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INVOLVEMENT OF APOPTOSIS AND CALCIUM ACCUMULATION THROUGH TRPV1 CHANNELS IN NEUROBIOLOGY OF EPILEPSY

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Abstract—Calcium ion accumulation into the cytosol of the hippocampus and dorsal root ganglion (DRG) are main reasons in etiology of epilepsy. Transient receptor potential vanilloid type 1 (TRPV1) channel is a cation-permeable calcium channel found in the DRG and hippocampus. Although previous studies implicate TRPV1 channels in the generation of epilepsy, suppression of ongoing seizures by TRPV1 antagonists has not yet been investigated. We tested the effects of TRPV1-specific antagonists, capsazepine (CPZ) and 5′-iodoresiniferatoxin (IRTX) on the modulation of calcium accumulation, apoptosis and anticonvulsant properties in the hippocampus and DRG of pentylentetrazol (PTZ) and capsaicin (CAP) administrated rats. Forty rats were divided into five groups as follows; control, PTZ, CAP + PTZ, IRTX, and IRTX + PTZ. Fura-2 and patch-clamp experiments were performed on neurons dissected from treated animals by CAP and CPZ. PTZ and CAP + PTZ administrations increased intracellular free Ca^{2+} concentrations, TRPV1 current densities, apoptosis, caspase 3 and 9 values although the values were reduced by IRTX and CPZ treatments. Latency time was extended by application CPZ and IRTX although CAP produced acceleration of epileptic seizures. Taken together, these results support a role for TRPV1 channels in the inhibition of apoptosis, epileptic seizures and calcium accumulation, indicating that TRPV1 inhibition may possibly be a novel target in the DRG and hippocampus for prevention of epileptic seizures and peripheral pain.

Key words: apoptosis, calcium ion, epilepsy, hippocampus, TRPV1 channels, seizures.

INTRODUCTION

Epilepsy is affecting approximately 50 million (> 2–3%) of the population worldwide and represents acute transient complex neurobehavioral disorder. Increased excitability of epileptic neurons in various brain regions induces acute transient complex neurobehavioral disorders (Iannotti et al., 2014). Although considerable research is being conducted on long-term changes associated with epileptogenesis, certain cellular and molecular mechanisms involved in epileptogenesis are still unclear (Gonzalez-Reyes et al., 2013). To date, the condition cannot be cured although antiepileptic drugs provide partial control of seizures (Nazıroğlu and Yürekli, 2013). Molecular targets of most of these antiepileptic drugs are restricted to ion channel activity such as glutamate and transient receptor potential (TRP) channels that induce epileptic seizures and peripheral pain (Naziroglu, 2009, 2012a; Nazıroğlu, 2012). The drugs are also widely used in the treatment of neuropathic pain resulting from peripheral nerve damage of various origins (Moore et al., 2014). Chronic neuropathic pain resulting from cancer chemotherapeutic drugs, including paclitaxel and cisplatin, is also treated with antiepileptic drugs (Fallon, 2013). The antiepileptic drugs have also scarce effect on peripheral pain. For these reasons, novel drugs to treat epilepsy and peripheral pain are highly necessary.

Calcium ion (Ca^{2+}) is an important second messenger that has been shown to be responsible for a number of signal transduction pathways including neuronal excitability, metabolism, cell proliferation, and cell death (Nazıroğlu, 2007; Kumar et al., 2014). It is well known that Ca^{2+} is involved in the induction of epilepsy. One family of calcium channels comprises TRP cation channels that were first described in photoreceptors of *Drosophila* flyers (Nazıroğlu, 2011). The TRP vanilloid type 1 (TRPV1) cation channels have been identified as a polymodal activator molecule on sensory neurons such as the dorsal root ganglion (DRG) and trigeminal ganglion neurons which respond to various stimuli including noxious heat ($\geqslant$42 °C), acids and vanilloids such as capsaicin (hot chili pepper component) (Nagy et al., 2014). When TRPV1 channels are activated, Na^{+} and Ca^{2+} entry occurs resulting in increased neuronal excitability (Nazıroğlu, 2012). Initially the TRPV1 channel was expressed in the DRG neurons which have critical role in mediating peripheral pain (Nagy et al., 2014). Furthermore, TRPV1 channels are expressed in the hippocampus and dental gyrus regions of the brain and these areas are critical regions in epileptogenesis (Manna and

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Abbreviations: $[Ca^{2+}]_i$, intracellular Ca^{2+}; CAP, capsaicin; CHAPS, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate; CPZ, capsazepine; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; DTT, Dithiothreitol; EEG, electroencephalography; EGTA, ethylene glycol-bis[2-aminoethyl-ether]-*N,N,N,N*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IRTX, 5′-iodoresiniferatoxin; $NMDG^{+}$, N-methyl-D-glucamine; OLDA, N-oleoyldopamine; PTZ, pentylentetrazol; ROS, reactive oxygen species; SDU, Suleyman Demirel University; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid type 1.

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Umathe, 2012; Gonzalez-Reyes et al., 2013). Capsazepine (CPZ) is the first reported antagonist of the TRPV1 cation channels that has been extensively used as a standard competitive antagonist in pharmacological studies (Nagy et al., 2014).

Recent studies have reported that TRPV1 channels may be a novel antiepileptic potential target (Chen et al., 2013; von Rüden et al., 2014). In fact, it was reported that expression of TRPV1 has been increased in the dentate gyrus of mice with temporal lobe epilepsy (Bhaskaran and Smith, 2010) supporting to this epileptogenesis role. 5'-Iodoresiniferatoxin (IRTX) is a selective TRPV1 channel antagonist and it was recently observed that epileptiform neuronal spike activity was increased in hippocampal slices of rats by capsaicin incubation although the activity was blocked by IRTX incubation (Iannotti et al., 2014). Recent papers (Manna and Umathe, 2012; Chen et al., 2013; von Rüden et al., 2014) provide recent observation of CPZ antiepileptic action but they report only behavioral and expression observations instead of molecular analyses such as calcium ion accumulation and apoptosis.

The current study was conducted to investigate involvement of apoptosis and Ca^{2+} accumulation through TRPV1 channels in the pentylentetrazol (PTZ)-induced rat model by measuring intracellular free calcium ($[Ca^{2+}]_i$) concentrations, current densities, apoptosis, and latency time, plus caspase 3 and caspase 9 values.

EXPERIMENTAL PROCEDURES

Chemicals

Trypsin solution, sodium hydroxide, PBS buffer, dimethyl sulfoxide (DMSO), RPMI 1640 buffer, 5′-iodoresiniferatoxin (IRTX), capsaicin, PTZ, and EGTA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). APOPercentage assay kit was purchased from Biocolor (Belfast, Northern Ireland). Collagenase IV was purchased from Worthington Inc. (USA). All organic solvents such as *n*-hexane and ethyl alcohol) were purchased from Millipore (Darmstadt, Germany). Fura-2 acetoxymethyl ester was purchased from Invitrogen (Carlsbad, CA, USA). N-acetyl-Leu-Glu-His-Asp-7-amino-4-methylcoumarin (AC-LEHD-AMC) was purchased from Bachem (Bubendorf, Switzerland). Capsaicin was purchased from Santa Cruz Biotechnology (Istanbul, Turkey). All others reagents were of analytical grade. Reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagent containers were refilled. Buffers were stable at +4 °C for 1 month.

Animals

Forty male adult (12 weeks old) Wistar albino rats, weighing 200 ± 20 g, were used in the experiment. The rats were allowed 1 week to acclimatize to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages with bedding. The ambient temperature and relative humidity of the animal room were 21 ± 1 °C and 60 ± 5%, respectively and were subjected to an automatically controlled 12-h/12-h light/dark cycle (lights on at 7.00). Epilepsy induction and drug administrations were performed between a.m. 9.00 and 12.00 each day. The animals were allowed free access to standard pelleted food and tap water.

All behavioral experiments were carried out between 900 h and 1500 h. The experimental protocol was approved by the ethics committee of Suleyman Demirel University (SDU) (Protocol number and date: 2013-02). Additionally, all efforts were performed to minimize animal suffering and to use only that number of animals necessary to produce reliable statistical analyses.

Preparation of hippocampal neuron samples

The animals were killed by ether asphyxiation and cervical dislocation in accordance with SDU Experimental Animal legislation. Primary cultures of rat hippocampal neurons were prepared as previously described (Ghazizadeh and Naziroglu, 2014; Nazıroğlu et al., 2014). The neurons were plated at a density of $< 1 \times 10^6$ cells/ ml on culture dishes.

Preparation of DRG neuron samples

DRG neurons were prepared as described previously (Nazıroğlu et al., 2012a, 2012b; 2013a). The DRG neurons (T13-L5) were carefully dissected from peripheral nerve roots of anesthetized animals. The dissociated DRG neurons were plated in 3.5-cm culture dishes and incubated in a cell culture atmosphere containing 95% air and 5% CO_2 for at least 1 h before analyses.

Experimental design

There are different convulsive drug models for induction of epilepsy in experimental animals. One of the drug models is PTZ and it is the accepted valuable model animal model for studying epilepsy and its consequences on memory. The rats were divided into five groups as follows:

I. *Group control (n = 8):* Placebo was supplemented to the first group.
II. *Group PTZ (n = 8):* PTZ (60 mg/kg) administrated intraperitoneally to rats for induction of epilepsy (Kutluhan et al., 2009; Nazıroğlu et al., 2013b).
III. *Group capsaicin + PTZ (n = 8):* Capsaicin (120 mg/kg) was injected subcutaneously to animals in this group 30 min before administration of PTZ (60 mg/kg).
IV. *Group IRTX (n = 8):* IRTX (100 μg/kg) was intraperitoneally administered to these animals (Maione et al., 2007).
V. *Group IRTX + PTZ (n = 8):* IRTX (100 μg/kg) was intraperitoneally administered to these animals consisting the group 30 min before administration of PTZ (60 mg/kg).

The DRG and hippocampal neurons were dissected from *in vivo* treated animals and examined afterward as