대 한 체 질 인 류 학 회 지
Korean J Phys Anthropol
22(4): 295 ~ 304, 2009

The Effect of 835 MHz Radiofrequency Radiation Exposure on the Immunohistochemical Distribution of Calbindin D28k and Calretinin in the Mouse Cerebellar Cortex

Dhiraj Maskey, In-young Choi, Ki-Sup Park, Jonu Pradhan[1], Young Bok Yoo

Department of Anatomy, [1]Department of Physiology, College of Medicine,
Dankook University, Cheonan, Chungnam 330-714. Korea

Abstract : Widespread use of mobile phones and subsequent electromagnetic field (EMF) exposure have raised crucial question of their possible biological effects on the nervous system. The study on the effect of radiofrequency (RF) radiation on the nervous system, however, did not precede enough to determine the biological hazard to brain. Until now, several studies have reported decreases in neuron number and neuronal damage in the cortex, hippocampus, and basal ganglia in the brains of animals exposed to RF radiation. However, there were few reports about the cerebellum, the main voluntary motor control center. In this regard, by using immunohistochemisty, current study intended to investigate the changes in the calbindin D28k (CB) and calretinin (CR)-immunoreactivity (IR) in the mouse cerebellar cortex after EMF exposure at 835 MHz for different exposure times and absorption rates, 1 h/day for 5 days at 1.6 W/kg, 1 h/day for 5 days at 4.0 W/kg, 5 h/day for 1 day at 1.6 W/kg, 5 h/day for 1 day at 4.0 W/kg, daily exposure for one month at 1.6 W/kg. Among groups, most prominent CB IR was observed in the Purkinje cell layer followed by molecular and granular layer. The highest CB IR was noted in 5 h/day for 1 day at 1.6 W/kg in the entire three layers while the lowest was noted in one month at 1.6 W/kg. Similarly CR IR was maximum in one month at 1.6 W/kg whilst the lowest was observed in 1 h/day for 5 days at 4.0 W/kg. EMF exposure for 5 days at 1.6 W/kg reduced CB-IR. The CR-IR was mainly localized in small cells in the granular layer, with maximum IR observed after one month exposure. Therefore, the present study suggest the possibility of alterations of calcium ion concentration, which play a role in maintaining metabolic homeostasis, in the cerebellum after long-term exposure to 835 MHz of RF radiation, which might lead to the disruption of normal trait.

(Received 6 November 2009, revised 1 December 2009, accepted 8 December 2009)

Key words : Radiofrequency, Calbindin D28k, Calretinin, Calcium, Cerebellum

Introduction

Radiofrequency (RF) energy occupies a part of the electromagnetic spectrum typically between 10 kHz and 300 GHz, known as microwaves (about 1 ~ 300 GHz). Microwaves are a form of non-ionizing radiation because it does not hold enough energy to displace electrons from the outer shell of an atom but can still induce molecular responses, leading to cell proliferation or cell death in vitro or in vivo (Moulder et al. 1999).

*This work was supported by the research fund of Dankook University in 2005.
Correspodence to : Young Bok Yoo (Department of Anatomy, Dankook University, College of Medicine)
E-mail : pppp@dankook.ac.kr

Interest in the biological effects and possible health outcomes of electric and magnetic fields have been steadily increasing over the past three decades. Furthermore, owing to the electromagnetic field (EMF) exposure, most mobile phone users raise the matter of their possible biological effects (Mausset et al. 2001) particularly on the central nervous system (CNS), due to its close vicinity to the brain, which is the most important organ to investigate the biological effect of RF radiation (Moulder et al. 2005). Investigations with animal models and humans have indicated that even extremely low frequencies can alter the activity of the central and peripheral nervous systems. Neuronal function is influenced by these changes, including regulation of synaptic plasticity, neurotransmitter release, neuronal survival, learning, and memory (Manikonda et al. 2007). Despite numerous studies in cellular biology, epidemiology, and toxicology, the potential adverse effects of EMF exposure on the CNS are still controversial (Hietanen 2006).

To investigate the effect of radiofrequencies emitted by mobile phones, several studies have reported decreases in neuron number and neuronal damage in the cortex, cerebellum, hippocampus, and basal ganglia in the brains of animals exposed to 900 MHz EMF (Mausset et al. 2001, Salford et al. 2003) though there was a contradiction by others (Joubert et al. 2007). Furthermore, several studies has been reported that RF radiation altered cognitive function of brain as well as physiological homeostasis when frequently exposed to mobile phone by studies (Hamblin et al. 2004; Curcio et al. 2005; Huber et al. 2005). These studies indicated that radiation can affect the electrical activity in the brain according to the various RF exposure level and is subsequently able to cause the alteration in cognition and behavior by a certain successive molecular events. The plausible hypothesis for the biological mechanism of RF radiation effect might be attributed to the alteration of the intracellular ionic distribution (Hossmann and Hermann 2003) or change calcium (Ca^{2+}) permeability across cell membranes (Adey 1981) according to the RF radiation exposure intensity and duration.

RF could also alter the conformational energy of glycoproteins in the cell membrane to open Ca^{2+} channels (Thomas et al. 2000). RF causes Ca^{2+} efflux from brain tissue in vitro (Blackman et al. 1979), and affects ion channels, such as decreasing single-channel formation and openings and increasing the rate of rapid burst-like firing (D'Inzeo et al. 1988). RF radiation alters Ca^{2+}-binding in the membrane, Na^{+}-K^{+}-ATPase activity (Behari et al. 1998), Ca^{2+}-ATPase activity, cell permeability, and central cholinergic activity (Kunjilwar and Behari 1993). These changes can result in neural degeneration, and cognition or behavior changes. Calcium binding proteins (CaBP) like calbindin D28k (CB) and calretinin (CR) buffer intracellular Ca^{2+} to help regulate Ca^{2+} homeostasis (Blaustein 1988).

As the major modulation center for motor activity by sensory inputs, the cerebellum (Doya 2000), whose gross, microanatomy and its connections with the rest of the brain have been well known for long period is best-studied. Considering the crucial role of CaBP such as CB, and CR to keep calcium homeostasis in numerous processes for cell viability, signal cascades, modulation of neurotransmitter transfer, and intracellular calcium concentration, CaBP could play a crucial role in preserving cerebellar motor coordinate function. However, only little studies in the cerebellum have been reported as a part of their data to investigate the effect of radiofrequencies radiation, yet.

Methods applicable for the quantification requiring chromatographic separation by high performance liquid chromatography are not efficient as they do not present precise information on particular brain localization of CaBP and is only applicable to determine CaBP concentrations in tissue homogenates. Immunohistochemistry on the other hand provides quantitative approach and allows semi-quantification of CaBP and discloses cru-

cial information on its localization either at macroscopic or microscopic level in brain structures (Mausset et al. 2001).

Therefore, present study firstly investigated the effects of RF radiation (CDMA mobile phone type) according to the various RF exposure intensity and duraion on the expression of CB and CR in the mouse cerebellum by using immunohistochemistry.

Materials and Methods

1. Animals

Male mice, six weeks old (n=60) weighing 20～30 g, were obtained from ICR (Orientbio Inc). Following arrival, they were acclimated for one week before handling and maintained under a 12-12 h day/night cycle in a temperature-controlled animal room (20 to 25°C) and received food and water *ad libitum*. All animal procedures were performed according to the NIH guidelines of animal research.

2. Electromagnetic field application

Wave Exposer V20, an apparatus designed by the Division of Information Technology Engineering, Soonchunhyang University, was used. The apparatus was designed for radiation of mobile phone waves to mice to study electromagnetic fields and radiation effects. The experimental group was exposed to 835 MHz, which is equivalent to the Korean CDMA mobile phone frequency. The specific absorption rates (SAR) value was divided into two groups, 1.6 W/kg and 4.0 W/kg. Waves were generated and amplified in an electronic unit, and eventually were radiated by a pyramidal rectangular horn antenna connected by waveguide to coaxial transition. The 22 inches of a standard mouse cage was used for the apparatus.

3. Experimental groups

The mice were divided randomly into 6 groups (n=10) as follows. Group A: Control group, Group B: 1 hour at low energy for 5 days (1.6 W/kg SAR), Group C: 1 hour daily at high energy (4.0 W/kg SAR) for 5 days, Group D: 5 hour exposure for 1 day at low energy (1.6 W/kg SAR), Group E: 5 hour exposure for 1 day at high energy (4.0 W/kg SAR), Group F: 8 hour exposure for one month at low energy (1.6 W/kg SAR). The control group was kept under the same laboratory conditions as the exposed groups but not subjected to radiation exposure.

4. Brain preparation

After sacrificing the animal with an overdose of ether, the mice were perfused transcardially with phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA) solution at a flow rate of 30 mL/min. The brain was dissected and post-fixed overnight in 4% PFA followed by a sucrose series (10%, 20% and 30%) solution at 4°C for cryoprotection. Using a cryostat microtome, 40-μm coronal brain slices of the cerebellar cortical region were obtained and processed as free floating sections for CB and CR immunodetection.

5. Immunohistochemistry

CB and CR were detected by free floating Immunohistochemistry. 40-μm sections of the cerebellar region were incubated for 48 h at 4°C with primary antibodies, polyclonal anti-rabbit calbindin D28k (1 : 5,000; AB-1778; Millipore, CA, USA) and polyclonal anti-goat calretinin (1 : 15,000; AB1550; Millipore, CA, USA) in PBS based blocking buffer containing 1% bovine serum albumin, 0.3% Triton X-100, and 1% normal horse serum. After three washes with PBS, sections were incubated with the biotinylated secondary antibodies at 1 : 250 for 1.5 h at room temperature. Sections

were then treated with an avidin-biotin-peroxidase complex (Vectastain ABC mouse Elite kit; Vector Laboratories, Burlingame, CA, USA) following the manufacturer's manual. The reaction was visualized using a solution containing 0.0125% diaminobenzidine (DAB) and 0.005% hydrogen peroxide. Sections from each group were stained together to minimize variability. Following additional washes, sections were mounted on gelatin coated slides, dehydrated in ethanol, cleared in xylene and cover slipped with DPX (Distrene Dibutyl-phthalali Xylene) .

6. Image analysis

Olympus BX 51 microscope was utilized for analysis, and pictures of the sections were taken by a microscope digital camera system (DP50, Olympus, Japan). Staining densities was determined by using the NIH image program (Scion Image). The sum of the gray values of all pixels in a selected region was divided by the total number of pixels in the selected region to determine the mean density of immunoreactivity (IR) per unit area (mm^{-2}). Unpaired *Student's t-test* was used for the statistical analysis and the data were expressed in terms of mean ± SD. Differences were considered significant when $P < 0.05$ at 95% confidence interval (CI) of mean.

Results

1. CB (Calbindin D28k) IR in the cerebellum

CB IR was present in the cerebellum of all groups, particularly in the Purkinje cell layer, with a uniform pattern of distribution in all six groups (Fig. 1). CB IR was also noted in branching Purkinje dendrites ascending vertically into the molecular layer (Fig. 1) as well as the distal axon that descended into the white matter. CB IR was not found in the stellate and basket cells in the molecular layer or the granule and Golgi cells in

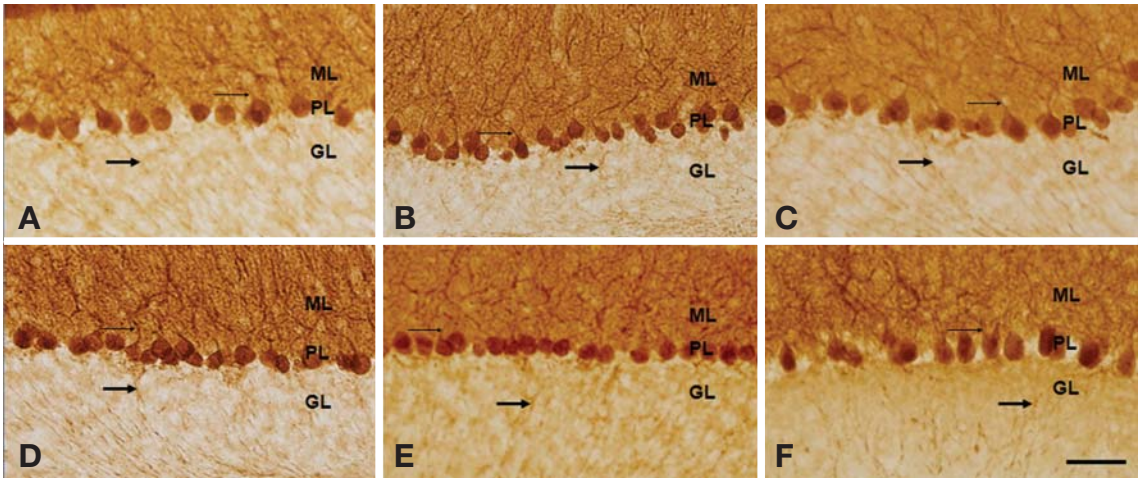

Fig. 1. Photomicrograph showing the immunohistochemical localization of calbindin D28k (CB) immunoreactivity in the cerebellar cortex of 5 wk old mice after radiofrequency exposure varying in SAR values and exposure duration: A: Control, B: 1 hour daily for 5 days at low energy (1.6 W/kg SAR), C: 1 hour daily for 5 days high energy (4.0 W/kg SAR), D: 5 hour exposure for 1 day at low energy (1.6 W/kg SAR), E: 5 hour exposure for 1 day at high energy (4.0 W/kg SAR), F: 8 hour exposure for one month at low energy (1.6 W/kg SAR). CB IR Purkinje cells can be seen arranged in a monocellular layer with immunoreactive dendrites (thin arrows) ascending vertically into the molecular layer. Axons of the Purkinje cells (thick arrows) descending into the granular layer. ML=Molecular layer, PL=Purkinje cell layer, GL=Granular layer; Scale bar=100 μm.

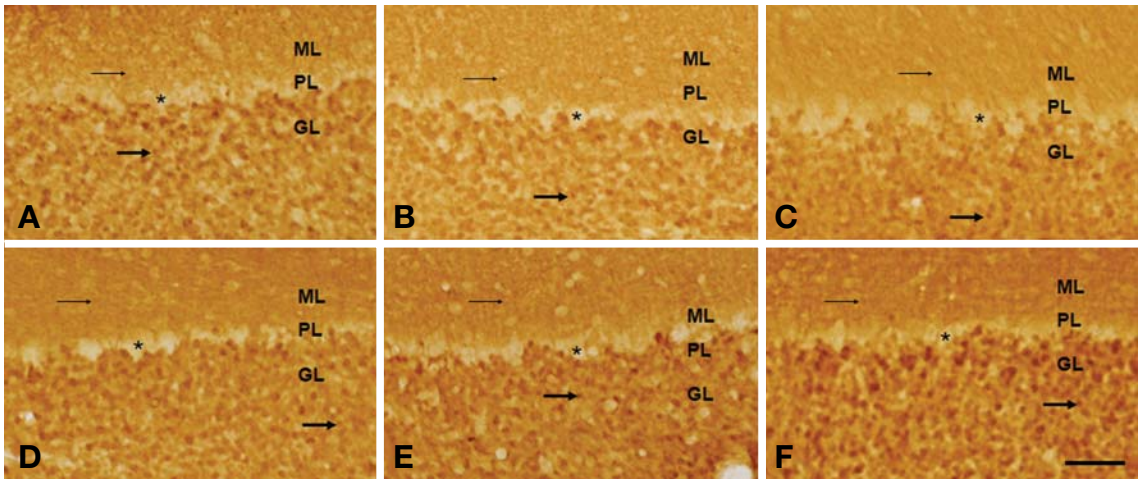

Fig. 2. Photomicrograph showing the immunohistochemical localization of calretinin (CR) immunoreactivity in the cerebellar cortex of 5 wk old mice after radiofrequency exposure varying in SAR values and exposure duration: A: Control, B: 1 hour daily for 5 days at low energy (1.6 W/kg SAR), C: 1 hour daily for 5 days high energy (4.0 W/kg SAR), D: 5 hour exposure for 1 day at low energy (1.6 W/kg SAR), E: 5 hour exposure for 1 day at high energy (4.0 W/kg SAR), F: 8 hour exposure for one month at low energy (1.6 W/kg SAR). Granular layer contains numerous small CR IR granule cells (thick arrows). CR negative Purkinje cells (asterisks) and immunostained horizontally coursing fibers (thin arrows) can be visualized in the molecular layer. ML= Molecular layer, PL=Purkinje cell layer, GL=Granular layer; Scale bar=100 μm.

the granular layer (Fig. 1). The dendritic trees of Purkinje cells are intensely stained for CB and are major contributors to the CB staining in the molecular layer.

The relative mean density of CB IR was highest in the Purkinje cell layer followed by the molecular layer and the granular layer (Fig. 3A). The mean density of the molecular layer in group A, group B, group C, group D, group E and group F was 126.48±2.39, 151.1±4.30, 131.64±2.14, 156.99±1.61, 147.85±2.96 and 115.04±11.52, respectively, with differences from controls for group B ($P<0.01$), group D ($P<0.005$), and group E ($P<0.005$). In the Purkinje cell layer, the mean density of CB IR was 145.79±3.94, 178.30±5.54, 148.63±4.72, 196.28±1.49, 179.11±4.54, and 161.82±5.58 in group A, group B, group C, group D, group E, and group F, respectively (Fig. 3A), with all groups significantly different from control (group B ($P<0.01$), group D ($P<0.0001$), group E ($P<0.0001$) and group F ($P<0.05$). In the granular layer, the mean density was 64.40±2.92, 74.68±2.42, 68.39±2.41, 77.85±1.38, 72.44±1.07, and 70.78±2.99, respectively, in group A, group B, group C, group D, group E, and group F (Fig. 3A).

2. CR IR in the cerebellum

The CR IR was most prominent in the granular layer, followed by the molecular and the Purkinje cell layer while the Purkinje cells did not show CR IR (Fig. 2). In the molecular layer, the stellate and basket cells were not conspicuously stained but a neuropil was observed (Fig. 2). In the molecular layer adjacent to the Purkinje cell layer, numerous immunoreactive fibers were found running in parallel. CR IR was mainly localized in the small cells in the granular layer, which are probably granule cells (Fig. 2).

The maximum relative mean density of CR IR was

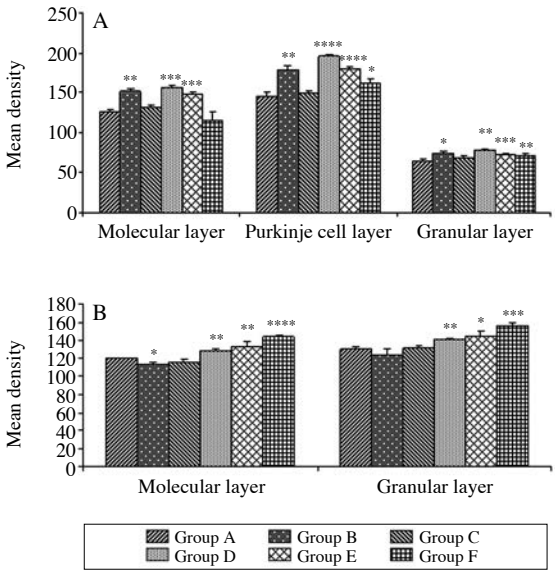

Fig. 3. Mean density of calbindin D28k (A) and calretinin (B) in the cerebellar cortex of the mice due to radiofrequency exposure at various SAR value and exposure duration: A: Control, B: 1 hour daily for 5 days at low energy (1.6 W/kg SAR), C: 1 hour daily for 5 days high energy (4.0 W/kg SAR), D: 5 hour exposure for 1 day at low energy (1.6 W/kg SAR), E: 5 hour exposure for 1 day at high energy (4.0 W/kg SAR), F: 8 hour exposure for one month at low energy (1.6 W/kg SAR). $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$.

highest in the granular layer followed by the molecular layer and Purkinje cell layer, with uniform distribution (Fig. 3B). Image analysis of the granular layer revealed the mean density of CR IR to be 130.66±2.25, 123.48±6.80, 131.55±1.84, 140.71±0.77, 144.10±6.30 and 156.83±2.95 in group A, group B, group C, group D, group E, and group F, respectively, with differences from control in group D ($P<0.01$), group E ($P<0.05$), and group F ($P<0.005$) (Fig. 3B). The mean density in the molecular layer was 120.26±0.67, 113.46±2.13, 115.81±2.89, 127.71±2.31, 133.13±4.92, and 143.69±2.53, in group A, group B, group C, group D, group E, and group F, respectively (Fig. 3B), and with significance from controls for group B ($P<0.05$), group D ($P<0.01$), group E ($P<0.01$) and group F ($P<0.001$) in the molecular layer.

Discussion

We exposed mice to 835 MHz RF at two different SARs (1.6 W/kg and 4.0 W/kg) for 1 h/day for 5 days at low (1.6 W/kg SAR) or high (4.0 W/kg SAR) energy, 5-h exposure for 1 day at low (1.6 W/kg SAR) or high energy (4.0 W/kg SAR), and one month exposure at low energy (1.6 W/kg SAR) to study the expression of CaBP in the cerebellar cortex.

Exponential growth in mobile communication has generated intense scientific interest, accompanied by a parallel increase in the density of EMF (Dubreuil et al. 2002). The effects of EMF emitted by mobile phones on the CNS are concerns because of their close proximity (Mausset et al. 2001, Dubreuil et al. 2002, Dubreuil et al. 2003, Odaci et al. 2008).

The adverse effect of mobile phones varies with the frequency of the radio wave, and can lead from simple headaches to brain tumors (Bolzano et al. 1995, Rothman et al. 1996, McKinlay 1997, Hardell et al. 1999, Maier et al. 2000). Although another study (Inskip et al. 2001) reported the absence of link bewteen brain tumors and RF exposure, brain damage or tumors are possible in the temporal area on the side used to talk on the phone (Hardell et al. 1999).

Animal and human studies have tested the effects of EMF on neurotransmitters, blood-brain barrier permeability, or behavior after exposure to global systems for mobile communication (D'Andrea et al. 2003, Brillaud and de Seze 2006). EMF can influence neuronal function, including regulation of synaptic plasticity, neurotransmitter release, neuronal survival, and learning and memory (Manikonda et al. 2007). The quantification of health hazards due to continuous exposure to microwaves can be quantified using a linear density of Purkinje cells (Albert et al. 1981). Changes in cerebellar Purkinje

cell somata and dendritic arbors are early markers of neuronal damage.

Exposure of mice to 16 Hz amplitude-modulated 2.45 GHz microwaves (SAR 1.64 W/kg) decreased Ca^{2+} precipitates containing synaptic vesicles within nerve terminals and increased precipitates in the synaptic clefts and on non-synaptic surfaces of neuronal plasma membranes (Kittel et al. 1996). These observations were related to the extremely low frequency component of the radiation signal and indicated increased neuronal Ca^{2+} release, which is in line with similar observations in vitro.

Cytoplasmic CaBPs regulate cytosolic Ca^{2+}, leading to specific adjustments in neuronal signaling. CB, a member of a large family of intracellular calcium binding proteins containing EF-hand Ca^{2+} binding motifs and related to calmodulin and troponin-C, has a biological role that includes Ca^{2+} regulation and Ca^{2+}-dependent signaling in neurons and during development (Nägerl et al. 2000, Berggard et al. 2002). CB is exclusively expressed in Purkinje cells and is often used as a marker for cerebellar Purkinje cells. CR, structurally related to CB, is the only CaBP expressed in cerebellar granule cells (Resibois and Rogers 1992). Because of high CR expression in granule cells, and because the parallel fiber-Purkinje cell synapse is central to cerebellar physiology, changes in CR could perturb cerebellar function and cause impaired motor coordination and alterations in Purkinje cell activity (Schiffmann et al. 1999). Here, longer exposure (5 h/day and 1 month) increased CB IR in the Purkinje cell layer. Similarly CR IR also increased with longer duration, peaking at 1 month, in all three layers of the cerebellar cortex. Similar studies have demonstrated a significant difference between the control and the exposed in the number as well as the density of the Purkinje cell layer (Albert et al. 1981a, b). Another study has noted a decrease in the Purkinje cell number with restoration after recovery time (Albert and Sherif 1988). This difference in the observation could be due to difference in the exposure time and wavelength which is a vital component which could elucidate different observations.

Extended exposure to 835 MHz RF radiation (CDMA type) at 1.6 W/kg and 4.0 W/kg could induce disruption of Ca^{2+} homeostasis by increasing Ca^{2+} efflux to produce neurobehavioral changes. The effects of long-term RF exposure should be elucidated further.

References

Adey WR : Tissue interactions with non-ionizing electromagnetic fields. Physiol Revs 61: 435-514, 1981.

Albert EN, Sherif M : Morphological changes in cerebellum of neonatal rats exposed to 2.45 GHz microwaves. Prog Clin Biol Res 257: 135-151, 1988.

Albert EN, Sherif MF, Papadopoulos NJ : Effect of nonionizing radiation on the Purkinje cells of the uvula in squirrel monkey cerebellum. Bioelectromagnetics 2: 241-246, 1981a.

Albert EN, Sherif MF, Papadopoulos NJ, Slaby FJ, Monahan J : Effect of nonionizing radiation on the Purkinje cells of the rat cerebellum. Bioelectromagnetics 2: 247-257, 1981b.

Behari J, Kunjilwar KK, Pyne S : Interaction of low level modulated RF radiation with Na^{+}-K^{+}-ATPase. Bioelectrochemistry and Bioenergetics 47: 247-252, 1998.

Berggard T, Miron S, Onnerfjord P, Thulin E, Akerfeldt KS, Enghild JJ, Akke M, Linse S : Calbindin D28k exhibits properties characteristic of a Ca^{2+} sensor. J Biol Chem 277: 16662-16672, 2002.

Blackman CF, Elder JA, Weil CM, Benane SG, Eichinger DC, House DE : Induction of calcium ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. Radio Sci 14: 93-98, 1979.

Blaustein MP : Calcium transport and buffering in neurons. Trends Neurosci 11: 438-448, 1988.

Bolzano Q, Garay O, Jr Manning TJ : Electromagnetic energy exposure of simulated users of portable cellular telephones. IEEE Trans Veh Technol 44: 390-403, 1995.

Brillaud E, de Seze R : Telephones mobiles et neurotoxicity pour le systeme nerveux central. Environnement risques & santé 5: 107-119, 2006.

Curcio G, Ferrara M, Moroni F, D'Inzeo G, Bertini M, De Gen-

naro L : Is the brain influenced by a phone call? An EEG study of resting wakefulness. Neurosci Res 53: 265-270, 2005.

D'Andrea JA, Chou CK, Johnston SA, Adair ER : Microwave effects on the nervous system. Bioelectromagnetics Suppl 6: 107-147, 2003.

D'Inzeo G, Bernardi P, Eusebi F, Grassi F, Tamburello C, Zani BM : Microwave effects on acetylcholine induced channels in cultured chick myotubes. Bioelectromagnetics 9: 363-372, 1988.

Doya K : Complementary roles of basal ganglia and cerebellum in learning and motor control. Curr Opin Neurobiol 10: 732-739, 2000.

Dubreuil D, Jay T, Edeline JM : Does head-only exposure to GSM-900 electromagnetic fields affect the performance of rats in spatial learning tasks. Behav Brain Res 129: 203-210, 2002.

Dubreuil D, Jay T, Edeline JM : Head-only exposure to GSM 900-MHz electromagnetic fields does not alter rat's memory in spatial and non-spatial tasks. Behav Brain Res 145: 51-61, 2003.

Hamblin DL, Wood AW, Croft RJ, Stough C : Examining the effects of electromagnetic fields emitted by GSM mobile phones on human event-related potentials and performance during an auditory task. Clin Neurophysiol 115: 171-178, 2004.

Hardell L, Nasman A, Pahlson A, Hallquist A, Hansson MK : Use of cellular telephones and the risk for brain tumors: a case-control study. Int J Oncol 15: 113-116, 1999.

Hietanen M : Establishing the health risks of exposure to radiofrequency fields requires multidisciplinary research. Scand J Work Environ Health 32: 169-170, 2006.

Hossmann KA, Hermann DM : Effects of electromagnetic radiation of mobile phones on the central nervous system. Bioelectromagnetics 24: 49-62, 2003.

Huber R, Treyer V, Schuderer J, Berthold T, Buck A, Kuster N, Landolt HP, Achermann P : Exposure to pulse-modulated radio frequency electromagnetic fields affects regional cerebral blood flow. Eur J Neurosci 21: 1000-1006, 2005.

Inskip PD, Tarone RE, Hatch EE, Wilcosky TC, Shapiro WR, Selker RG, Fine HA, Black PM, Loeffler JS, Linet MS : Cellular-telephone use and brain tumors. N Engl J Med 344: 79-86, 2001.

Joubert V, Leveque P, Cueille M, Bourthoumieu S, Yardin C : No apoptosis is induced in rat cortical neurons exposed to GSM phone fields. Bioelectromagnetics 28: 115-121, 2007.

Kittel A, Siklós L, Thuróczy G, Somosy Z : Qualitative enzyme histochemistry and microanalysis reveals changes in ultrastructural distribution of calcium and calcium-activated ATPases after microwave irradiation of the medial habenula. Acta Neuropathol 92: 362-368, 1996.

Kunjilwar KK, Behari J : Effect of amplitude modulated radiofrequency radiation on cholinergic system of developing rats. Brain Res 601: 321-324, 1993.

Maier M, Blakemore C, Koivisto M : The health hazards of mobile phones. BMJ 320: 1288-1289, 2000.

Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya RS, Sashidhar RB, Subramanyam C : Influence of extremely low frequency magnetic fields on Ca^{2+} signaling and NMDA receptor functions in rat hippocampus. Neurosci Lett 413: 145-149, 2007.

Mausset AL, de Seze R, Montpeyroux F, Privat A : Effects of radiofrequency exposure on the GABAergic system in the rat cerebellum: clues from semiquantitative Immunohistochemistry. Brain Res 912: 33-46, 2001.

McKinlay A : Possible health effects related to the use of radiotelephones: recommendations of a European Commission Expert Group. Radiol Prot Bull 187: 9-16, 1997.

Moulder JE, Erdreich LS, Malyapa RS, Merritt J, Pickard WF, Vijayalaxmi : Cell phones and cancer: what is the evidence for a connection?. Radiat Res 151: 513-531, 1999.

Moulder JE, Foster KR, Erdreich LS, McNamee JP : Mobile phones, mobile phone base stations and cancer: a review. Int J Radiat Biol 81: 189-203, 2005.

Nägerl UV, Novo D, Mody I, Vergara JL : Binding kinetics of calbindin-D28k determined by flash photolysis of caged Ca ($^{2+}$). Biophys J 79: 3009-3018, 2000.

Odaci E, Bas O, Kaplan S : Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Res 1238: 224-229, 2008.

Resibois A, Rogers J : Calretinin in rat brain: an immunohistological study. Neuroscience 46: 101-134, 1992.

Rothman KJ, Chou CK, Morgan R, Balzano Q, Guy AW, Funch DP, Preston-Martin S, Mandel J, Steffens R, Carlo G : Assessment of cellular telephone and other radio frequency exposure for epidemiologic research. Epidemiology 7: 291-298, 1996.

Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR : Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ Health Perspect 111: 881-883, 2003.

Schiffmann S, Cheron G, Lohof A, D'Alcantara P, Meyer M, Parmentier M, Schurmans S : Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. Proc Natl Acad Sci USA 96: 5257-5262, 1999.

Thomas D, Tovey SC, Collins TJ, Bootman MD, Berridge MJ, Lipp P : A comparison of fluorescent Ca^{2+} indicator properties and their use in measuring elementary and global signals. Cell Calcium 28: 213-223, 2000.

Korean J Phys Anthropol
22(4): 295 ~ 304, 2009

835 MHz 전자기파 방출에 노출된 생쥐 소뇌겉질에서의 Calbindin D28k와 Calretinin의 면역염색성 분포의 변화

마스키 디라즈, 최인영, 박기섭, 프라단 조누[1], 유영복
단국대학교 의과대학 해부학교실, [1]생리학교실

간추림 : 현대 널리 보급된 무선 휴대폰의 사용은 이로 인해 발생하는 전자기파가 뇌와 신경계통에 미칠 생물학적 영향은 중요 관심사이지만, 아직까지 많은 연구가 진행되지 않았다. 전자기파의 방출로 인해 신경계통에 미치는 영향으로 여러 연구에서 뇌의 여러 부분에서의 신경세포수의 감소, 신경세포의 손상 등을 보고하였다. 그러나, 아직까지 행동을 관장하는 소뇌부분에 관한 어떤 연구도 진행되지 않아 본 연구에서 생체 항상성을 유지하는 데 중요한 이온인 칼슘의 물질대사에 중요한 역할을 하는 calbindin D28k (CB)와 calretinin (CR)의 항체를 이용하여 면역염색학적인 방법으로 생쥐의 소뇌에서 835 MHz 전자기파 방출이 미치는 영향을 조사하였다.

CB와 CR 특이 항체에 대한 면역염색성은 835 MHz의 전자기장에 서로 다른 시간 동안 노출시킨 후 시행하였는데, 사용한 실험조건은 1.6 W/kg 에서 하루 1시간씩 5일 동안 노출된 군, 4.0 W/kg에서 하루 1시간씩 5일 동안 노출된 군, 1.6 W/kg에서 하루 5시간씩 하루 동안 노출된 군, 4.0 W/kg에서 하루 5시간씩 하루 동안 노출된 군, 그리고 1.6 W/kg 에서 한달 동안 8시간씩 매일 노출된 군을 이용했다. CB의 면역반응성은 조롱박세포에서 현저하게 관찰되었으며, 가지돌기와 먼쪽 축삭들에서도 관찰되었다. 1.6 W/kg에서 5일 동안 전자기파에 노출된 경우 CB의 면역반응성이 감소하였고, CR의 면역반응성은 주로 과립층의 작은세포들에 국한되었으며, 면역반응성은 한 달간 노출된 군에서 염색성이 증가되었으며, 조롱박세포층에서는 어떤 세포도 염색된 것을 관찰할 수 없었다. 따라서, 본 연구는 835 MHz 전자기파에 장기간 지속적으로 생체가 노출되면 신경계통에서 물질대사의 항상성을 유지하는 칼슘농도의 변화를 초래해, 소뇌에서의 칼슘대사 이상으로 인한 병적인 행동을 유발할 수 있는 가능성을 제시하였다.

찾아보기 낱말 : 전자기파, Calbindin D28k, Calretinin, 칼슘, 소뇌

교신저자 : 유영복(단국대학교 의과대학 해부학교실)
전자우편 : pppp@dankook.ac.kr