J Membrane Biol (2015) 248:83–91
DOI 10.1007/s00232-014-9744-y

Epilepsy But Not Mobile Phone Frequency (900 MHz) Induces Apoptosis and Calcium Entry in Hippocampus of Epileptic Rat: Involvement of TRPV1 Channels

Mustafa Nazıroğlu · Fatma Feyza Özkan · Seher Rabia Hapil · Vahid Ghazizadeh · Bilal Çiğ

Received: 26 May 2014 / Accepted: 23 October 2014 / Published online: 9 November 2014

Abstract Electromagnetic radiation (EMR) and epilepsy are reported to mediate the regulation of apoptosis and oxidative stress through Ca^{2+} influx. Results of recent reports indicated that EMR can increase temperature and oxidative stress of body cells, and TRPV1 channel is activated by noxious heat, oxidative stress, and capsaicin (CAP). We investigated the effects of mobile phone (900 MHz) EMR exposure on Ca^{2+} influx, apoptosis, oxidative stress, and TRPV1 channel activations in the hippocampus of pentylenetetrazol (PTZ)-induced epileptic rats. Freshly isolated hippocampal neurons of twenty-one rats were used in study within three groups namely control, PTZ, and PTZ + EMR. The neurons in the three groups were stimulated by CAP. Epilepsy was induced by PTZ administration. The neurons in PTZ + EMR group were exposed to the 900 MHz EMR for 1 h. The apoptosis, mitochondrial membrane depolarization, intracellular reactive oxygen species (ROS), and caspase-3 and caspase-9 values were higher in PTZ and PTZ + EMR groups than in control. However, EMR did not add additional increase effects on the values in the hippocampal neurons. Intracellular-free Ca^{2+} concentrations in fura-2 analyses were also higher in PTZ + CAP group than in control although their concentrations were decreased by TRPV1 channel blocker, capsazepine. However, there were no statistical changes on the Ca^{2+} concentrations between epilepsy and EMR groups. In conclusion, apoptosis, mitochondrial, ROS, and Ca^{2+} influx via TRPV1 channel were increased in the hippocampal neurons by epilepsy induction although the mobile phone did not change the values. The results indicated that TRPV1 channels in hippocampus may possibly be a novel target for effective target of epilepsy.

Keywords Apoptosis · Electromagnetic radiation · Epilepsy · Hippocampus · TRPV1 channels

Abbreviations

$[Ca^{2+}]_i$	Intracellular Ca^{2+}
CAP	Capsaicin
CPZ	Capsazepine
DDT	Dithiothreitol
DHR123	Dihydrorhodamine 123
DMSO	Dimethyl sulfoxide
EMR	Electromagnetic radiation
NP40	Nonidet-P-40 substitute
PTZ	Pentylentetrazol
ROS	Reactive oxygen species
TRP	Transient receptor potential
TRPV1	Transient receptor potential vanilloid 1

Introduction

The effects of electromagnetic radiation (EMR) on humans and the relationship of EMR with various diseases including neurological disorders have been investigated

M. Nazıroğlu (✉) · V. Ghazizadeh
Neuroscience Research Center, University of Suleyman Demirel, TR-32260, Isparta, Turkey
e-mail: mustafanaziroglu@sdu.edu.tr

M. Nazıroğlu · B. Çiğ
Department of Neuroscience, Institute of Health Science, University of Suleyman Demirel, Isparta, Turkey

F. F. Özkan · S. R. Hapil
Faculty of Medicine, University of Suleyman Demirel, Isparta, Turkey

(Nazıroğlu et al. 2012 a, b; Kesari et al. 2013). As a result of advances in technology, people are constantly exposed to EMR. Common cell phone frequencies as 900 and 1800 MHz are examples of sources of electromagnetic fields (Kahya et al. 2014). At the same time, EMR exposure as cell phone use is becoming more and more common in our world. Limited animal studies have suggested effects of 900 MHz EMR on apoptosis and oxidative stress values in hippocampus (Köylü et al. 2006) and epilepsy (Erdinc et al. 2003; López-Martín et al. 2006). EMR exposure affects also various cell functions via actions exerted on intracellular and molecular membrane proteins, including ion channels, Ca^{2+} influx, and enzymes (Piacentini et al. 2008; Nazıroğlu and Gümral 2009; Nazıroğlu et al. 2012 b, c). Hence, the GSM-modulated 900 MHz EMR may aggregate hippocampal oxidative stress, apoptosis, and Ca^{2+} influx in epilepsy through activation of cation channels. This subject needs to be clarified by further research.

Calcium ion (Ca^{2+}) is an important second messenger that has been shown to be responsible for a number of physiological pathways including neuronal excitability and cell proliferation (Nazıroğlu 2007; Espino et al. 2011; Nazıroğlu et al. 2012d). It is well known that the Ca^{2+} is involved in the induction of epilepsy. Some studies (Manikonda et al. 2007; Ammari et al. 2008; Ghazizadeh and Nazıroğlu 2014) with hippocampal neurons indicated a correlation between Ca^{2+} influx, oxidative stress, and EMR although results of some studies did not confirm the results of the authors (Platano et al. 2007; O'Connor et al. 2010). One cell membrane Ca^{2+} channel family is a transient receptor potential (TRP), and the family includes seven different subfamilies including TRP vanilloid (TRPV) (Nazıroğlu 2011; Nazıroğlu et al. 2013a). The TRPV1 type of TRP channels is a non-selective ion channel and is highly expressed in central and peripheral neurons (Caterina et al. 1997). Oxidative stress occurs during the physiological activities such as phagocyte and mitochondrial functions, and it induces apoptosis, mitochondrial membrane depolarization, and Ca^{2+} influx through activations of cation channels and cytosolic second messengers (Nazıroğlu 2009; Espino et al. 2012). TRPV1 channel is activated by physical stimuli such as high heat ($\geq$43 °C), oxidative stress, and capsaicin (CAP, the pungent ingredients of hot chili peppers) (Susankova et al. 2006; Nazıroğlu 2011). Recently, we observed that activation of TRPV1 induced increase of cytosolic-free Ca^{2+} $[Ca^{2+}]_i$ concentration, oxidative stress, and apoptotic cell injury in dorsal root ganglion (DRG) neurons (Nazıroğlu et al. 2013b). Increased expression and function of the TRPV1 channels has been recently reported in hippocampus of patients with epilepsy (Sun et al. 2013). Results of recent animal studies indicated also the importance of TRPV1 channels in induction of epilepsy (Bhaskaran and Smith 2010; Manna and Umathe 2012). The increase of Ca^{2+} influx through overproduction of ROS in hippocampus is a main cause of epileptic seizures (Nazıroğlu 2009). The 900 MHz EMR exposure induces also over production of ROS and Ca^{2+} influx in neuronal cells (Piacentini et al. 2008) although their molecular mechanisms are not clarified yet. There are some reports that 900 MHz EMR can increase the temperature of skin (Anderson and Rowley 2007) and tympanic temperature (Bortkiewicz et al. 2012). Hence, it is possible that 900 MHz EMR can stimulate increased Ca^{2+} influx through heat-activated TRPV1 channels. Therefore, the oxidant effects of EMR may induce apoptosis, oxidative stress, and Ca^{2+} influx through activation of TRPV1 channels in hippocampus of epileptic animals.

To our knowledge, there is no report of effect of mobile phone-induced EMR (900 MHz) on TRPV1 cation channel activation, oxidative stress, and apoptosis in hippocampal neurons of epileptic rats. In the present study, we have evaluated, for the first time, the possibility that the exposure to GSM-modulated 900 MHz EMR could induce TRPV1 cation channel activation, oxidative stress, and apoptosis in hippocampal neurons of epileptic rats.

Materials and Methods

Chemicals

Ethylene glycol-bis(2-aminoethyl-ether)-N,N,N′,N′-tetra-acetic acid (EGTA), dimethyl sulfoxide (DMSO), capsaicin (CAP), capsazepine (CPZ) and RPMI 1640 medium, ficoll-histopaque separating medium, N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (ACDEVD-AMC), nonidet-P-40 substitute (NP40), 2-(N-morpholino) ethanesulfonic acid hydrate (MES hydrate), PEG, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-[(3-chomalidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and dithiothreitol (DTT) were obtained from Sigma-Aldrich Chemical (Istanbul, Turkey). Dihydrorhodamine 123 (DHR123/N-acetyl-Leu-Glu-His-Asp-7-amino-4-methylcoumarin (AC-LEHD-AMC) was purchased from Bachem (Bubendorf, Switzerland). A mitochondrial stain 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was purchased from Santa Cruz (Dallas, Texas, USA). All organic solvents were purchased from Merck Chemicals (Darmstadt, Germany). Fura-2/AM was purchased from Promega (Eugene, Oregon, USA). The reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagent containers were refilled.

Animals

All experimental procedures were approved by the Medical Faculty Experimentation Ethics Committee of Süleyman

Demirel University (Protocol Number; 2013-02/06). Twenty-one male Wistar Albino rats (aged 16 weeks and weighed 160–180 g) were used in the current study.

Study Groups

The animals were randomly divided into four groups as follows:

I. *Control group* ($n = 7$) The rats did not receive exposure and/or administrations. However, hippocampal cells of the groups were kept in the same culture medium without exposure and administrations.
II. *PTZ groups* ($n = 14$): PTZ (60 mg/kg) was intraperitoneally administered to rats for induction of epilepsy (Nazıroğlu et al. 2013b).

Hippocampal neurons of the second group were divided into two subgroups as follows:

II-A *PTZ + 1 h 900 MHz exposure* The hippocampal neurons of the groups were exposed to 900 MHz EMR for 1 h after induction of epilepsy.
II-B *PTZ + 1 h 900 MHz exposure + capsazepine (CPZ)* The hippocampal neurons of the groups were exposed to 900 MHz EMR for 1 h after induction of epilepsy and incubation of CPZ (0.1 mM and 30 min incubation).

Epilepsy was induced in group II, II-A, and II-B by intraperitoneal administration of PTZ (60 mg/kg). After 1 h of PTZ administration all rats were sacrificed and brain samples were taken.

Seizure intensity was evaluated according to a scale as described in our previous studies (Nazıroğlu et al. 2008, 2013b) during the seizure attack. Video recording was taken during the seizure attack.

Preparation of Hippocampal Samples

Rats were deeply anesthetized with ethyl ether and decapitated. The brains were removed and the hippocampus was dissected. Preparation of hippocampal was performed essentially as described elsewhere (Senol et al. 2014). Hippocampus samples were immediately dissected and fragmented and placed in Hank's buffered salt solution (HBSS) and incubated for 30 min with trypsin and mixed every 10 min. It was centrifuged (at 500 g for 5 min) and the supernatant was discarded and replaced by HBSS for two times and then used in assays.

Exposure System and Design

Details of the exposure system have been described in detail elsewhere (Özorak et al. 2013). The cells were kept in a circulatory water bath (Fig. 1). The neurons were attachable cell to walls of the tube. The cells were floating at the surface of the medium during the exposure system. The exposure system was kept in a specific room which included plastic furniture such as tables and chairs to prevent possible radiation reflection. Walls of the room were covered by chromium–nickel metals (diameter: 1 mm) for protecting the cells from possible outside electromagnetic interference exposure. The continuous wave of radiofrequency signal (900 MHz with 217 Hz pulses) emitted by the generator was amplified initially and then fed into the cancer cells in the water bath through an antenna (Biçer Electronic, Sakarya, Turkey). This antenna has a special Falcon holder designed to accommodate the cells for appropriate exposure conditions. The repetition time, frequency, and amplitude of the radiofrequency energy spectrum was monitored by a satellite level meter (PROMAX, MC-877C, Barcelona, Spain). Radiation reflection and exposure were measured with a Portable RF Survey System (HOLADAY, HI-4417, Eden Prairie, MN, USA) with a standard probe. The EMR dose was calculated from the measured electric field strength (V/m). Distance was arranged as zero cm between the falcon tubes and probe of the exposure system. Six falcon tubes each containing $1x10^6$ cells/ml (5 ml total medium) were placed on a non-conductive plexi glass table at a height of 110 cm at precise location where required power density was measured (Fig. 1). The distance was 5 mm between the tubes. The radiofrequency field inside the specific room was probed using a strength meter and the precise positions which provided power densities of 1.2, 12, or 120 $\mu W/cm^2$ were determined (Jin et al. 2012). The required power density (≤ 12 $\mu W/cm^2$) was continuously recorded every 5 min using a satellite level meter (EXTECH-480836, Extech Instruments, Nashua, NH, USA). At the top of the tube, the average specific absorption rate (SAR) estimated for 900 MHz exposure at 12 $\mu W/cm^2$ power flux density was 0.023 ± 0.001 mW/kg. The water bath was (Water Bath 601, Jiangsu Zhengji Instruments, Jiangsu, China) installed in the chromium–nickel-covered room which maintained 37 °C temperature (relative humidity of 83 %) and the inside temperature of the tube was also the same.

The SAR values were calculated by using electric frequency properties of cell culture medium samples and measured electric field intensities for every distance at a certain frequency. The SAR values were calculated using Burkhardt's formula (Burkhardt et al. 1996).

Measurement of Intracellular-Free Calcium Concentration ($[Ca^{2+}]_i$)

The hippocampus cells were loaded with 4 μM fura-2/AM in loading buffer with 1×10^5 cells per ml for 45 min at

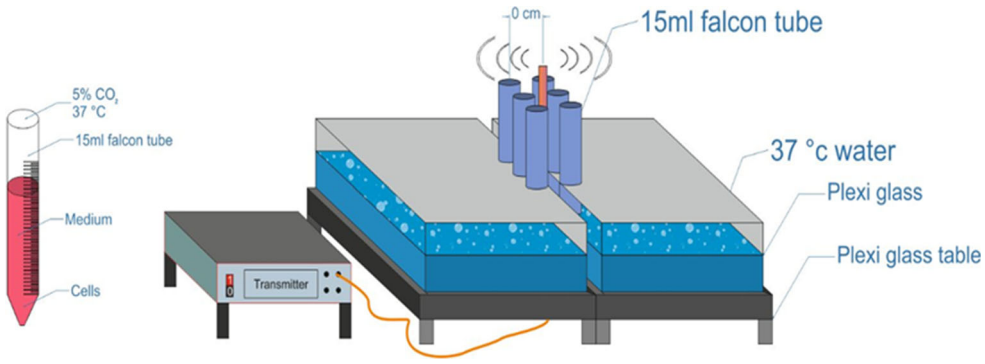

Fig. 1 Schematic diagram of 900 MHz RF exposure device

37 °C in the dark, washed twice with phosphate buffer then incubated for an additional 30 min at 37 °C to complete probe de-esterification, and re-suspended in loading buffer at a density of 1×10^5 cells per ml according to a procedure published elsewhere (Nazıroğlu et al. 2011, 2014). All groups were exposed to capsaicin (CAP) for stimulation of $[Ca^{2+}]_i$ influx. Fluorescence was recorded from 2 ml aliquots of magnetically stirred cellular suspension at 37 °C by using a spectrofluorometer (Cary Eclipsys, Varian Inc, Sydney, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored by using the fura-2 340/380 nm fluorescence ratio and were calibrated according to the method of (Grynkiewicz et al. 1985).

Apoptosis Level and Caspase Activity Assays

The APO Percentage assay was performed according to the instructions provided by Biocolor (Belfast, Northern Ireland) and described elsewhere (Uğuz and Nazıroğlu 2012; Kahya et al. 2014).

The determination of caspase-3 and caspase-9 activities was based on a method previously reported (Espino et al. 2009, 2010, 2011) with minor modifications. Stimulated or resting cells were washed once with PBS. After centrifugation, cells were re-suspended in PBS at a concentration of 10^5 cells/ml. Fifteen micro-liters of the cell suspension were added to a microplate and mixed with the appropriate peptide substrate dissolved in a standard reaction buffer that was composed of 100 mM HEPES, pH 7.25, 10 % sucrose, 0.1 % CHAPS, 5 mm DTT, 0.001 % NP40 and 40 µm of caspase-3 substrate (AC-DEVD-AMC) or 0.1 m MES hydrate, pH 6.5, 10 % PEG, 0.1 % CHAPS, 5 mm DTT, 0.001 % NP40, and 0.1 mM of caspase-9 substrate (AC-LEHD-AMC). Substrate cleavage was measured with the microplate reader (Infinite pro200; Tecan Austria GmbH, Groedig, Austria) with excitation wavelength of 360 nm and emission at 460 nm. The data were calculated as fluorescence units/mg protein and presented as fold-increase over the pretreatment level (experimental/control).

Intracellular Reactive Oxygen Species (ROS) Measurement

DHR123 is a non-fluorescent, non-charged dye that easily penetrates cell membrane. Once inside the cell, DHR123 becomes fluorescent upon oxidation to yield rhodamine 123 (Rh 123), the fluorescence being proportional to ROS generation. The fluorescence intensity of Rh123 was measured in an automatic microplate reader (Infinite pro200). Excitation was set at 488 nm and emission at 543 nm (Espino et al. 2009, 2010); Nazıroğlu et al. 2013b). Treatments were carried out in triplicate. The data are presented as fold-increase over the pretreatment level (experimental/control).

Mitochondrial Membrane Potential Determination

Cells were incubated with 1 µM JC-1 (a cationic dye) for 15 min at 37 °C as previously described (Espino et al. 2011). The JC-1 exhibits potential-dependent accumulation in the mitochondria. It indicates mitochondrial depolarization by a decrease in the red to green fluorescence intensity ratio. After incubation with JC-1, the dye was removed and the cells were washed in phosphate buffered saline (PBS). The green JC-1 signal was measured at the excitation wavelength of 485 nm and the emission wavelength of 535 nm; the red signal at the excitation wavelength of 540 nm and the emission wavelength of 590 nm. Fluorescence changes were analyzed using a fluorescence spectrophotometer. The data are presented as fold-increase over the pretreatment level (experimental/control).

Statistical Analyses

All results were expressed as mean ± SD. Significant values in the four groups were assessed with an unpaired Mann–Whitney *U* test. Data were analyzed using the SPSS statistical program (version 17.0, Chicago, Illinois, USA). *P* values of less than 0.05 were regarded as significant.

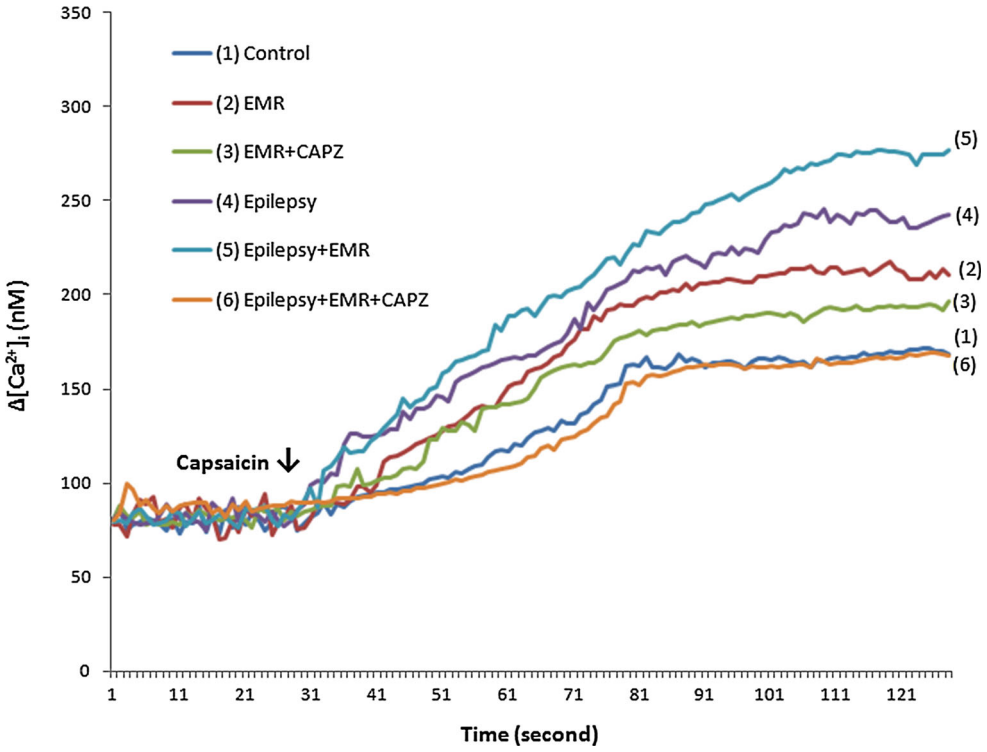

Fig. 2 Effects of EMR (900 MHz) exposure on intracellular-free Ca^{2+} concentrations of hippocampal neurons in control and PTZ-induced epileptic rat. ($n = 6$ and mean ± SD)

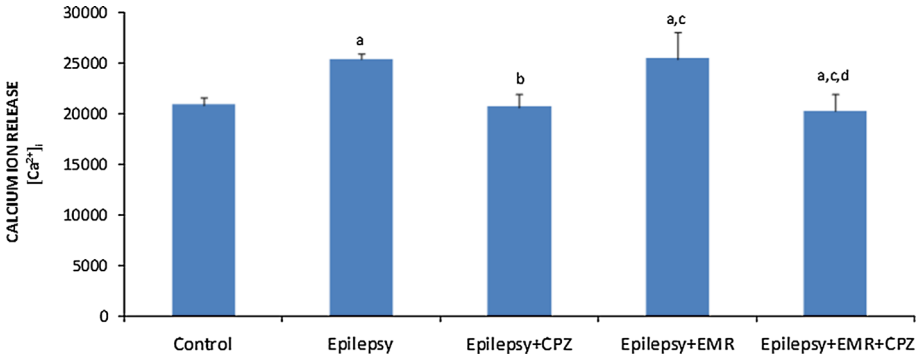

Fig. 3 Effects of EMR (900 MHz) exposure and capsazepine (CPZ and 0.1 mM) incubations on intracellular-free Ca^{2+} concentrations of hippocampal neurons in control and PTZ-induced epileptic rat. ($n = 6$ and mean ± SD). Fura-2-loaded rat hippocampal neurons were stimulated with capsaicin (CAP and 0.1 mM) in the presence of normal extracellular calcium ($[Ca^{2+}]_o = 1.2$ mm for 150 s. The traces shown are representative of eight separate experiments. (mean ± SD). $^{a}p < 0.05$ versus control. $^{b}p < 0.05$ versus epilepsy. $^{c}p < 0.05$ versus epilepsy + CPZ. $^{d}p < 0.05$ versus epilepsy + EMR

Results

Effects of 900 MHz EMR on Intracellular-Free Calcium ($[Ca^{2+}]_i$) Concentration

The results of $[Ca^{2+}]_i$ concentrations in control, epilepsy, epilepsy + CPZ, epilepsy + EMR, and epilepsy + EMR + CPZ are shown in Figs. 2, 3. The $[Ca^{2+}]_i$ concentration was significantly ($p < 0.05$) higher in epilepsy and epilepsy + EMR groups than the control groups. CPZ is a non-specific TRPV1 channel blocker. The hippocampal neurons were incubated for 30 min before one hour EMR exposure and CAP stimulation. After the incubations, the $[Ca^{2+}]_i$ concentration was significantly ($p < 0.05$) lower in epilepsy + CPZ and epilepsy + EMR + CPZ groups than the epilepsy and epilepsy + EMR groups. Hence, epilepsy induced Ca^{2+} influx in the neurons through TRPV1 channel activation.

Effects of 900 MHz EMR on Apoptosis and Caspase Values

We investigated the effects of 900 MHz EMR exposure on the rate of programed cell death as apoptosis and caspase

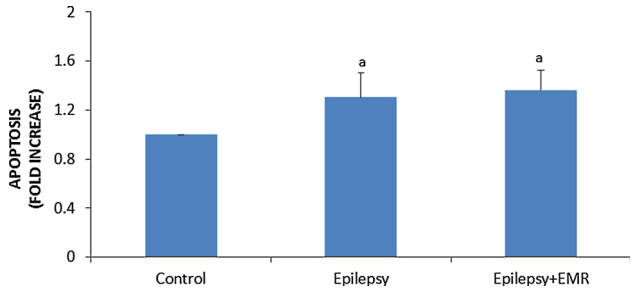

Fig. 4 Effects of EMR (900 MHz) exposure on apoptosis levels in hippocampus of control and epileptic rats. Values are presented as mean ± SD of 6 separate experiments and expressed as fold-increase over the pretreatment level (experimental/control). $^{a}p < 0.05$ versus control

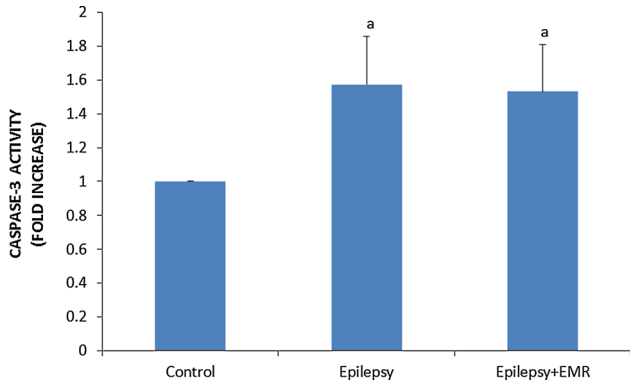

Fig. 5 Effects of EMR (900 MHz) exposure on hippocampus caspase-3 activity in control and PTZ-induced epileptic rats. Values are presented as mean ± SD of 8 separate experiments and expressed as fold-increase over the pretreatment level (experimental/control). $^{a}p < 0.01$ versus control

values in the hippocampal neurons. The results of apoptosis, caspase-3, and caspase-9 values in control, epilepsy, and epilepsy + EMR groups are shown in Figs. 4, 5, 6, respectively. The apoptosis ($p < 0.05$), caspase-3 ($p < 0.01$), and caspase-9 ($p < 0.05$) values in the epilepsy and epilepsy + EMR groups were significantly higher than in the control group. There was no statistical significance in the values between epilepsy and epilepsy + EMR groups.

Effects of 900 MHz EMR on Intracellular ROS Production and Mitochondrial Depolarization Values

The ROS and mitochondrial membrane depolarization values results in control, epilepsy, and epilepsy + EMR groups are shown in Figs. 7, 8 respectively. The ROS and mitochondrial membrane depolarization values were increased by the epilepsy induction and EMR exposure. The ROS ($p < 0.05$) and mitochondrial membrane depolarization values ($p < 0.01$) values in the epilepsy and epilepsy + EMR groups were significantly higher than in the control group. There was no statistical significance in the values between epilepsy and epilepsy + EMR groups.

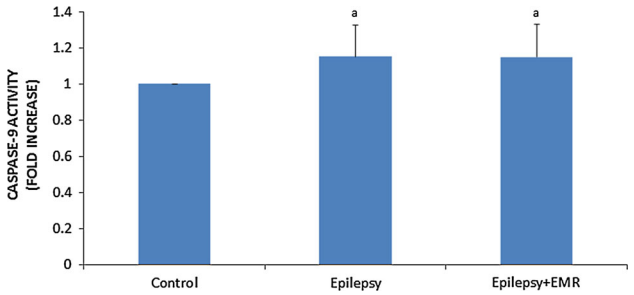

Fig. 6 Effects of EMR (900 MHz) exposure on hippocampus caspase-9 activity in control and PTZ-induced epileptic rats. Values are presented as mean ± SD of 8 separate experiments and expressed as fold-increase over the pretreatment level (experimental/control). $^{a}p < 0.05$ versus control

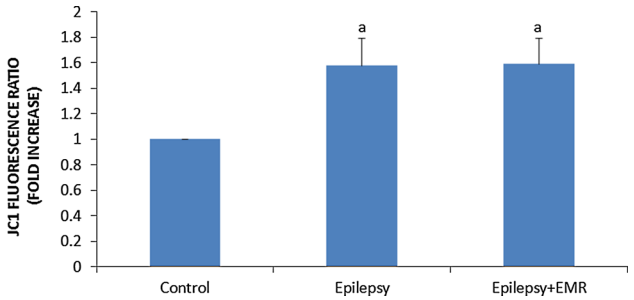

Fig. 7 Effects of EMR (900 MHz) on intracellular ROS level in hippocampus of control and PTZ-induced epileptic rats. Values are presented as mean ± SD of 9 separate experiments and expressed as fold-increase over the pretreatment level (experimental/control). $^{a}p < 0.05$ versus control

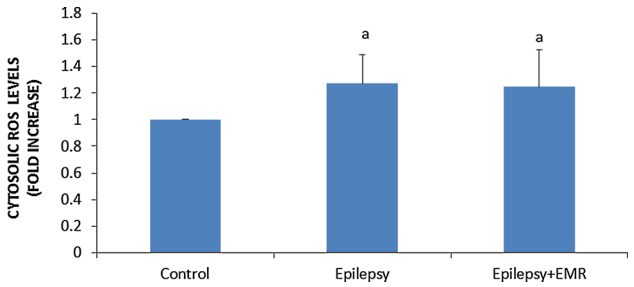

Fig. 8 Effects of EMR (900 MHz) exposure on hippocampus mitochondrial membrane depolarization in control and PTZ-induced epileptic rats. Values are presented as mean ± SD of 3 separate experiments and expressed as fold-increase over the pretreatment level (experimental/control). $^{a}p < 0.01$ versus control

Discussion

We found that hippocampal apoptosis, caspase-3, caspase-9, ROS, mitochondrial depolarization, and $[Ca^{2+}]_i$ concentration were increased by epilepsy induction. Hence,

PTZ administrations to the rats are characterized by increased oxidative stress, Ca^{2+} influx, and apoptosis. Administration of TRPV1 channel blocker, CPZ, caused a decrease in $[Ca^{2+}]_i$ concentration. To the best of our knowledge, the current study is the first to compare the epilepsy and 900 MHz EMR with particular reference to its effects on oxidative stress, Ca^{2+} signaling and apoptosis redox system in PTZ-induced hippocampal injury in rats.

Results of some studies indicate that electromagnetic waves emitted from mobile phone (900 MHz) affect physiological and biochemical responses, including cell proliferation (Erdinc et al. 2003; Köylü et al. 2006; Nazıroğlu et al. 2013c). It is possible that non-thermal effects of electromagnetic waves from mobile phones increase the ROS in tissues and cells. It is well known that ROS leads to oxidative damage in major cell macromolecules, such as lipids, proteins and nucleic acids, and proposed to be the cause in tissue injury. The ROS is also responsible for dramatically altered neuronal function injury in epilepsy. PTZ has been used in experimental epilepsy for inducing generalized seizures. The current study indicated that a convulsive dose (60 mg/kg) of PTZ administration induced a significant increase in cytosolic ROS production levels of hippocampal neurons. We did not observe additional effects of the EMR exposure on the cytosolic ROS production. Our results are confirmed by results of previous reports of oxidative stress increment in brain and hippocampus during epileptic seizures (Erdinc et al. 2003; López-Martín et al. 2006; Nazıroğlu et al. 2008, 2013c).

A large number of studies linked seizure-induced cell damage to excitotoxic mechanisms (Heinemann et al. 2002). Convulsions can result in augmented neurotransmitter (glutamate) release, leading to Ca^{2+} uptake through voltage gated and chemical calcium channels. In fact during convulsions, extracellular Ca^{2+} decreases while cytosolic Ca^{2+} concentration increases (Nazıroğlu 2009, Nazıroğlu and Yürekli 2013). Mitochondria were reported to accumulate Ca^{2+} provided cytosolic Ca^{2+} rises or provided mitochondrial uptake dominates mitochondrial Ca^{2+} extrusion (Heinemann et al. 2002), thereby leading to depolarization of mitochondrial membranes (Hansford 1994). Uptake of Ca^{2+} into mitochondria stimulates the tricarboxylate cycle resulting in augmented reduction of pyridine nucleotides, which may be one of the mechanisms of the coupling of neuronal and metabolic activity. Exposure of mitochondria to high cytosolic-free Ca^{+2} was shown to increase formation of ROS (Espino et al. 2009, 2011). In the current hippocampal ROS, mitochondrial depolarization and $[Ca^{2+}]_i$ values were increased by PTZ administration although administration of TRPV1 channel blocker, CPZ, decreased the $[Ca^{2+}]_i$ concentration in the neurons. Modulation of TRPV1 in hippocampal cells by means of the treatment of with the CPZ might be caused decrease in mitochondrial ROS productions, apoptosis, and cell membrane Ca^{2+} influx.

Examination of the response of $[Ca^{2+}]_i$ concentration to the 900 MHz-induced EMR showed in hippocampal cells that there was significant difference between the EMR-exposed and sham-exposed neurons through activation of TRPV1 channels. In radiofrequency range, the main established quantitative effect of 900 MHz EMR on biological tissues is heating due to vibrational movements of water molecules. The temperature changes induced in tissues also constitute the basis for the setting of radiofrequency exposure limits and recommendations (Matikka Virtanen et al. 2010). The TRPV1 cation channel plays a key role in the perception of thermal pain; however, its molecular role in hippocampus is extensively unexplored (Susankova et al. 2006; Nazıroğlu 2011). In the current study, $[Ca^{2+}]_i$ concentration in the hippocampus was not higher in PTZ + 900 MHz EMR group than PTZ group. Hence, we did not observe additional effects of 900 MHz EMR on the Ca^{2+} entry through thermal sensitive TRPV1 channels.

Apoptosis is programed death and it is mediated by specific proteinases namely caspases. There are two major pathways for apoptosis (Hansford 1994). One involves death receptors and is marked by Fas-mediated caspase-8 activation, and the other is the stress or mitochondrial-mediated caspase-9 activation. Both pathways induce caspase-3 activation (Hansford 1994; Espino et al. 2009). In the current hippocampal apoptosis, caspase-3 and caspase-9, values were increased by PTZ administration. Similarly, (Carballo-Quintás et al. 2011) found c-fos and glial markers were increased by the combined stress of non-thermal irradiation and the toxic effect of picrotoxin on cerebral tissues exposed to 900 MHz. In study of (López-Martín et al. 2006) 900 MHz GSM radiation stimulated c-fos expression in different areas of the limbic system and triggered a marked increase in neuronal excitability in seizure-prone rats (Ammari et al. 2008) reported that subchronic exposures to a 900 MHz EMF signal for 2 months could adversely affect rat brains (indicating potential gliosis). It was reported that ROS production and mitochondria membrane depolarization may play an important role in the process of apoptosis in human cell which is induced by the radiation of 900 MHz EMR (Lu et al. 2012) although the hypothesis on the apoptosis and mitochondrial membrane depolarization values was not support by results of many studies (Capri et al. 2004; Joubert et al. 2008). We were not able to see additional effects on the hippocampal apoptosis, caspase, and mitochondrial membrane depolarization values in the current study. Hence, the results were confirmed by results of (Capri et al. 2004 and Joubert et al. 2008).

In conclusion, we found that hippocampal apoptosis, caspase-3, caspase-9, ROS, and mitochondrial depolarization were increased by induction of epilepsy but not the EMR exposure. Hence, we observed striking correlations between the effects of epilepsy on the values in hippocampal neurons of the epileptic rats. However, epilepsy and the EMR interacted with TRPV1 cation channel permeability, and $[Ca^{2+}]_i$ concentration is increased through activation of TRPV1 channels by epilepsy induction and EMR exposure. The presence of a biological effect of the EMR and epilepsy on the TRPV1 channels in the hippocampal neurons may be due in part to thermal exposure of these cells to the EMR, which may permit enough time for the plasma membrane changes to occur.

Acknowledgments Vahid Ghazizadeh (M.D.) was partially supported for the project by Free Oxygen Radical Society, Isparta, Turkey. Abstract of the manuscript submitted on 5th Neuroscience Days Meeting, 1–2 June 2013, Isparta, and the abstract was published in Cell Membr Free Radic Res. MN formulated the present hypothesis and was responsible for writing the report. ÖFF, HSR, VG, and BÇ were responsible for the analyses.

Declaration of interest There is no conflict of interest in the study. All authors approved the final manuscript.

References

Ammari M, Lecomte A, Sakly M, Abdelmelek H, de-Seze R (2008) Exposure to GSM 900 MHz electromagnetic fields affects cerebral cytochrome c oxidase activity. Toxicology 250:70–74

Anderson V, Rowley J (2007) Measurements of skin surface temperature during mobile phone use. Bioelectromagnetics 28:159–162

Bhaskaran MD, Smith BN (2010) Effects of TRPV1 activation on synaptic excitation in the dentate gyrus of a mouse model of temporal lobe epilepsy. Exp Neurol 223:529–536

Bortkiewicz A, Gadzicka E, Szymczak W, Zmyślony M (2012) Changes in tympanic temperature during the exposure to electromagnetic fields emitted by mobile phone. Int J Occup Med Environ Health 25:145–150

Burkhardt M, Poković K, Gnos M, Schmid T, Kuster N (1996) Numerical and experimental dosimetry of petri dish exposure setups. Bioelectromagnetics 7:483–493

Capri M, Scarcella E, Fumelli C, Bianchi E, Salvioli S, Mesirca P, Agostini C, Antolini A, Schiavoni A, Castellani G, Bersani F, Franceschi C (2004) In vitro exposure of human lymphocytes to 900 MHz CW and GSM modulated radiofrequency: studies of proliferation, apoptosis and mitochondrial membrane potential. Radiat Res 162:211–218

Carballo-Quintás M, Martínez-Silva I, Cadarso-Suárez C, Alvarez-Figueiras M, Ares-Pena FJ, López-Martín E (2011) A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains. Neurotoxicology 32:478–494

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Erdinc OO, Baykul MC, Ozdemir O, Ozkan S, Sirmagul B, Oner SD, Ozdemir G (2003) Electromagnetic waves of 900 MHz in acute pentylenetetrazole model in ontogenesis in mice. Neurol Sci 24:111–116

Espino J, Mediero M, Lozano GM, Bejarano I, Ortiz A, García JF, Pariente JA, Rodríguez AB (2009) Reduced levels of intracellular calcium releasing in spermatozoa from asthenozoospermic patients. Reprod Biol Endoc 7:11

Espino J, Bejarano I, Redondo PC, Rosado JA, Barriga C, Reiter RJ, Pariente JA, Rodríguez AB (2010) Melatonin reduces apoptosis induced by calcium signaling in human leukocytes: evidence for the involvement of mitochondria and bax activation. J Membr Biol 233:105–118

Espino J, Bejarano I, Paredes SD, Barriga C, Rodríguez AB, Pariente JA (2011) Protective effect of melatonin against human leukocyte apoptosis induced by intracellular calcium overload: relation with its antioxidant actions. J Pineal Res 51:195–206

Espino J, Pariente JA, Rodríguez AB (2012) Oxidative stress and immunosenescence: therapeutic effects of melatonin. Oxid Med Cell Longev 2012:670294

Ghazizadeh V, Nazıroğlu M (2014) Electromagnetic radiation (Wi-Fi) and epilepsy induce calcium entry and apoptosis through TRPV1 channel in hippocampus and dorsal root ganglion of rats. Metab Brain Dis 29:787–799

Grynkiewicz C, Poenie M, Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450

Hansford RG (1994) Physiological role of mitochondrial Ca^{2+} transport. J Bioenerg Biomembr 26:495–508

Heinemann U, Buchheim K, Gabriel S, Kann O, Kovacs R, Schuchmann S (2002) Cell death and metabolic activity during epileptiform discharges and status epilepticus in the hippocampus. Prog Brain Res 135:197–210

Jin Z, Zong C, Jiang B, Zhou Z, Tong J, Cao Y (2012) The effect of combined exposure of 900 MHz radiofrequency fields and doxorubicin in HL-60 cells. PLoS ONE 7:e46102

Joubert V, Bourthoumieu S, Leveque P, Yardin C (2008) Apoptosis is induced by radiofrequency fields through the caspase-independent mitochondrial pathway in cortical neurons. Radiat Res 169:38–45

Kahya MC, Nazıroğlu M, Çığ B (2014) Selenium reduces mobile phone (900 MHz)-induced oxidative stress, mitochondrial function, and apoptosis in breast cancer cells. Biol Trace Elem Res 160:285–293

Kesari KK, Siddiqui MH, Meena R, Verma HN, Kumar S (2013) Cell phone radiation exposure on brain and associated biological systems. Indian J Exp Biol 51:187–200

Köylü H, Mollaoglu H, Ozguner F, Naziroglu M, Delibas N (2006) Melatonin modulates 900 MHz microwave-induced lipid peroxidation changes in rat brain. Tox Ind Health 22:211–216

López-Martín E, Relova-Quinteiro JL, Gallego-Gómez R, Peleteiro-Fernández M, Jorge-Barreiro FJ, Ares-Pena FJ (2006) GSM radiation triggers seizures and increases cerebral c-Fos positivity in rats pretreated with subconvulsive doses of picrotoxin. Neurosci Lett 398:139–144

Lu YS, Huang BT, Huang YX (2012) Reactive oxygen species formation and apoptosis in human peripheral blood mononuclear cell induced by 900 MHz mobile phone radiation. Oxid Med Cell Longev 2012:740280

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

Manna SS, Umathe SN (2012) Involvement of transient receptor potential vanilloid type 1 channels in the pro-convulsant effect of anandamide in pentylenetetrazole-induced seizures. Epilepsy Res 100:113–124

Matikka Virtanen H, Keshvari J, Lappalainen R (2010) Temperature changes associated with radiofrequency exposure near authentic metallic implants in the head phantom–a near field simulation study with 900, 1800 and 2450 MHz dipole. Phys Med Biol 55:5867–5881

Nazıroğlu M (2007) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res 32:1990–2001

Nazıroğlu M (2009) Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. Neurochem Res 34:2181–2191

Nazıroğlu M (2011) TRPM2 cation channels, oxidative stress and neurological diseases: where are we now? Neurochem Res 36:355–366

Nazıroğlu M, Gümral N (2009) Modulator effects of selenium and L-carnitine on wireless devices (2.45 GHz) induced oxidative stress and electroencephalography records in brain of rat. Internat J Radiat Biol 85:680–689

Nazıroğlu M, Yürekli VA (2013) Effects of antiepileptic drugs on antioxidant and oxidant molecular pathways: focus on trace elements. Cell Mol Neurobiol 33:589–599

Nazıroğlu M, Kutluhan S, Yilmaz M (2008) Selenium and topiramate modulates brain microsomal oxidative stress values, Ca^{2+}-ATPase activity, and EEG records in pentylentetrazol-induced seizures in rats. J Membr Biol 225:39–49

Nazıroğlu M, Özgül C, Çelik Ö, Çiğ B, Sözbir E (2011) Aminoethoxydiphenyl borate and flufenamic acid inhibit Ca^{2+} influx through TRPM2 channels in rat dorsal root ganglion neurons activated by ADP-ribose and rotenone. J Membr Biol 241:69–75

Nazıroğlu M, Ciğ B, Doğan S, Uğuz AC, Dilek S, Faouzi D (2012a) 2.45 GHz wireless devices induce oxidative stress and proliferation through cytosolic Ca^{2+} influx in human leukemia cancer cells. Int J Radiat Biol 88:449–456

Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA (2012b) 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. Physiol Behav 105:683–692

Nazıroğlu M, Dikici DM, Dursun S (2012c) Role of oxidative stress and Ca(2 +) signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels. Neurochem Res 37:2065–2075

Nazıroğlu M, Tokat S, Demirci S (2012d) Role of melatonin on electromagnetic radiation-induced oxidative stress and Ca2 + signaling molecular pathways in breast cancer. J Recept Signal Transduct Res 32:290–297

Nazıroğlu M, Akay MB, Celik O, Yıldırım MI, Balcı E, Yürekli VA (2013a) Capparis ovata modulates brain oxidative toxicity and epileptic seizures in pentylentetrazol-induced epileptic rats. Neurochem Res 38:780–788

Nazıroğlu M, Çiğ B, Özgül C (2013b) Neuroprotection induced by N-acetylcysteine against cytosolic glutathione depletion induced-Ca^{2+} influx in dorsal root ganglion neurons of mice: role of TRPV1 channels. Neuroscience 242:151–160

Nazıroğlu M, Yüksel M, Köse SA, Ozkaya MO (2013c) Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signaling pathways in females and males. J Membr Biol 246:869–875

Nazıroğlu M, Sahin M, Ciğ B, Aykur M, Erturan I, Ugan Y (2014) *Hypericum perforatum* modulates apoptosis and calcium mobilization through voltage-gated and TRPM2 calcium channels in neutrophil of patients with behcet's disease. J Membr Biol 247:253–262

O'Connor RP, Madison SD, Leveque P, Roderick HL, Bootman MD (2010) Exposure to GSM RF fields does not affect calcium homeostasis in human endothelial cells, rat pheocromocytoma cells or rat hippocampal neurons. PLoS ONE 5:e11828

Özorak A, Nazıroğlu M, Çelik Ö, Yüksel M, Özçelik D, Özkaya MO, Çetin H, Kahya MC, Kose SA (2013) Wi-Fi (2.45 GHz)- and mobile phone (900 and 1800 MHz)-induced risks on oxidative stress and elements in kidney and testis of rats during pregnancy and the development of offspring. Biol Trace Elem Res 156:221–229

Piacentini R, Ripoli C, Mezzogori D, Azzena GB, Grassi C (2008) Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Ca(v)1-channel activity. J Cell Physiol 215:129–139

Platano D, Mesirca P, Paffi A, Pellegrino M, Liberti M, Apollonio F, Bersani F, Aicardi G (2007) Acute exposure to low-level CW and GSM-modulated 900 MHz radiofrequency does not affect Ba^{2+} currents through voltage-gated calcium channels in rat cortical neurons. Bioelectromagnetics 28:599–607

Senol N, Nazıroğlu M, Yürüker V (2014) N-acetylcysteine and selenium modulate oxidative stress, antioxidant vitamin and cytokine values in traumatic brain injury-induced rats. Neurochem Res 39:685–692

Sun FJ, Guo W, Zheng DH, Zhang CQ, Li S, Liu SY, Yin Q, Yang H, Shu HF (2013) Increased expression of TRPV1 in the cortex and hippocampus from patients with mesial temporal lobe epilepsy. J Mol Neurosci 49:182–193

Susankova K, Tousova K, Vyklicky L, Teisinger J, Vlachova V (2006) Reducing and oxidizing agents sensitize heat-activated vanilloid receptor (TRPV1) current. Mol Pharmacol 70:383–394

Uğuz AC, Nazıroğlu M (2012) Effects of selenium on calcium signaling and apoptosis in rat dorsal root ganglion neurons induced by oxidative stress. Neurochem Res 37:1631–1638