; Lin-Liu and Adey, 1982; Litovitz et al., 1997a; Markkanen et al., 2004; Penafiel et al., 1997; Persson et al., 1997; Veyret et al., 1991). Comprehensive reviews on the role of modulation in appearance of the NT RF are available (Belyaev, 2010a; Blackman, 2009). Recent studies provided new evidence for dependence of the NT RF effects on modulation (Lustenberger et al., 2013; Schmid et al., 2012; Valbonesi et al., 2014).

5.2.5 Polarization

Our research group have consistently reported data showing that the NT MW effects depend on polarization (Alipov et al., 1993; Belyaev et al., 1992a–c, 1993a,b; Belyaev and Kravchenko, 1994; Shcheglov et al., 1997a; Ushakov et al., 2006, 1999). Usually, only one of the two possible circular polarizations, left-handed or right-handed, was effective at each frequency window/resonance. The sign of effective circular polarization (left or right) alternated between frequency windows. The difference between effects of right and left polarizations cannot be explained by heating or by the mechanism of the so-called *hot spots* due to unequal SAR distribution. The varying effects of differently polarized MW and the inversion of effective circular polarization between resonances and after irradiation of cells with X-rays provided strong evidence for the NT mechanisms of MW effects. The data by others supported our findings on the role of polarization in the NT MW effects (Polevik, 2013; Shckorbatov et al., 2009, 2010).

5.2.6 Electromagnetic Noise

Litovitz and colleagues found that ELF magnetic noise inhibited the effects of MW on ODC in L929 cells (Litovitz et al., 1997a). Following studies confirmed that NT MW effects may depend on electromagnetic noise (Burch et al., 2002; Di Carlo et al., 2002; Lai, 2004; Lai and Singh, 2005; Litovitz et al., 1997a; Sun et al., 2013).

5.2.7 Static Magnetic Field

Dependence of MW effects on SMF during exposure has been described (Belyaev et al., 1994a; Gapeev et al., 1997, 1999; Ushakov et al., 2006). SMF is considered as one of the physical variables, which may be important for the NT MW effect (IARC, 2013).

5.2.8 E and H Fields

It is particularly interesting to know whether the NT biological effects of MW are induced by the electric (E) or the magnetic (H) field component of EMF. In fact, separating E from H could be potentially important for evaluating the mechanisms underlying the effects of RF radiation on biological systems. Recent studies have indicated that the E component of the EMF may be more significant than H component in NT MW effects (Schrader et al., 2011).

5.2.9 Near and Far Fields

MW sources close to the body surface as in case of exposure to MP produce near-field exposures. The emitted field is magnetically coupled directly from the antenna into the tissues. The most agreed-upon definition for the near field admits that the near field is less than one wavelength (λ) from the antenna. In the near fields, the E and H fields are not necessarily perpendicular. They are often more nonpropagating in nature and are therefore called fringing fields or induction fields. At increasing distances from the source, the human body progressively takes on properties of a radio antenna, with absorption of radiated energy determined by physical dimensions of the trunk and limbs. This is a far-field exposure, defined as fully developed at 10–30 wavelengths from the source. These fields are approximately spherical waves that can in turn be approximated in a limited region of space by plane waves.

In dependence on type and location of transmitter, exposure may occur in far or near field. Kamenetskii et al. have shown that field structures with a local coupling between the time-varying E and H fields differing from the E–H coupling in regular-propagating free-space electromagnetic waves in a source-free subwavelength region of MW fields can exist (Kamenetskii et al., 2013).

To distinguish such field structures from regular EMF structures, they termed them magnetoelectric (ME) fields. They show existence of sources of MW ME near fields—the *ME particles*. These particles can be represented by small quasi-2D ferrite disks with magnetic-dipolar-oscillation spectra. The near fields originating from such particles are characterized by topologically distinctive power-flow vortices, nonzero helicity, and a torsion degree of freedom. The authors provided a theoretical analysis of properties, including chirality and helicity, of the E and H fields inside and outside of a ferrite particle with magnetic-dipolar-oscillation spectra resulting in the appearance of MW ME near fields. Based on the obtained properties of the ME near fields, the authors suggested possibilities for effective MW sensing of natural and artificial chiral structures.

In specially designed experiments, Gapeev et al. have compared effects in near and far field (Gapeev et al., 1996). These authors exposed mouse peritoneal neutrophils to RF using different types of antenna and wide-band coupling with the object both in near-field and far-field zones. They found that low-intensity RF in the near-field zone modifies the activity of peritoneal neutrophils in a frequency-dependent manner. The RF exposure inhibited luminol-dependent chemiluminescence of neutrophils activated by opsonized zymosan. This effect was not found in the far-field zone.

5.3 BIOLOGICAL SYSTEMS ARE NONLINEAR AND NONEQUILIBRIUM OPEN THERMODYNAMIC SYSTEMS

All objects, whether living or nonliving, are continuously generating EMF due to the thermal agitation of their charged particles. The EMF spectrum that is generated is described by Planck's law for the ideal case of a black body in thermal equilibrium. Thermally generated EMF has a random, noncoherent character. Historically, biology has been steeped in the biochemistry of equilibrium thermodynamics. Heating and heat exchange have been viewed as measures of essential processes in the brain and other living tissues, and intrinsic thermal energy has been seen as concept setting an immutable threshold for external MW stimulation (Adair, 2003). From many studies on the NT MW biological effects, it is clear that heating is not the basis of a broad spectrum of biological phenomena incompatible with this concept. They are consistent with processes in nonequilibrium thermodynamics. There is an emerging notion that the physical mechanisms of the NT MW effects must be based on physics of nonequilibrium and nonlinear systems (Binhi, 2002; Binhi and Rubin, 2007; Bischof, 2003; Brizhik et al., 2009a; Frey, 1974; Frohlich, 1968; Grundler et al., 1992; Kaiser, 1995; Scott, 1999; Srobar, 2009b). Physically, living biological systems are not at equilibrium (they have different energy levels than their surroundings) and are open thermodynamic systems (they can exchange energy and matter with the surroundings) (Trevors and Masson, 2011). Such systems may locally decrease entropy (increase order). Spontaneous nonlinear oscillations occur in biological systems (Kruse and Julicher, 2005; Nakahata et al., 2006). Since living systems are not in thermal equilibrium, their electromagnetic (or generally, vibration) spectrum may also deviate from the thermal spectra given by Planck's law (Cifra et al., 2011). It is generally accepted that physical theories on interactions between MW at low intensities and biological tissues must account for the facts that biological systems do not exist at equilibrium, that the dynamic nature of these systems is controlled by enzyme-mediated reactions, and that primary effects may be amplified by nonlinear biological processes (IARC, 2013).

5.4 κT PROBLEM

A number of subcellular organelles (membrane, mitotic spindle, nucleus, DNA-domain) or even a single molecule (protein, DNA) was considered as the target of interaction with NT MW. Regardless of the target, one of the major problems in explaining NT MW effects is seen in the fact that the quantum of energy of an EMF with a frequency lower than a few THz in most cases is less than the average energy of thermal noise (kT constraint), providing limits for the effects of a single quantum (Adair, 2003). However, the kT problem is based on assumption that primary

absorption occurs by the atomic or molecular target under thermal equilibrium conditions in a single-quantum process. This assumption is not scientifically justified. In particular, besides atomic/molecular targets, relatively large particles with almost macroscopic magnetic moment, the so-called magnetite (Binhi, 2002), or charged macromolecular DNA–protein macrocomplexes such as nucleoids and nuclei (Matronchik and Belyaev, 2008) can be a sensitive target for NT MW effects. Moreover, even cell ensembles may be responsible for primary interaction with MW (Belyaev et al., 1994a; Shcheglov et al., 1997a). The interaction with EMF can be of multiple-quantum character and may develop in the absence of thermal equilibrium (Binhi and Rubin, 2007; Panagopoulos et al., 2000, 2002).

The kT problem has recently been challenged by Binhi and Rubin (2007). These authors stress that the notion of a *kT constraint* originates from statistical physics and is only applicable to systems near thermal equilibrium. In such systems, NT MW cannot change the mean energy of cellular structures or, more precisely, the vibration energy of their molecules stored in their degrees of freedom. Degrees of freedom characterize the ways in which a molecule or structure can move (vibrate, rotate, etc.). The energy absorbed by a degree of freedom from MW at certain frequency cannot be *stored* or accumulated at this frequency if this degree of freedom is coupled to other degrees strongly. In that case, the energy at this frequency will be redistributed to other degrees of freedom (frequencies) and will dissipate very rapidly. However, biological systems are thermodynamically far away from equilibrium, and some of their degrees of freedom are weakly coupled to others or to the surrounding heat bath. Therefore, thermalization time (time needed to redistribute energy into other degrees of freedom) may be significantly higher as compared to systems in thermal equilibrium. Thus, it is not surprising that NT MW can induce a significant change in energy in some degrees of freedom before dissipation or redistribution of the energy. In accordance with this concept, recent study has revealed an amazing feature of the Fröhlich's systems, namely, that with increasing total pumping, the incoming energy is deposited almost entirely in the lowest-order (fundamental) mode, even though its modal pumping rate becomes less than that of the higher-order modes (Srobar, 2009a).

The kT problem can also be addressed in the frames of stochastic resonance as a possible process involved in EMF nonlinear interactions with biosystems. In stochastic resonance, the sensitivity of a system to a weak periodic signal is actually increased when the optimal level of random noise is added (Hanggi, 2002; McDonnell and Abbott, 2009).

Given classical considerations of electromagnetic waves, another question with respect to NT MW effects has to do with the coupling of energy. The absorption of EMF energy is inefficient when the receiving antenna (molecules, cells, and their ensembles) is considerably smaller than the EMF wavelength. However, coupling and energy transfer to subcellular structures may become greater if there is resonance interaction of NT MW with vibration modes of the cellular structures (Adair, 2002). As already mentioned earlier, cell ensembles may be responsible for primary interaction with MW (Belyaev et al., 1994a; Shcheglov et al., 1997a). Coupling of EMF in the MW region to vibrations of cellular structures is consistent with the fundamentals of Fröhlich's theory that predicts resonant interaction of biomolecular structures with external EMFs (Frohlich, 1980). Theoretical analysis of available experimental data on the MW effects at superlow intensities concluded that these effects must be considered using a quantum-mechanical approach (Belyaev et al., 1994a; Binhi, 2002). In quantum-mechanical consideration, the vector potential that interacts with the wave function altering its phase appears to be the coupling pathway (Trukhan and Anosov, 2003, 2007).

5.5 FRÖHLICH'S THEORY

A fundamental theory suggested by Fröhlich postulated that biological systems exhibit coherent longitudinal vibrations of electrically polar structures (Frohlich, 1968). In the Fröhlich's theory, when the energy supply exceeds a critical level, the polar structure enters a condition in which a steady state of nonlinear vibration is reached and energy is stored in a highly ordered fashion.

While the Fröhlich's theory has been criticized, often by using unjustified assumptions such as that biological system is in thermal equilibrium, it has never been dismissed because it does not contradict the basic physical principles. Fröhlich's condensation of oscillators in vibration mode of lowest frequency is usually compared with Bose–Einstein condensation, superconductivity, lasing, and other unique phenomena involving macroscopic quantum coherence (Reimers et al., 2009). Fröhlich's condensates were classified into three types: weak condensates in which profound effects on chemical kinetics are possible, strong condensates in which an extremely large amount of energy is channeled into one vibrational mode, and coherent condensates in which this energy is placed in a single quantum state (Reimers et al., 2009). According to Reimers et al., from those three types, only weak condensates may have profound effects on chemical and enzyme kinetics and may be produced from biochemical energy or from RF, MW, or terahertz radiation (Reimers et al., 2009). The Fröhlich theory has predicted the existence of selective resonant EMF interactions with biosystems (Frohlich, 1970, 1975). In line with Fröhlich theory, multiple experimental studies have shown dependence of NT MW effects on frequency (Belyaev, 2010a). As far as the frequency is concerned, Fröhlich's original estimate was in the vicinity of 10^{11} Hz, but considering the diversity of oscillating species, both lower and higher frequencies may also be expected. It has been shown that energy can be condensed into Fröhlich's fundamental mode responsible for resonance interaction at the frequency of 0.1–1 GHz (Srobar, 2009b). Such condensation of metabolic energy into fundamental mode results in a significant deviation from thermal equilibrium (Srobar, 2009b).

The results of studies with different cell types indicated that narrowing of the resonance window upon decrease in PD is one of the general regularities in cell response to NT MW (Belyaev et al., 1996; Belyaev and Kravchenko, 1994; Grundler et al., 1992; Shcheglov et al., 1997a). This regularity suggests that many coupled oscillators are involved nonlinearly in the biological effects or the response of living cells to MW as has been predicted by Fröhlich (1968).

While the original Fröhlich model considered excitation in membranes, it is not mechanistically limited to any particular cellular structure. Basically, Fröhlich's condensates may form in any biological structure consisting of electrically polar oscillators, such as microtubules (important elements of the cytoskeleton), actin filaments, or DNA. Pokorny considered oscillations in microtubules that are electrically polar structures with extraordinary elastic deformability at low stress and with energy supply from hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) (Pokorný, 2004). At least a part of the energy supplied from hydrolysis can excite vibrations. The author's calculation shows that some forms of such energy are not thermalized but are instead condensed into a pattern of oscillations. In this context, the structure of the cell's cytoskeleton, which is based on microtubules, is stated to satisfy the basic requirements for an oscillating electric field. Author considers an ionic charge layer or cylindrical envelope around the microtubule. Near the surfaces of microtubules, there may be layers of ordered water as much as 200 nm thick. The effects of the ion layer and bound water would be to reduce viscosity and thus promote the survival of excitations within the microtubule. Losses to viscosity in the inner cavity of the microtubule might be particularly low, as it is thought that all the water in this area could be ordered. Srobar has further considered damping in the Fröhlich's theory (Srobar, 2009b).

5.6 SPIN STATES, RADICAL PAIR MECHANISM

Even small EMF effects on radical concentration could potentially affect multiple biological functions (IARC, 2013). Effects of MW on spin-dependent recombination of radicals via the free radical pair mechanism (RPM) have been established in multiple studies and have recently been reviewed (Belyaev, 2010a; Georgiou, 2010). The reported effects include increased production of reactive oxygen species, enhancement of oxidative stress–related metabolic processes, an increase in DNA single-strand breaks, increased lipid peroxidation, and alterations in the activities of enzymes associated with antioxidative defense. Furthermore, many of the changes observed in RF-exposed cells were prevented by pretreatment with antioxidants and radical scavengers (Belyaev, 2010a;

IARC, 2013). Recent review has summarized studies on EMF exposure and oxidative stress in brain (Consales et al., 2012). While the data from different studies should be compared with care in view of variation in physical and biological parameters, most part of collected data have shown effects of ELF and RF EMF on oxidative stress in brain (Consales et al., 2012).

Keilmann has proposed a theory based on molecular spin (Keilmann, 1986). Spin is a kind of intrinsic degree of freedom of molecules and particles, which, in simple words, characterizes rotation of particles around their own axis. Spin is weakly coupled to other degrees of freedom and thus can be nonthermally populated. In other words, NT MW can influence the population of spin states. Triplet and radical molecules are the special target of this theory since they have nonzero spin. Hence, if these molecules belong to a reaction chain where the reactivity is dependent on spin, EMF can influence the reaction rate. This triplet mechanism may also account for frequency dependence of the NT MW effects (resonance dependency on EMF frequency) affecting biochemical reactions involving radicals.

Woodward et al. calculated magnetic RF (10–150 MHz) resonances among the electron–nuclear spin states of the radical pair, which produces a change in the yield of the product formed by recombination of singlet radical pairs (Woodward et al., 2001). If the radical pairs are sufficiently long-lived (>100 ns), a weak RF magnetic field can enhance the singlet ↔ triplet interconversion and so significantly alter the fractions of radical pairs that react via the singlet and triplet channels. This can take place even though the applied field may be much smaller than the hyperfine couplings, and all magnetic interactions are much weaker than the thermal energy per molecule, kT (Woodward et al., 2001). If spin relaxation and diffusive separation of the radicals are slow enough, effects approaching this size might even be seen for fields as weak as the Earth's (Woodward et al., 2001). In general, larger RF effects are expected if the diffusion of radicals is restricted, for example, in more viscous cell nuclei (Brocklehurst and McLauchlan, 1996). The effect of the RF field on recombination of radicals depends strongly on its frequency and orientation of ambient SMF (Henbest et al., 2004). In dependence on Zeeman and hyperfine interactions, complex response to weak static and RF fields can be anticipated in which the number and position of the resonances and the selection rules for RF-induced transitions are determined by the combined effects of the two interactions (Henbest et al., 2004). Radical pair effects were usually studied using organic radicals in isotropic solution with hyperfine interactions smaller than ~80 MHz. However, this frequency range might be considerably extended using radicals with much larger hyperfine interactions, for example, when the unpaired electron is centered on a phosphorus atom, for which resonant effects might be seen at frequencies up to ~1 GHz (Stass et al., 2000). The frequency range of resonant behavior can be also extended under conditions of restricted molecular rotation when anisotropy of the hyperfine interactions may become important (Stass et al., 2000).

Radicals were considered as the part of a system described by nonlinear equations of biochemical kinetics with bifurcations (Grundler et al., 1992). Structured organization of radicals in biological systems may have an important role for estimation of power levels and comparison with sensitivity of chemical reactions in vitro. The best known example suggesting this structural organization in biology is magnetic compass of birds, which is believed to be based on RPM and sensitive to weak magnetic fields (Ritz et al., 2009).

The long-lived radicals with lifetime of hours have been described in leaving cells (Feldermann et al., 2004; Gudkov et al., 2010; Hafer et al., 2008; Hausser, 1960; Held, 1988; Kumagai et al., 2003; Warren and Mayer, 2008). Because RF effects on conversation to triplet depend on the lifetime of radicals, long-lived radicals may underlie the NT RF effects.

5.7 ELECTROSOLITON

The other theory of EMF generation relates to the electrosoliton (Brizhik et al., 2003, 2009a,b). The soliton is a self-reinforcing solitary wave (a wave packet or pulse) that maintains its shape while it propagates. The electrosoliton is the electrical counterpart of a soliton. Electrosolitons can

be viewed as moving charges that provide transport of a charge in biological systems and can be considered an important part of EMF generation in the MW frequency region. In their theoretical analysis, the authors showed that the spectrum of biological effects of EMF can be divided into two major bands. The lower-frequency band is connected with an intense form of EMF energy absorption and consequent emission of sound waves of solitons. In contrast, the higher-frequency bands induce soliton transitions to delocalized states and thus are able to destroy the soliton and disrupt the transfer of energy and information.

5.8 MW HEARING

A well-characterized physical mechanism of MW effects is the so-called MW hearing or Frey effect discovered by Alan Frey (1961). Human beings can *hear* MW energy if MWs are specifically modulated. MW hearing depends on several physical parameters and individual traits. Among physical parameters, it is mostly dependent on shape of pulses and energy in one pulse. If the PD at peak value was relatively high, 267 mW/cm^2 at the duty cycle of 0.0015 and a frequency of 1.3 GHz fitting into the range of mobile phone carrier frequencies, threshold value expressed in units of average incident PD was 0.4 mW/cm^2 (Frey, 1962). At first, MW hearing was repeatedly dismissed as an artifact until it was demonstrated in rats in a carefully controlled study by King et al. (Justesen, 1975; King et al., 1971). Frey and Messenger (Frey and Messenger, 1973) demonstrated and Guy et al. (1975) confirmed that an MW pulse with a slow rise time is ineffective in producing an auditory response; only if the rise time is short, resulting in effect in a square wave with respect to the leading edge of the envelope of radiated RF energy, does the auditory response occur. Thus, the rate of change (the first derivative) of the wave form of the pulse is a critical factor in perception. Given a thermodynamic interpretation, it would follow that information can be encoded in the energy and *communicated* to the *listener.* Dependence of MW hearing effects on type and parameters of modulation has been theoretically described and experimentally confirmed (Elder and Chou, 2003). MW hearing occurs in the frequency range from 2.4 MHz to 10 GHz (Elder and Chou, 2003). The threshold for RF hearing of pulsed 2450 MHz fields was related to an energy density of 40 mJ/cm^2 per pulse or energy absorption per pulse of 16 mJ/g, regardless of the peak power of the pulse or the pulse width (less than 32 ms); calculations showed that each pulse at this energy density would increase tissue temperature by about 5×10^{-6}°C (Elder and Chou, 2003). Evidently, any pulsed MWs will generate acoustic transients in brain tissue, which may or may not be audible.

5.9 PLASMA MEMBRANE AND IONS

The cellular membrane has long been considered as a target for interaction with MW (Adey, 1981, 1999; Desai et al., 2009; Frohlich, 1968). Kaiser suggested coupling of EMF to intracellular Ca^2 oscillations as a possible mechanism (Kaiser, 1995). In his model, nonlinear oscillators were considered, which manifested specific phenomena including synchronization, sub- and superharmonic resonances, and sensitivity of biosystems to MW at various frequencies and intensities. A cell membrane–related theory for MW effects has been proposed by Devyatkov and his coworkers (Devyatkov, 1973; Devyatkov et al., 1994). These scientists considered deformations and asymmetries of polar cellular membrane as a mechanism for generation of acoustoelectric waves whose electric component depends on deviation from the healthy state. A normal (healthy) physiological state was characterized by the lowest sensitivity to MW.

It should be stressed that uncoupled membrane channel in the presence of thermal noise and a general *white noise* intensity (in thermodynamic equilibrium) cannot be reckoned as a relevant target for NT MW effects (Astumian et al., 1995). Instead, the response of coupled nonlinear oscillators should be considered. In recent experimental study, response of neurons to applied electric field resulted to changes in somatic potential of 70 μV, below membrane potential noise levels for

neurons, demonstrating that emergent properties of neuronal networks can be more sensitive than measurable effects in single neurons (Deans et al., 2007). A theoretical model by Panagopoulos et al. has analyzed effects of ELF fields and ELF-modulated RF on collective behavior of intracellular ions that affects membrane gating (Panagopoulos et al., 2000, 2002; Panagopoulos and Margaritis, 2010).

5.10 DNA, CHROMATIN, NUCLEI

The number of credible possible sources for electromagnetic signals in cells includes DNA (Liboff, 2012). The experimental data by Belyaev and coworkers provided strong evidence that DNA is a target for NT MW effects: (1) changes in supercoiling of DNA loops and helicity of DNA in leaving cells induced by DNA intercalator ethidium bromide and ionizing radiation correlated with changes in response of these cells to differently polarized NT MW (Alipov et al., 1993; Belyaev et al., 1992a–c; Ushakov et al., 1999); (2) direct prove was obtained by exposing cells with genetically increased length of genome and comparing the obtained resonance responses at differently polarized MW with theoretical predictions (Belyaev et al., 1993a). Possibility that DNA may be a target for MW effects was questioned in study by Prohovsky (Prohofsky, 2004). This study analyzed vibration modes in DNA double helix considered as stretched-out molecule under conditions of thermal equilibrium, in Debye approximation for solids. However, this is an unjustified assumption for modeling of DNA in a leaving cell for the fundamental reasons already discussed earlier. The DNA of each human cell would reach about 2 m in length if it would be stretched out, while it is condensed mostly by proteins, RNA, and ions in chromatin by a factor of about 1000 inside the nucleus of about 5 μm in diameter. Chromatin is organized at several levels of organization in discrete units, one of which represents charged domain of DNA supercoiling (DNA loop or chromatin domain). The net charge of nucleus/DNA loop and chromatin condensation ratio is dynamically changed because of dynamic interactions of DNA with other molecules and ions displaying a spectrum of natural oscillations—acoustic, mechanic, and electromagnetic (Belyaev, 2010b; Belyaev et al., 1993a; Matronchik and Belyaev, 2008). Associations of multiple chromatin binding proteins with DNA are transient and last for only a few seconds (Pliss et al., 2010). Emerging evidence indicates the presence of natural oscillations in charged chromatin (Belyaev, 2010b; Binhi et al., 2001; Matronchik and Belyaev, 2008). Natural rotation of entire nucleus in leaving cells including neurons was found (Brosig et al., 2010; Ji et al., 2007; Lang et al., 2010; Levy and Holzbaur, 2008; Park and De Boni, 1991). These effects provide clear evidence for structured dynamic organization of DNA in leaving cells and provide further basis for NT mechanism of EMF effects.

Importantly, local concentration of macromolecules in human nuclei is very high and may reach 160, 210, and 14 mg/mL for proteins, RNA, and DNA, correspondingly (Pliss et al., 2010). This concentration fits to those at which macromolecules crystallize in solutions. Whether chromatin in nuclei is organized as a liquid crystal remains to be investigated (Leforestier and Livolant, 1997). However, it is clear that DNA in the living cell cannot be considered as an aqueous solution of DNA molecules in thermodynamic equilibrium.

Recent study by Pliss et al. provided clear evidence for asymmetric oscillatory movement of the chromatin domains in human cells over short time periods (<1 s) (Pliss et al., 2013). These oscillations were energy independent and characteristic for early, mid-, and late S-phase of cell cycle. In contrast, fluorescent beads of similar dimensions (100 nM) as chromatin domains displayed random Brownian motion following microinjection into the cell nucleus. Slow nonuniform rotation of nuclei in different cell types has been documented using time-lapse microscopy (Gerashchenko et al., 2009; Ji et al., 2007; Levy and Holzbaur, 2008).

Matronchik and Belyaev have considered the effects of ELF and MW on slowly rotating chromatin domain/nucleus in the frame of their model of phase modulation (Matronchik and Belyaev, 2008). In particular, frequency dependency (of resonance type) of the ELF/MW effects and their

dependence on SMF have been considered by modeling slow nonuniform rotation of the charged DNA-domain/nucleoid under combined effects of ELF/MW and SMF. This model suggested that the MW exposure results in slow nonuniform rotation of the nucleoid with angular speed that depends on Larmor frequency. The model predicts also that the NT effects of MW are dependent on carrier frequency and SMF in the place of MW exposure.

An important finding for evaluation of mechanisms is dependence of MW resonance spectra on PD during exposure (Shcheglov et al., 1997a). These data on rearrangement of resonance response of cells to MW in dependence on its PD have been interpreted in the framework of the model of electron-conformational interactions (Belyaev et al., 1996).

The Ising model has been applied for primary interaction of MW with DNA (Arinichev et al., 1993). Arinichev et al. explained resonance absorption and effect of MW by the collective long-relaxation optical vibrations within the same frequency range. The primary structure of double-stranded DNA was considered as three tied chains: two sugar–phosphate chains and one chain of complimentary nucleotides linked by hydrogen bonds. One sugar–phosphate chain was considered a 1D lattice whose unit cell consists of two compact molecular groups, that is, deoxyribose (D) and a residue of phosphoric acid (P). Given the polarity of this 1D lattice's unit cell, the optical branch in the law of dispersion corresponds to polar phonons. Radically new features in the spectrum of optical vibrations of the sugar–phosphate chain appeared when the influence of the nucleotide chain was taken into account. This chain was interpreted as a 1D order–disorder ferroelectric-like system due to the double-wall potential of hydrogen bonds between the complimentary nucleotides. The regulatory proteins and ions are bonded to certain DNA sequences in the processes of transcription, replication, repair, and recombination, producing the polarization of DNA and the ordering of proton tunneling. This kind of ordering was interpreted as a local order–disorder phase transition. To describe this phase transition, the solution for the Ising model in the transverse field was obtained with the self-consistent field method. Because of the obtained relationship between vibrations of the sugar–phosphate chain and the collective modes in the chain of complimentary nucleotides, the external MW may affect the local phase transition in hydrogen bonds of nucleotide pairs (partial unwinding of the DNA) and therefore, the process of DNA–protein interactions. The partially unwound DNA regions may be cleaved by endonucleases, resulting in DNA damage. The suggested model explained the different efficiency of right- and left-handed polarized MW (Belyaev et al., 1992a–c, 1993a,b, 1994a, 1996; Belyaev and Kravchenko, 1994; Ushakov et al., 1999). Recent experimental study by Vishnu et al. (Vishnu et al., 2011) confirmed the Arinichev's theoretical model (Arinichev et al., 1993). Vishnu et al. investigated the effect of mobile phone radiation on DNA by using fluorescence technique. Absorption spectra showed increased absorption of DNA after exposure to radiation from mobile phones with different SAR values up to 1.4 W/kg and MW frequencies, 900 MHz–3.2 GHz, which characterizes unwinding of the DNA double strand. Fluorescence intensity of dye-doped DNA solution was reduced suggesting that absorbed energy is used in unwinding the double strand of DNA after exposure to MW.

5.11 MW/ELF MECHANISMS

Some physical mechanisms consider effects ELF and MW in frame of the same physical models (Binhi, 2002; Chiabrera et al., 1991, 2000; Matronchik et al., 1996; Matronchik and Belyaev, 2005, 2008; Panagopoulos et al., 2002).

Binhi's mechanism of quantum interference considers effects of magnetic field on distribution of probabilities of coordinate of charged particle or, in other words, on wave function of charged particle (Binhi, 2003). The effect is based on shift in the phase of wave function and appearance of nonlinear interference. This mechanism has especially interesting application for rotating charged particles like ions in the pockets of rotating proteins. Based on quantum-mechanical calculations stemming from this mechanism, ELF and MW can result, under specific parameters of these fields

such as intensity and frequency, in a shift in kinetics of ion binding into the protein pockets, affecting activity of this protein and related biochemistry (Binhi, 2003). Importantly, effective conditions of exposure include SMF at the place of exposure similar to the model of Matronchik and Belyaev, which is also based on combined EMF effects on phase (Belyaev et al., 1994b; Matronchik et al., 1996; Matronchik and Belyaev, 2008).

A recently suggested model considers effects of magnetic vector potential **A** (Trukhan and Anosov, 2003, 2007). Magnetic vector potential, which was initially introduced as value for calculation of magnetic field, **B** (**B** = rot**A**), was considered by analogy with the well-known Aharonov–Bohm and Josephson's effects as primary mechanism underlying the effect on charge transport (electrons in macromolecules or protons in water clusters). The effect is based on shift in the wave functions of charges and their interference. Importantly, the effect may appear at very low **A**, about 3×10^{-5} T m (**B** ~ 50 μT). In contrast to **B**, which decreases by $\sim 1/R^3$ with a distance from elementary magnetic dipole, **A** decreases as $\sim 1/R^2$, which may provide better interaction between cells in response to EMF. This interaction between cells in response to weak EMF was experimentally established in repeated experiments both with MW (Belyaev et al., 1994a, 1996; Shcheglov et al., 2002, 1997a) and ELF exposure (Belyaev et al., 1995, 1998).

5.12 CONCLUSIONS

Significant progress has been achieved in understanding the mechanisms for NT biological effects of MWs. However, this understanding is not comprehensive. It is generally accepted that more than one physical theory may describe the same phenomena (compare, e.g., Debye model of phonons in a box and Einstein model of quantum harmonic oscillators for solids). Thus, a variety of physical mechanisms may explain interaction of biosystems with NT MW and its important characteristics such as dependence on frequency, polarization, and modulation.

ACKNOWLEDGMENTS

Financial support from the National Scholarship Programme of the Slovak Republic and the Russian Foundation for Basic Research is gratefully acknowledged.

LIST OF ABBREVIATIONS

EMF electromagnetic field
ELF extremely low frequency
MW microwaves
PD power flux density
RF radiofrequency
SAR specific absorption rate
SMF static magnetic field

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