The Clustering of Growth Factor and Cytokine Factor Receptors Was Induced by 50 Hz Magnetic Field and Blocked by Noise Magnetic Field

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Abstract To study the possible effects of 50Hz magnetic field (MF) exposure on clustering of cell surface receptors, explore the mechanism of signal transduction of ELF-MF and confirm whether or not the noise magnetic fields (MF) interfere these effects induced by 50 Hz MF. Chinese Hamster Lung (CHL) cells were respectively exposed to 50Hz MF, noise MF with 0.4 mT, and combined MF which was superstition of 50 Hz MF with noise MF for different exposure durations (5, 15 and 30 min). Cells treated with 100ng/ml epidermal growth factor (EGF) or 10ng/ml tumor necrosis factor (TNF) for 15 min served as positive control. The clustering of EGF and TNF receptor was analyzed with confocal microscope. The results showed that, like the EGF and TNF, 50 Hz MF at 0.4 mT could obviously induce the clustering of cell surface receptors after exposure for 5 min, while the noise MF alone with the same intensity did not induce receptor clustering. When surperposed of noise MF with the same intensity, the receptor clustering induced by 50Hz MF was inhibited. It suggested that the membrane receptors would be the target sites where MF interacts with cell, and the noise MF could interfere these effects.

Keywords: 50 Hz magnetic fields; receptor; clustering; noise magnetic field; interference

That the electricity was widely used in our society made the extremely low frequency electromagnetic fields (ELF-EMF) in environment more and more high. Evidence is accumulating that exposure to ELF-EMF may produce a lot of biological effects. Especially, some reports showed that ELF-EMF such as those from electric power transmission and distribution lines have been associated with increased risk childhood leukemia, cancer of the nervous system, lymphomas and breast cancer [1~6]. However, the mechanism of ELF-EMF biological effects is still unclear. Our previous study showed that 50 Hz magnetic field (MF) with 0.4 mT could phosphorylate and activate the stress-activated protein kinase (SAPK) and P38 mitogen-activated protein (MAP) kinase (P38 MAPK) [7, 8]. Kie et al. [9] also showed that ELF-EMF induced the mitogen-activated protein (MAP) kinase (Erk1/2) activity. These are evidences that ELF-MF exposure may activate signal transduction pathways. Yet, where and how the EMF signal transfers into biological signal in cells is unknown. Since many low-energy electromagnetic fields have little energy to directly traverse the membrane, it is possible that they may modify the existing signal transduction procession in cell membrane, thus producing both transduction and biochemical amplification of the effects of the field itself [10]. In the present study, we explored if exposure cells to 50Hz MF can induce clustering of cell surface receptors like their ligands, and also investigate whether noise MF

can interfere the receptor clustering caused by 50 Hz MF, as we found that the noise MF block the SAPK activation[11].

1 Materials and methods

(i) Exposure system. The single 50 Hz MF exposure system consists of three groups of Helmholtz coils with 36cm width, 8cm height, two power regulators and a set of CO_2 incubator (Model 3164, Forma). Three square coils which the upper, middle, and lower coils are 168, 60, 168 turns separately were in series connection, and were set up in an iron-shielded box. The iron box (containing three square coils) was put into CO_2 incubator, and three coils contacted with two power regulators outside the CO_2 incubator in series connection. A very uniform 50Hz sinusoidal MF was generated in the center of the coils ($10\times10\times10cm^3$ of three dimensional space) when the coils are energized. The combined exposure system of 50 Hz sinusoidal and noise MF has the same components as the single MF system, but Helmholtz coils are circled with crewel brass wire, which connect with different MF signal, one for 50 Hz sinusoidal MF and the other for noise signal. The noise signal was supplied by Litovitz Lab (USA). The MF signal was monitored with oscillograph. Magnetic flux density in the central area of exposure system was measured by a power frequency field meter. Cell dishes were placed in the central area of the coils and the MF was perpendicular to the dishes. The AC background field was 1~2 μT, and static magnetic field was 18 μT with a 14.1 μT horizontal and 12.0 μT vertical component.

(ii) Antibody and Chemicals. EGF and TNF-α (Calbiochem) , Anti-EGFR and Anti-TNF-R1 (Santa Cruz) , NP40 (Fluka) , Propidium iodide (SIGMA) , RPMI medium 1640 (Gibco BRL) , Goat anti-rabbit IgG-FITC (Sino-American Biotech. Co.).

(iii) Cell Culture, Group and Treatment. Chinese hamster lung line (CHL) cells were cultured on glass cover slips in RPMI medium 1640 containing 15%FCS 100U/ml of penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin, at 37±0.5°C with 95% air and 5% CO_2. Three days (72h) late, all cells were deprived of serum and cultured in serum-free medium for 12 hours. Then CHL cells were treated. The cells in the experiment were divided into five groups: a) positive control (with ligands), b) sham exposure, c) 0.4mT 50 Hz MF exposure, d) 0.4mT noise MF exposure, and e) 0.4 mT 50 Hz MF combined with 0.4 mT noise MF exposure. The positive control was treated with 100ng/ml EGF or10ng/ml TNF for 15 min. Exposure cells were cultured in

exposure system for various times (5, 15, and 30min) with the same condition, and the MF was perpendicular to the dishes. Following different treatment, cells were rinsed with phosphate saline buffer, fixed with paraformaldehyde, treated with NP-40, sealed with goat serum, incubated with antibodies of receptors, goat anti rabbit IgG-FITC and propidium iodide. Finally, the clustering of EGF or TNF receptors was analyzed with confocal microscope (Leica, TCS-SP). Experiments were repeated more than three times.

2 Results

The results showed that the EGF and TNF could cluster their receptors. Like EGF and TNF, 50 Hz MF at 0.4 mT also obviously induced the clustering of EGF and TNF receptors after exposure for 5 min. However, the noise MF with the same intensity didn't induce receptor clustering. When superposed of noise MF, the receptor clustering induced by 50Hz MF was inhibited (fig. 1 and 2).

3 Discussion

The receptor on the cell surface is one of important action sites for the extracellular signals, such as hormones, cytokines, et al., and the specific binding between extracellular signals and receptors is usually the beginning for signal transduction. Normally, some ligands (such as EGF) binding to corresponding receptors could induce the receptor clustering, and then activate the cellular signal transduction pathway. So the receptor clustering is usually the initial process of cell signal transduction, and it becomes the index that shows whether extracellular factors interact with receptors. Some studies showed ELF-MF could activate the signal transduction pathways [7~9]. But the initial site for ELF-MF interacting with cell is also unclear. Devary [12] found Ultraviolet (UV) light maybe activated the SAPK pathway through cellular membrane, and Rosette[13] confirmed the growth factor and cytokine receptor is the site from which the UV light activates the SAPK cascade. ELF-MF is similar with UV in essence. So we supposed the receptor maybe one of the targets for ELF-MF. The results of the present study showed exposure to 0.4mT 50 Hz MF could induce the clustering of EGF and TNF receptors. It indicates that 50 Hz MF may interact with signal pathways normally used by growth factors and cytokines. The cellular membrane may be the initial sites where EMF acts with cell and transfer its signal into biological signal. But exactly how the 50 Hz MF lead to multimerization of cell surface receptor is not clear. Physical perturbation of the plasma membrane or a conformational change caused by 50 Hz MF may be the mechanism. The significance of clustering of cell receptors induced by MF is being studied. Litovitz [14] first found that the superposition of incoherent MF could block the enhancement of ODC activity by a coherent MF in L929 cells if the incoherent field is equal to or greater than that of the coherent field, and thought that the superposition of an incoherent field on a coherent would lead to a total field that is incoherent, and the degree of the incoherent was dependent on the relative amplitudes of the noise and coherent components. The experiment was repeated in developing chick embryos [15]. The noise

MF with the same intensity didn't induce the receptor clustering, and inhibited the sinusoidal MF effects while combined with sinusoidal MF. Based on the present study, we concluded that the receptors on cell surface are the possible target sites that EMF acts on organism. We also confirmed the noise MF could interfere the effects of ELF-MF.

References

1. Wertheimer, N., Leeper, E., Electrical wiring configurations and childhood cancer. Am J Epidemiol, 1979, 109: 273~284.
2. Miller, A.B., To, T., Agnew, D.A. et al., Leukemia following occupational exposure to 60 Hz electric and magnetic fields among Ontario electric utility workers. Am J Epidemiol, 1996, 144: 150~160.
3. Coogan, P.E, Clapp, R.W., Newcomb, P.A. et al., Occupational exposure to 60 hertz magnetic fields and risk of breast cancer in women. Epidemiol, 1996, 7: 459~464.
4. Kheifets, L.I., Afifi, A.A., Buffler, P.A. et al., Occupational electric magnetic field exposure and brain cancer: a meta-analysis. J Occup Environ Med, 1995, 37: 1327~1341.
5. Savitz, D.A., Wachtel, H., Barnes, F.A. et al., Case-control study of childhood cancer and exposure to 60 Hertz magnetic fields. Am J Epidemiol, 1988, 128: 21~38.
6. Demers, P.A., Thomas, D.B., Rosenblatt, K.A., Occupational exposure to electromagnetic fields and breast cancer in men. Am J Epidemiol, 1991, 134: 340~347.
7. Sun, W.J., Chiang, H., Fu, Y.T. et al., Exposure to 50Hz Electromagnetic Fields Induces the Phosphorylation and activity of Stress-activated Protein kinase in Cultured Cells. Electro- and Magnetobiology, 2001, 20: 415~423.
8. Sun, W.J., Yu, Y.N., Chiang, H. et al., Exposure to Power-Frequency Magnetic Fields Can Induce Activation of P38 Mitogen-Activated Protein Kinase. Chinese Journal of Industrial Hygiene and occupational Diseases, 2002, 20(4): 252~255.
9. Nie, K., Martirosyan, V., Henderson, A., EMF induces low levels of MAP kinase. Abstract Book of Twenty-third Annual Meeting, St. Paul, Minnesota, June 10-14, 2001, 75.
10. Luben, R.A., Effects of low-energy electromagnetic fields (pulsed and DC) on membrane signal transduction processes in biological systems. Health-Phys, 1991, 61: 15~28.
11. Sun, W.J., Chiang, H., Fu, Y.D. et al., Effects of Noise Magnetic Fields on the Enhancement of SAPK Phosphorylation Induced by 50 Hz Magnetic Fields. Chinese Journal of Industrial Hygiene and occupational Diseases, 2002, 20(4): 246~248.
12. Devary, Y., Rosette, C., DiDonato, J.A. et al., NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. Science, 1993, 261: 1442~1445.
13. Rosette, C., Karin, M., Ultraviolet light and osmotic stress: activation of the JNK cascade

through multiple growth factor and cytokine receptors. Science, 1996, 274: 1194~1197.

14. Litovitz, T.A., Krause, D., Montrose, C.J. et al., Temporally incoherent magnetic fields mitigate the response of biological systems to temporally coherent magnetic fields. Bioelectromagnetics, 1994, 15: 399~409.
15. Farrell, J.M., Barber, M., Krause, D., et al., The superposition of a temporally incoherent magnetic field inhibits 60 Hz-induced changes in the ODC activity of developing chick embryos. Bioelectromagnetics, 1998, 19: 53~56