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Mechanism for action of electromagnetic fields on cells

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Abstract

A biophysical model for the action of oscillating electric fields on cells, presented by us before [Biochem. Biophys. Res. Commun. 272(3) (2000) 634–640], is extended now to include oscillating magnetic fields as well, extended to include the most active biological conditions, and also to explain why pulsed electromagnetic fields can be more active biologically than continuous ones. According to the present theory, the low frequency fields are the most bioactive ones. The basic mechanism is the forced-vibration of all the free ions on the surface of a cell's plasma membrane, caused by an external oscillating field. We have shown that this coherent vibration of electric charge is able to irregularly gate electrosensitive channels on the plasma membrane and thus cause disruption of the cell's electrochemical balance and function [Biochem. Biophys. Res. Commun. 272(3) (2000) 634–640]. It seems that this simple idea can be easily extended now and looks very likely to be able to give a realistic basis for the explanation of a wide range of electromagnetic field bioeffects.

Keywords: Oscillating electric–magnetic fields; Biological effects; Action mechanism; Ions' forced-vibration

At least until the first publication of the present theoretical model [1] there was not any generally accepted mechanism to explain the action of weak electric fields on cells [2]. Several mechanisms that had been proposed for the explanation of the biological action of ELF (''extremely low frequency'') magnetic fields, either face objections on energy levels and other issues [2,3] or [4], do not take into account friction forces and thus are found to have deficiencies. Our theoretical model, some extensions of which we are going to present here, seems to explain well the biological action of ELF and VLF (''very low frequency'') fields even at very low intensities of several V/m, in the case of electric fields, without the deficiencies of other proposed mechanisms [2].

As we have described [1], our model is based on the simple hypothesis that an oscillating, external electric field, will exert an oscillating force on each of the free ions that exist on both sides of all plasma membranes and that can move across the membranes, through transmembrane proteins. This external oscillating force will cause, to each free ion, a forced-vibration. When the amplitude of the ions' forced-vibration transcends some critical value, the oscillating ions can give a false signal for gating channels that are electrically sensitive (or even mechanically sensitive) disordering in this way the electrochemical balance of the plasma membrane and therefore the whole cell function. Since the amplitude of the forced-vibration is found to be inversely proportional to the field's frequency [Eq. (4)] low frequency fields appear to be more bioactive according to the present theory.

The same idea can be extended now to include oscillating magnetic fields as well, since such fields would also exert forces on the free ions, with the same mechanism and with similar results as the ones described in the case of oscillating electric fields.

According to the present mechanism, there is also an explanation why pulsed electromagnetic fields can be more bioactive than continuous fields of the same characteristics, or why the greatest effects of a continuous field may occur with onset or removal of exposure to this. Such phenomena have been observed in several experiments [5–8] and until now there was not any theoretical explanation.

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Finally the described mechanism is extended now to include the most active biological conditions, since in its first presentation [1] only the mildest conditions were discussed.

It is well known that on both sides of every cell membrane, there are large numbers of free ions (mainly K^+, Na^+, Cl^-, Ca^{2+}, etc.), which control the cell volume, play an important role in signal transduction processes, and create an intense electric field that exists between the two sides of all cell membranes [9].

Ion fluxes through cell membranes are caused by forces due to concentration and voltage gradients, between the two sides of the membrane. Under equilibrium conditions, the net ion flux through a membrane is zero and the membrane has a voltage difference $\Delta\Psi$ of the order of 100 mV, between its external and internal surfaces, with the internal always being negative in relation to the external, which is called the "membrane's electrical potential." Cation electrosensitive channel-proteins seem to be the main cause for the generation of this voltage gradient [10].

The potential difference across the plasma membrane, under equilibrium conditions, caused by a certain type of ion, is given by the Nernst equation [1,9]. The total electrical potential difference across the membrane is the sum of the contributions from all types of existing ions.

An oscillating, external electric or magnetic field will exert an oscillating force on every free ion on both sides of the plasma membrane, as well as on the ions within channel proteins, while they pass through them. This external oscillating force will cause on every ion, a coherent forced-vibration, superimposed on the ion's random thermal motion [11].

The mechanism

Forces exerted on a free ion. As we have described in detail [1], if we consider the simplest case of an external, alternating electric field, of intensity: $E = E_0 \sin \omega t$ and circular frequency: $\omega = 2\pi v$ (v, the frequency), then on every free ion in the vicinity of a cell's plasma membrane will be exerted: (a) An alternating force of magnitude: $F_1 = Ezq_e = E_0 zq_e \sin \omega t$ (z, the ion's valence and $q_e = 1.6 \times 10^{-19}$ Cb, the electron's charge). (b) A restoration force: $F_2 = -Dx$, proportional to the displacement distance x. ($D = m_i\omega_0^2$, the restoration constant, with m_i the ion's mass and $\omega_0 = 2\pi v_0$, with v_0 the ion's oscillation self-frequency, if the ion were left free after its displacement x.) In our case, this restoration force is found to be very small compared to the other forces and thus does not play any important role. (c) A damping force, $F_3 = -\lambda u$, where u, is the ion's velocity and λ is the attenuation coefficient for the ion's movement, which for the cytoplasm or the extracellular medium is calculated to be $\lambda \cong 10^{-12}$ kg/s, while for ions moving inside channel proteins is calculated to have a value: $\lambda \cong 6.4 \times 10^{-12}$ kg/s (for the case of Na^+ ions, moving through open Na^+ channels) [1].

Forced-vibration equation for a free ion. Each ion, because of the above forces, will obtain an acceleration a and its movement equation (let us say for the x direction) will be

$$\begin{aligned} & m_i \mathsf{a} = -\lambda u - Dx + E_0 z q_e \sin \omega t \\ & \Rightarrow m_i \frac{d^2x}{dt^2} + \lambda \frac{dx}{dt} + m_i \omega_0^2 x = E_0 z q_e \sin \omega t. \end{aligned} \tag{1}$$

Eq. (1) is the movement equation of a free ion in the vicinity of a cell's plasma membrane, under the influence of an external, alternating electric field.

As we have shown in detail [1], the general solution of Eq. (1) is

$$x = \frac{E_0 z q_e}{\lambda \omega} \cos \omega t - \frac{E_0 z q_e}{\lambda \omega}. \tag{2}$$

As we can see, the term $-E_0 zq_e/\lambda\omega$ of the solution displaces the ion's forced-vibration, at a constant distance: $-E_0 zq_e/\lambda\omega$, from its initial equilibrium position, but has no effect on the vibrational term, which is: $(E_0 zq_e/\lambda\omega)\cos\omega t$ and thus plays no role in the ion's vibrational movement.

As we shall discuss later on, this constant displacement of the whole vibrational movement, represented by the term $-E_0 zq_e/\lambda\omega$, at the moment when the external field is applied and during its first period (onset of the field), when the total ion displacement will be twice the amplitude $E_0 zq_e/\lambda\omega$ of the forced-vibration, is able to double the effect of the external field. The same happens at the moment when the external field is interrupted. This suggests that pulsed fields can be twice more drastic than continuous, non-interrupted, fields of the same rest characteristics.

Nevertheless, the vibrational movement is described by the equation

$$x = \frac{E_0 z q_e}{\lambda \omega} \cos \omega t. \tag{3}$$

Eq. (3) represents a harmonic oscillation of constant amplitude independent of any initial conditions.

As we can see, the amplitude of the forced-vibration is

$$A = \frac{E_0 z q_e}{\lambda \omega} \tag{4}$$

and the forced-vibration is in phase with the external force.

Thus, an external alternating electric field will cause on every free ion, in the vicinity of the plasma membrane, a forced-vibration of the same frequency as that of the external field and with vibrational amplitude inversely proportional to the frequency. The ions will oscillate in phase with the field.

Irregular channel gating, due to the free ions' forced-vibration. The oscillating ions will then represent a periodical displacement of electric charge, able to exert forces on every fixed charge of the membrane, like the charges on the voltage sensors of voltage-gated channels. In this way, the oscillating ions could be able to upset the membrane's electrochemical balance, by gating such channels.

Additionally, ions already inside voltage-gated channels, while they pass through them, are able, because of the forced-vibration, to move into another position than the one, if there were not any external field, giving with their charge, a false signal for gating such channels, also by exerting forces on the channels' voltage sensors.

The channel proteins on the cell membranes are constructed by several parallel transmembrane α-helixes and it is not clear yet whether they form aqueous pores [12], or "condensed state pathways" [13]. In any case, the ions seem to pass dehydrated through the channels [12]. There are "voltage-gated channels," "mechanically gated channels" (gated by ion pressure), and "ligand-gated channels" (chemically sensitive) [9].

Voltage-gated channels are mainly cation channels. The state of these channels (open or closed) is determined by the electrostatic interaction between the channels' voltage sensors and the transmembrane voltage. They interconvert between open and closed states, when the electrostatic force, acting on the electric charges of their voltage sensors, transcends some critical value. The voltage sensors of these

channels are four symmetrically arranged, transmembrane, positively charged helical domains, each one designated S4 [14–19].

It is known that changes in the transmembrane potential of the order of 30 mV are able to gate electrosensitive channels [20,21].

We have shown [1] that a single ion's displacement ∂r, of about 10^{-12} m (for ions moving inside channels), in the vicinity of S4, can generate a force on each S4, equal to that, generated by a change of 30 mV, in the membrane's potential, and thus gate a cation channel.

The effective charge of each S4 domain is found to be: $q = 1.7q_e$ [20]. The force on this charge, generated by a change $\partial\Delta\Psi = 30$ mV of the membrane potential, is calculated [1] to have a magnitude: $\partial F = 8.16 \times 10^{-13}$ N.

This is the force, on the voltage sensor of a voltage-gated channel, required normally, to interconvert the channel between closed and open states.

The force acting on the effective charge of an S4 domain, via an oscillating, z-valence free cation, is

$$F = \frac{1}{4\pi\varepsilon\varepsilon_0} \cdot \frac{q \cdot zq_e}{r^2}$$

and thus

$$\partial r = -\frac{2\pi\varepsilon\varepsilon_0 \partial F \cdot r^3}{q \cdot zq_e}. \quad (5)$$

This is the displacement of one, z-valence cation, in the vicinity of S4, able to generate the force ∂F necessary to gate the channel. Where r is the distance between the free ion and the effective charge on the S4 domain, which can be conservatively taken as 1 nm [9,14,18] and $\varepsilon_0 = 8.854 \times 10^{-12}\,\mathrm{N^{-1}\,m^{-2}\,Cb^2}$ is the dielectric constant of vacuum. The relative dielectric constant ε can have a value of 80 for a water-like medium (cytoplasm, or extracellular space), or a value as low as 4, for ions moving within channel-proteins [22].

From Eq. (5) and for $\partial F = 8.16 \times 10^{-13}$ N, $z = 1$, we get

$$\partial r \cong 80 \times 10^{-12}\,\mathrm{m} \quad (\text{for } \varepsilon = 80)$$

and

$$\partial r \cong 4 \times 10^{-12}\,\mathrm{m} \quad (\text{for } \varepsilon = 4).$$

(For double-valence cations and $\varepsilon = 4$, we get $\partial r \cong 2 \times 10^{-12}$ m.)

As we can see, a single cation's displacement of only few picometers from its normal position is able to interconvert voltage-gated channels, between open and closed states (for cations moving already within channels).

Therefore, any external field, which can cause a forced-vibration of the ions, with amplitude

$$A \geqslant \partial r \quad (6)$$

is able to alter the function of a cell.

Free ions move anyway because of thermal activity, with kinetic energies larger normally than the ones got by an external electromagnetic field [24]. But as we have explained [1], thermal motion is a random motion, in every possible direction, different for every single ion, causing no displacement of the ionic "cloud" and for this does not play any important role in the gating of channels, or in the passing of ions through them. In contrary, forced-vibration is a coherent motion of all the ions together in phase, which when superimposed to thermal motion can cause the effects described above.

If two or more cations interact (in phase), with an S4 domain, from 1 nm distance, ∂r in Eq. (5) decreases proportionally. The concentration of free ions on both sides of mammalian cell membranes is about one ion per nm^3 [9] and, this is why, we have conservatively calculated ∂r for one cation, interacting with an S4 domain, although it is very likely that several ions interact simultaneously each moment with an S4 domain from a distance of the order of 1nm. This is also true for ions moving within a channel, since it is known that although they pass through the narrowest part of the channel in single file [12,23], several ions fill the pore each moment as they pass sequentially and several ion-binding sites (three in potassium channels) lie in single file through the pore, close enough that the ions electrostatically repel each other [12].

In the mildest case, if we consider only one ion interacting with an S4 domain, this ion moving with a drift velocity, $u = 0.25$ m/s [1], it needs a time interval $\delta t = \partial r/u \cong 1.6 \times 10^{-11}$ s, in order to be displaced at the necessary distance $\partial r = 4 \times 10^{-12}$ m and this time interval is considerably smaller than the duration of channel opening or closing which is about 2.5×10^{-5} s [25]. During the same time interval δt, this ion will be displaced at a total distance X_{kT}, ranging from 1.5 to 4×10^{-10} m, because of thermal motion, according to the relation: $X_{kT} = \sqrt{2kT\delta t/\lambda}$, for human body temperature, 37 °C or $T = 310$ °K. (X_{kT} in m, δt in sec, λ in kg/sec, $k = 1.381 \cdot 10^{-23}\,\mathrm{J \cdot K^{-1}}$ is the Boltzmann constant) [1]. The mean free path of the ions in the aqueous solutions around the membrane is about 10^{-10} m [26] and it is certainly smaller within the channels, (the diameter of a potassium ion is 2.66×10^{-10} m and the diameter of the narrowest part of a potassium channel is about 3×10^{-10} m, thereby the mean free path of a potassium ion within the channel must be of the order of 10^{-11} m) [12,25]. Therefore, the ion within the above time interval δt will run because of its thermal activity several mean free paths, each one in a different direction, resulting in mutually extinguishing opposing forces on the channel's sensors, while at the same time the ion's displacement because of the external field is in a certain direction, exerting on each S4 domain a force of constant direction.

In the most realistic case, if we consider several ions interacting simultaneously with an S4 domain, then the effect of the external field is multiplied by the number of ions, whereas the effect of their random thermal motions becomes even more negligible.

Results and discussion

Substituting A from Eq. (4) in (6), it comes to the fact that a bioactive, external, oscillating electric field, of intensity amplitude E_0 and circular frequency ω, which causes a forced-vibration on every single-valence ion ($z = 1$), must satisfy the relation

$$\frac{E_0 q_e}{\lambda\omega} \geqslant 4 \times 10^{-12}\,\mathrm{m}. \quad (7)$$

We can call Eq. (7) bioactivity condition. Since we adopted the smaller value for ∂r ($\cong 4 \times 10^{-12}$ m), which is valid for cations moving within channels ($\varepsilon = 4$), we will use the corresponding value for λ that we have also calculated for cations moving within channels ($\lambda \cong 6.4 \times 10^{-12}$ kg/s) [1]. Thereby, the last relation becomes

$$E_0 \geqslant \omega \times 1.6 \times 10^{-4} \quad (8)$$

or

$$E_0 \geqslant \nu \times 10^{-3} \quad (\nu \text{ in Hz}, E_0 \text{ in V/m}). \quad (9)$$

Relation (9) gives the bioactive intensity amplitudes E_0 of an oscillating electric field in response to the frequency ν of the field. This relation is represented in Fig. 1, in arbitrary logarithmic scale (in other words, the equivalent relation

$$\log E_0 \geqslant \log \nu - 3 \geqslant 0 \quad (10)$$

is represented), by the region above line 1 (line included).

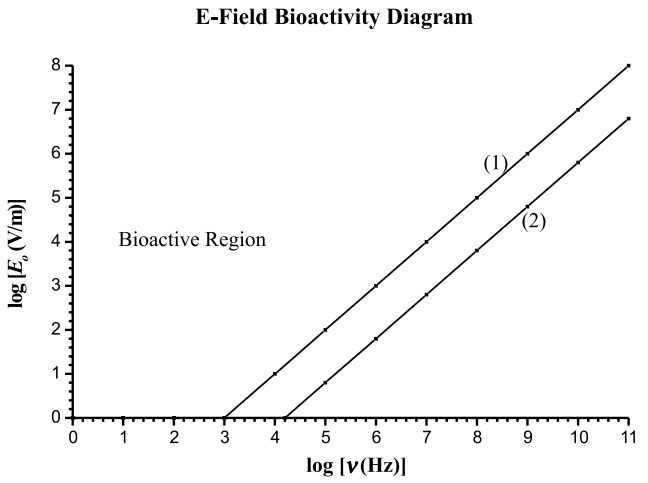

Fig. 1. The region above line 1 (line included) represents the bioactive combinations (v, E_0) between the frequency and the intensity of a continuous oscillating electric field acting on single-valence cations, considering one cation interacting with the channel's sensor. The region above line 2 (line included) represents the bioactive combinations (v, E_0) between the frequency and the intensity of a pulsed oscillating electric field acting on double-valence cations, considering two cations interacting in phase with the channel's sensor from 1 nm distance.

Conditions (7)–(10) and line 1 in Fig. 1, refer to one single-valence oscillating ion interacting with the channel's sensor and to continuous (uninterrupted) oscillating electric fields (mildest case).

As we have already said, the ability of an oscillating electric field to cause biological effects will be maximum at the moment when it is applied or interrupted or during its first and last periods, when the ions' displacement will be twice the amplitude of the forced-vibration [as denoted by Eq. (2)]. For pulsed fields this will be taking place constantly with every repeated pulse. Thereby in the case of pulsed electric fields, the left parts of the conditions: (7)–(9) are multiplied by 2.

Therefore, it is theoretically proved that pulsed electromagnetic fields can be twice more effective biologically than continuous electromagnetic fields and this explains the results of several published experiments which have reported such an observation and also that the greatest effects seem to occur with onset or removal of the exposure to the field [5–8].

If additionally we take into account double-valence ions (e.g., Ca^{2+}), then the left parts of the above relations are multiplied by 4 and the right parts are divided by 2 (∂r is divided by 2). The bioactivity of the field is then multiplied by 8.

Finally, since it is very likely that several ions interact simultaneously each moment with S4 from 1 nm distance and considering very conservatively two ions interacting simultaneously, the bioactivity of the field is multiplied by 16.

Hence, for the most drastic case of pulsed-electric fields acting on double-valence ions, the bioactivity condition (7) becomes

$$\frac{E_0 q_e}{\lambda \omega} \geqslant 0.25 \times 10^{-12}\,\mathrm{m}. \tag{11}$$

Correspondingly, conditions (9) and (10) become

$$E_0 \geqslant v \times 0.625 \times 10^{-4} \quad (v \text{ in Hz}, E_0 \text{ in V/m}) \tag{12}$$

and

$$\log E_0 \geqslant \log v - 4.2 \geqslant 0. \tag{13}$$

Condition (13) is represented in the "E-field bioactivity diagram" in Fig. 1, by the region above line 2 (line included).

The "E-field bioactivity diagram" above gives the (v, E_0) combinations which can be bioactive on cells. As for whole organisms, it has been claimed that the conductivity of their bodies shields the interior of the body from external electromagnetic fields, especially at low frequencies [2,24,27]. Even if this is true for the inner tissues of a living organism, what about the skin cells, the eyes, or the brain. We would not be very sure that what is valid for a piece of dielectric material with the same conductivity as the average of a biological tissue would be as valid for living organisms and humans especially. Even more when there is quiet strong evidence suggesting that electromagnetic fields of all frequencies (especially at ELF and microwave frequencies) and even at very low intensities can be bioactive on cells and whole organisms [5,28–58].

The present theoretical model can be extended to explain the biological action of oscillating magnetic fields as well, if we replace the electric force $F_1 = E_0 z q_e \sin \omega t$, by the expression

$$F_1' = B_0 u z q_e \sin \omega t, \tag{14}$$

which is the force exerted by an alternating magnetic field, $B = B_0 \sin \omega t$, with intensity amplitude B_0, on an ion with charge zq_e, moving with velocity u, vertically to the direction of the magnetic field.

The relative magnetic permeability of biological tissues is

$$\mu_{\text{biological material}} \cong 1$$

[27]; therefore, the magnetic field's intensity within the biological material will be almost equal to the intensity outside (in the air). In this way, according to the same reasoning as with the electric field, we get corresponding bioactivity conditions for an oscillating magnetic field.

For ions moving through a channel vertically to the direction of the external magnetic field, for $u = 0.25$ m/s, the velocity that we have calculated for Na^+ ions moving through an open Na^+ channel [1] and for the mildest case of a continuous oscillating magnetic field, acting on

single-valence ions, the corresponding to condition (7), bioactivity condition, is

$$\frac{B_0 u q_e}{\lambda\omega} \geqslant 4\times 10^{-12}\,\mathrm{m} \quad (\omega \text{ in rad/s},\ u \text{ in m/s},\ B_0 \text{ in T}), \tag{15}$$

from which, we get

$$B_0 \geqslant 40\nu \quad (\nu \text{ in Hz},\ B_0 \text{ in G}) \tag{16}$$

or

$$\log B_0 \geqslant \log \nu + 1.6. \tag{17}$$

In Fig. 2, condition (17) is represented by the region above line 1 (line included).

Conditions (15)–(17) and line 1 in Fig. 2 refer to one single-valence cation, interacting with the channel's sensor from 1 nm distance.

Correspondingly, for the most drastic case of pulsed magnetic fields, acting on double-valence ions and considering very conservatively two ions interacting simultaneously and in phase with S4 from 1 nm distance, we get

$$\frac{B_0 u q_e}{\lambda\omega} \geqslant 0.25\times 10^{-12}\,\mathrm{m} \quad (\omega \text{ in rad/s},\ u \text{ in m/s},\ B_0 \text{ in T}) \tag{18}$$

or

$$B_0 \geqslant 2.5\nu \quad (\nu \text{ in Hz},\ B_0 \text{ in G}) \tag{19}$$

or

$$\log B_0 \geqslant \log \nu + 0.4. \tag{20}$$

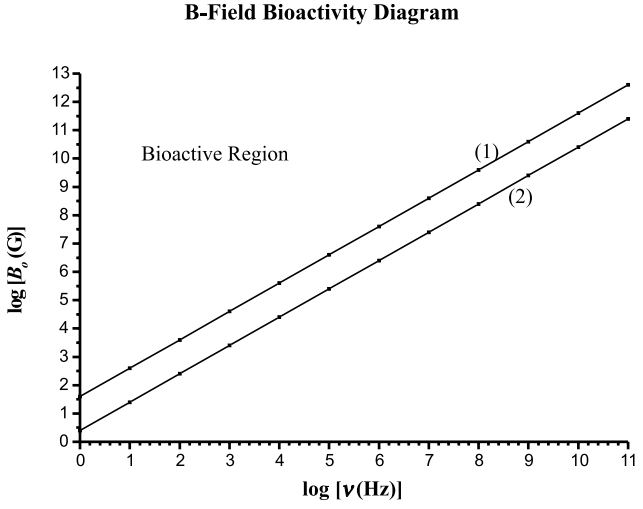

Fig. 2. The region above line 1 (line included) represents the bioactive combinations (ν, B_0) between the frequency and the intensity of a continuous oscillating magnetic field acting on single-valence cations, considering only one cation interacting with the channel's sensor. The region above line 2 (line included) represents the bioactive combinations (ν, B_0) between the frequency and the intensity of a pulsed, oscillating magnetic field acting on double-valence cations, considering two cations interacting in phase with the channel's sensor from 1 nm distance.

In Fig. 2, condition (20) is represented by the region above line 2 (line included).

If we finally take into account an induced electric field E_{ind}, generated by the pulsed magnetic one, as it always happens, for which we can conservatively accept a typical value of the order of 1 V/m [5,59,60] and if we assume that the induced electric field is in the same direction with the magnetic force F_1' (vertically to $\vec{B}$), then, for the most drastic case of pulsed-magnetic fields acting on double-valence ions, the bioactivity condition becomes

$$\frac{(B_0 u + E_{\mathrm{ind}})q_e}{\lambda\omega} \geqslant 0.25\times 10^{-12}\,\mathrm{m} \quad (E_{\mathrm{ind}} \text{ in V/m},\ B_0 \text{ in T}) \tag{21}$$

$$\Rightarrow B_0 \geqslant 2.5\nu - 4\times 10^4 \quad (\nu \text{ in Hz},\ B_0 \text{ in G}). \tag{22}$$

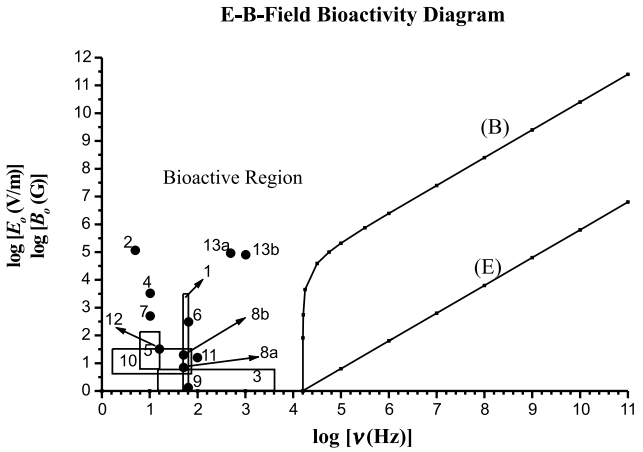

Fig. 3. The diagram depicts the bioactive regions for oscillating electric (E) and magnetic (B) fields in the most drastic case of pulsed fields acting on double-valence cations, considering two cations interacting in phase with the channel's sensor from 1 nm distance. The numbered spots and rectangular areas correspond to the frequency and intensity values of the fields used in the following reports: (1) epidemiological studies [33–37], connecting power line fields with cancer. The plotted area corresponds to electric field intensity. (2) Effect of 5 Hz, 116.6 kV/m pulsed electric field on DNA synthesis in chick chondrocytes [38]. (3) Effect of alternating magnetic fields 15–4000 Hz, 0.023–5.6 G, on DNA synthesis in human fibroblast cells [39]. (4) Effect of 10 Hz, 3.19 kV/m pulsed electric field on DNA synthesis and calcium concentration in mouse bone cells [40]. (5) Effect of alternating electric fields 6–16 Hz, 5–100 V/m on calcium concentration in chick and cat cerebral cells [41]. (6) Effect of 60 Hz, 220 G (rms) alternating magnetic field on calcium concentration in rat thymocytes [42]. (7) Effect of 10 Hz, 500 V/m alternating electric field on bone cell proliferation and DNA synthesis [43–45]. (8) Effect of 50 Hz alternating fields, 7 V/m electric (8a) and 20 G magnetic (8b), on cell proliferation and DNA synthesis in cultured mammalian cells [46]. (9) Effect of 60Hz alternating fields, 1 V/m electric and 1 G magnetic, combined or separately, on the cell membranes of the slime mold Physarum polycephalum [47–50]. (10) Effect of pulsed and alternating magnetic fields, 1.5–72 Hz, 3.8–35 G, on RNA and protein synthesis in Sciara coprophila salivary gland cells [51–53]. (11) Effect of 100 Hz, 17 G pulsed magnetic field on oviposition and development of Drosophila [54]. (12) Mutagenic action of 16 Hz, 35 G pulsed magnetic field on Drosophila sperm [55]. (13) Induction of hypotension in rats by 0.5 kHz, 93 kV/m (13a) and 1 kHz, 85 kV/m (13b) pulsed electric fields [56].

Since we are only concerned about the absolute value of the magnetic field intensity magnitude, it must be: $B_0 \geqslant 0$. Thereby condition (22), is anyway satisfied for every v that: $2.5v - 4 \times 10^4 < 0$ and thus we are concerned about the values of v that satisfy the condition:

$$2.5v - 4 \times 10^4 \geqslant 0 \Rightarrow v \geqslant 1.6 \times 10^4$$
$$\Rightarrow \log v \geqslant \log 1.6 + 4$$
$$\Rightarrow \log v \geqslant 4.20412.$$

In Fig. 3 we represent relation (22) (B_0 versus v), in arbitrary logarithmic scale, (in other words we represent $\log B_0$ versus $\log v$). In the diagram, these most active conditions for pulsed magnetic field are represented by the region left and above the B-line (line included). Correspondingly, the most active conditions for pulsed electric field are represented by the region above the E–line (line included). Thereby in Fig. 3, the most active conditions, both for pulsed electric and magnetic fields, are resumed.

As is evident from the "*E–B*-field bioactivity diagram" (Fig. 3), there are many combinations of (v, E_0) and (v, B_0) values, able to produce biological effects on cells. According to the diagram, oscillating electric or magnetic fields, with frequencies lower than 1.6×10^4 Hz (ELF and VLF fields), can be bioactive, even at very low intensities of several V/m or Gauss correspondingly. The majority of published reports with positive results on biomolecules, cells, and whole organisms have been performed with ELF fields. The frequency and intensity values of oscillating electric and magnetic fields used in several important experimental and epidemiological published studies with positive results are plotted in the diagram.

As the frequency of the field increases more than 1.6×10^4 Hz, the minimum intensity of the field, able to cause biological effects on cells, with the described mechanism, increases linearly with frequency, in the case of electric fields. An RF ("radio frequency") field of 10^8 Hz (FM-band) must have an intensity amplitude of at least 6.3×10^3 V/m or 63 V/cm, while a microwave field of 10^{10} Hz must have an intensity amplitude of at least 6.3×10^5 V/m or 6.3 kV/cm, in order to cause biological effects according to the described mechanism.

Since in several published experiments with RF and microwave fields, biological action is recorded at much lower intensities [28–32,57,58], it seems that either these fields act on living matter according to additional mechanisms yet to be found, or the recorded biological effects are due to low-frequency harmonics of the RF fields, or due to the pulse repetition frequency in the case of pulsed RF fields.

Actually, there is some experimental evidence suggesting that the most bioactive components of complex electromagnetic signals containing both low and high frequencies are the low frequency ones [57,58], and this is obviously in complete agreement with our theory. Thereby such experimental observations find now for the first time a theoretical explanation, by means of our theory.

Magnetic fields with frequencies higher than 1.6×10^4 Hz seem to be less bioactive than electric ones of the same frequencies, according to the present mechanism.

We believe that the present theoretical model provides a realistic explanation for the action of electromagnetic fields on cells, in actual biological conditions.

It seems possible that the oscillating ions during forced-vibration can also exert mechanical forces-pressure, on the plasma membrane, able to upset the membrane's electrochemical balance, under certain conditions, by opening or closing mechanically gated channel proteins, like some Ca^{+2} influx channels [61]. But of course this can be a subject of a separate research, based on the present theoretical model of the ions' forced-vibration which we have presented here.

In any case, irregular gating of ion channels, caused by the forced-vibration of the free ions, under the influence of an external oscillating electromagnetic field, can certainly upset the electrochemical balance of the plasma membrane and, consequently, disrupt the cell's function.

The present theoretical model demonstrates that pulsed fields can have increased biological action in relation to continuous (uninterrupted) fields and thus such observations can now be theoretically explained for the first time.

The present theoretical model seems to explain well the biological action of ELF and VLF fields (electric and magnetic). As for RF and microwave fields, in the case that these fields are pulsed on ELF frequencies or include ELF harmonics as it usually happens, their biological action is again well explained with the described mechanism. Otherwise like we said before, we have to seek for complementary mechanisms. Such a complementary mechanism can certainly be temperature increase within the tissue, in the case of microwave fields.

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