Dariusz SZTAFROWSKI, Zbigniew WRÓBLEWSKI[1], Marcin ŁUKASZEWICZ[2]

Institute of Electrical Power Engineering Wrocław University of Technology Wrocław, Poland (1)
Faculty of Biotechnology, University of Wroclaw (2), Faculty of Chemistry, Wrocław University of Technology Wrocław, Poland (3)

Evaluation of how low frequency magnetic field 50 Hz affect living cells

Abstract. *The mechanism of ELF-MF impact on the metabolic processes occurring in cells of the living organisms is discussed. Existing research suggests that biological membranes may be composite antenna for stimulation by an electromagnetic field. To further elucidate this mechanism the use of fluorescent probes is suggested.*

Streszczenie. *W referacie przedstawiono postulowany mechanizm oddziaływania ELF-MF na procesy metaboliczne zachodzące w komórkach organizmów żywych. Błony biologiczne mogą być anteną zbiorczą dla bodźca jakim jest pole elektromagnetyczne. Postuluje się wykorzystanie sond fluorescencyjnych do dalszych badań. (**Wpływ pola magnetycznego 50 Hz na komórki organizmów żywych**)*

Keywords: ELF field, in vitro research, gene expression, fluorescent probes.
Słowa kluczowe: pole magnetyczne, badania *in vitro*, ekspresja genów, sondy fluorescencyjne.

Introduction

Among the various sources of electromagnetic fields, high tension power cables cause the greatest concern. People more and more often have questions about whether being within an electromagnetic field has an influence on their health or may have in the future. It is certain that greater needs for electric energy will also mean greater exposure of people to electromagnetic fields. Scientific experiments undertaken over many years in various research and development centres all over the world show that magnetic fields with industrial frequencies induce various responses in living organisms.

Biological mechanisms of this type are not yet well understood. In published research we can find likely but not fully proven hypotheses concerning possible mechanisms for the influence of magnetic fields on biological structures. Because of our incomplete understanding of the effect that electromagnetic process has on living organisms, the construction of new electro-energetic objects such as high tension power lines often causes concern and protest by local inhabitants. In addition mass media often highlight only sensational news (for example the threat of cancer) which exacerbates public concern. Such selective reporting of the arguments creates the feeling that living and working near to objects emitting EMF is unsafe [3,4,20,21]. It makes it difficult not only to build new high tennsion power lines but also remodernize the existing ones. A good example of that could be the fragment of a single-track power line 220 kV being converted into four-track power line (2 x 220 kV and 2 x 400 kV) near Poznań.

On the other hand, there is no doubt that the influence of EMF on the human body is not indifferent. EMF in the neighbourhood of some industrial tools, for example a welder, may produce metamorphopsia, temporary blindness (during the exposure to the field of the induction value equal a few mT - self-research) [23]. It can also have a positive influence on a human organism, contributing to the fight against some of the illnesses (e.g. healing of wounds and bone fractures).

Among such extreme possibilities, there is an abundance of situations, in which it is crucial to determine the level of safe exposure to the above mentioned factors for the sake of professional, health and many other reasons. This issue is still poorly understood and there should be more research in these areas. That is why specific and objective analyses of these problems should be the task of teams of scientists with different specialisations. Producing credible results is possible only through interdisciplinary research [8].

Living organisms consist of solid and liquid substances. In the majority they are organic substances being the subject of biochemical and biodynamical processes. All the tissues from which the living organisms are composed have both electric and magnetic properties.

The nature of the effect of the EMF on living organisms depends on its frequency. For high frequency EMF (e.g. microwaves) the major influence is thermal causing an increase in the temperature of the exposed objects.

At the low frequency the changing EMF generates the flow of electric current in the living organisms. The flowing current results form the components: the current density vector induced by alternating electric field and the current density vector induced by alternating magnetic field. The total current is a sum of both of those components.

Furthermore, under the influence of the external electromagnetic field on the living organisms we can observe as follows [15,16,23]:
- magnetization,
- effects on diamagnetic molecules,
- effects on liquid crystals in organisms,
- effects on water,
- effects on piezoelectric elements.

There is no doubt, as it is stated in the results of the numerous research, that 50 Hz frequency electromagnetic fields produced by overhead high tension power lines, may cause various measurable changes (so called, biological effects) in living organisms or even the symptoms of the body response to those effects.

Although it is clear that EMF does cause a change in the organisms, it is not clear that the change is necessarily negative.

During planning and conducting research on the effect of EMF on living organisms we should clearly distinguish the biological effects and health risks.

Biological effects and health risks

Biological effect is the reaction of the organism or a cell to stimulation. Biological effect does not mean illnesses. Mainly, it is a sign of triggering the adaptation processes.
Disturbance of physiological balance and development of illness occur only after exceeding the efficiency levels of the adaptation, compensatory mechanisms and regenerative mechanisms. Therefore, the risk to health leading to the occurrence of illness arises only after the rupture of the state of functional equilibrium of the living organism. The

role of the health risk is to determine the probability of occurrence of the state of such disequilibrium.

In the living and working environment the human being is exposed to the various factors which can affect his/her health conditions. Some of those factors are characterized by having a high biological activity and even after a short time of exposure they may significantly harm health or cause the development of certain diseases.

In the case of the low biological activity environment factors, such as 50Hz frequency electromagnetic field, it is extremely difficult to establish the cause of biological effects or the disease development. Finding a cause-effect relation between the affection of a physical or chemical factor and the occurrence of the disease is easy only if the biological activity of that particular factor is well defined. In the conditions of existence of many environmental factors, the most difficult problem is establishing the real cause-effect relation between factors and the effect they cause. This relation is presented on the illustrations 2 and 3.

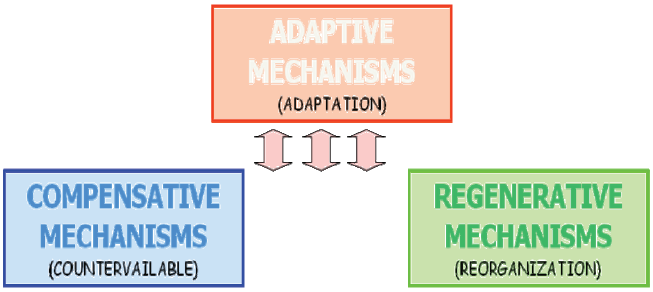

Fig 1. Diagram of the organism's reaction to the effect of the external factor

Due to high economic importance, a board range of epidemiological research is being conducted to establish the relation between the intensity of 50 Hz frequency electromagnetic field and the increased risk of disease incidence. Although those types of studies are very expensive and time consuming they do not clarify the mechanism of electromagnetic field influence on living tissues.

Thus, it is worthwhile to conduct research at a molecular level which will enable us to understand the mechanisms by which these physical factors affect the living organisms.

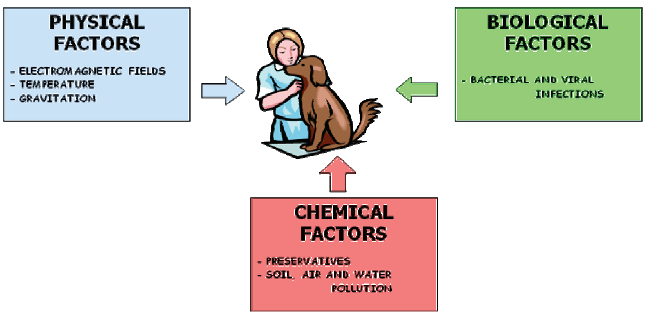

Fig.2. Finding a cause-effect relation between the affect of a stressogeneous factor and the occurrence of the dieses is easy only if the biological activity of that particular factor is well defined

At this same time, it is important to stress that too few studies concerning cells and tissues have been conducted so far to be able to determine if the exposure of such biological structures to the electromagnetic field observed in the proximity of overhead high tension power transmission lines causes permanent and harmful effects.

On the basis of the research conducted so far, it is impossible to determine either the value of health risk nor the value of the current intensity causing that risk. An extra difficulty is the fact that in their natural habitats living organisms are the subjects to multiple environmental factors with various biological activities.

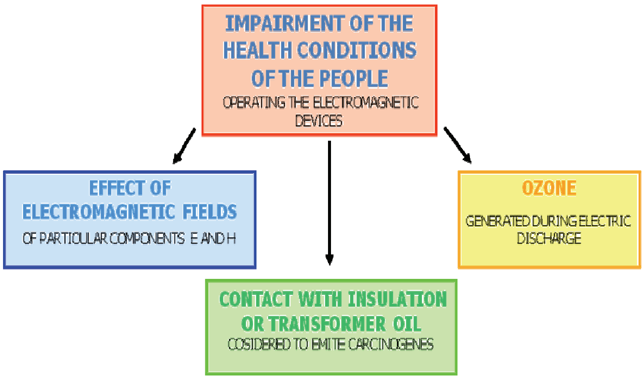

Fig 3. In the conditions of existence of many environmental factors, the most difficult problem is establishing the real cause-effect relation between factors and the effect they cause

Thus, the most difficult issue is to determine whether the occurrence of some phenomena (e.g. biological effect or a health disorder) is a consequence of a real cause-effect relation of that phenomenon and the particular environmental factor, rather than random coincidence (e.g. the reason for developing a cancer could have been electromagnetic field or any other external factor). That is why before confirming the results on animals it is necessary to conduct multidirectional research on cell cultures and plants and then intensive epidemiological study.

Free radicals and reactive oxygen species

Nowadays most of the research suggests that the factors responsible for most of the observed biological effect induced by low frequency 50 Hz EMF are free radicals and reactive oxygen species [2,16]. Free radicals (or radicals) are atoms, molecules or ions with unpaired electrons on an external shells. They are highly reactive as they tend to be pared by taking part in chemical reaction generating secondary free radical. Such a chain reaction involving free radicals may lead to harmful breakdown of important macromolecules for example DNA, resulting in mutations that can be potentially malignant.

Free radicals are necessary in some biological processes. As they are generally highly reactive, even very small concentrations may have great impacts on the homeostasis of redox reactions being fundamental for living cell biochemistry. Homeostasis is kept both by production of radicals and antioxidants which deactivates radicals. Cell generates various kinds of antioxidants which could be divided in low and high molecular mass.

Antioxidants of low molecular mass such as vitamin C, flavonoids or caretonoids by quick reaction with radicals protect other important molecules within living cell.

High molecular weight antioxidants are proteins with enzymatic activities such as super oxide dysmutase (SOD) catalysing chemical reactions neutralising free radicals, for example:

$$2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2$$

In such a reaction two molecules (oxygen and hydrogen peroxide) with pared electrons are generated from two radicals with unpaired electrons. Although hydrogen peroxide is not radical, but has strong oxidising properties, thus other enzymes such as catalase degrade H_2O_2 into water and oxygen:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

As not only free radicals are highly reactive but also other molecules containing oxygen such as H_2O_2 which are very important for the living cell to maintain redox homeostasis, often in published results the term ROS (**R**eactive **O**xygen **S**pecies) is used. It is generally assumed that even small changes in the ROS concentration within a cell triggers several signalling cascades enabling organisms to adapt to environmental changes such as temperature, light, nutrients availability etc. Biological systems like others are not perfect and even though there is very efficient control of production and deactivation of ROS, some of them affect macromolecules leading to unwanted changes such as mutations. As inappropriate level, homeostasis in some cases is the source of pathological changes. That is why the antioxidants are often used in food supplementation.

As far as the impact of EMF on ROS level within the cell is rather well documented, molecular mechanisms of observed changes need to be elucidated [17].

Generation of free radicals within the cell

Coupling by enzymes various biochemical reactions within the cell often is based on coenzymes – the universal transmitters of electrons and protons such as NAD and NADP. Special acceptor of electrons from coenzymes is respiratory chain localised in membranes, where electrons are separated from protons. The respiratory chain is built from complexes with increasing redox potential which transfers only electrons. The final electron acceptor is usually oxygen. Energy resulting from electron transfer is used to build up electrochemical potential between two sides of membrane. In some situations electron transport may be disturbed resulting in so called electron leakage, which instead of completing chain and being transfer on final acceptor, are transferred on other compounds resulting in generation of free radicals. In eukaryotic organisms the respiratory chain is localised in mitochondria. An extra organelle producing free radicals in plant is chloroplast where, as in mitochondria, energy conversion reaction generates majority of ROS. We can summarize that the main resource of the free radicals are the reactions taking place inside of membranes or in between the edge of the membranes and the environment surrounding them.

Biological membranes

Cell structures gathering EMF induced primary signal which triggers cascade of reactions could be biological membranes. Biomembranes have very complex selectively-permeable structure. Their appropriate liquid crystal organisation in space is crucial for maintenance of functions such as electrochemical gradient, compartmentalisation of both cell and processes as well as sensing and transmitting various signals enabling adaptation to dynamic changes of environmental conditions.

Thanks to their molecular structure and anchored proteins in a certain stereochemical configuration, the membrane maintains its cell potential, which consist the base for any change of energy and active transport of chemical substances. The membrane also contains the receptors responsible for detecting the changes in the environment and triggering the cascade of reactions resulting in adapting the cell metabolism to the changed conditions. The cascade of reactions often results in a change of the genes expression.

Many molecules forming the biomembrane as well as the membrane itself have a dipole structure and may be charged. Under influence of EMF the above mentioned molecules may change position within the membrane affecting its structure. It may lead to a change in transmission of various compounds and the enzymatic activity of the enzymes located within the membrane. The change of the membrane structure may allow the compounds hidden within the membrane to contact with the external compounds, leading to the chemical reaction that may permanently damage the molecules of the membrane causing its further malfunction of structure and functionality.

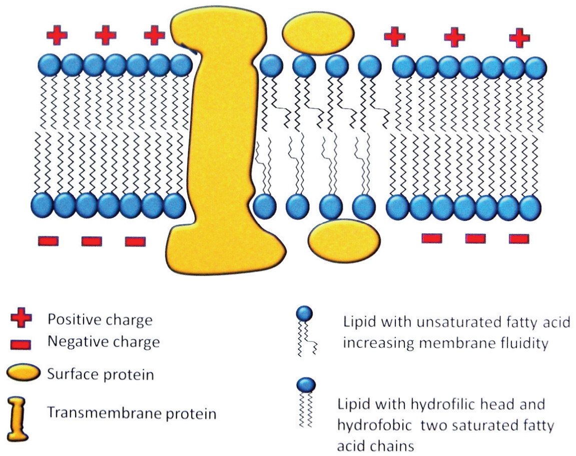

Fig.4. Organisation of semi liquid crystal structure of biological membrane composed of phospholipids bilayer matrix with transmebrane and surface proteins and electrochemical gradient

These are the reasons why a lot of research is directed in basic molecular mechanism how EMF affect biological membranes in vivo and in vitro. Unfortunately, many experiments on membranes are very difficult to re-perform in different laboratories. The observed effects are insignificant and the standard deviation high. A solution to this problem could be sensitive markers enabling qualitative and quantitative observation of EMF impact on biochemical reactions within the living cell [1]. Very promising for this purpose are fluorescent probes enabling a very sensitive measurement in real time of such parameters as membrane potential and synthesised thanks to it ATP, a level of free radicals concentration and ion concentration, for example Ca^{2+} which is universal signal of biochemical reaction signalling cascades.

Methods of free radical detection

The discovery of the influence of the changes of redox homeostasis and ROS on triggering various control and adaptation processes as well as the harmful effect of free radicals on the living organisms, they all caused the very intensive progress in development of the research methods. As ROS are often short lived they are very hard to measure in real time. So most of experimental detection methods are based on the products of reactions of various compounds with ROS.

Detection of biological membranes degradation products

Biological membranes separating cell compartments are very first to be affected by ROS. Semi liquid crystal structure of biological membranes matrix, in which there are anchored other components such as proteins, is provided by phospholipids bilayer. Phospholipids have polar head groups and non-polar fatty acid tails. Fatty acids tails are arranged so that their fatty acid tails face one another to form an inner oily core held together by the hydrophobic effect, while their charged heads face the aqueous solutions on either side of the membrane. To maintain appropriate fluidity some of fatty acid tails must be unsaturated (having double carbon-carbon bounds). This double bound twists the molecule resulting in relaxation of the whole structure. In such less packed structure interaction between molecules is weaker increasing fluidity. Unsaturated hydrocarbon chains are prone to rapid oxidation thus are first to react with ROS. That is why cell is producing hydrophobic antioxidants such as carotene or vitamin A which have many linked double carbon-carbon bonds. Thus methods aiming to detect destructive effect of ROS on membranes are often based on measurements of products resulting from degradation of unsaturated fatty acids.

Measurement using fluorescent probes

Detection of lipids degradation end products, such as saturated fatty acids, does not enable observation of dynamic changes in real time. Such cumulative observation does not reveal more subtle dynamic fluctuations in ROS concentration which are the result of regulatory adaptation of the cell. In consequence many new methods seek to use fluorescent probes enabling observation of subtle *in vivo* changes. Depending on their chemical structure, fluorescent probes may react specifically with various groups of ROS. As a result of such reaction modification of chemical structure of the probe results in fluorescence characteristic (they become active or inactive fluorophore, change absorption or emission maxima).

Beside substrate specificity fluorescent probes may be preferentially accumulated in various cell compartments, such as organelles (chloroplasts or mitochondria). Fluorescence may be also dependent on H^+ concentration which theoretically enables observation of very subtle localised reactions such as membrane polarisation, which is substantial for all energetic of the cell.

Another advantage of fluorescent probes is potentially very high sensitivity of measurements. A molecule of fluorescent probe may emit many photons after excitation with appropriate light. There is signal amplification after just one reaction between fluorescent probe and ROS. Unfortunately the amplification process is often short. Fluorescent probes are prone to photooxidation (destruction of chemical structure in consequence of absorbed light). That is why there is extensive research on new more efficient fluorescent probes.

The biggest advantage of fluorescent probes is the possibility of their use in *in vivo* non destructive experiments practically in a real time. To react within the cell, the probe has to cross barrier of biological membranes. Another condition is that probes may not be harmful or interfere with cell metabolism to reflect real changes in studied parameter.

Until now fluorescent probes have been used in only few studies on the impact of EMF on living organisms. With development of new probes these experimental approaches seems to be very promising for this type of studies.

Gene expression

Revealing whole genomes sequences enables holistic experimental approaches. For model organism such as yeast (*Saccharomyces cerevisiae*), *Arabidopsis thaliana* or *Escherichia coli* microchips with all genes have already been created. They enable observation of whole genes expression changes in response to given stimulus. Modification of given gene expression results at first in a modified amount of the gene product – messenger RNA (mRNA). mRNA is transported from nucleus to cytoplasm where it is used as template for the production of encoded by the mRNA protein. After appropriate folding, maturation and delocalisation generated protein is catalysing specific chemical reaction resulting in generation of given metabolite. In recent years there is intensive research focused on holistic approaches enabling measurement in one experiment all mRNA molecules (genomics), all proteins (proteomics) and all metabolites (metabolomics) produced within a cell.

The microchips, elaborated for the model organisms with well known genome, enable recording total messenger mRNA molecules within an entire cell. Thus, the complex study of various factors, both biotical and abiotical (such as electromagnetic field) is possible.

Experiments with DNA microchips provided a huge amount of experimental data. It appeared that rate limiting step is analysis and interpretation of such vast amount of information. In fact even factors of small intensity cause expression change of many genes. It is a consequence of very complex network of metabolic interactions. For example Lupke et al. [6] have shown that low frequency 50 Hz EMF with intensity of 1 mT affects expression of over 900 genes but for many of these genes influence of ELF-MF could not be confirmed by other methods. It is not an easy task to determine threshold of important changes for whole genes. It may vary for the various genes due to the absolute amount of mRNA molecules, which may be very low for the regulatory genes and very high for the constitutive genes, necessary to maintain functions of leaving cell. Regardless of the significance threshold level, other methods confirm impact of ELF-MF on genes expression and increase of ROS level. Primary receptor inducing observed changes under influence of ELF-MF remains to be uncovered. Understanding the relationships within whole network at various levels (mRNA, proteins or metabolites) will require a lot of effort especially, that the function of about 17% of genes that may be affected by ELF-MF has not been defined yet. Classification of genes according to the functional groups suggests that ELF-MF affect mostly transport, cell cycle, metabolism (macromolecules, in particular), cell division, signal transduction and immunologic response. These functional groups should become the starter point for a marker genes search, used to study the intensity of ELF-MF and their potential impact on the health condition of humans.

Model of influence on cell by ELF-MF

The mechanism of influence of ELF-MF on increase of free radicals level is unknown. It is suggested that there is (are) receptor(s) in biological membranes sensing ELF-MF and transmitting signal. However the direct interaction of ELF-MF on ROS by their stabilisation for example, may not be excluded. Due to low energy of 50 Hz magnetic fields, to which living organisms living near high tension lines are exposed to, in order to be sensed, the signal must be cumulated. This could be performed by biological membranes which could act as satellite antenna cumulating signal and transmitting trough various transduction pathways leading to observed changes in gene expression and ROS level.

Conclusions

It should be experimentally feasible to determine the impact of ELF-MF on biological membranes using fluorescent probes. The measurable effect of that impact may be observed as:

1. Changes in membrane electrochemical potential
2. Changes in membranes permeability

3. Electron leakage leading to ROS increase
4. Sub cellular localisation of ROS level
5. Activity changes of some membrane transporters
6. Delocalisation and concentration changes of signalling molecules such as Ca^{2+}

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***Autorzy**: dr hab. inż. Marcin Łukaszewicz, Uniwersytet Wrocławski, Wydział Biotechnologii ul. Przybyszewskiego 63/77, 51-148 Wrocław, Politechnika Wrocławska, Wydział Chemii, ul. Wybrzeże Wyspiańskiego 27, 50-370 Wrocław E-mail: marcin.lukaszewicz@pwr.wroc.pl, Prof. dr hab. inż. Zbigniew Wróblewski E-mail: zbigniew.wroblewski@pwr.wroc.pli, mgr Dariusz Sztafrowski, E-mail: dariusz.sztafrowski@pwr.wroc.pl Politechnika Wrocławska, Instytut Energoelektryki, ul. Wybrzeże Wyspiańskiego 27, 50-370 Wrocław.*