Model analysis of nonlinear modification of neutrophil calcium homeostasis under the influence of modulated electromagnetic radiation of extremely high frequencies

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Abstract. The problem of resonance effects of electromagnetic radiation (EMR) on biological objects remained unsolved till now. Previously we demonstrated that low-intensity amplitude-modulated EMR of extremely high frequencies (EHF) modified the activity of mouse neutrophils in the synergistic reaction of calcium ionophore A23187 and phorbol ester PMA. The EHF EMR influence on the neutrophils was significant at the carrier frequencies of radiation within a narrow range of 41.8–42.05 GHz and at the modulation frequency of 1 Hz. The purpose of the work was the analysis of frequency-dependent modification of intracellular free calcium concentration ($[Ca^{2+}]_i$) by modulated EHF EMR on the basis of a special model for $[Ca^{2+}]_i$ oscillations in the neutrophils. The calcium channels of plasma membrane were chosen as the action target of external modulation in the model. The computer simulation demonstrated the rise in $[Ca^{2+}]_i$ at the influence of the external field with a threshold dependence on the modulation amplitude. The effect depended heavily on a sequence of delivery of the chemical and electromagnetic stimuli. The narrow-band rise in $[Ca^{2+}]_i$ had a phase-frequency dependence. With the modulation amplitudes exceeding the threshold value, the rise in $[Ca^{2+}]_i$ of more than 50% of the initial level was observed at the frequency of about 1 Hz and in the phase range of 0.3–2.5 radians. The results of the model analysis are in good correspondence with the experimental data obtained before, namely, with the resonance modification of the neutrophil activity at the modulation frequency of 1 Hz and with the presence of the effect only at high concentrations of calcium ionophore.

Key words: Computer simulation, Calcium oscillations, Calcium homeostasis, Effects of electromagnetic fields, Phase-frequency dependence.

1. Introduction

The weak electromagnetic fields (EMFs) of various frequency ranges cause significant modifications of physiological status of living systems [1–3]. Ca^{2+} ions are of importance in the biological effects of the EMFs [2, 4, 5]. Some biological effects of the EMFs are considered as a result of the influence on Ca^{2+} -dependent processes of intracellular signalling. It has been demonstrated that weak EMFs

can influence processes of the Ca^{2+} transport through plasma membrane [6, 7], modify activity of Ca^{2+} -dependent membrane-associated proteins [8, 9], induce Ca^{2+} mobilisation [10], change intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$) [11, 12] and affinity of intracellular proteins to Ca^{2+} [13], resulting in essential modifications at the level of functions of cells of different types. In particular, the cells of the immune system are shown to be responsive to the EMF influence [4, 14–20]. It is supposed that the EMF effects are conditioned by the influence on Ca^{2+} -dependent signalling systems of these cells.

One of peculiarities of the EMF influence on biological objects is the frequency dependence of the effects. Considering the EMF influence on an object, the modification of a registered parameter is exhibited only in narrow bands of the field frequencies. The shape of the frequency dependence of the effect is close to the resonance characteristics of an oscillatory circuit, therefore such effects can be qualified as resonance or quasi-resonance effects. The band width of the effect manifestation can account for less than 10^{-3} from the central frequency. This ‘band’ can be adjacent with frequency intervals, in which no essential modification of the registered parameter is observed at the action of the EMF [21, 22]. The problem of the resonance interaction between the EMFs and the biological objects still remains to be solved. It has become increasingly evident that quasiperiodic processes play an important role in formation, stability, development and functioning of living systems [23]. The oscillations of $[\text{Ca}^{2+}]_i$, found in several cell types [24–26], can serve as a good example of oscillatory processes in biological objects. Such processes can show high responsivity to the influence of weak external periodic signals [27, 28]. When characteristic frequencies of the oscillatory processes and frequencies of external action coincide, a significant modification of functional activity of a biological system is likely.

By this means, experimental investigations show that: a) the weak EMFs can influence Ca^{2+} -dependent systems of intracellular signalling by a modification of $[\text{Ca}^{2+}]_i$ and/or affinity of proteins to Ca^{2+} , b) the EMF effects have frequency dependencies, being strongly marked, which can indicate high responsivity of quasiperiodic processes of intracellular signalling to the influence of the EMF with frequencies, being close to characteristic frequencies of such processes.

The purpose of the work was the analysis of frequency-dependent modifications of $[\text{Ca}^{2+}]_i$ under the influence of the modulated electromagnetic radiation (EMR) of extremely high frequencies (EHF) on a basis of the model developed for $[\text{Ca}^{2+}]_i$ oscillations in the cells of the immune system, these are the neutrophils.

2. Experimental Background

To investigate the EHF EMR effects on calcium signalling in the cells of the immune system, we have created an experimental model of the synergistic reaction of calcium ionophore A23187 and phorbol ester PMA in activation of the respiratory burst in the mouse neutrophils [17]. The EHF EMR effect on production of reactive

Figure 1. Resonance-like modification of the synergistic reaction of calcium ionophore A23187 and PMA in the neutrophils exposed to the amplitude-modulated EHF EMR ($50 \mu\text{W cm}^{-2}$) at the fixed carrier frequency of 41.95 GHz and different modulation frequencies. Concentrations of A23187 in these experiments were $7.5\text{--}10 \mu\text{M}$. The extremum of the dependence corresponds to the modulation frequency of 1 Hz. The direction of the effect depends on the used frequency of modulation. At the modulation frequency of 1 Hz, the activation of the synergistic reaction was observed. Confidence intervals calculated by the Student *t*-test ($p < 0.05$) are shown.

oxygen species (ROS) by the neutrophils in the synergistic reaction was found only in the presence of Ca^{2+} in the extracellular medium and at high concentrations of A23187 (of $5\text{--}20 \mu\text{M}$), ensuring Ca^{2+} influx from the extracellular space and raised $[\text{Ca}^{2+}]_i$ concentration of more than 600 nM during all time of the EHF EMR influence [29]. Starting from these indirect data, we suppose that the radiation modifies Ca^{2+} -dependent signalling systems in the neutrophils.

The effect of continuous EHF EMR had a resonance dependence on carrier frequency of the radiation [15, 16]. The maximum inhibition of the ROS production by the neutrophils, making about 25% from the control, was observed at the radiation frequency of 41.95 GHz. The low-intensity amplitude-modulated EHF EMR also modified the intensity of the respiratory burst of neutrophils in the synergistic reaction of calcium ionophore A23187 and phorbol ester PMA [15, 16]. It has been brought out that the resonance-like inhibition or activation of the respiratory burst was observed at the modulation frequency of 1 Hz and at fixed carrier frequencies of 41.85 GHz or 41.95 GHz (Figure 1). Thus, the significant modifications of the

Figure 2. Schematic representation of calcium transport processes, underlying the considered model. $X = [Ca^{2+}]_i$ – concentration of free calcium in the cytosol, $Y = [Ca^{2+}]_s$ – concentration of calcium in the intracellular calcium store. The cell was stimulated by calcium ionophore A23187, changing Ca^{2+} influx into the cell (βv_a) by the change of the extent of stimulation (β). The terms v_i ($i = 0, 1, 2, 3$), kX and $k_f Y$ represent different calcium fluxes (see text for details).

neutrophil activity were presented at the influence of the EHF EMR with carrier frequencies of 41.8–42.05 GHz and at the modulation frequency of 1 Hz.

We suppose that the basal modifications of cell metabolism under the influence of the EHF EMR occurred in our experiments in the course of the neutrophil activation by calcium ionophore A23187, forasmuch as the effect was not present at exposure of intact cells [15, 16]. With the irradiation switched off, the subsequent activation of the respiratory burst of neutrophils by PMA revealed the radiation effect at a level of functional activity and affected biochemical and morphological modifications, caused by the irradiation, not immediately. Therefore, the offered model should include intracellular signalling pathways, participating in the neutrophil activation by calcium ionophore A23187, and explain peculiarities of the modulated EHF EMR influence [15, 16].

3. The Model

To develop our model for Ca^{2+} oscillations, we have taken as a basis the model described in [30], where it is entitled as ‘the minimal model for signal-induced Ca^{2+} oscillations’. The choice was founded on the analysis of existing models for Ca^{2+} oscillations [30–38]. We have taken into account the fact that ‘the minimal model for Ca^{2+} oscillations’ is one of the most known models, which predict the occurrence of $[Ca^{2+}]_i$ oscillations without inositol 1,4,5-trisphosphate (IP_3) oscil-

lations. In our experimental conditions the neutrophils were activated by calcium ionophore A23187. The mechanism of the action of A23187 on the neutrophils is complicated enough and is not investigated completely. Therefore, within the framework of our model, we took into consideration solely the well known mechanism of the action of A23187 on the cells. Calcium ionophore A23187, greatly increasing the ability of divalent ions to cross biological membranes, can increase $[Ca^{2+}]_i$, giving it properties as an ionophore [39]. Forasmuch as we did not register changes of $[IP_3]$ in our experiments, the intracellular signalling pathways, connected with activation of phospholipase C (PLC), hydrolysis of phospholipids and their further metabolism, was not taken into account at the model development. It is obvious that Ca^{2+} -dependent intracellular signalling systems in the neutrophils include a great number of biochemical reactions with positive and negative feedbacks. Other mechanisms of rise in $[Ca^{2+}]_i$ are not ruled out at the action of calcium ionophore A23187. For example, the so-called ‘capacitative’ or store-regulated mechanism of Ca^{2+} influx into the cell [40] is potential. It is also possible that the rise in $[Ca^{2+}]_i$ affects the activity of different enzymes (PLC, protein kinase C (PKC), phospholipase A_2 , etc.) through the cascade of feedbacks [41]. Within the limits of the work, we modified ‘the minimal model for Ca^{2+} oscillations’ [30], excepting IP_3 -dependent signalling systems. Thus, we have restricted our attention by the following key processes in our model: a) the rise in $[Ca^{2+}]_i$ triggered by the ionophore mechanism, depending on the extent of stimulation β (concentration of A23187), b) the activation influence of the initial rise in $[Ca^{2+}]_i$ on the Ca^{2+} influx through non-selective cation channels of the neutrophil plasma membrane, which also pass Ca^{2+} and are not activated by IP_3 directly [42], c) processes of Ca^{2+} -induced Ca^{2+} release from intracellular stores to the cytosol [30]. The basic concept of considered processes of calcium homeostasis, included into the model, is shown in Figure 2. It is necessary to note once again that the schematic model submitted in Figure 2 is not full for the neutrophils. However, the model does not contradict the data obtained in other investigations and is sufficient for verifying the approach to the model analysis of the influence of weak low-frequency signals on calcium homeostasis of cells, including neutrophils.

Considering denotements shown in Figure 2, the following set of differential equations is proposed for the model:

$$\begin{cases} \dot{X} = v_0 + \beta v_a + v_1 - v_2 + v_3 + k_f Y - kX & (1) \\ \dot{Y} = v_2 - v_3 - k_f Y & (2) \end{cases}$$

In these equations, variables X and Y relate, respectively, to intracellular free calcium concentration ($[Ca^{2+}]_i$) and calcium concentration in the intracellular calcium store ($[Ca^{2+}]_s$). Rates of transport processes v_1 , v_2 and v_3 are described by Hill functions whose threshold constants are taken as K_1 , K_2 , K_R and K_A ; h , n , m and p are the cooperativity coefficients implying positive cooperativity of trans-

Figure 3. The mean level of $[Ca^{2+}]_i$ (filled circles) and frequency of oscillations (open circles) as functions of the extent of stimulation β . Arrows indicate the range of values of the parameter β , at which $[Ca^{2+}]_i$ oscillations appear. Parameter values of the model: $v_0 = 1 \mu M s^{-1}$, $v_a = 5.5 \mu M s^{-1}$, $V_{M1} = 30 \mu M s^{-1}$, $V_{M2} = 65 \mu M s^{-1}$, $V_{M3} = 500 \mu M s^{-1}$, $K_1 = 2 \mu M$, $K_2 = 1 \mu M$, $K_R = 2 \mu M$, $K_A = 0.9 \mu M$, $h = 3$, $n = 2$, $m = 2$, $p = 4$, $k = 10 s^{-1}$, $k_f = 1 s^{-1}$.

port processes. All concentrations and rates are defined with respect to the total intracellular volume.

$v_1 = V_{M1} \frac{X^h}{K_1^h + X^h}$ – the rate of Ca^{2+} influx through plasma membrane channels.

Here V_{M1} denotes the maximum rate of Ca^{2+} transport, limiting v_1 at high $[Ca^{2+}]_i$. To approximate the Ca^{2+} influx through plasma membrane to non-zero level at low $[Ca^{2+}]_i$, the constant rate v_0 characterises the leakage rate and is introduced into Equation (1).

$v_2 = V_{M2} \frac{X^n}{K_2^n + X^n}$ – the rate of ATP-driven pumping of Ca^{2+} from the cytosol into the intracellular store. In this equation, V_{M2} denotes the maximum rate of pumping, limiting v_2 at high $[Ca^{2+}]_i$.

$v_3 = V_{M3} \frac{Y^m}{K_R^m + Y^m} \times \frac{X^p}{K_A^p + X^p}$ – the rate of Ca^{2+} transport from the intracellular calcium store into the cytosol. In this equation, V_{M3} denotes the maximum transport rate.

In Equations (1,2), terms v_0 and kX characterise the leakage rate of Ca^{2+} influx into the cytosol and active Ca^{2+} pumping from the cell, respectively. These processes take place even in the absence of the cell stimulation. The term $k_f Y$

characterises the passive Ca^{2+} efflux from the intracellular store, stabilising the amplitude of Ca^{2+} transients at different levels of stimulation [30]. For simplicity, terms kX and $k_f Y$ are taken as linear. We adopted model parameter values used for numerical simulation (see the legend to Figure 3), so that they match to parameter values in models [30, 38] and do not contradict functional peculiarities of neutrophils. It is marked in [35] that similar kinetics of $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_s$ modifications may be observed at the appropriate variation of parameter values in the limits of 25–400% from the specified values. At the stimulation of the cell by A23187, the rate of Ca^{2+} transport by ionophore is determined by term βv_a , where parameter β characterises extent of stimulation (concentration of A23187), $0 < \beta < 1$; v_a is the maximum rate of Ca^{2+} influx, that might be provided by the A23187 concentration, which is the highest of the used ones. The values of v_a were adopted so that the mean levels of $[\text{Ca}^{2+}]_i$, resulting from numerical simulations at different values of the extent of stimulation β , corresponded those found experimentally in [29]. Thus, we could compare A23187 concentrations used in experiments with values of β , used at numerical simulations. The dependence of the mean level of $[\text{Ca}^{2+}]_i$ on the extent of stimulation is depicted in Figure 3. Figure 4 indicates time dependencies of $[\text{Ca}^{2+}]_i$ for some values of parameter β and conditions of the cell stimulation. In the range of values of parameter β from 0 to 0.35 the model has a stable steady state. The $[\text{Ca}^{2+}]_i$ oscillations are absent at such a level of stimulation (Figures 3 and 4,a), and $[\text{Ca}^{2+}]_i$ does not exceed $0.3 \mu\text{M}$, that corresponds to the raised level, which was obtained in experiments by the use of A23187 in concentrations from 0.1 to $0.5 \mu\text{M}$. At further increasing of β from 0.36 to 0.8, the steady state becomes unstable. The limit cycle becomes the model attractor, i.e. there are sustained oscillations of $[\text{Ca}^{2+}]_i$ at the specified range of parameter β (Figure 4,b). Further increasing of β of more than 0.85 results in that the steady state becomes again stable, the oscillations of $[\text{Ca}^{2+}]_i$ vanish (Figure 4,c), and $[\text{Ca}^{2+}]_i$ achieves about $0.75 \mu\text{M}$; in experiments, this level corresponds to A23187 concentrations of more than $5 \mu\text{M}$ [29]. The analysis of these results allows to collate the model values of the extent of stimulation β and A23187 concentrations. The reliable effect of both continuous and modulated EHF EMR has been found only at high A23187 concentrations [15–17]. To analyse the EHF EMR effects on the synergistic reaction of A23187 and PMA in neutrophils, the range of values of parameter β has been confined by 0.85–1, which corresponds to A23187 concentrations from 5 to $20 \mu\text{M}$. To investigate frequency-dependent properties of the model at the influence of the modulating signal, we have chosen the value of the extent of stimulation $\beta = 0.9$, at which oscillations of $[\text{Ca}^{2+}]_i$ were absent.

Experimental data on the EHF EMR influence on cells of different types show that low-intensity EHF EMR can influence the activity of Ca^{2+} -dependent plasma membrane channels [8, 9]. In the work [8], performed on *Characean* alga cells exposed to the EHF EMR with frequencies of 38–78 GHz, the frequency-dependent modifications of the transient chloride current through Ca^{2+} -activated chloride channels were found. Using patch voltage-clamp method in single Ca^{2+} -activated

potassium channels of cultured kidney cells *Vero*, it was shown that the EHF EMR greatly modifies both the Hill coefficient and the apparent affinity of the channels to Ca^{2+} [9]. Basing on these data and the results of works [2–4, 6, 43], which have evidenced that the EHF EMR effect on cellular systems can be conditioned by the influence on signalling pathways of membrane-associated processes, we have chosen cation channels of plasma membrane as the action target of external modulation in the model. These channels also pass Ca^{2+} and are activated by the rise in $[\text{Ca}^{2+}]_i$ [42]. Then, the rate of Ca^{2+} transport v_1 through plasma membrane channels is substituted by the following term:

$$v_1 \rightarrow v_1[1 + F_1 \cos(2\pi \nu t + \varphi)],$$

where ν is the frequency of the external field (Hz), φ is the phase, and F_1 is the modulation amplitude ($F_1 < 1$). We consider only the influence of the low-frequency modulating signal. In our opinion, the mechanisms of biological effects of the two frequencies of amplitude-modulated EHF EMR, the carrier and modulating ones, distinguished by the factor of 10^{10} , is apparently different [15, 16].

4. Results

Some authors noted that the EMF effect can be dependent on the functional status of a biological object [1, 17, 36, 37, 44]. Performing numerical experiments, we analysed two possible schemes of the influence of the modulating electromagnetic signal. First, the influence begins at the instant the system comes into a stable steady state after chemical stimulation. Second, the chemical stimulation occurs on the background of the electromagnetic signal already involved. The numerical integration of the set of differential equations (1,2) took into account the modulation of the rate of Ca^{2+} transport v_1 through plasma membrane channels and demonstrated the difference of modifications of the mean level of $[\text{Ca}^{2+}]_i$ at the influence of the external modulation in two considered cases. We shall note at once that all numerical experiments carried out in the presence of the external modulating field revealed only the rise in the mean level of $[\text{Ca}^{2+}]_i$ at certain parameters of the external field. The rise in the mean level of $[\text{Ca}^{2+}]_i$ of more than $1.2 \mu\text{M}$ was considered as significant. This rise is by 50% greater than the mean level of $[\text{Ca}^{2+}]_i$ in the absence of the external modulation, making up about $0.75 \mu\text{M}$ at $\beta = 0.9$. This rise has been considered hereinafter as the effect of external modulation.

If the external field was switched on at the instant $[\text{Ca}^{2+}]_i$ was at steady level, the modulating signal caused induced $[\text{Ca}^{2+}]_i$ oscillations (Figure 4,d). The effect had a threshold dependence on the modulation amplitude and occurred at modulation amplitudes $F_1 > 0.25$ and at frequencies of the external field of less than 1.5 Hz (Figure 5).

With the chemical stimulation of the cell occurred on the background of the external field already involved, the modulating signal also caused induced $[\text{Ca}^{2+}]_i$

Figure 4. Time evolution of the $[Ca^{2+}]_i$ excepting the influence of external modulation for fixed values of the extent of stimulation: a) $\beta = 0.3$; b) $\beta = 0.6$; c) $\beta = 0.9$; and at $\beta = 0.9$ at the influence of external modulation: d) the field was switched on, when $[Ca^{2+}]_i$ came into a stable steady state after chemical stimulation (the moment of switching is indicated by an arrow), parameter values of the signal are $F_1 = 0.3$, $\nu = 1$ Hz, $\varphi = 0$ radians; e–g) the chemical stimulation was on the background of the influencing external field with $\nu = 1$ Hz, $\varphi = 1.3$ radians and amplitudes: e) $F_1 = 0.3$; f) $F_1 = 0.4$; g) $F_1 = 0.6$.

Figure 5. Dependence of the mean level of $[Ca^{2+}]_i$ on amplitude and frequency of the external modulating field. The field was switched on, when $[Ca^{2+}]_i$ came into a stable steady state after chemical stimulation. A – three-dimensional representation of the dependence; B – area of amplitudes and frequencies, for which the rise in $[Ca^{2+}]_i$ exceeds $1.2 \mu M$. An arrow and letter 'd' on the graph indicate amplitude and frequency of the external field, for which time evolution of $[Ca^{2+}]_i$ is submitted in Figure 4 under corresponded letter (d).

Figure 6. Dependence of the mean level of $[Ca^{2+}]_i$ on amplitude and frequency of the external modulating field. The chemical stimulation of the cell was on the background of the influencing external field. A – three-dimensional representation of the dependence; B – areas of amplitudes and frequencies, for which the rise in $[Ca^{2+}]_i$ exceeds $1.2 \mu M$. Phase of the external field was 1.3 radians. Arrows and letters on the graph indicate amplitudes and frequencies of the external field, for which time dependencies of $[Ca^{2+}]_i$ are submitted in Figure 4 under corresponded letters (e–g).

Figure 7. Dependence of the mean level of $[Ca^{2+}]_i$ on frequency and phase of the external modulating field. The chemical stimulation of the cell was on the background of the influencing external field. *A* – three-dimensional representation of the dependence; *B* – areas of frequencies and phases, for which the rise in $[Ca^{2+}]_i$ exceeds $1.2 \mu M$. The modulation amplitude was 0.3.

oscillations (Figure 4,e–g). However, in contradistinction to the case submitted in Figure 5, additional narrow frequency bands of the rise in $[Ca^{2+}]_i$ were observed on amplitude-frequency dependence of the effect (Figure 6). Figure 6,B allows to estimate ranges of amplitudes and frequencies of the external field, at which the mean level of $[Ca^{2+}]_i$ exceeds $1.2 \mu M$ (more than 50% of the initial value), which is significant for the development of the effect of modulated EHF EMR at high

concentrations of calcium ionophore A23187 [29]. The effect depended on the phase of the external field at the moment of chemical stimulation at such sequence of delivery of the actions. The study of the effect dependence on the phase of the modulating signal was carried out at fixed value of modulation amplitude $F_1 = 0.3$. The narrow-band rise in the mean level of $[Ca^{2+}]_i$ was found at certain frequencies and phases of the external field (Figure 7). Thus, in case of the chemical stimulation of the cell on the background of the influencing external field, the narrow-band rise in $[Ca^{2+}]_i$ was observed at certain values of modulation amplitudes, having the phase-frequency dependence. The frequencies of the external field from 0 to 0.15 Hz for the whole area of phases may be considered as effective, alike frequencies from 0.7 to 1.3 Hz for phases of 0.3–2.5 radians and frequency band with central frequency of about 2.5 Hz for phases of 0–4.5 radians (Figures 6,B and 7,B).

We also investigated the external modulation influence at the extent of stimulation $\beta < 0.9$. The rise in $[Ca^{2+}]_i$ was found only at $\beta > 0.85$, that proves a correct choice of parameter value $\beta = 0.9$ in numerical experiments. The choice of v_1 as the action target of the modulating signal is not specific. We have carried out the qualitative analysis of dynamics of the given set of equations at the modulation of other rates of Ca^{2+} transport processes. Results of the numerical simulation are analogous with the results obtained before, and their quantitative analysis can be the object of a separate research.

5. Discussion

The model analysis of modifications of neutrophil calcium homeostasis at the joint action of calcium ionophore A23187 and low-intensity amplitude-modulated EHF EMR on the cells has shown the presence of narrow-band rise in $[Ca^{2+}]_i$ at high A23187 concentrations and certain values of amplitude, frequency and phase of the external field. The parameter values of the model were adopted in such a manner that they do not contradict functional peculiarities of the neutrophils. The effect of external modulation was found only at large values of the extent of stimulation β , at modulation amplitudes exceeding the threshold value ($F_1 = 0.25$), and only in narrow bands of external field frequencies. These results are in good agreement with experimental data obtained previously: a) the manifestation of the EHF EMR effect on the synergistic reaction of calcium ionophore A23187 and phorbol ester PMA in the neutrophils only at high A23187 concentrations [17], b) threshold dependence of the effect on an absorbed energy flux density of the radiation [15, 16], c) resonance-like modifications of the neutrophil activity at the modulation frequency of 1 Hz [15, 16]. The accomplished model analysis allowed us to find both frequency and phase dependence of the effect. The dependence of the rise in $[Ca^{2+}]_i$ on the phase of the external field could mean that the effect manifestation needs a certain order of synchronisation of processes of the intracellular signalling with the parameters of the external field. Whether the synchronisation occurs at

the EMF interaction with a biological object or the effectiveness of the influence strongly depends on the phase of the influencing signal, it is anything but clear. It should become a subject for further researches.

The frequency-dependent rise in $[Ca^{2+}]_i$ triggered by the modulating signal can lead to a frequency-dependent modification of cell activity at a level of whole cell. The rise in $[Ca^{2+}]_i$ above some level can modify activity of Ca^{2+} -dependent enzymes, such as PKC, being in the cytosol of the cell, and, consequently, change their activity in phosphorylation of membrane proteins (one of the targets of PKC is NADPH-oxidase). As a result, we registered the frequency-dependent modification of the ROS production by the neutrophils under the influence of modulated EHF EMR [15, 16]. Both the direction and the effect magnitude can depend not only on the frequency of external modulation, but also on a series of other parameters, for example, on the carrier frequency of the radiation. Consequently, the effect direction and magnitude can be changed, and a shift of the effective frequency of the modulating signal can occur. The effective frequencies of the external modulation of 0.7–1.3 Hz (Figure 7) are in the range of the frequencies of $[Ca^{2+}]_i$ oscillations for the used values of the model parameter (see Figure 3). It can testify that the nonlinear system effectively responds only to those frequencies of the influencing signal, which are close to the frequencies of intracellular signalling processes.

The analysis of properties of nonlinear models, constructed on the basis of experimental data on the intracellular signalling processes, is an effective tool for studying the mechanisms of the influence of external stimuli of different nature, including weak EMFs. The results of numerical experiments are able to explain a series of experimentally established peculiarities of the EMF influence on living systems. For example, the possible mechanism of a delay of the onset of calcium oscillations in the presence of external modulation in a model of IP_3 -induced calcium oscillations was explained in [33]. However, the consideration of possible frequency peculiarities of the external modulation influence was absent in the cited work. Our results show that a simple model enables the demonstration of the mechanism of threshold dependence of the effects on intensity of the modulating signal and frequency dependence of the effects. The model can predict new properties of the effect, such as the phase-frequency dependence. One of the basic results of our model analysis of the effects of the modulating signal on calcium homeostasis is the determination of the effect dependence on a sequence of the influence of chemical stimulation and electromagnetic signal on the cell. As we have shown, the character of the effect depended on a sequence of delivery of the stimuli. The considered model predicts the effect dependence on the phase of the modulating signal, dependence of resonance characteristics of the effects on a system status. Using new data in the field of both intracellular signalling systems and the influence of weak EMFs on these systems, a further modification of the model is possible, which allows us to expand understanding of the mechanisms of the influence of weak periodic stimuli on cell calcium homeostasis.

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References

1. Devyatkov, N.D., Golant, M.B. and Betskii, O.V.: *Millimeter waves and their role in vital activity*, Moscow, Radio i Svyas', 1991 (in Russian).
2. Adey, W.R.: Biological effects of electromagnetic fields, *J. Cell. Biochem.* **51** (1993), 410–416.
3. Grundler, W., Kaiser, F., Keilmann, F. and Walleczek, J.: Mechanisms of electromagnetic interaction with cellular systems, *Naturwissenschaften* **79** (1992), 551–559.
4. Walleczek, J.: Electromagnetic field effects on cells of the immune system: The role of calcium signaling, *FASEB J.* **6** (1992), 3177–3185.
5. Karabakhtsian, R., Broude, N., Shalts, N., Kochlatyi, S., Goodman, R. and Henderson, A.S.: Calcium is necessary in the cell response to EM fields, *FEBS Lett.* **349** (1994), 1–6.
6. Adey, W.R.: Physiological signalling across cell membranes and cooperative influences of extremely low frequency electromagnetic fields, in H. Frohlich (ed.), *Biological coherence and response to external stimuli*, Springer, Berlin, 1988, 148–170.
7. Yost, M.G. and Liburdy, R.P.: Time-varying and static magnetic fields act in combination to alter calcium signal transduction in the lymphocyte, *FEBS Lett.* **296** (1992), 117–122.
8. Kataev, A.A., Alexandrov, A.A., Tikhonova, L.I. and Berestovsky, G.N.: Frequency-dependent electromagnetic millimeter-wave effects on ionic currents in the cell membrane of *Nitellopsis*: Nonthermal action, *Biophysics* **38** (1993), 445–460.
9. Geletyuk, V.I., Kazachenko, V.N., Chemeris, N.K. and Fesenko, E.E.: Dual effect of microwaves on single Ca^{2+} -activated K^{+} channels in cultured kidney cells, *Vero*, *FEBS Lett.* **359** (1995), 85–88.
10. Kullnick, U.: Do weak, low-pulsed frequency, high-frequency electromagnetic or magnetic-fields alter the basic bioelectrical parameters of nerve-cells in vineyard snails (*Helix-pomatia* L) 1. Electromagnetic-fields, *Bioelectrochem. Bioenerg.* **37** (1995), 39–45.
11. Carson, J.J., Prato, F.S., Drost, D.J., Diesbourg, L.D. and Dixon, S.J.: Time-varying magnetic fields increase cytosolic free Ca^{2+} in HL-60 cells, *Am. J. Physiol.* **259** (1990), C687–C692.
12. Lindstrom, E., Lindstrom, P., Berglund, A., Lundgren, E. and Mild, K.H.: Intracellular calcium oscillations in a T-cell line after exposure to extremely-low-frequency magnetic fields with variable frequencies and flux densities, *Bioelectromagnetics* **16** (1995), 41–47.
13. Chiabrera, A., Bianco, B., Moggia, E. and Tommasi, T.: Basal cell metabolism and sensitivity to low-intensity AM RF fields, in Proc. of the COST 244 meeting *Biomedical effects of electromagnetic fields; biological effects relevant to amplitude modulated RF fields*, Kuopio, September 3–4, 1995, 7–16.
14. Gapeyev, A.B., Safronova, V.G., Chemeris, N.K. and Fesenko, E.E.: Inhibition of the production of reactive oxygen species in mouse peritoneal neutrophils by millimeter wave radiation in the near and far field zones of the radiator, *Bioelectrochem. Bioenerg.* **43** (1997), 217–220.
15. Gapeyev, A.B., Yakushina, V.S., Chemeris, N.K. and Fesenko, E.E.: Modulated low-intensity EHF EMR activates or inhibits neutrophil respiratory burst depending on modulation frequency, *Biofizika* **42** (1997), 1125–1134 (in Russian).

16. Gapeyev, A.B., Yakushina, V.S., Chemeris, N.K. and Fesenko, E.E.: Modification of production of reactive oxygen species in mouse peritoneal neutrophils on exposure to low-intensity modulated millimeter wave radiation, *Bioelectrochem. Bioenerg.* **46** (1998), 267–272.
17. Safronova, V.G., Gapeyev, A.B., Alovskaya, A.A., Gabdulhakova, A.G., Chemeris, N.K. and Fesenko, E.E.: Millimeter waves inhibit the synergistic reaction of calcium ionophore A23187 and phorbol ester in the respiratory burst of neutrophils, *Biofizika* **42** (1997), 1267–1273 (in Russian).
18. Elekes, E., Szabo, L.D. and Thuroczy, G.: Effect on the immune system of mice exposed chronically to 50 Hz amplitude-modulated 2.45 GHz microwaves, *Bioelectromagnetics* **17** (1996), 246–286.
19. Cleary, S.F.: A review of in vitro studies: low-frequency electromagnetic fields, *Am. Ind. Hyg. Ass. J.* **54** (1993), 178–185.
20. Rojavin, M.A., Tsygankov, A.Y. and Ziskin, M.C.: In vivo effects of millimeter waves on cellular immunity of cyclophosphamide-treated mice, *Electro- and Magnetobiology* **16** (1997), 281–292.
21. Grundler, W. and Keilmann, F.: Sharp resonance in yeast growth prove nonthermal sensitivity in microwaves, *Phys. Rev. Lett.* **51** (1983), 1214–1216.
22. Grundler, W. and Keilmann, F.: Resonant microwave effect on locally fixed yeast microcolonies, *Z. Naturforsch.* **44** (1989), 863–866.
23. Goldbeter, A. (ed.): *Cell to cell signalling: From experiments to theoretical models*, Academic Press, London, 1989.
24. Berridge, M.J. and Galione, A.: Cytosolic calcium oscillators, *FASEB J.* **2** (1988), 3074–3082.
25. Berridge, M.J.: Calcium oscillations, *J. Biol. Chem.* **265** (1990), 9583–9586.
26. Tsunoda, Y.: Oscillatory Ca^{2+} signaling and its cellular function, *New Biolog.* **3** (1991), 3–17.
27. Kaiser, F.: Non-linear dynamics and biophysical systems. I. Interaction of static and periodic fields with nonlinear periodic processes, *Kleinheubacher Berichte* **37** (1994), 763–769.
28. Kaiser, F.: Coherent oscillations – their role in the interaction of weak ELM-fields with cellular systems, *Neural Network World* **5** (1995), 751–762.
29. Alovskaya, A.A., Gabdulhakova, A.G., Gapeyev, A.B., Dedkova, Ye.N., Safronova, V.G., Fesenko, E.E. and Chemeris, N.K.: Biological effect of the EHF EMR depends on the functional status of neutrophils, *Herald New Med. Techn.* **5** (1998), 11–15 (in Russian).
30. Goldbeter, A., Dupont, G. and Berridge, M.J.: Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation, *Proc. Natl. Acad. Sci. USA* **87** (1990), 1461–1465.
31. Swillens, S. and Mercan, D.: Computer simulation of a cytosolic calcium oscillator, *Biochem. J.* **271** (1990), 835–838.
32. Meyer, T. and Stryer, L.: Calcium spiking, *Annu. Rev. Biophys. Biophys. Chem.* **20** (1991), 153–174.
33. Eichwald, C. and Kaiser, F.: Model for receptor-controlled cytosolic calcium oscillations and for external influences on the signal pathway, *Biophys. J.* **65** (1993), 2047–2058.
34. Stucki, J.W. and Somogyi, R.: A dialogue on Ca^{2+} oscillations: An attempt to understand the essentials of mechanisms leading to hormone-induced intracellular Ca^{2+} oscillations in various kinds of cells on a theoretical level, *Biochim. Biophys. Acta* **1183** (1994), 453–472.
35. Rotnes, J.S. and Rottingen, J.-A.: Quantitative analysis of cytosolic free calcium oscillations in neutrophils by mathematical modelling, *Cell Calcium* **15** (1994), 467–482.
36. Eichwald, C., Kaiser, F. and Walleczek, J.: Nonlinear dynamics and biophysical systems: II. Theoretical modeling of intracellular calcium-signaling pathways under the influence of external electromagnetic fields, *URSI-Kleinheubacher Berichte* **37** (1994), 771–778.
37. Eichwald, C. and Kaiser, F.: Model for external influences on cellular signal transduction pathways including cytosolic calcium oscillations, *Bioelectromagnetics* **16** (1995), 75–85.

38. Kaimachnikov, N.P. and Lisnichuk, L.Ya.: A model for Ca^{2+} oscillations in lymphocytes based on the regulation of Ca^{2+} influx into the cell, *Biol. Membr.* **12** (1995), 105–112 (in Russian).
39. Campbell, A.K. and Hallet, M.B.: Measurement of intracellular calcium ions and oxygen radicals in polymorphonuclear leukocyte-erythrocyte ‘ghost’ hybrids, *J. Physiol.* **338** (1983), 537–550.
40. Demaurex, N., Monod, A., Lew, D.P. and Krause, K.-H.: Characterization of receptor-mediated and store-regulated Ca^{2+} influx in human neutrophils, *Biochem. J.* **297** (1994), 595–601.
41. Lew, D.P.: Receptor signalling and intracellular calcium in neutrophil activation, *Eur. J. Clin. Invest.* **19** (1989), 338–346.
42. Tscharner von, V., Prod’hom, B., Baggiolini, M. and Reuter, H.: Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration, *Nature* **324** (1986), 369–372.
43. Grundler, W. and Kaiser, F.: Experimental evidence for coherent excitations correlated with cell growth, *Nanobiology* **1** (1992), 163–176.
44. Eichwald, C. and Walleczek, J.: Activation-dependent and biphasic electromagnetic field effects: Model based on cooperative enzyme kinetics in cellular signaling, *Bioelectromagnetics* **17** (1996), 427–435.