EXPOSURE TO NON-IONIZING RADIATION AND CELLULAR SIGNAL TRANSDUCTION

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Introduction

The debate on the possible health hazards in connection with residential powerlines, use of cellular telephones and especially the growing number of base stations in the living environment has been raging for many years. Various effects after exposure to radiofrequency/microwave electromagnetic fields from cellular telephones have been reported from laboratory, clinical and epidemiological studies. However, until now very few studies have appeared that offer an explanation for these effects.

This study is part of our on-going work to find a mechanism for the biological effects of electromagnetic fields (EMF).

In our work we have chosen cell proliferation as the parameter to follow the changes in mammalian cell tissue cultures after exposure to EMF. So we studied changes in cell proliferation after exposure to both extremely-low-frequency (ELF) and radiofrequency (RF) electromagnetic fields [1-3]. As cell proliferation is the target of our studies, the most obvious thing was to look for a mechanism that could be connected to cell cycle regulation.

When conditions are not favourable for successful cell proliferation e.g. high level of damaged DNA, low nutrients, hypoxia, activation of viral genomes, the cell pauses in either G1, G2, or M phase of the cell cycle. Such arrest provides a cell with extra time for activation of the repair, defensive or survival machinery. If a severe incompatibility of the intracellular conditions with progression through cell cycle is signalled, apoptosis is initiated. One group of proteins that is known to affect cell cycle progression are *heat-shock proteins* (HSP). Several families of HSPs are known, their main function being chaperones for newly synthesized proteins. However HSPs can also have other functions, depending on cell type. For example will moderate heat shock arrest the cell cycle transiently at the G1/S and G2/M check points, due to the increased release of HSPs. This is due to the regulation of various cell cycle reactions by HSPs at these transitions points and so the heat-shock response serves to protect the cell from exposure to harmful stress. However, the release of HSPs is not only triggered by a raise of temperature, but also by any form of cellular stress, such as environmental or oxidative stress [4]. But could non-ionizing radiation not be a stress factor as well?

Indeed it has already been found that EMF, both ELF and MW radiation, can stimulate transcription and generation of HSP in cells and organisms [5-10].In case of ionizing radiation it is already known that it effects cell cycle progression by delay in the G1, S and G2 phases [11]. Also 50 Hz EMF has been found to cause changes in the cell cycle and the levels of certain cell cycle proteins in human cells [12].

Children are shown to be more vulnerable to non-ionizing radiation from both ELF and RF. A review concluded that there is definitely a connection between leukaemia in children and exposure to EMF from e.g. powerlines (13). In a recent epidemiological study it was recorded

that the highest incidence of braincancer was found in the age group of 20-29 years after the use of cellular telephones for 10 years (14). We have studied the effect of exposure to EMF in primary cell cultures with respect to age.

Experimental

Cell lines: transformed human amnion cells (AMA), human skin fibroblasts (K14) and primary cell cultures of human amnion cells and fetal skin fibroblasts.

Exposure systems: the ELF field was a sinusoidal 50 Hz electromagnetic field. The MW field was generated by signal simulation of the Global System for Mobile communications (GSM) of 960 MHz, modulated with a 217 Hz pulse, resulting in SAR values of 0.021, 0.21 and 2.1 $mW.kg^{-1}$ respectively; cells were exposed in a TEM cell at 37^0C and temperature controlled within $\pm$ $0.1\ ^0C$.

The cells were exposed at varying field strengths, times and temperatures. Cell proliferation was determined by a colorometric assay and cell cycle proteins by a modified immunofluorescence method. Further details of the experimental protocol have been described before [1-3, 9].

Results and discussion

ELF: exposure to ELF electromagnetic fields resulted in significant changes in cell proliferation [1]. Maximum effects were found at 30 min exposure and at a field density of 80 μT. A linear field density dose response was only found in the region before the peak value. Longer exposure times resulted in adaptation, so that no higher effects or even no effects at all were detected.

RF: exposure resulted in a significant change in cell growth, highest at the maximum SAR of 2.1 $mW.kg^{-1}$ [2]. The minimum exposure time at all power levels had to be 30 min to obtain a peak effect . Repeated periods of exposure did not seem to change the effects. The linear correlation between power level, exposure time and growth change was not distinct as in the case of ELF exposure and there was no lineariy in certain regions of the radiofrequency field density spectrum. This can be related to the oscillatory nature of cell growth. Apparently the interaction of RF radiation with cellular oscillators contributes more to these effects than in case of ELF.

Non-thermal effects: experiments, done at two different temperatures: 35 or 39 ± 0.1^0 C under similar conditions, showed a significant higher change in cell proliferation in the exposed cells, whereas the change in proliferation rate in the sham exposed cells was not significant. Obviously, a temporary change in temperature alone did not seem to affect cell proliferation, contrary to exposure to RF. These experiments show that changes in cell proliferation due to exposure to RF fields, could not be the result of heat generation, if any [15].

Heat-shock proteins: A significant increase was found in Hsp-70 concentrations after exposure of the cells to both MW and ELF electromagnetic fields. After ELF exposure there was also a small increase in HSP-90 [3]. Contrary to others who found high levels of HSP-27 [8, 10] after exposure to RF, we only found insignificant amounts [9]. However, this is possibly due to different cell types and exposure conditions. In our case the lifetime of the RF generated HSP-70 was found to be 3-4 hrs.

Cyclin/PCNA: this S-phase specific protein was used in monitoring cells in the S-phase by following the changes in concentration of this protein after RF exposure.

In synchronized cells the result of exposure to the RF field was that cells were delayed in the S-phase. As HSP release arrests the cell cycle at the G1/S and the G2/M check points the S-

phase is then extended in the RF exposed cells until HSP-70 had disappeared. In our experiment normal cell cycle progression was resumed after 3- 4 hafter disappearance of the RF generated HSP-70.
In non-synchronized cells RF exposure resulted in an initial decrease of cells in the S-phase as a result of the G1/S block triggered by HSP-70 release, which prevented new cells from entering the S-phase. After the decline in HSP-70 levels both blocks are released, resulting in a quasi-synchronous transition from G1 into S and G2 into M. This is then measured as the increase in cells in the S-phase. This maximum then folllows the same pattern as for normal synchronously proliferating cells [16]. So the result of the cell cycle arrests at the G1/S and G2/M check points is an apparent synchronization of the cells upon release of the 2 blocks.
Primary cells: ELF exposure resulted in an increase in growth, but mainly in the younger and vigorously growing cells. With increasing age and increasing number of passages, the effect of EMF exposure diminished gradually and disappeared completely in the older cells. The same effect of age was also seen with respect to the effect of certain growth factors in combination with ELF, whereas growth inhibitors had the same effect at all ages.

Conclusion

We have restricted our studies to short-time effects and to only one cell cycle. Long time, repeated, or chronic exposure will certainly result in adaptation, so that EMF effects may often not be detected. So in a simplified manner we can summarize that generally RF exposure will cause a change in the cell cycle and ultimately in cell proliferation. Through RF generation of heat-shock proteins, the cell cycle will temporarily be arrested at the 2 checkpoints G1/S and G2/M. This will keep the cell cycle in the S-fase until the excess of HSP has disappeared and then the normal cell cycle will be resumed. In synchronized cells this will result in a delay of the cell cycle that can give the faulty impression that proliferation is decreased, when this time-lag is not taken into consideration. In non-synchronized cells this will result in a apparent cell synchronization, detected as an increase in cell proliferation. This might explain the varying experimental results of different groups. However, only when any damaged DNA has been repaired, the RF generated HSP will be broken down and the normal cell cycle is resumed. Another way to resume the normal cell cycle is to dispose of cells with faulty DNA through apoptosis by the p53 activated pathway. However it is known from many cancer cell types that their p53 activity is inhibited, preventing apoptosis of faulty cells. Several studies seem to indicate that also EMF exposure can deactivate the p53 apoptosis pathway. Moreover it has been shown that in the case of generation of HSP through oxidative stress or other stress factors, the HSP response is diminishing upon repeated exposure and so increasingly stronger stress amounts are required to obtain the same HSP response. So repeated exposure to RF/EMF can also result in proliferation of faulty cells due to a failing HSP response. At the same time cell proliferation can also be affected by the RF induced changes in the levels of other proteins. Therefore it will be necessary to study RF/EMF effects on other cellular proteins, such as DNA repairing enzymes and also the effects of chronic and repeated exposure. However it will be necessary not to look at RF induced changes on transcription level only, as done by most, but also to study the changes in the protein levels themselves, as we have choosen to do. A change in transcription level of certain genes is not always certain to lead to a change in protein synthesis and to a detectable change, since only through the protein itself an effect will be executed.

The growth promoting effect of ELF which we found to be greatest in younger and vigorously

growing primary cell cultures, is certainly another verification of the results of the epidemiological studies on children and younger people.

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