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Short communication

Problems of weak electromagnetic field effects in cell biology [1]

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Abstract

Electrostimulations of cells by weak electric or electromagnetic LF and HF-fields are applied widely today; capacitively or inductively coupled, however, they are seldom applied for cell-free and membrane-free solutions of enzymes. First, the detection of a response of the cells ('electrical window') is a prerequisite for testing at least three parameters: frequency, amplitude and treatment time, besides reproducible biological conditions. The 'state-of-the-art' of this fast developing direction of bioelectrochemistry can be characterized in the following way: the results from several laboratories of (a) cell proliferation, (b) ion transport, (c) activation of several enzymes (Na,K-ATPase), (d) increase of certain protein concentrations (heat-shock protein hsp70) are more or less in agreement. Unfortunately, there are discrepancies between no less than 7 labs in the gene expression of c-*myc*, c-*fos* histone 2B, -actin, URA-3 and others, especially for low fields (< 0.05 mT), e.g., in HL60 cells! The reason why seems to be: (1) differences in the most suitable isolation procedure, (2) interferences in the case of too low magnetic flux and (3) too small ranges of parameters have been measured. Today, three open problems must be pointed out: (A) What is the physiological causality for specific 'electrical windows' and their positive or negative efficacy? (B) What are the biochemical targets for either magnetic or electric fields or both? (C) What is the influence of electrical and (or) thermal noise on field efficiency?

Keywords: Electromagnetic field effects; Cell biology; Electrical window

1. Introduction

Up to now, hundreds of electric and electromagnetic field effects on human beings, animals, organs, cells and homogeneous enzyme reactions have been described either in the laboratory or under environmental conditions, e.g., Refs. [1–5]. However, there are only few presentations of reproducible electromagnetic 'field-windows', e.g., Refs. [6–10], namely the response of membrane transport and some cell processes by certain 'windows' of the 3 parameters: (a) frequency, (b) amplitude, and (c) duration of LF a.c. treatment besides of environmental conditions (temperature, conductivity, osmolarity, nutrition medium, special additives etc.). The reason why an inductive or capacitative coupling to cells causes such responses by positive or negative (inhibition) electrostimulation is mostly unknown in spite of a dozen hypotheses and theories [6] (Table 1). Experimental verification has been claimed by all authors, however, it depends on the intensity of treatment, e.g., $E = 20$ V/cm for the ECC-model [7] whereas the radical recombination [8,9] seems to be sensitive for lowest fields and moreover valid in general including cell-free systems.

The purpose of this evaluation is to discuss the basic problems in electrostimulation of cells by ELF weak fields.

The advantages of cellular studies include: (a) experimental variables can be controlled precisely, (b) accurate dosimetric data, (c) relatively simple cell geometry amenable for modeling of field energy transfer, and (d) morphological and biochemical changes which can be detected easily.

2. Experimental techniques

Nowadays various fields and wave forms are being used in medicine, biology, and bioelectrochemistry. Three main LF techniques can be distinguished.

(1) Direct (dc) or alternating (ac) current application via inert electrodes (platinum, stainless steel, etc.). Field strengths E up to 25 V/cm, frequencies $f < 1000$ Hz and current density $j < 0.25$ mA/cm^2 have been used after

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Table 1
Current theories and models on electrostimulation of cells [1]

Model (starting)	Keywords	Authors
(a) Electroconformational coupling (ECC)	Change of transmembrane voltage by higher pulses (20 V/cm) shifts the equilibrium of enzyme conformations having different electric moments; extended by ion rectification.	R. Astumian, T. Tsong
(b) Oscillatory activation barrier (OAB)	Decrease of the activation barrier for product dissociation from an enzyme by small amplitude alternating fields.	V. Markin, T. Tsong
(c) Zeeman three state coulombic (ZTS)	Zeeman–Stark effect on ligand-receptor binding parameters by sinusoidal electromagnetic exposure.	A. Chiabrera, B. Bianco
(d) Larmor precession (LP)	Change of motion of a charged particle (Ca^{2+} ion) at the active side of an enzyme (e.g. calmodulin cavity).	D. Edmonds
(e) Cyclotron resonance (CR)	Cyclotron resonance frequency of 16 Hz for Ca^{2+} affects calmodulin dependent phosphorylation.	A. Liboff, B. McLeod
(f) Parametric resonance (PR)	Change of subharmonics of Ca^{2+} (meta-stable bound at calmodulin) parametric resonance frequences activates or inhibits cell proliferation and motility.	V. Lednew, J. Blanchard, C. Blackman
(g) Polarization force (PF)	Changes of polarization forces in the field gradient at the cell surface.	K. McLeod
(h) Cell array impedance (CAI)	Hyperbolic amplification of an external field effect, if >100 cells are in an array positioned.	A. Pilla
(i) Free radicals (FR)	Unpaired electrons, triplets, acceleration of recombination >50 μT, >10 μV/cm.	J. Walleczek, C. Timmel [9]
(j) Parametric amplification (PA)	Coherence conditions for energy transfer according Fröhlich rate equation.	J. Pokorny [16]

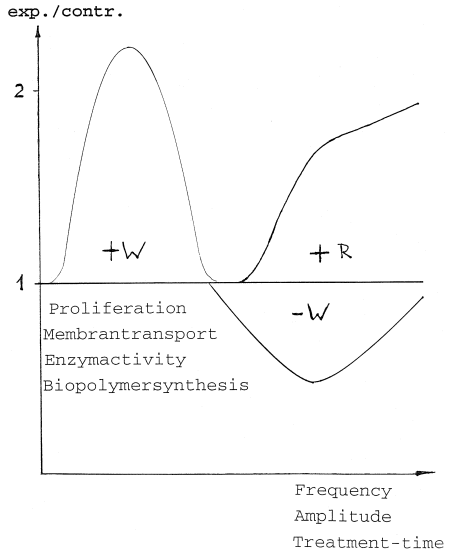

Fig. 1. Scheme of + and − windows and + region for main cell processes.

insertion into cell suspensions, tissues or single cells (microelectrodes). However, some electrolytic reactions at electrode surfaces may be induced.

(2) Capacitively coupled EM fields, e.g., sine waves $E < 100$ mV/cm, $f < 100$ Hz, $j > 1$ μA/cm^2 applied via electrode plates on either side of a shielded cell suspension by agar bridges or artificial membranes.

(3) Inductively coupled ELF electromagnetic fields (sinusoidal or other types of pulsating induced currents (PEMIC) by solenoids or Helmholtz coils around the vessel inducing small electric currents in the probe ($E < 1$ mV/cm, $f < 100$ Hz, $j > 1$ μA/cm^2, $B < 20$ mT).

3. Results and discussion

Different kinds of response curves have been described for frequency, amplitude and treatment time: either positive or negative 'windows,' or rising and decreasing dependencies shown in the scheme (Fig. 1). Examples for proliferation, membrane transport, enzyme activity, biopolymer syntheses are given in Refs. [1–5]. However, the reproducibility is not always sufficient and statistics [10] until 1997 shows for a dozen processes the results of different laboratories (Fig. 2). The reasons are not clear; it may be either the fermentation conditions of cell strains, or the parameters of the experimental set up. According to the critical review of Adair [11], it is unlikely that < 0.05 mT magnetic fields at 50 or 60 Hz can affect other processes than free radical reactions—during their sufficient cage containment time of about 50 ns—suppressing recombination rate by 10 of 40%. Besides the ECC model (a) for high field strength (compare Table 1), the magnetic field theories (c), (d) and (i) can describe and even predict [8,9] a wide range of effects, whereas (b), (g), (h) are of limited value whereas (e) and (f) seem to have physical difficulties [11]. The coherence mechanism (j) [16] has some probability by internal weak electric fields of cells [19] and chemi-

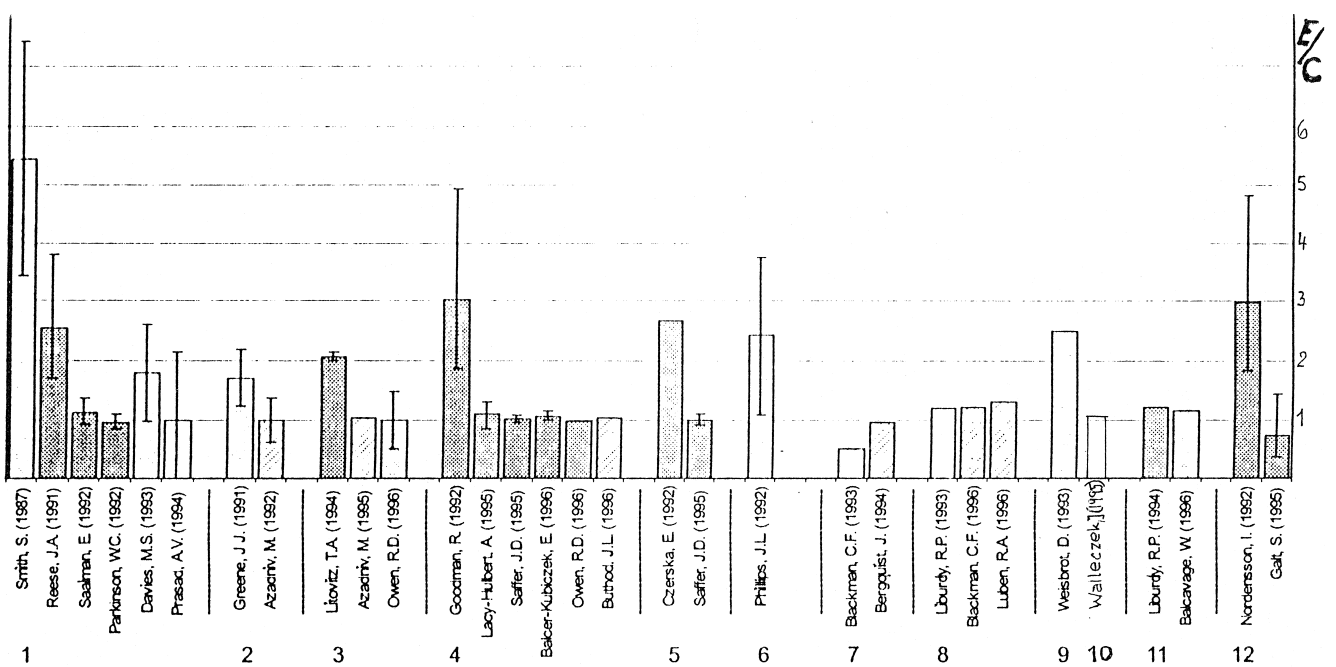

Fig. 2. Comparison for some selected results according to Ref. [10] simplified: (1) Motility of diatoms; (2) Uridine uptake of HL-60; (3) ODC activity of L929; (4) Gene expression in HL-60; (5) Gene expression in D audi cells; (6) Gene expression in CEM-CM3; (7) Neurite outgrowth of PC-12; (8) Melatonin suppression in MCF-7; (9) Gene expression in Yeast; 10 Ca^{2+} oscillations in Jurkat; 11 CD3-binding of Jurkat; 12 Chromosome aberrations in Amniotic cells. E/C: experiment/control.

Table 2
Comparison of some parameters in the original and replication experiments of gene expression in HL-60 cells [2,4,5] and personal communication of E. Balcer–Kubiczek

Parameter	O: Goodman	R1: Lacy–Hulbert	R2: Saffer	R3: Balcer–Kubiczek
Orientation B_{AC}	Horizontal	Horizontal[a]	Vertical[b]	horizontal[a]
Ambient B_{AC}	< 0.1 μT	< 5 nT[a]	< 0.1 μT[a]	0.1 μT (maximum value) to 0.01 μT (average value)[a]
Ambient B_{DC}	2 μT (shielded)	< 1 μT (shielded)[b]	< 33 μT (not shielded)[b]	42.55 μT (environmental)
Equilibration time	60 min	60 min[a]	20 min[b]	less than 0.4 nT inside the mu-metal shield
Cell origin	Columbia University	European Collection of Animal Cell Cultures[b]	American Type Culture Collection[b]	15 to 30 min (prior to inserting flasks into the energized coils)[b]
Cell density	$5 \cdot 10^5$ ml^{-1}	$(8–10) \cdot 10^5$ ml^{-1}[a]	$(2–10) \cdot 10^5$ ml^{-1}[a]	University of Maryland stock originally from the Institute of Human Virology (Dr. Robert Gallo's lab)[b]
Cell culture medium	RPMI 1640	RPMI 1640[a]	RPMI 1640[a]	5×10^5 cells/ml in 15 ml of full medium[a]
Medium manufacturer	Gibco	(no data)	Life Technologies[b]	RPMI 1640[a]
Serum	PCS	PCS[a]	FBS[b]	Life Technologies/Gibco BRL[a] Fetal bovine serum (FBS)[b]
Serum manufacturer	Sigma	(no data)	HyClone, Logan[b]	HyClone (Logan, UT)[b]
Cell container	T-25 flasks	T-25 flasks[a]	organ culture dishes[b]	25-cm^2 (125) flask[a]
Blindness	Not blinded	Blinded[b]	Blinded[b]	Yes[b]

[a] Parameter same as in original experiment.
[b] Parameter has been varied.

Table 3
EMF field effects on activity of Ornithin decarboxylase (ODC)

Cells	Author original	Year	Author replication	Year	Pos./neg.,zero
L929 and others	Litovitz, T.	1991	Azadniv, M.	1995	+
			Byus, C.	1987	+
			Litovitz, T. > until	1997	7 +
			Mattson, M.	1997	3 +
			Kaicer, E.	1992	+
			Mevissen, M.	1995	+
			Byus, C.	1998	0
			Kumlin, T.	1998	0
			Balcer–Kubiczek, E.	1996	−

luminescence patterns [20]. Anyway, there are more phenomena than explanations and moreover too many contradictions. Fig. 2 presents a series of results from 12 processes by several authors [10]. Mostly studied have been the motility of diatoms and the gene expression in HL-60 cells. In the former case, the conditions are too different, whereas in the latter they are very similar (Table 2); however, blindness and biochemical treatment are not exactly the same. The clarification of this problem is of greatest importance, because it concerns fundamental life processes!

The examples for positive electrostimulation of ODC (Table 3, [12]) must be extended opposite to Fig. 2 as well as for gene expression (CEM-CMB), neurite outgrowth [13] and Ca-oscillations [14,15], where significant increases are predominant.

One of the most essential consequences of a change in cellular signalling is the altered proliferation. In most cases, according to Table 4, an electrostimulation has been found likewise surprisingly for extremely low values (0.0012 mT!) claimed by R. Liburdy's group [5], however, some zero effects were described also. Of course, the experimental conditions are too different for detailed conclusions; nevertheless, a tendency for electrostimulation of proliferation is obvious.

In any case, it is necessary to repeat several times by different experimentalists in relation to frequency and amplitude changes. Such example is demonstrated in Fig. 3 exhibiting a steep positive window (+25%) around 0.5 mT for 50 Hz, followed by a broad negative window (−20%) for B > 1 mT,determined independently by three authors.

The crucial question is: why are 50/60 Hz responsible for this and most other windows? The half-life times of many metabolic reactions are in the order of these ms field

Table 4
Electrostimulation of cell proliferation

Cells	Conditions		Author	Year	Pos., zero, neg.
	Hz	mT			
Lymphoc., human	50	5	Antonopoulos	1995	+
Lymphoc., human	50	5	Rosenthal	1989	+
Lymphoc., human	100	1.3	Scarfi	1997	+
Lymphoc., human	50	0.03–1	Paile	1995	0
Lymphoc., human	60	0.2	Cohen	1986	0
Lymphoc., human	60	0.22	Livingstone	1991	0
Ovary, Chin, H.	60	0.2	Livingstone	1991	0
Fibrobl., human	50	0.02–20	Cridland	1996	0
Fibrobl., mouse	50	20	Kula	1996	−
Cancer, colon	60	0.1	Phillips	1986	+
Cancer, breast	20	2	Johann	1993	+
K562	50	< 0.2	Fiorani	1992	+
	50–100	0.1–0.7	Katsir	1998	+
MCF-7	60	0.0012	Liburdy	1993	+
T47B	60	0.0012	Harland	1998	+
SF-757	60	0.0012	Afzal	1998	+
Amnion, human	50	0.08	Kwee	1995	+
Epider., mouse	60	1.1	West	1994	+
E. coli	50	0.48	Aarholt	1981	+
E. coli K12	2–50	1–10	Mittenzweig	1996	(+)
E. coli	9	0.03	Alipov	1994	+
Yeast	80	1.8	Bolognani	1994	+
Yeast, S.C.	15	0.5	Berg	1997	+
	50	0.5	Berg	1997/98	+
	50	> 1	Berg	1997	−

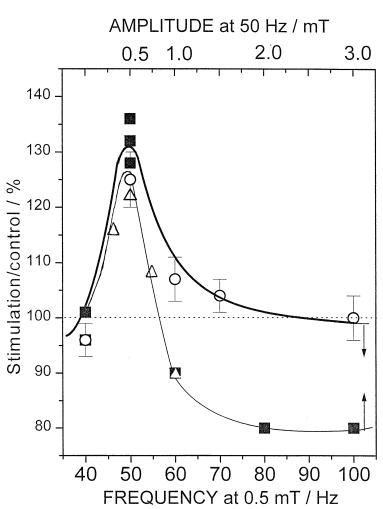

Fig. 3. Stimulated yeast proliferation dependences after 5 to 7 h of fermentation and exposure: on frequency at 0.5 mT, measured by M. Mehedintu and H. Berg ○ [17] and X. Wang ■ (unpublished); on amplitude at 50 Hz, measured by Fiedler et al. △ [18] and X. Wang ■ (unpublished).

changes, but the radial life times are only in the order of ns [9]—a big time gap in between! By way of contrast HF stimulation can act in coherence with unpaired electrons or cellular HF emissions. After all, it must be realized that our knowledge on causality of weak field effects is still in the beginning!

4. Conclusions

Today's situation of weak ELF-field effects shows mostly unsatisfactory results. Now authors must be aware of the necessary to determine reproducible relations of effects at least for frequencies < 100 Hz, amplitudes < 12 mT and treatment times < 2 h in order to promote an objective development. For our deeper knowledge on life processes it will be of great importance to analyze such responses of cells and tissues unambiguously!

It has to be borne in mind that ''This situation strongly advocates unprejudiced, multidisciplinary, professional efforts and scrutiny in the near future''
(''Provando—riprovando'' according to Galilei).

References

[1] H. Berg, Possibilities and problems of low frequency weak electromagnetic fields in cell biology, Bioelectrochem. Bioenerg. 38 (1995) 153–159.
[2] H. Berg, Elektrostimulation in der Zellbiologie—'Feldfenster' und ihre Bedeutung für Umweltfelder. Sitzungsberichte der Sächs. Akad. Wiss. zu Leipzig, Math.-nat. Klasse Bd. 126, Heft 4 (1997) 1-37; Hirzel V. Stuttgart/Leipzig.
[3] I. Hönes, A. Pospischil, H. Berg, Electrostimulation of denitrifying bacterium Pseudomonas stutzeri, Bioelectrochem. Bioenerg. 44 (1998) 275–277.
[4] J. Dennis, J. Stather (Eds.), Radiation Protection Dosimetry. Special issue: Non-ionising radiation, Vol. 72, Nuclear Technology Publishing, Ashford, 1997.
[5] C. Portier, M. Wolfe (Eds.), NIEHS Working Group Report: Assessment of Health Effects from Exposure to Power Line Frequency Electric and Magnetic Fields. NIH Publication No. 98-3981, 1998.
[6] H. Berg, L. Zhang, Electrostimulation in cell biology by low frequency electromagnetic fields, Bioelectrochem. Bioenerg. 12 (1993) 147–163.
[7] D. Astumian, H. Berg, Direct electric field effects and sequential processes in biosystems, Bioelectrochem. Bioenerg. 25 (1991) 455–462.
[8] J. Walleczek, Magnetokinetic effects on radial pairs: a paradigm for magnetic field interactions with biological systems at lower than thermal energy, in: M. Blank (Ed.), Electromagnetic fields: Biological Interactions and Mechanisms, Vol. 250, American Chem. Soc., Washington, 1995, 395–420.
[9] C. Timmel, U. Till, B. Brocklehurst, K. McLauchlan, P. Hore, Effects of weak magnetic fields on free radical recombination reactions, Mol. Phys. 95 (1998) 71–89.
[10] M. Gustavson, M. Lindgren, S. Galt, Y. Hamnerius, Independently replicated biological effects of ELF electromagnetic fields: a literature study, Proceedings from the 2nd World Congress for Electricity and Magnetism in Biology and Medicine, 1997, Plenum, New York, 1999.
[11] R. Adair, Hypothetical biophysical mechanisms for the action of weak low frequency electromagnetic fields at the cellular level, Radiation Protect Dosim. 72 (1997) 271–278, see [4].
[12] T. Litovitz, Can electromagnetic fields modify the activity of ODC? Coherence time effect? Reply to R. Glaser, Bioelectrochem. Bioenerg. 46 (1998) 303–306.
[13] B. Sisken, E. Leman, P. Resig, M. Markov, A. Pilla, Evaluation of two PhE-EMF amplitudes and melatonin concentrations on Neurite outgrowth in dorsal root ganglia. Abstract Book p. 46–47 of the Second World Congress for Electricity and Magnetism in Biology and Medicine, Bologna, June 8–13, 1997.
[14] R. Liburdy, Calcium signalling in lymphocytes and ELF fields, FEBS Lett. 301 (1992) 53–59.
[15] E. Lindström, P. Lindstrm, A. Berglund, E. Lundgren, K. Hansson, Mild, Intracellular calcium oscillations in a T-cell line after exposure to ELF magnetic fields with variable frequencies and flux densities, Bioelectromagnetics 16 (1995) 41–48.
[16] J. Pokorny, T. Wu, Biophysical Aspects of Coherence and Biological Order, Springer, Heidelberg, 1998.
[17] M. Mehedintu, H. Berg, Proliferation response of yeast Saccharomyces cerevisiae on electromagnetic field parameters, Bioelectrochem. Bioenerg. 43 (1997) 67–70.
[18] U. Fiedler, U. Gröbner, H. Berg, Electrostimulation of yeast proliferation, Bioelectrochem. Bioenerg. 38 (1995) 423–425.
[19] H.A. Pohl, Dielectrophoresis. Cambridge Univ. Press, Cambridge, 1978.
[20] R. Vogel, X. Guo, R. Süsmuth, Chemiluminescence patterns from bacterial cultures undergoing bacteriophage induced mass lysis, Bioelectrochem. Bioenerg. 46 (1998) 59–64.