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International Workshop on Action Mechanisms
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Rapporteur's Report
Dr. Vijayalaxmi, Department of Radiation Oncology
University of Texas Health Science Center
San Antonio, TX 78229, USA.

Welcome address: Dr. Weiss

"This is the 4th meeting of the German Mobile Telecommunication Programme. The final international meeting will be held in summer 2008. There will be a public meeting with the minister and other officials followed by a scientific meeting together with world health organization (WHO) and international commission on non-ionizing radiation protection (ICNIRP) to discuss the science and other things in a larger context. Hopefully, answers to some of the questions (what was the situation before the start of the program, what has been achieved, what are the lessons learned, where do we still have gaps, can we define minimum standards for future work, how safe are electromagnetic field exposures, how safe is our regulatory system, do we need to change or modify anything, what are the consequences of our policies, and the findings that have impact on guidelines and standard settings) will emerge at the end of this process. Keep all this in mind for the next two days. I encourage you to have open exchange of scientific discussions. We hope to get new ideas to take home".

Ministry for environment/nature conservation and Nuclear Safety: Dr. Böttger

"During the last years, especially in late 1990s, we had intense discussion on health effects of mobile telecommunication systems. To make the discussions more objective, the Federal government asked the Radiation Protection Commission (RPC) to prepare an evaluation on the knowledge of health effects which may occur below the safety standards. So, in 2001, the RPC completed the recommendation on limit values and precautionary measures for the protection of general public against EM Fields. One of the major recommendations of RPC was to intensify research to find answers to open questions on possible health effects. This recommendation was the basis of different measures of the network providers at the federal government. In December 2001, the mobile telephony network providers signed a self-commitment and one of these commitments was to support a research program of the Federal Ministry of Environment with additional 8.5 million Euros. So we had, for this program, 17 million Euros. The Federal office of Radiation Protection started to organize and to coordinate a research program on behalf of Ministry of Environment. Different steps were implemented in the program to make sure that the interests of all stake holders were considered. At least 50 projects were elected to cover the entire field to evaluate possible health effects of EMF. It is a big step forward to give us much more solid risk assessment on possible health effects. This will be the basis for the Federal government to decide in which way we have to change the national legislation in this field. The program has an important contribution to prepare much more solid basis for the discussion on possible health effects, and for the ministry, it is important that the program to have good scientific discussion and transparency. The results of this program are of great interest to the Federal Government of Germany. The purpose of the workshops is to discuss the results of German Telecommunications Research Programme.

Last year, we had 3 very successful workshops on dosimetry, risk communications, and acute health effects. There will be another workshop in October 2007 on long term effects".

Session 1: Functional Aspects

Chair: Asmuß

Genotoxic effects of HF-signals on human cells *in vitro*.

Pollet, D and Waldmann, P. University of Applied Sciences, Darmstadt, Germany.

Dr. Pollet described the controversial nature of results from the investigations on genotoxic effects of exposure to radiofrequency radiation reported in peer-reviewed scientific publications. While the data from a majority of the studies indicated no significant increase in genotoxicity in cells exposed to radiofrequency radiation as compared with sham-exposed control cells, the results from some in vitro studies clearly showed such effects in different cell types. In particular recent studies from the REFLEX project (5th Framework Program of the European Union) report of DNA strand-breaks, chromosomal aberrations and micronuclei induced by experimental RF-EMF exposure. Because of the relevance of potentially adverse effects on DNA integrity to human health, a multi-centered study is funded by the German Federal Office for Radiation Protection to determine possible genotoxic effects of radiofrequency radiation generated by mobile telecommunication equipment on DNA, chromosomal and genomic level.

Dr. Waldmann described the general protocol used for the proposed multi-centered study. Peripheral blood lymphocytes from 20 healthy, non-smoking donors (10 donors of 50-60 years age and 10 donors <18 years age) will be exposed in active phases of cell cycle (G_0, G_1, S, G_2 and if possible in mitosis) to 1800 MHz GSM intermittent signal – 5 minutes On and 10 minutes OFF at 0 (sham), 0.2, 2 and 10 W/kg specific absorption rate (SAR). Four genotoxicity assays will be used – single strand breaks using alkaline comet assay, chromosomal aberrations (CA), micronuclei (MN) and sister chromatid exchanges (SCE). Cells exposed to gamma radiation will be used as positive controls for CA and MN while mitomycin C will be used for SCE. The radiofrequency radiation exposure will be conducted 'blind' in wave guides supplied by IT'IS, Zurich under controlled temperature conditions. The data will be decoded only after completion of the microscopic evaluation of the slides in 3 separate centers. The initial problems encountered with the wave guides in the IT'IS exposure system are resolved now. To date, pre-test results are obtained and detailed genotoxicity experiments with one donor were completed. The project shall be completed by august 2008.

Questions and Answers:

1. MN test may not be sensitive - Four genotoxicity assays are included in the project.
2. Neutral comet assay and H2AX techniques should have been included to measure double strand breaks in the DNA - Since the project was aimed as a confirmation study, new methods and other end-points were not included in the proposed project.

Rapporteur (Vijayalaxmi) had more discussions with the investigators involved in the project at dinner and break-fast time. Dr. Asmuß and Dr. Ziegelberger (chair-persons at Bundesamt für Strahlenschutz) were also included in some of these discussions. Since there is very little data in the published scientific literature on the incidence of MN in lymphocytes harvested at 52 hours (for final comparison), it was suggested to use the "classical" method for MN assay in which cytochalasin-B is added between 40-44 hours and the cells harvested at 72 hours.

Functional and molecular investigations after 1.8 GHz radiofrequency electromagnetic fields exposure in different immune relevant cells.

Simko, M. University of Rostock, Germany.

The project is completed. Investigations were conducted to determine whether the radiofrequency radiation fields emitted from mobile phones induce free radicals (superoxide radical anion and ROS) in different immune relevant cells. The radiofrequency radiation signals used were either continuous wave or pulse modulate wave (217 Hz, GSM-nonDTX which is speaking and GSM-DTX which is non-speaking) at 0.5, 1.0, 1.5, 2.0, 5.0 and 10 W/kg SAR. A statistically significant elevation of free radical release was observed after 45 minutes exposure to GSM-DTX signals in primary human monocytes and Mono Mac 6 cells but not in primary lymphocytes and K562 cells: however, this change could be detected only when radiofrequency radiation-exposed cells were compared with sham-exposed control cells and not with incubator controls. Positive control cells exposed to PMA or treated with heat (42^{o}C) exhibited a significant elevation in free radical levels, but no additive effect was observed when radiofrequency radiation exposure was combined with PMA. The phagocytic uptake was not affected after exposure to any RF signals in Mono Mac 6 cells. Cell proliferation, cell cycle and the induction of apoptosis or necrosis showed no differences in sham-exposed and control conditions. Also, there were no significant effects of radiofrequency radiation exposures on different stress response relevant parameters such as heat shock protein expression (HSP) or changes in cell physiological processes.

A protein micro-array analysis was performed in primary human monocytes exposed for 45 minutes to GSM-DTX signal to understand the underlying mechanism(s). Several alterations in the expression of numerous proteins were observed after radiofrequency radiation exposure. Also, an activation of cellular metabolism and cell proliferation/differentiation in radiofrequency radiation-exposed cells and a decreased cell metabolism in the sham-exposed cells was detected. With respect to the expression of certain specific genes (PIK3R1, CCNC, Raf 1, HPRT) there was no evidence of alterations after 45 minutes exposure to GSM-DTX in human umbilical cord blood derived monocytes when compared with sham-exposed and incubator control cells. The overall data demonstrated that GSM-DTX exposure at high peak SAR levels induced alterations in free radicals release and changes in numerous protein expression levels. However, these alterations did not induce changes in physiological processes such as phagocytosis, cell proliferation, apoptosis and necrosis.

Questions and Answers:

1. Method to measure ROS effects - Free radical release was measured using a flow cytometric method.
2. Any temperature increase during radiofrequency radiation exposure - Temperature measurements could not be made during radiofrequency radiation exposure; they were measured before and after exposure and they did not indicate any significant increases.
3. Possibility of evaporation of the medium during prolonged radiofrequency radiation exposures - Evaporation of medium was not noticed. However, similar wave guides are used in Dr. Waldmann's laboratory and they appear to have problems with air conditioning and evaporation of medium.
4. How many times the experiments with sham-/sham-exposure were conducted - sham-/sham-experiments were conducted only once.

5. Statistical methods used to analyze the results - *t*-test was not used for the data expressed in ratios. The ratios are used for the presentation.
6. Need to conduct more experiments since the number of experiments in different exposure conditions is not given for all data – all experiments were performed in triplicates and in three independent experiments

Comments:
There was an extensive discussion on the effects reported in sham-/sham-exposure conditions. From the large amount of data presented, the sham-effect may be real. The general impression is that the radiofrequency radiation exposure equipment supplied by IT'IS in Zurich is the best that is available in the market and is used by several groups, including the participants in REFLEX program. If there was something wrong with the equipment, a lot of money might have been spent in a wrong way. If there were questions about the exposure system, biologists may not be able to answer the questions. The answer must come from those people who designed and constructed the equipment (these people were not present during the discussion). If the equipment did not work well, this must be checked. So, it is important to ask the technical experts to check whether there is some kind of interference between sham and non-sham-exposure, over-coupling of field generating unit in the incubator itself and electromagnetic compatibility problem.

PANEL DISCUSSION

1. Genotoxicity studies are necessary? Other genotoxicity tests recommended were mutation assays in bacteria (*Salmonella typhimurium*) and mammalian cells.
2. Is induction of ROS a possible mechanism for indirect action of RF field exposure? This needs to be examined further.
3. Do we need more standardization of exposure system? Biologists will not be able to check the exposure systems properly. There are wonderful exposure equipments but biologists will never know whether the exposure is what it is expected to be. These equipments need to be checked and subjected to independent verification.
4. Statistical analyses of all studies are correct? Multiple comparisons are done and whether they are all adjusted for multiplicity is not always clear. If one has hundreds of statistical comparisons, then by chance, 5% will certainly show significant difference.

Session 2: Gene Expression
Chair: Ziegelberger

Effects of HF-signals on the melatonin synthesis in isolated pineal organs of Djungarian Hamsters.

Lerchl, A. Jacobs University Bremen, Bremen, Germany.

The investigations are complete. The data are already published. The radical scavenging activities of melatonin are well-known. According to the "melatonin hypothesis", exposure to EMF reduced the synthesis of melatonin. Lowered melatonin levels may induce damage to bio-molecules and can lead to cancer, stroke, coronary infarction, neuronal degeneration and other diseases caused by reactive oxygen species. Melatonin is now in clinical use to prevent some of these diseases. In order to investigate the possible effects of radiofrequency radiation exposure on melatonin synthesis, an experimental system was developed. Melatonin synthesis was measured in isolated pineal organs under radiofrequency radiation-exposed and sham-exposed conditions. Pineal organs of Djungarian hamsters (*Phodopus sungorus*) were continuously perfused with Krebs-Ringer buffer, stimulated with the beta-adrenergic receptor agonist isoproterenol to induce melatonin synthesis, and exposed for 7 hours to 1800 MHz continuous-wave or pulse modulate wave (GSM) at 8, 80, 800 and 2700 mW/kg SAR. Sham-exposed pineal organs were used as controls. Experiments were performed in a blind fashion. The perfused samples were collected every hour and melatonin concentrations were measured using a radioimmunoassay. As compared with sham-exposed controls, both types of radiofrequency radiation signals had no effect on melatonin synthesis at 8 and 80 mW/kg SAR. However, a significant increase in melatonin synthesis was observed when the pineal glands were exposed to radiofrequency radiation at 800 mW/kg SAR. At 2700 mW/kg SAR, melatonin levels were elevated when exposed to continuous wave, but significantly decreased in GSM-exposed pineal glands: the latter could be due to a thermal effect where an elevation in temperature, approximately 1.2^{o}C, was observed. The overall data indicated no adverse effects in the synthesis of melatonin by pineal gland at SAR levels which are relevant to human exposure. Thus, the results do not support the "melatonin hypothesis".

Questions and Answers:

1. Instead of using isolated pineal glands, Djungarian hamsters could have been exposed (whole-body) to radiofrequency radiation in radial transmission wave guides - The aim of the project was to determine the effect of precise radiofrequency radiation exposure on the pineal gland itself. Therefore the study was conducted in isolated pineal glands instead of using non-restrained animals in which SAR levels can vary widely.
2. Do the Djungarian hamsters exhibit circadian rhythm – Yes, melatonin levels are high during night and almost nothing during day time.
3. When were the pineal glands isolated, during night or day and what is the purpose of using beta-adrenergic receptor agonist - Pineal glands were isolated during the day when the levels of melatonin were low. Hence, an external substance, beta-adrenergic receptor agonist, was used to stimulate melatonin synthesis, and this is to simulate the situation during the night.

Influence of GSM-signals on differential gene expression in isolated human blood cells: Differential gene expression – first results.
Halter, R. Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany.

This is an on-going investigation. Lymphocytes isolated from the whole blood collected from 20 adult (50-60 years age) and 20 juvenile (18-21 years age) healthy volunteers of both genders were exposed for 1 and 48 hours to 1800 MHz pulse modulate wave (GSM) signals at 0, 0.2, 2.0 and 5 W/kg SAR. All exposures were conducted blind. The expressions of HSP-70, HSP-27, HSP-27-P, p38MAPK, C-myc, C-Jun and p-21 genes were analyzed by Western blotting. As positive controls cultured Balb3T3 cells were used for p38MAPK and C-Jun, mouse lung adenocarcinoma cells for C-myc and lymphocytes which were cultured at 42°C for 2 hours for the HSPs. The affymetrix microarray gene expression analysis of 320 lymphocyte samples, hierarchical gene cluster analysis, self-organizing map and annotation of altered regulated genes will be completed in June 2008. Microarray gene expression analysis was done with sham-exposed and exposed cells.

In addition, 10 selected proteins based on the results by the array analysis which were reported in regulation in various test systems will be analyzed by Western blotting and 20 genes by real time qPCR to validate the array data.

Questions and Answers:

1. The durations of radiofrequency radiation exposure (1 and 48 hours) and the selected SAR levels may not be suitable for all gene-expression analysis since the expression of different genes may peak at different times and at different SAR levels. Hence, a duration- and SAR-response studies would be more appropriate - At 1 hour exposure time point, the early response genes will be expressed. The data are not yet decoded for SAR dose-dependency effects since the experiments are still ongoing. Also, examination of whole genome wide expression for radiofrequency radiation exposure duration- and SAR-dependency will require many more experiments and will be extremely expensive.
2. Why then use whole genome arrays at all? Looking for certain target genes or using arrays concentrating on certain questions, e.g. on cancer markers, is more straight forward. – Whole genome arrays provide the possibility to find new, so far unknown molecular targets. Other approaches are restricted to known targets. Even cancer is too complex to cover all possibly involved candidate genes by “hypothesis driven” tests. In the range of the research programme both approaches are used.

Effects of mobile phone signals (GSM and UMTS) on the blood-brain barrier *in vitro*.
Franke, H. University Hospital Münster, Department of Neurology, Münster, Germany.

The aim of the study is to determine alterations in gene expression to gain insights into potential molecular targets. Endothelial cells were isolated from rat brain (RBEC), cultured, characterized and used as *in vitro* model for blood-barrier investigations (endothelial cells of the cerebral capillaries form the permeability barrier in the brain). Five days after isolation and culturing, the cells were exposed to 1800 MHz radiofrequency radiation (either UMTS or GSM 1800) for 72 hours at 0.4, l.0, 3.0 and 8.0 W/kg SAR. Two radial wave guides kept in an incubator were used for radiofrequency radiation exposures. Each wave guide had 6 positions to keep cell culture dishes: five positions were used for cell exposures while the sixth one was used to keep the temperature probe. Temperature surveillance revealed negligible heating effect for the lower SAR levels whereas exposure at 8 W/kg SAR elevated the culture medium

temperature to 38°C. Sham-exposed control cells as well as those maintained at 38°C were used as positive controls in quintuplicate experiments. The integrity of each cell monolayer was verified by examination under a phase contrast microscope prior to and after 72 hour exposure. Immediately after the exposures, RNA was extracted from each exposed sample using the commercial Qiagen RNAase Micro Kit, stored at -70°C and later hybridized to the Affymetrix gene chip® rat genome 230 2.0 array. Quality control of RNA samples was carried out on Agilent Bio-analyzer micro chip kits. The hybridizations on the Affymetrix chip allowed the identification of perfect match and mismatch gene pairs in the entire rat genome. Among the 28000 genes on the chip, after normalization of signal levels, 18663 altered genes were reliably detected. In the next filtering step, 14287 genes showed at least 1.4-fold change in the expression compared with sham-exposed cells. There were 8900 genes that were differentially exposed in cells exposed to UMTS and 11488 genes that were differentially expressed in cells exposed to GSM 1800. These changes were not due to temperature increase ($p<0.05$) and indicated that cells reacted differently to UMTS and GSM exposures. The next RT-PCR investigations will verify the up- or down- regulation of genes. Further investigations will include enriched pathway analysis (gene ontology in cell communications, signal transduction, gap junction proteins, protein binding, focal adhesion, cell-matrix junction, cell-substrate adherens junction, etc) and biological annotations. The selection of specific candidate genes for this purpose has not yet been made. The high efficiency of detecting changes with gene chip arrays will not only help to identify these molecular targets which are difficult to be predicted entirely by other methods, but also provide an excellent basis for analyses addressing changes in protein expression, functional assays of BBB related transporters, determination of dose-response ratios and a threshold of the biophysical interaction.

Questions and Answers:

1. Will it be possible to use another type of grouping and filtering - In general, this is possible. A different algorithm can be used for different grouping and filtering. An intelligent guessing and a long list of candidate genes that are likely involved in BBB were used and will be used for further detailed analysis.
2. Can the *in vitro* system be used to study the functional aspects of BBB - A group in London is currently working with these cell culture models for substance transport, permeation and building of resistance, etc. The real goal of this research project was to select targets to find genes/proteins which may be affected and differentially expressed. The next step is to investigate whole animal model. One major question is that whether we should have a target first and then look for functional aspects or vice versa.
3. Is the data from 2 control chips in each experiment enough to draw conclusions - The opinion of bioinformatics experts is that the data from 2 chips is similar and adequate. Also, there is back-up data for further examination.
4. What are the inter- and intra-chip variations in the expression of genes between sham- and radiofrequency radiation-exposed cells - There must be some biological variation.
5. Are there any overlaps in the differential expression of genes between UMTS and GSM? - Yes, there were genes that overlapped between UMTS and GSM signals.
5. How do you determine the false positives and false negatives - The mining tools in algorithms for normalization procedures and cross-wise comparison between radiofrequency radiation- and sham-exposures exclude false positive observations. The statistical analysis part of this project is based on an extremely complex scheme.
6. Has any one any where in the world or the group in London is replicating this work. Is the response of endothelial cells from human and rat the same? - So far, no one has replicated

this work. The group in London is working on functional aspects of pharmaceutical substances and their target has nothing to do with radiofrequency radiation.

7. What is the status of endothelial cells in the cultures and this would make a difference - The status of the cells is extremely important. The cells were confluent when the radiofrequency radiation exposure started.

Comments:
The grouping and filtering of certain types of genes is interesting and exclusion of the genes from the beginning is important. The expression analysis gives information on which genes/proteins are reacting to radiofrequency radiation exposure. Based on this, it is possible to select targets for further studies. Many unexpected targets can be detected with this type of screening process. A hypothesis can be generated from which a specific pathway in a cell/animal will react. Then, the whole animal physiology needs to be evaluated. It is important to do a pilot experiment using cells in different stages, i.e., growing, semi-confluent and fully confluent stages since they may respond differently to UMTS and GSM. A bridge between in vitro and in vivo studies for real-time BBB may be built by taking the cells from cranial window experiments performed in Japan.

PANEL DISCUSSION

1. Further studies on melatonin are required? Not for radiofrequency radiation exposures. Where as for ELF, there is still some controversy and further studies may be needed.
2. Do the existing and currently running studies on possible effects of RF-EMF on the blood-brain barrier cover the field? Bundesamt für Strahlenschutz has two in vivo BBB projects and the results will be discussed in October meeting. The expression of gene work needs to be replicated.
3. Are screening arrays the adequate method to detect relevant markers/end-points? (Risk of false positives overlook fail to notice the weak effects on the other hand)? There is an extensive discussion on the screening of gene expression. Focusing on certain molecular targets/topics is troubling because there is no biophysical reason to expect radiofrequency radiation exposure can elicit such effects. However, it is frequently the case that the effects appear initially at molecular level. There may not be a significant increase in temperature to cause the effects but temperature gradients and convected currents in the culture medium around the cells which may cause the observed effects. These are not artifacts. These are macroscopic effects but not molecular effects.
4. What are the essentials and limitations for the interpretation of results from screening arrays? What if certain regulatory changes do not lead to detectable further effects in vitro and in vivo?
5. Should/can further research be focused on certain molecular targets)? If so – which were relevant candidates)? – No suggestions.

Session 3: Sensory Systems
Chair: Pophof

Effects of HF-signals on retinal ganglion cell activity.
Ammermüller, J. Department of Neurobiology, University of Oldenburg, Germany.

The aim of this double-blind study is to measure putative, direct effects of high-frequency electromagnetic fields (HF-EMF) on neuronal signal processing in the retina. Isolated mouse retinae were exposed, inside a calibrated exposure system, to GSM and UMTS signals (900 and 1800 MHz) at 0.02, 0.2, 2, and 20 W/kg SAR. Changes in the response rates and latency period to different intensities of light stimuli (ON and OFF) were recorded extracellularly from retinal ganglion cells before, during, and 30 minutes after exposure. Sham-exposed retinae were included in the experiment. The temperature of the perfusion solution surrounding the retina was measured and regulated to remain at a constant value. Separate experiments were performed to determine the effects of temperature alone. Multiple means comparisons (>300 Dunnett's t-tests) and the general linear model statistical analyses suggested some significant differences. However, the overall data indicated that the response of radiofrequency radiation-exposed cells did not differ significantly and systematically from the sham-exposed cells. Temperature increase alone induced increased response rate and decreased latency periods. These results suggested that putative effects can result from thermal warming but not from HF-EMF effects *per se*.

Questions and Answers:
1. How could you measure separately the ON response and OFF response of the electrodes - This is done through spike sorting. We have 2 electrodes. The cells that are nearer to the electrode produces larger signals and the cells that are further away produce smaller signals. The shapes of the signals are different.
2. Have you used any other radiofrequencies - No.

Ionic currents through Ca^2+ channels in mature mouse inner hair cells under mobile phone field exposure.
Engel, J. Universitat Tübingen, Tübingen, Germany.

Mobile phones, when are in use, are kept in close proximity to the ear and most of the radiofrequency radiation energy of the signals is directly absorbed by the area around the ear. When the study started, relatively few studies were conducted with respect to the effects of mobile phone use on hearing. The ear is composed of 3 parts. The outer ear is comprised of pinna and ear canal which guides the sound and helps in localizations. The middle ear consists of ear drum and transforms the air-borne sound into fluid-based sound (otherwise the sound will be reflected by more than 90%). The inner ear consists of the vestibulum, semi-circular vestibular canals and the cochlea where the sound is analyzed by transforming the fluid signals into neuronal signals. The inner hair (IHC) cells in the inner ear transform the external sound into neuronal signals whereas the outer hair cells (OHCs) actively amplify the acoustical signals. The aim of this investigation is to determine the effect of mobile phone field signals on the functioning of the voltage-activated L-type Ca^{2+} channel Ca_v 1.3 in mouse IHCs. The specific Ca^{2+} channel is driven by the receptor potential of the hair cells which triggers exocytosis via Ca^{2+} influx and thus plays a crucial role in the signal transduction process of hearing. Two different exposure signals were used and simulated 1800 MHz GSM and UMTS

at 0.02, 0.2, 2, 20 W/kg SAR. Sham-exposed cells were included in the experiments. Temperature was constantly monitored and measured but not controlled. Under randomized blinded exposure conditions patch-clamp recordings of the currents through Ca^{2+} channels were made in acutely explanted organs of Corti of mice (~18 days after birth when the IHCs are mature) during 5 minutes pre-exposure period, 20 minutes exposure and 10-15 minutes post-exposure.

Using the patch-clamp method a small glass pipette (1 micron tip diameter) filled with intracellular solution was attached to the cell membrane so that an electrically tight seal was produced. Application of small negative pressure ruptured the cell membrane, such that voltage-activated currents through the cell membrane could be recorded. Ca^{2+} ions were not used in the bath solution, because it would create too much stress on the cells during experiment. Instead a Ba^{2+} solution was used which gives larger Ca^{2+}-channel currents and also blocks K^+ channels. Intracellular cesium was also used in the patch pipette and extra-cellular TEA and 4AP to block all residual K^+ currents because these currents are 100-1000 times larger than Ca^{2+} currents. Under these conditions, currents can be recorded for 40 minutes. I-V-relationships were calculated from current traces and different parameters were extracted from the I-V relationships: maximum current, voltage of half-activation, steepness of activation, series-resistance and leak-resistance. The data were analyzed using Dunnett's t-test. So far, no statistically significant effect of radiofrequency radiation exposure was observed on Ca^{2+}-currents.

Questions and Answers:

1. The pipettes might have a significant impact on cellular and extra-cellular recordings. Were they used and considered when the SAR values were calculated and temperature effects were determined - The same capillary pipettes made of excellent quality quartz glass (quite expensive) were used for all experiments. There was no change in the temperature during exposure period. Although the randomization of groups was not optimal, no significant effects between the groups were observed.
2. How many cells were examined for each data point - The intention was to analyze 25 cells for each measurement condition. However, for each exposure intensity and signal type 15 cells only survived the entire 40 minutes recordings. The data from 2 additional cells are available but the tight seal was broken after exposure. Hence, it was considered not appropriate to include them in the statistical analysis since these 2 cells were not monitored during the entire (exposure and post-exposure) period.
3. Did you expect to observe an effect because of electric, magnetic or electromagnetic fields - Ca^{2+} channels are very sensitive molecules. Ca^{2+} functions as a signal ion in cells and modulates 1000s of processes in the cells. Whenever the metabolic state of cells is not really very good, Ca^{2+} channels are degraded to prevent too much Ca^{2+} influx into the cell. In my experience over the last 10 years, whenever the cell preparations and the solutions used are not perfect, measurements of Ca^{2+}/Ba2+ currents fail – there are small or no currents left. Ca^{2+} channels are very susceptible molecules for exposures to electric, magnetic and electromagnetic fields but they are essential for the hearing process.
4. Did you ever use another radiation quality like UV or gamma radiation to see their effect in this test system - Other radiations were not used in these experiments.
5. Did you use positive control - The best positive control is the induction of electric currents by voltage steps generated during the experiments which elicit a positive response.

PANEL DISCUSSION

1. Is electrophysiology a reliable method to investigate possible action of EMF on sensory systems? Is another approach necessary? – Yes, this is an extremely sensitive system used in very special type of cells. The data agree with the results meanwhile obtained in *in vivo* studies in animals and humans.
2. Were the study design, the number of replications, the statistical approach and the power appropriate to achieve reliable results? - The data from only 15 cells per exposure per stimulation type and intensity were presented. Care must be taken however, to extrapolate these data to the whole tissue with millions of cells.
3. What are the underlying mechanisms of the described effects? Have other than thermally evoked responses been found? - Basic neuroscience studies are not screening studies – they are more like mechanistic studies. Other non-physiological changes in parameters (e.g. pH value) could potentially change sensory responses..
4. What is the thermal response threshold of sensory cells and neuronal network? – There exists no thermal threshold, since physical and chemical processes depend gradually on temperature.
5. What are the physiological and health consequences of the effects observed under 3) and (4)? – To keep the thermal changes to a minimum.
6. Are any questions on the influence of MEF on sensory systems still open, is further research needed? – We have to wait for the final statistical analysis. At the moment it seems that further research is not needed.

Session 4: Action Mechanisms
Chair: Geschwentner

Dielectric properties of tissues and cells.
Loidl, A. Experimental Physics University of Augsburg, Germany.

In this project bulk dielectric measurements for frequencies from 100 MHz up to 40 GHz and temperatures ranging between 20-50°C using varying excitation voltages were performed and combined with single-cell investigations utilizing patch-clamp techniques which can directly be compared with dielectric spectroscopy. In these measurements, the permittivity and conductivity are the determining factors in the absorption, reflection and transmission of electromagnetic radiation. Theoretical calculations (modern theories on charge transfer in biological materials) and dielectric spectroscopy should provide insights into the nature of complex absorption processes. Measurements of electrolytic solutions with different types of ions and different concentrations provide precise information on the influence of dissolved ions on the main water relaxation and on possible AC and DC conductivity contributions. Broadband dielectric spectra were obtained in model systems in human peripheral whole blood and also in different concentrations of cultured cells such as fibroblasts, melanoma cells, PC12 nerve cells and keratinocytes. Detailed analyses indicated that even at GHz frequencies, in addition to the contribution of water, AC and DC conductivities as well as additional relaxation processes play a role. In the frequency range between 100 MHz and 40 GHz the absorption of electromagnetic radiation as a function of frequency, temperature and ion concentration can be described by a universal set of parameters.

Questions and Answers:
1. There was a rise in conductivity in fibroblasts, red cells and whole blood at 1-2 GHz exposures. Is this correct? - It is known for a long time that there is an increase in conductivity at GHz frequencies.
2. Can the model be used for cells that are different shapes and at different temperatures? - Red cells are not spherical in shape and polarization will depend on their concentration in suspension.

Comments:
There was an extensive discussion on electroporation.

Sub-cellular RF-filed distribution and absorption depend on the dielectric properties of biological membranes.
Gimsa J. University of Rostock, Germany.

The presentation is on the sub-cellular distribution of radiofrequency radiation fields inside the membranes and also inside the cells. The philosophy of modeling of membranes and cells is to: (i) translate the published data on molecular properties into electric properties in sub-cellular compartments, (ii) set up geometric models for cells/vesicles, (iii) calculate induced dipole moment, i.e., Clausius Mossotti Factor (CMF), (iv) check the CMF by single particle AC-electro-kinetic measurements and finally, (v) consider the field strength and absorption at exceptional model sites. The model that is used in this study is human red cells since they are homogeneous, abundant in blood and are well investigated. The presentation

was summarized as follows. Molecular properties strongly influence the absorption in various cell compartments. Anisotropy effects are probably underestimated due to possible differences in potential distributions within the membrane or the volume of the objects remain hidden in the relaxations-like behavior of the frequency-dependence of the conductivities of the equivalent bodies. Hidden “anisotropy dispersions” may result in erroneous interpretation of the data. Membrane anisotropy may induce higher local currents leading to higher energy dissipation. Dispersion of the permittivity anisotropy will lead to conductivity anisotropy. All anisotropies are probably dispersed above 500 MHz. Averaging the absorption over the membrane layers may lead to higher energy dissipation (up to 10-times) than averaging over membrane properties. In layered membrane models, the absorption in the outermost layer is dominating. Effects of membrane proteins on membrane-fields distribution remain to be elucidated.

Questions and Answers:

1. How much energy is necessary to induce rotation and dispersion of molecules in sub-cellular structures and membranes? - Basically the thermal motion reduces the friction.
2. There is a speculation over the years that electrically induced forces on particles being significant, at 1 GHz frequency, to cause some biological effects. Do you have any thoughts? - There are some experiments to indicate the possibility of electroporation at higher field strength. This may be due to dielectric effects.

Comments:

There was a good amount of discussion on Antoniety’s publication dealing with local increases (100- to 1000-fold) in absorption in colloidal solutions and appear to be wrongly interpreted due to increase in temperature.

PANEL DISCUSSION

1. Are the applied experimental and mathematical approaches appropriate to investigate the interaction of EMF with living matter? - No new approaches are proposed.

2. Do the measurements and calculations suggest any new, still unknown, interaction mechanism between EMF and tissues or cells? - A theory is developed on the models based on biophysical mechanisms for the observed effect. However, it is important to include realistic and natural situations like skin in the modeling of anisotropic effects where a simple explanation can be found for the observed effects.

3. What are the physiological and health consequences of the interactions discussed? - There is lot of work on membranes. Calculations done so far are complicated but are not based on a real natural biological experimental system. The models have mostly used membrane systems which include lot of charge transport. No attempt has been made to determine how external fields interfere with such transport systems.

4. Are there suggestions for further research or new hypothesis concerning action mechanism of EMF, which could be tested? - Mitochondria are much smaller than the cells and there is lot of charge transport within mitochondria. The possibility of electrical interference for the charge transport in mitochondria could be examined. The dielectric theory is not sophisticated but very much used. It is good enough for exposure systems but may not be good enough for

mechanistic understanding of dielectrical properties. Also, it is important to understand the temperature effects which cause heating and thus biological effects.

WORKSHOP CONCLUSIONS

General consensus of the workshop:
The investigations are elegant and conducted well.
The presentations are excellent.
The studies on sensory systems gave new insights.
All data raised more questions which need answers. Good science always raises more questions than answers.
“We may close some doors but we should not throw away the key.”
“More doors may need to be opened to get into the big room”.
Do all the overall results justify the revisions of present guidelines? The answer is NO and they are adequate for the purpose for which they are developed.
The debate on long-term effects may not be resolved from the data presented in this workshop - it has to come in the context of human epidemiological studies.
It is not possible to convince the people who believe that mobile phones are detrimental to health.
Standardization and verification of exposure systems is extremely important for good research on radiofrequency radiation effects.
The results from the entire program will be discussed 2008 in Berlin.