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Immunohistochemical localization of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in the superior olivary complex of mice after radiofrequency exposure

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HIGHLIGHTS

- BDNF IR were decreased in the auditory brainstem nuclei after 3 months RF exposure.
- GDNF IR were decreased in the auditory brainstem nuclei after 3 months RF exposure.
- RF exposure may result in detrimental effect of central auditory function via BDNF and GDNF.

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ABSTRACT

Raising health concerns about the biological effects from radiofrequency exposure, even with conflicting results, has prompted calls for formulation of a guideline of the biological safety level. Given the close proximity between a mobile phone and the ear, it has been suggested that the central auditory system may be detrimentally influenced by radiofrequency exposure. In the auditory system, neurotrophins are important in the regulation of neuron survival, especially mammalian cochlear neurons. Neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) present in the auditory system are responsible for the maintenance of auditory neurons. BDNF and GDNF may protect against acoustic trauma and prevent from hearing defect. The present study applied radiofrequency at a specific absorption rate (SAR) of 1.6 W/kg (E1.6) or 0 W/kg group to determine the distribution of BDNF and GDNF in the nuclei of superior olivary complex (SOC). In the E1.6 group, significant decrements of BDNF immunoreactivity (IR) were noted in the lateral superior olive, medial superior olive, superior paraolivary nucleus and medial nucleus of the trapezoid body. GDNF IR was also significantly decreased ($p < 0.001$) in all SOC nuclei of the E1.6 group. The decrease in the IR of these neurotrophic factors in the SOC of the E1.6 group suggests a detrimental effect of RF exposure in the auditory nuclei.

1. Introduction

Over 1.4 billion people, about 20% of the world's population, utilize and depend on mobile communication [30]. The World Health Organization and other health agencies have concluded that there is no threat to health for children and adults posed by electromagnetic fields (EMFs) generated by cell phone use [22]. However, increased risk of cancer has prompted calls for safety guidelines and a determination of permissible radiofrequency (RF) levels in humans.

Due to close physical proximity of a mobile phone to the head, a possible biological effect of RF exposure and the potential effect have been of interest. A meta-analysis of 19 studies conducted from 1999 to 2007 on the EMFs emitted by Global System for Mobile Communications phones [4] concluded that attention and working memory are affected. Neural damage involving decreased number of neurons has been reported in the hippocampus [16], cerebellum [17] after RF exposure. Loss of pyramidal cells in the cornu ammonis and dentate gyrus has also been reported [16]. Increased oxidative stress due to *in vitro* and *in vivo* EMF exposure in various organ

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systems has been proposed [2]. Given the explosive popularity of cellular phones and the aforementioned proximity of cell phones to the ear, little is known about the influence of RF on brain auditory function and physiology. If there is a relationship between RF exposure and diminished central auditory system function, cell phone use could have an effect on auditory function.

Neurotrophins are important in the regulation of neuron survival in the auditory system, particularly survival of mammalian cochlear neurons [13]. Neurotrophin withdrawal may harm neurons due to oxidative stress, which leads to apoptosis [12]. One of the neurotrophin family members is brain-derived neurotrophic factor (BDNF) [13], which is abundant in the brain and participates in survival, differentiation, and functional maintenance of specific neurons in the central nervous system (CNS) as well as influences synaptic strength and neuronal plasticity [29]. BDNF is reportedly essential for neuron survival [24], exists in peripheral and central auditory nuclei [9]. BDNF expression changes after unilateral cochlear ablation [28] suggesting the effect of BDNF on neuronal survival and differentiation. Decreased BDNF expression in the postnatal cochlea leads to loss of outer hair cells in the cochlea, which diminishes hearing [24].

Auditory system damage can also occur due to a variety of stressors such as noise, drugs, infection, mechanical injury, and aging [26]. Glial-derived neurotrophic factor (GDNF), a glycosylated, disulfide-bonded homodimeric protein, which is a member of the transforming growth factor-beta superfamily [11], protects sensory cells of the auditory system against damage induced by noise [10] and cisplatin [7]. GDNF promotes survival and differentiation of a wide variety of neuronal cells including cerebellar Purkinje cells [20], central noradrenergic neurons [3], and subpopulations of sensory and sympathetic neurons [5]. GDNF effectively protects and restores toxically impaired neuronal systems [8].

The present study was planned to understand the potential effects of a 3-month RF exposure on the central auditory system of mice using immunohistochemistry with anti-BDNF and GDNF specific antibodies at 835 MHz at a specific absorption rate (SAR) of 1.6 W/kg.

2. Materials and methods

2.1. Animals

Six-week-old male ICR mice ($n = 10$; weight, 20–30 g; Orient Bio, Seongnam, South Korea) were used for the experiments. The animals were housed in a conventional state under adequate temperature ($23 \pm 1^{\circ}$) and humidity (50%) control with a 12 h light/12 h dark cycle, and provided free access to water and food. All procedures were carried out under a protocol approved by the Dankook University Institutional Animal Care and Use Committee in accordance with the NIH guidelines for the care and use of laboratory animals.

2.2. RF irradiation

The entire body of the mice was exposed to 835 MHz radiation using Wave Exposer V20 [16, Supplementary Data]. The mice were divided randomly into two groups ($n = 5$/group) comprising a sham control group (SC; SAR = 0 W/kg) and an experimental group with SAR exposure of 1.6 W/kg (E1.6; SAR = 1.6 W/kg). Both groups were exposed to 835 MHz of radiation with a SAR average of 1.6 W/kg and 0 W/kg 8 h/day for 3 months.

2.3. Immunohistochemistry

Brains were collected after perfusion with phosphate-buffered saline (PBS, pH 7.4) and fixation in 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were post-fixed in 4% PFA overnight, cryoprotected by infiltration with series of sucrose solutions (10%, 20%, and 30% consecutively) at 4 °C and embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, USA). The frozen brain blocks were coronally sectioned on a freezing, sliding microtome into 40 μm thick slices, and free-floating immunohistochemistry was performed as described previously [16]. Briefly, sections including the superior olivary complex (SOC) were used for staining with polyclonal anti-rabbit BDNF (AB6201-500; Abcam, Cambridge, UK), and polyclonal anti-rabbit GDNF (AB18956; Abcam, Cambridge, UK) antibodies at a 1:2000 dilution for both. The sections were incubated without the primary antibody as a negative control. Specificity of the primary antibodies was confirmed with Western blots of mouse brain extracts (Supplementary Data).

2.4. Image and statistical analysis

Ten sections per animal were randomly selected to quantitatively analyze Immunoreactivity in each group for each experiment. Digital images of each nuclear subregion were captured with a light microscope (Olympus, Tokyo, Japan) equipped with a DP50 digital camera system (Olympus). Using the Image J program (NIH, Bethesda, MD, USA), the density of immunoreactivity for a specific antibody was evaluated based on optical density (OD). The background OD was taken from areas that were unstained but adjacent to the measured area. After background density was subtracted, the results were expressed as the mean ± standard deviation. A comparison of the mean density of the SOC subfields, which included the lateral superior olive (LSO), superior paraolivary nucleus (SPN), medial superior olive (MSO), and medial nucleus of the trapezoid body (MNTB) between the SC and E1.6 groups was done by one-way analysis of variance using SPSS software (SPSS Inc., Chicago, IL, USA). A $p < 0.05$ was considered significant.

3. Results

3.1. BDNF staining

BDNF immunoreactivity (IR) was observed in the SC group ($n = 5$) in the different nuclei of the SOC. Prominent BDNF IR was overall observed in the nuclei of the LSO (Fig. 1A–D), MSO (Fig. 1E–H), and MNTB (Fig. 1I–L), whereas moderate BDNF IR was observed in the SPN (Fig. 1E,F). A number of neurons as well as the neuropil were prominently stained by the specific anti-BDNF antibody in the LSO of the SC group (Fig. 1C). The loss of BDNF immunoreactive neurons was noted in the E1.6 group ($n = 5$) compared with that in the SC group (Fig. 1D) and BDNF IR was decreased in the neuropil. The SPN of the SC group was comprised of prominent BDNF immunoreactive somata surrounded by puncta possibly representing presynaptic contacts with dendrites (Fig. 1G). Prominent loss of BDNF IR was noted in the neuronal somata of the SPN in the E1.6 group, as well as in the neuropil of MSO (Fig. 1G,H). BDNF IR neurons were prominently observed as horizontal rows in the MNTB of the SC group, which decreased significantly in the E1.6 group (Fig. 1K,L).

3.2. GDNF staining

GDNF immunopositive neurons as well as neuropil were prominently observed throughout LSO in the SC group, in which some neuronal somata contained GDNF immunoreactive granules (Fig. 2A,C). GDNF IR in the neurons of the E1.6 group decreased severely though the GDNF IR neuropil, and IR granules were observed in the LSO (Fig. 2B,D). A number of GDNF immunoreactive neurons were observed in the SPN of the SC group, as small round,