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Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca^{2+} channels in brain and dorsal root ganglion in rat

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ABSTRACT

We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca^{2+} influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR + melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca^{2+} values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyldiphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca^{2+} influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca^{2+} channels.

1. Introduction

In present times there is widespread use of 2.45 GHz irradiation-emitting devices in industrial, scientific, medical, military and domestic applications, with potential leakage of such radiation into the environment [1]. Several studies have suggested that biological systems might be sensitive to such form of radiation [2,3]. Today there is widespread use of 2.45 GHz radiation from common household devices like microwave ovens, wireless access points, and computers, which in some cases were shown to be carcinogenic [4].

Reactive oxygen substances (ROS) are produced by a free radical chain reaction, which can also be initiated by ROS [5]. ROS also cause injury by reacting with biomolecules such as lipids, proteins and nucleic acids as well as by depleting enzymatic antioxidant such as glutathione peroxidase (GSH-Px) and/or nonenzymatic antioxidants such as reduced glutathione (GSH), vitamins A, C, E and β-carotene in the brain and neuronal cells [6]. Pain and brain diseases are impaired in individuals with brain and sensory neuron-related neurodegenerative diseases; this is believed to be, in part, the result of excessive production of ROS [7]. The brain and neurons consume the highest amount of oxygen in the human body [6] although most of the oxygen used in brain tissues is converted to CO_2 and water, small amounts of oxygen form ROS [5]. The existence of polyunsaturated fatty acids which are targets of the ROS in the brain makes this organ more sensitive to oxidative damage [8]. ROS may be involved in the action of cell phone-induced electromagnetic radiation (EMR) on biological systems [2,9–11].

Neuropathic pain states severely limit the quality of life. There are several types of sensory neurons in dorsal root ganglion (DRG) neurons with responsiveness to different kinds of external and internal stimuli. These stimuli such as nociceptive, thermal and mechanical activate different receptors and ion channels that are present in the nerve terminals at the sensory receptive fields. Their expression in selective subsets of DRG neurons determines the response profile of individual neurons to a given stimulus [12]. Ca^{2+} homeostasis is one of the most important factors of cellular physiological function. It

Abbreviations: 2-APB, 2-aminoethyldiphenyl borinate; DRG, dorsal root ganglion; EEG, electroencephalography; EMF, electromagnetic fields; EMR, electromagnetic radiation; FFA, flufenamic acid; GSH, glutathione; GSH-Px, glutathione peroxidase; LP, lipid peroxidation; ROS, reactive oxygen species; SAR, specific absorption rate.

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is involved in such diverse function as cellular proliferation, apoptosis, physiological signal transductions and production of oxidative stress. Cytosolic free Ca^{2+} $[Ca^{2+}]_i$ concentration is controlled by a number of membrane bound ion channels located both on the plasma and intracellular membranes. Transient receptor potential (TRP) channels are a group of non-selective cation channels that have important functions in sensory neurons [13]. One subgroup of TRP melastatin is TRP melastatin 2 (TRPM2). The TRPM2 channel protein has two distinct domains with one functioning as an ion channel and the other as an ADP-ribose (ADPR)-specific pyrophosphatase [14]. The TRPM2 channel is also a redox-sensitive Ca^{2+}-permeable cation channel, and the Ca^{2+} influx through TRPM2 induced by H_2O_2 mediates necrotic cell death [15]. The channel in neuronal cells such as rat striatal neurons [14], organotypic hippocampal culture [16] and DRG neurons [17,18] can also be gated by oxidative stress. 2.45 GHz EMR induced oxidative stress may induce Ca^{2+} influx in DRG neurons through activation of TRPM2 channels and the subject should investigate in DRG neurons of rats.

Melatonin, the main secretary neurohormone of the pineal gland, has been considered a potent antioxidant that detoxifies a variety of ROS in many pathophysiological states [19]. Melatonin as a hormone primarily acts also through G-protein coupled receptors [19]. Melatonin plays also a significant role in neuroprotection against a variety of neurodegenerative diseases whose pathogenesis involves damage of ROS [8]. For examples, melatonin modulated mobile phone EMR-induced oxidative stress in brain of rat [2]. It has been also shown that melatonin is superior to vitamin E as peroxyl radical scavenger [20]. However, mechanisms of melatonin involved in neuroprotection are continued to be explored. We hypothesized that, if melatonin is a potent scavenger, it could prevent or ameliorate the experimental EMR-induced DRG neuron and brain oxidative injury through regulation of TRPM2 channels and Ca^{2+} influx, counteracting the impairment on antioxidant endogenous system.

The present study was designed to determine the effects of 2.45 GHz exposure on the brain cortex and DRG neuron oxidant, antioxidant redox systems and cytosolic Ca^{2+} release through TRPM2 and voltage gated Ca^{2+} channels, as well as the possible protective effects of melatonin on the brain and DRG injury induced by EMR.

2. Materials and methods

2.1. Chemicals

All chemicals were analytical grade, obtained from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA) and all organic solvents from Merck Chemical Inc. (Istanbul, Turkey). All solutions, except phosphate buffers, were prepared daily and stored at +4.0 °C. The reagents were allowed to equilibrate at room temperature for at least 30-min before used for analysis. The phosphate buffers were stable at +4.0 °C for 1 month.

2.2. Animals

This study is planned and organized as completely double-blind. All experimental procedures had been approved by Medical Faculty Experimentation Ethics Committee of Süleyman Demirel University (Protocol Number; 2011-01/02). Male Wistar Albino (n = 32) rats were used in the current experiment. At the start of the experiment the rats were 4 months old and weighed 237 ± 31 g. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Suleyman Demirel University. The rats were housed individually in stainless-steel cages in a pathogen-free environment our laboratory at +22 °C ± 3 with light from 08.00 to 20.00 and free access to water and fed a commercial diet (Korkuteli Yem Ltd., Antalya, Turkey). Environmental average light intensity was 4000 lux and humidity was 40 ± 10%.

2.3. Study groups

After one week adaptation process, the animals were randomly divided into four equal groups as follows:

Group A_1: Cage-control rats,
Group A_2: Sham-control rats,
Group B: Rats exposed to 2.45 GHz during 60 min/day for 30 days
Group C: Same as group B, treated with ip injections of melatonin at a dose of 10 mg/kg/day.

The one-hour exposure to irradiation in groups B–C took place between 9 AM and noon each day. The first dose of melatonin administration was performed 24 hours prior to exposure. Sham-control rats received ip injections of isotonic saline solution at an equal volume to that of melatonin used in group C.

Melatonin was dissolved in a small (100 μl) amount of dimethyl sulfoxide and then diluted with physiological saline solution. The volume of melatonin solution injected daily was 0.1 ml (2–2.5 mg/day). The melatonin dose used in this study was chosen on the basis of our previously published experiment [2].

2.4. Exposure system and design

Details of exposure system have been described in detail elsewhere [3,21]. However, we reduced power values as 1 mW/m^2 in the experiments as described below. A "SET ELECO" generator from Set Electronic Co, Istanbul (Turkey), provided with a half-wave dipole antenna system was used to irradiate the cells with a 2.45 GHz radio frequency with 217 Hz pulses. The electric field density was set at 11 V/m in order to get a 0.1 W/kg whole-body average specific absorption rate (SAR).

Radiation reflection and exposure were measured with a Portable radio frequency Survey System (HOLADAY, HI-4417, Minnesota, USA) with a standard probe. The electromagnetic radiation dose was calculated from the measured electric field density (V/m). SAR values were calculated by using electric properties of tissue sample and measured electric field intensities for every distance in certain frequency. These values were shown in Table 1.

We used eight rats in the exposure system at the same time (Fig. 1). This device is organized with a special cylindrical strainer which is appropriate for exposure condition and physical size of one rat (length: 15 cm, diameter: 5 cm). The noses of the rats were positioned in close contact to monopole antenna and the tube was ventilated from head to tail to decrease the stress of the rat while in the tube. The repetition time, frequency, and amplitude of the radio

Table 1
The effects of melatonin on glutathione peroxidase (GSH-Px) activity, reduced glutathione (GSH) and antioxidant vitamin concentrations on brain of 2.45 GHz induced EMF. (Mean ± SD).

Parameters	Control (n = 8)	Sham control (n = 8)	2.45 GHz (n = 8)	2.45 GHz + Melatonin (n = 8)
LP (μmol/g protein)	69.69 ± 6.74	73.06 ± 7.48	73.06 ± 5.43	72.19 ± 8.44
GSH (μmol/g protein)	9.10 ± 1.31	9.42 ± 1.35	9.21 ± 1.38	9.15 ± 1.10
GSH-Px (IU/g protein)	34.78 ± 2.35	36.30 ± 2.75	36.65 ± 2.48	34.80 ± 3.13
Vitamin A (μmol/g tissue)	1.69 ± 0.11	1.64 ± 0.19	1.67 ± 0.07	1.68 ± 0.15
Vitamin C (μmol/g tissue)	34.78 ± 6.39	33.96 ± 6.39	30.52 ± 6.02	38.32 ± 6.61
Vitamin E (μmol/g tissue)	21.00 ± 1.49	20.70 ± 1.52	19.90 ± 1.45	23.20 ± 0.95[a]
β-carotene (μmol/g tissue)	1.08 ± 0.12	1.07 ± 0.12	0.99 ± 0.07	1.15 ± 0.19

[a] $p < 0.05$ versus 2.45 GHz group.