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Does electromagnetic therapy meet an equivalent counterpart within the organism?

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Abstract

This review bundles all available new information about intrinsic electrical phenomena in many types of cells (also non-neural) and tissues and shows that exogenously applied electric fields (as DC – EF, EMF or pulsed electromagnetic fields PEMF) can couple to the endogenous electrical phenomena of the body. These endogenous fields are generated ubiquitously in all tissues via cellular ion pumps and transporters and these ion gradients can be transferred by gap junctions. Such electric phenomena can now be monitored in living cells and tissues by electro-sensitive markers. Observations are now available from early embryonic development on to wound healing and regeneration within the adult organism. Based on these grounds we demonstrate that endogenous electric fields act directly on classical pathways of molecular and cell biology. Adequately applied frequencies and pulsing from outside can couple to these endogenous fields or to the receptors of classical biochemical pathways and manifest in positive "reprogramming" of tissue functions. In sum, this new analytical approach to the bodies´ own electrical information processes opens up new avenues for adequate EMF and PEMF therapy.

Introduction

In the last decades many clinical studies were published which show beneficial effects of electric field (EF), electromagnetic field (EMF) or pulsed electromagnetic field (PEMF) therapy. If one looks over the vast amounts of publications, it is hard to find a common scientific base for the frequencies, time sequences of applications and intensities used. And often this therapy is questioned in principle because of a supposed lack of an adequate counterpart for EMF therapy from outside.

So we address in the present review following questions:

1) Do the EMF/PEMF triggers used in therapy meet an equivalent counterpart in the biological tissue? – Means, are similar electric fields generated endogenously or is no counterpart existing?

2) Which studies in literature show interactions of EMF/PEMF with the "classical" cell biology – and which mechanisms of coupling and which effects show in vitro and in vivo animal experiments as well as clinical studies?

Equivalent counterpart in the organism?

Elaborated studies could show that electrical and ion gradient phenomena are intrinsic to biological systems. These electric field gradients ("bioelectricity") are not only created by small ions but are also driven by larger biomolecules. These charge driven electric fields trigger pathways such as cell signaling, tissue factors, growth hormones, transmitters etc [1-7]. Since about ten years from now, new methods like membrane potential– and ion– sensitive *in vivo* dyes as well as constructs for imaging and molecular tracing are available, allowing a direct observation of the mentioned processes in cells, tissues and living systems. Thus, also effects of electric fields coming from outside as influencing factors to this endogenous bioelectricity and other targets can be studied adequately.

In living organisms, electromagnetic fields are generated endogenously mostly as direct current fields (DC) or ultra-low frequency (ULF)-EMF [6]. These fields arise from the segregation of charges by molecular pumps, transporters and ion channels situated in the plasma membrane [8]. Thus, they are not spikes of action potentials like in nerve (required field 10 – 20V/cm) cells but smoothly changing - like ULF – EMF.

It is important to look first at the resting potential of the cell. Means indeed each type of cell in the body has to maintain it specific level of resting potential at the cell membrane. Differentiated cells possess a high membrane potential with the highest for neurons (-75 mV), glia (-90 mV) and muscle cells (-50 mV). In embryogenesis resting potential values range from -8.5mV in the fertilized egg to -23mV in the four-cell till -25mV in the 16-cell frog embryo [5,9]. It is intriguing that, in general, malignant cancer cells (0- 10 mV) as well as proliferating cells (CHO, 3T3, etc., -12 to -25 mV) have low cell membrane (resting) potentials [10,11].

Recent studies imply that the resting potential is a key regulator of cell cycle as well as of proliferation. Depolarization of cell membrane potential by external changes in ion concentration inhibits G1/S progression of Schwann´ cells, astrocytes, fibroblasts and lymphocytes. This suggests that hyperpolarization should be important for initiating S – phase [12-14]. Many proteins are involved in this membrane potential triggered cell cycle control [14]. For G2/M transition, depolarization of the plasma membrane should be mandatory. In total

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a rhythmic change to hyperpolarization before DNA synthesis to longer depolarization during mitosis can be found as general pattern in tissue embryogenesis and regeneration [15].

For regenerative therapy the fact is important, that in normal human mesenchymal stem cells (hMSCs), cell differentiation is accompanied by a progressive hyperpolarization. Artificial depolarization holds these cells in an undifferentiated (stem - like) state, while artificial hyperpolarization accelerates differentiation [16]. In the next step of transduction from changes in resting membrane potential to intracellular mechanisms it is discussed an increasing Ca++ entry into the cell and a positive feedback loop between Ca++ entry and Ca++ dependent potassium channels [17]. In further signaling cascades till gene regulation, e.g. phosphatase and tensin homolog (PTEN) is involved as well as epigenetic regulators like histone decarboxylase (HDAC).

Cell cycle can also determine cell fate in diseases, means depending on outside conditions, the resting membrane potential level can switch in a flip flop manner into different states - especially if the order between the cells is perturbed during a diseased state. This may happen also between larger groups of cells; because ion transmitting gap junctions exist as well as other ways to convey information. Nowadays even computer modeling studies arise, showing how groups of cells with altered membrane potential level behave compared to normal cells [18].

On the other hand, relatively few papers exist how the resting potential in cells and group of cells is altered in pathogenesis, e.g. during inflammation. It is only known that inflammation causes a lowering of the threshold for action potentials [19]. Regarding inflammation-induced joint pain, Hatch et al., describe that hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are implicated [20].

In fact, the observation that the level of resting potential can switch from a diseased potential back to normal could be a very good argument for EMF / PEMF therapy [10]. Means, this therapy may trigger the tendency of the resting potential into the direction of a switch back from diseased to normal state. However, before we go to action mechanisms of PEMF therapy and to reactions of cells and tissues, let us first see how endogenous bioelectricity is working normally in healthy and wounded tissue as well as in a regenerating organism.

During early embryonic development, bioelectric fields are actively generated by passive Na+ uptake and by ion transporters (ion pumps). Differences in charge gradients between various regions are forming intraembryonic voltage gradients (about 1 - 5 V/cm). A depolarization of a neuron by surface electrodes requires a field of 10 - 20 V/cm. In contrast to these short - lived action potentials of neurons, small endogenous EF of all other cell types last very long and build up gradients that persist from UL- EMF to minutes and weeks, means [4].

Patterns of cell membrane potentials arise even in fertilized eggs and can be clearly demonstrated by electrosensitive dyes in life cell imaging [3]. Electric field gradients also influence larger charged molecules like growth factors or other charged signaling molecules - demonstrating significant links to classical biochemical signaling pathways (e.g. via serotonin) [21,22]. A network of open gap junctions then distributes signaling further [23]. After spreading the signal via gap junctions it is transmitted further into the classical signaling cascades. Ultimately, these signaling molecules control the expression of genes and also epigenetic mechanisms. Carneiro et al. found that these signaling molecules mentioned control the expression of genes (also epigenetics e.g. by a histone decarboxylase –HDAC- dependent intracellular receptor) [24]. This explains how electric gradient formation in single cells can lead to large-scale morphogenetic gradients.

In embryogenesis, during wound healing and regeneration as well as in vitro, many cell types normally migrate in along an electric field at field strengths of 0.1 - 10 V/cm, like neural crest cells, fibroblasts, osteoblasts, keratinocytes, chondrocytes, rat prostate cancer cells and many epithelial cell types [25-31]. Regarding sensing of such fields, our group found that the function of Na, K -ATPase and a Na+/H+ exchanger isoform (NHE3) could act also as directional sensors [29-31]. The information is transferred via a mechanism that involves PIP2 as a mediator and the cell membrane potential acts as a regulatory cue. This maintains persistent direction in electrotaxis. Also genes involved in these electrotaxis phenomena have been found during wound healing e.g. phosphatase and tensin homolog (PTEN) enzymes are involved [32].

In wound healing, in general, enhanced DC- EF are present especially in epithelial layers. Here, a transepithelial potential is generated with the cathode at the wound center. It is possible that EF are the earliest signals that an epithelial cell receives to initiate directional migration into the dermal wound bed [33,34]. This signal lasts for many hours and regulates different cell behaviors within 0.5 mm to 1mm from the wound edge. After complete re-epithelialization, the signal fades [4]. Kucerova et al. could also show that EFs after wounding arise initially and later on, other factors (like growth factors etc.) take over [35].

Regarding regeneration, the present models show that H+ pump (V-ATPases)- dependent changes in membrane voltage are an early mechanism inducing e.g. tail (spinal cord, muscle and vasculature) regeneration in Xenopus [36]. After amputation, the regeneration bud depolarizes, but after 24 h it repolarizes due to V-ATPase activity. The cell-surface V-ATPase is also up regulated at the mRNA and protein levels within 6h of amputation - an extremely early step in the regeneration process. More recently, Özkucur et al. could show that ion contents in the axolotl tail blastema change dynamically during regeneration and, in most cases, are still fluctuating at 48 h *post amputationem* whereas after 24 hours, downstream pathways (BMP, Notch, Msx, Wnt and Fgfs) are activated [37,38]. After 7 days this regeneration is completed.

Electromagnetic fields (EMF) are also produced endogenously within the organism. One should keep in mind that many rhythms are present in the nervous system, in the musculoskeletal system and within all connective tissue. Frequencies from 5 to 30Hz were found during postural muscle activity (quiet standing) and of 10Hz during walking [39]. So everything in living systems is in motion and changing magnetic fields are associated with changing EF. Thus, endogenous EMF and PEMF arise from the movement of muscles, tendons, etc. and the actions of the musculoskeletal system itself. Mechanical deformation of dry bone causes piezoelectricity. Furthermore, bending strain couples to the spatial gradients of permanent dipoles in collagen molecules [40,41]. At physiological conditions, mechanical stress-generated potentials are formed by the streaming potential, which is the electric potential difference between a liquid and a capillary, diaphragm, or porous solid through which it is forced to flow, or the electrokinetic processes, i.e. movement of ions because of fluid motion within tissues [42].

At the dimensions of single cells, enzymatic and metabolic activities of cells are mostly processed rhythmically. Thus, every substrate change and every small metabolic cycle has its own up and

down often in a sinus wave with a typical frequency [43]. However, the situation within cells and tissues is extremely complex and far from being completely understood. We have more than ten thousands of biochemical reactions happening simultaneously within a single cell [44]. Furthermore, coupling mechanisms of EMF and PEMF into the cascade of cell reactions are revealed only partially. On the other hand, our techniques to trace these coupling phenomena by modern cell biology now are far more sophisticated. So also the mechanistic impact of "electrotherapy" can be monitored more precisely - at least in standardized experimental situations in vitro and partly in vivo. In clinical situations it is more complicated again, however, also here relatively hard data now arise via many double blinded and randomized trials, showing the specific benefit of such therapies – especially for the use of PEMF as therapeutic trigger to enhance healing and regeneration processes.

Thus, recent in vitro studies begin to reveal how such EMF or PEMF stimuli are coupled or linked to the classical signaling pathways in molecular biology and genetics.

If the EMF or especially PEMF is strong enough, then Faraday coupling is the most plausible mechanism. Faraday coupling means magneto-electric induction or triggering surface charges on the cell membrane [45]. Here, charges and ions on the cell membrane can be moved. Furthermore, it is possible that receptors on the cell membrane can be set into motion if the EMF frequency hits the resonance frequency of a swinging molecular antenna within a receptor, transporter or another signaling element within the cell membrane [46,47]. It is clear that by this Faraday induction can influence also the resting potential of the cell.

Because the magnetic component of EMF can intrude into the cell, Faraday's induction law is also applicable within the cell, as demonstrated by reorganization of the electrostatically negative charged actin filaments. Cho et al. showed that a 1 or 10 Hz field changed microfilament structure from an aligned form to globular patches, whereas higher frequencies (20–120 Hz) had no effect [48]. Possibly, the moment of inertia in the actin fibers could not follow the changing field at higher frequencies, whereas at low frequencies the steady distortion inhibited formation of the typical cable-like structures.

In line with Faraday coupling is electroconformative coupling which means that periodic changes of an electric field can change the conformation of molecules in general and especially of cellular enzymatic systems, especially those within membrane structures[49,50]. Here, the EMF or PEMF pulses can be converted into chemical energy by enhancing the turn over speed of metabolically active systems, which can convert also signaling molecules and thus trigger cellular reactions. The sensitivity of this effect can be significantly enhanced by stochastic resonance, which means that a signal that is normally too weak to be detected by a sensor, can be boosted by addition of a "white noise" mixture of frequencies. By this "package" of white noise frequencies the original signal is amplified whereas the rest of the white noise remains in the same amount. So finally the signal overcomes the threshold to be detected by the sensor, which then can resonate with the original, previously undetectable signal [51].

EMF penetration of the cell can happen without massive attenuation. Thus, these fields can interact with cell organelles as well as with the DNA directly. In this context, Lin et al. found besides the heat sensitive region of heat shock protein gene (HSP 70) also an EMF sensitive region (electro responsive element – EMRE) [52,53]. Also the promotor of c-myc possesses such an EMRE [54]. Here we have a direct coupling of EMFs to classical cell biological signaling pathways.

Electrical fields induced by PEMF fields can be transported over larger distances by gap junctions. Thus, electric fields spread over distances up to millimeters in an embryonic or adult organism. Gap junctions are also termed "electrical synapses" in neurosciences [55]. Special proteins build up a certain channel or junction within neighboring cells membranes to form a gap junction. By gap junctions, groups of cells can exchange ions (e.g. Ca++, K+), however, also second messengers like cAMP, cGMP, IP3 or metabolites very rapidly, thereby building up gradients in their membrane potentials. Thus, EF can spread very quickly [55]. Gap junctions connect nearly all cell types, and interestingly connect also cells, which are not residing in close proximity, rather are connected by extended processes that often also possess gap junctions [56].

Finally, the most prevailing species of free radicals within a cell are radical oxygen species (ROS and hydroxyl radicals) and radicals formed by nitric oxide (NO) [57]. The lifetime of a free radical is varying dramatically - less than a nanosecond till 3–5 s in NO [57]. The radical pair phenomenon (NO, ROS) can thus interfere with endogenous oscillations in cell or metabolic systems. Resonance effects that greatly enhance (in phase), or diminish or extinguish (counter phase) characterize this interplay. Rosenspire et al. have shown in experiments with neutrophils and weak magnetic pulses that it is possible to modulate endogenous metabolic oscillations, which influence the production rate of reactive oxygen species and nitric oxide [58]. They propose an electrically sensitive membrane-embedded receptor complex, such as VSP, which transduces the signal to 1–25 Hz Ca2+ pulses. With regard to the release of NO a strong boost in blood circulation was evidenced [59,60]. Here, the activation of the vasodilatative component of NO may be of significance [61]. Indeed, our group could also show a direct increase in NO produced by endothelial cells in an *in vitro* study [59].

In many species the ROS triplet phenomenon is used for sensing magnetic field lines - mostly in birds. Triplets are generated by the radical producing impact of blue light within the photoreceptors. Then, the mechanism works as follows: the direction of the magnetic field lines interferes with triplet orientation and by aligning of the free triplet radical along the field lines the photoreceptor cells have get an orientation cue. And, because the photoreceptors are aligned in perfect hemispheres, retinae are ideal antennas. In an experiment with birds and yellow filter glasses upon their eyes, the birds had no blue light radicals anymore, and – consequently no directional information means the birds lost orientation [62].

Interactions of EMF/PEMF with "classical" cell biology - in vitro and in vivo animal experiments as well as clinical studies

Based on these new and consolidated cell and molecular biological grounds the "bioelectric" way can be applied to many clinical situations e.g. diabetic peripheral neuropathy.

In situations like diabetic peripheral neuropathy the literature shows that in the PEMF area many clinical observations exist, [59,63,64]; whereas the literature is very inconsistent for in vitro studies on neuronal cells as well as for studies with molecular- and cell biological background [65-72]. On the other hand, a profound understanding of the ongoing processes, especially of potential neuroprotective effects - see also such EF effects in patients of retinal degeneration would be very useful e.g. for the large number of diabetic

patients that have impaired axonal transport of transmitters within existing peripheral nerves as well as deficits in regrowth of impaired and damaged neuronal processes of autonomic nerves and wound healing [73-76].

For these situations DC EF can be applied with electrodes only in restricted areas whereas PEMF has the ability to penetrate deeper into the tissue [66] and to reach larger areas. Here, carrier frequencies in the kHz range were used to surmount possible higher impedances within the tissue. Other PEMF studies work in the low Hz range from 2 Hz on [64].

The cell biological effects described in the above mentioned studies reach from general enhancement of viability to increase of neurotrophic or other growth factors, influence of cytokines as well general cytoprotection. Activation of the vasodilatative nitrogen oxide (NO) may be of significance, too [61]. Indeed, our group could also show a direct increase in NOs produced by endothelial cells in an in vitro study [59]. The aforementioned impact mechanisms combined with increased blood circulation and the signal cascade of NO might be an important strand of the PEMF effect.

Osteogenic differentiation is enhanced in MSCs by PEMF only if the cells are pre-committed [77]. Means in detail, that MSCs derived from adipocytes differentiate faster and more expressed if they are cultured in a medium favoring osteogenic differentiation. Zhai et al. could show that PEMF stimulation with 15.38 Hz at 20 Gs (2 mT) for 2 h/day enhanced osteoblastic functions through amelioration of the cytoskeletal organization; increased proliferation-related gene expressions (including Ccnd 1 and Ccne 1) as well as upregulated gene and protein expressions of collagen type 1of the Runt-related transcription factor 2 and of Wnt/β-catenin signaling (Wnt1, Lrp6, and β-catenin) at proliferation and differentiation phases [78]. In a rat rotator cuff repair model PEMF therapy improves tendon to bone healing. Joint function was not altered; however, the bone quality was improved [79].

On the other hand neural differentiation was favored after PEMF treatment in human bone marrow MSCs [80]. The expression of neural markers such as NF-L, NeuroD1 and Tau was enhanced. Furthermore, a cell protective effect was found via the PI3K/Akt/Bad signaling pathway. In nerve crush experiments in rats, peripheral nerve regeneration could be enhanced by PEMF as well as by addition of Schwann – like cells derived from human dental pulp stem cells [81]. In guinea pigs, Veronesi et al. could show that PEMF (75 Hz) ameliorated all symptoms of knee osteoarthritis [82].

Positive PEMF influence is also reported on the anabolic activity of chondrocytes, a chondroprotective effect is observed on joint cartilage and on spontaneous osteoarthritis in animal models [83-91]. At the same time, the catabolic effect of IL – 1b is reduced [91,92]. Furthermore an increased gene expression in members of the Transforming Growth Factor (TGF – ß) family is effected [93]. The local expression of TGF – ß hereby also results in improved bone fracture healing [94], whereby the proliferation, differentiation and synthesis of cartilage matrix proteins are also improved [85,95].

In clinical studies applied PEMF at varying carrier (high) and modulation (low) EMF frequencies [96]. The treated knee of the osteoarthritis patients had less pain, less stiffness and an increased physical function. Two meta – analyses by Negm et al. [97] (7 studies analysed) and Ryang We et al. [98] (14 studies analysed) show very positive effects of PEMF in the management of knee osteoarthritis.

In our own randomized, placebo controlled PEMF study with an electrode-less device we found in osteoarthritis patients significant reduction in stiffness and a significant reduction in disability in daily activities [64]. The device (MagCell) -delivered a sinusoidal magnetic field with varying in a frequency of 4 and 12 Hz and a magnetic flux density of 105 mT even in 1 cm tissue depth.

As an overall conclusion of all clinical PEMF studies mentioned above, one can recommend the PEMF as mono – therapy or as additional therapy in combination with pharmaceutical therapy. This recommendation can now be fostered by reputable research in molecular and cell biology.

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