In vitro and

by Lutz Haberland

In *vitro* studies

The first session dealing in particular with in vitro studies was the tutorial session 1: "High-Throughput Screening Techniques in EMF Research". The lectures are paraphrased in detail in the **report of Vijayalaxmi**. In short: The relatively new technologies proteomics and transcriptomics seem to be a good tool to examine the effects of EMF on the expression and activation of (functionally partially unknown) proteins. However, the sensitivity of these methods is limited. Expected small effects have to be verified using other, more sensitive methods.

With the help of the above mentioned methods and other techniques, **R. Nylund** et al. (group D. Leszczynski) examined the influence of modulated (GSM) and unmodulated 1800 MHz fields (2 W/kg) on protein expression in a human endothelial cell line. They found an enhanced expression in 58 of 1476 examined proteins, however only in GSM-modulated field, not in unmodulated ones. Many of the increasingly expressed proteins are linked to the functioning of the cytoskeleton. When replicating tests one year after – this time using the more traditional method of Westernblotting – no effect could be found. The reason behind this discrepancy is still unclear, even to the authors, and they plan to repeat the tests, also in other cell lines.

Two posters are of interest in the context of increased expression and phosporylation of stress proteins (Hsp, presented at the BEMS meeting 2003) detected by the group around D. Leszczynski. **F. Poulletier de Gannes** and colleagues used the same human endothelial cell line as the group around Leszczynski, as well as a similar one, in order to examine the expression of Hsp27 at exposure to a 900 MHz field, SAR = 2 W/kg. Significant changes were not found; there

studies mechanisms

was, however, a trend towards enhanced expression after one-hour exposure in the cell line of Leszczynski. This finding had already been heavily discussed at the Helsinki Hsp workshop in April. The crucial question was "whether there would perhaps be an effect after all" which could become significant with a larger number of tests. The group around **J. Miyakoshi** examined the expression of stress proteins Hsp27 and Hsp70 in human glioma cells at exposure to a 1950 MHz CW field (SAR: 1, 2 and 10 W/kg). They found no change in protein expression; only the highest SAR showed a trend towards decreased phosporylation of Hsp27.

The influence of radiofrequency fields on the enzyme ornithine decarboxylase (ODC) has been a subject of research for several years; it was primarily the group around Litovitz that reported positive results. The enzyme ODC has been linked to the development of cancerous cells. **M. Taxile** and colleagues wanted to replicate these tests, once with 50 Hz modulated 835 MHz fields and, as an extension, with 217 Hz (GSM) modulated 1800 MHz fields. However, they could not determine any change in ODC activity, neither at 0.5, resp. 1, nor at 2.5 W/kg.

In 2001, J. Tattersall and colleagues detected an influence of 700 MHz fields on the electric activity of hippocampus tissue slices of rats. A poster of **A.J. Smith** (Tattersall group) demonstrated the results of similar investigations performed in mice, but this time at 900 MHz. There were no differences between exposure and sham (control) experiments, though. There was no explanation on whether the lack of effects possibly was due to the different frequency. But further tests with 400 MHz (Tetra) and 2200 MHz (UMTS) are planned.

Free radicals, and especially reactive oxygen species (ROS), have repeatedly been considered possible target points for electromagnetic fields. **M. Lantow** and **M. Simkó** observed the influence of 1800 MHz fields (SAR = 0.5 to 2 W/kg) in different cell lines. They could not find any significant change in ROS production (poster).

What strikes the eye when looking at the presented investigations is the large number of replication experiments (per se a positive development). The fact that results were mostly diametrically opposite to previous findings, once more indicates that the biological effects of EMF near or below limits either are very slight or are dependent on very many influential factors. The perfect methodology to examine this still eludes us.

Mechanisms

As last year, the topic of mechanisms was addressed in association with the issue of modeling, the latter clearly prevailing. Out of eight lectures in session 6, "Mechanisms and Modeling", only one actually dealt with a potential mechanism.

J. Carson (co-author: J. Walleczek) dealt with the light-dependent magnetic field influence on a periodic peroxidase-oxidase oscillator. Concentrations of the molecules NADH, oxygen, hydrogen peroxide and nitrogen periodically change in this artificial chemical oscillator. This system is also seen as a model of peridoc oscillations in biological systems. The authors now have found a light-dependent effect of static magnetic fields. Chemical oscillations were affected in their amplitude only when there was a simultaneous exposure of light, especially with wavelengths of 580 to 700 nm, and a magnetic field of approx. 80 mT. The effect was explained by the fact that light excites products of intermediate metabolism to a triplet state which then can be affected by the actually

quite strong magnetic field. The study is another contribution to the radical pair theory, but cannot explain phenomena emerging at magnetic field intensities as usually occur in the environment.

Other observations on potential mechanisms were hidden among the contributions of the Students Session, etc. Two lectures (and two posters) of the group around **G. D'Inzeo** described the efforts made to shed more light on the processes at the cellular level, especially around cell membranes (microdosimetry).

The lecture of **M. Gianni** addressed the influence of low-frequency fields on nerve fiber activity. According to the theory of stochastic resonance, the signal-noise ratio is enhanced when a given amount of stochastic noise, which contains frequency components of the periodic signal, is added to a periodic signal (here: EMF in the range of 20 to 100 Hz). Endpoint is the change in the membrane potential. The basis of calculations was the assumption that nerve fiber activity is quite constant at 83 Hz. In discussion, it was objected that there are no nerve fibers constantly firing at this frequency.

In the following, **M. Pellegrino** talked about microwave effects (0.8 to 2 GHz, SAR 1 W/kg, CW, GSM-modulation) on the function of ion channels. Experiments resulted in a decrease of opening probability of channels at exposure, whereas conductivity remained constant. Experimental results shall now be described and explained by mathematical models, as well as predicted in future. Unfortunately, the study is not finished yet.

A survey study on recently discussed hypotheses on mechanisms was part of session 7, "RF Studies". **A. Sheppard** summed up concepts of nonlinear reactions, short-term heatings in small areas (water layers surrounding macromolecules, macromolecules themselves, whole cells) and resonant excitation of macromolecules due to radiofrequency field exposure. He concluded that at intensities within the valid limits energy diffusion between molecules is too fast to cause noteworthy energy inputs, resp. temperature elevations. Moreover, excitation of macromulecules is not to be expected at frequencies below 10^{11} Hz (mobile radio transmits with 10^9 Hz), due to model calculations.

Two of the three lectures comprising session 11, "Biological Effects and Medical Applications", were also related to the topic area of mechanisms. D. Leszczynski tried to trace back radiofrequency field effects on protein expression reported by his group to an influence on the protein folding process. But a more detailed concept of this is still lacking.

The interaction of electromagnetic fields with electrons, especially those in the DNA, also called electron transfer, is the mechanism preferred by **M. Blank**. Since hydrogen bindings between DNA molecules are quite weak here, external fields are claimed to be perfectly able to shift electrons, and thus to weaken bindings. During the partially very lively discussion critics said that field effects on free electrons cannot be compared to effects on bound electrons, not even regarding the magnitude of order.

We should note that mechanisms for non-thermal (radiofrequency fields) and "non-stimulating" effects (low-frequency fields) that could explain the number of reported experimental results are still unknown.

From an organizational perspective, it surely would be preferable to bring the lectures on mechanisms together in a properly named session at the coming meeting.

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