Neuroscience 314 (2016) 170–178

TRPV1 RECEPTORS AUGMENT BASAL SYNAPTIC TRANSMISSION IN CA1 AND CA3 PYRAMIDAL NEURONS IN EPILEPSY

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Abstract—Temporal lobe epilepsy in human and animals is attributed to alterations in brain function especially hippocampus formation. Changes in synaptic activity might be causally related to the alterations during epileptogenesis. Transient receptor potential vanilloid 1 (TRPV1) as one of the non-selective ion channels has been shown to be involved in synaptic transmission. However, the potential role of TRPV1 receptors in synaptic function in the epileptic brain needs to be elucidated. In the present study, we used quantitative real-time PCR (qRT-PCR), western blotting, and immunohistochemistry to assess hippocampal TRPV1 mRNA expression, protein content, and distribution. Moreover, the effects of pharmacologic activation and inhibition of TRPV1 receptors on the slope of evoked field excitatory postsynaptic potentials (fEPSPs) were analyzed in CA1 and CA3 pyramidal neurons, after 3 months of pilocarpine-induced status epilepticus (SE). SE induced an upregulation of TRPV1 mRNA and protein content in the whole hippocampal extract, as well as its distribution in both CA1 and CA3 regions. Activation and inhibition of TRPV1 receptors (via capsaicin 1 μM and capsazepine 10 μM, respectively) did not influence basal synaptic transmission in CA1 and CA3 regions of control slices, however, capsaicin increased and capsazepine decreased synaptic transmission in both regions in tissues from epileptic animals. Taken together, these findings suggest that a higher expression of TRPV1 in the epileptic condition is accompanied by alterations in basal synaptic transmission.

Key words: temporal lobe epilepsy, TRPV1, hippocampus, CA1, CA3, synaptic transmission.

INTRODUCTION

Temporal lobe epilepsy (TLE) is a common neurological condition associated with spontaneous recurrent seizures. TLE is related to the structural and functional changes in the neuronal circuitry in temporal lobe structures which cause the brain to be more susceptible to seizure generation (Reid and Stewart, 1997; Leite et al., 2005; Bertram, 2009; Zhang et al., 2010; Upreti et al., 2012; Buckmaster, 2014). Transient receptor potential vanilloid 1 (TRPV1) has been recently demonstrated to participate in hippocampal synaptic function (Li et al., 2008; Gibson et al., 2008; Kauer and Gibson, 2009; Maione et al., 2009) and epileptic behavior (Nilius et al., 2007; Fu et al., 2009; Nazıroğlu and Övey, 2015), however its role in synaptic function of two related hippocampal subregions in an epileptic model is not clear.

TRPV1 as one of the non-selective ion channels is mostly permeable to Ca^{2+} ions and is activated by a wide range of stimuli, including heat above 42 °C, pH alterations, lipid ligands, and capsaicin (Szallasi and Blumberg, 1999; Clapham, 2003). Previous studies have thoroughly investigated the presence and distribution of TRPV1 receptors in the brain (Mezey et al., 2000; Roberts et al., 2004; Tóth et al., 2005; Cristino et al., 2006). Moreover, it has been shown that TRPV1 has critical roles in hippocampal synaptic transmission, neurotransmitter release, and plasticity (Li et al., 2008; Kauer and Gibson, 2009; Maione et al., 2009).

Recent evidences suggest that non selective ion channels, including TRPV1 play a significant role under pathological conditions in which excitability of neural cells in the brain, like seizures is subject to alterations (Nilius et al., 2007; Fu et al., 2009; Eslamizade et al., 2015). Interestingly, post-mortem studies showed that patients with epilepsy had a higher TRPV1 expression in their brain (Sun et al., 2013). Moreover, direct evidence implying contribution of TRPV1 receptors in epilepsy comes from a series of *in vivo* and *in vitro* studies. For instance, intracerebroventricular injection of anandamide,

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Abbreviations: ACSF, artificial cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; fEPSPs, field excitatory postsynaptic potentials; SE, status epilepticus; TLE, Temporal lobe epilepsy; TRPV1, Transient receptor potential vanilloid 1.

http://dx.doi.org/10.1016/j.neuroscience.2015.11.045
0306-4522/

an activator of TRPV1 evokes convulsions in PTZ model of epilepsy (Manna and Umathe, 2012). In contrary, systemic administration of activators of TRPV1, including piperine was demonstrated to have anti-seizure effects in mice (Chen et al., 2013). These findings indicate that different TRPV1 activators have potential impacts on the animal models of epilepsy. In addition, Bhaskaran and Smith (2010) showed that *in vitro* activation of TRPV1 receptors with capsaicin in dentate granule neurons provokes excitatory circuit activity and another study indicated that antagonizing TRPV1 receptors in CA3 region *in vivo* using capsazepine-induced antiepileptic effects (Gonzalez-Reyes et al., 2013). More recently we found that TRPV1 gained a different influence on the synaptic plasticity in the epileptic hippocampus (Saffarzadeh et al., 2015). In spite of these studies, a direct link between potential changes in TRPV1 and basal synaptic function in the epileptic hippocampus remained uninvestigated.

In the present study, we addressed changes in the expression and subsequently potential impact of TRPV1 activity in the basal synaptic transmission of two main vulnerable regions of the hippocampus in the pilocarpine model of epilepsy to compare it with normal tissues.

EXPERIMENTAL PROCEDURES

Animals and drug treatments

Male Wistar rats (100 ± 10 g) were kept at a 12-h light–dark cycle (lights on at 6 a.m.) and humidity-controlled animal house with water and food *ad libitum*. All experimental procedures have been done after proper animal handling to minimize stress. All experiments were approved by the animal care and use guidelines approved by the institutional ethics committee at Iran University of Medical Sciences and Shefa Neuroscience Research Center.

Pilocarpine injection

Rats received an intraperitoneal injection (i.p.) of methyl-scopolamine (1 mg/kg) 30 min prior to the injection of pilocarpine to reduce peripheral cholinergic effects of pilocarpine. A single dose of pilocarpine hydrochloride (380 mg/kg) was administrated i.p. After the pilocarpine injection, seizure behavior was observed for at least 2 h. Seizure behavior included being motionless after pilocarpine administration, forelimb clonus, rearing, and falling according to the categories 3–5 of Racine's classification (Racine, 1972). The animals experiencing status epilepticus (SE) were monitored through 3 days/week for 1 h in a period of 3 months. These animals were accepted as the epilepsy model when they experienced spontaneous seizure behavior 2–3 times per week. Control group was age-matched with 3-month epileptic rats.

Quantitative real-time PCR (qRT-PCR)

Tissue samples were obtained from isolated hippocampi of epileptic and control age-matched rats. Total RNA was extracted from tissue samples by the Gene ALL Ribospin Kit (GeneALL, South Korea). Total RNA (500 ng) was used for cDNA synthesis by Maxima first-strand cDNA synthesis kit (Thermo Scientific, MA, USA). The Maxima first-strand cDNA synthesis kit contains oligo $(dT)_{18}$ and random hexamer primers to prime synthesis of first-strand cDNA. β-actin primers were taken from previous studies (Peinnequin et al., 2004). The primers of TRPV1 designed by AlleleID® 7.50 (Premier Biosoft International, Palo Alto, Canada). The primer pairs used in this study span exon–exon junctions or are located on different exons. To generate the lowest C_t value and a sharp peak, the optimal primer concentration was determined for each primer pair. The efficiency of qRT-PCR was approximately two for each pair of primers which was evaluated using serial 1:2 dilutions of template cDNA, on a CFX 96 Real-Time System (Bio-Rad, CA, USA). Real-time amplifications, using Eva Green detection chemistry, were run in duplicate with the CFX 96 Real-Time System (Bio-Rad). The primer sequences for the gene and internal control were as follows: β-actin Forward primer 5′-AAG TCC CTC ACC CTC CCA AAA G-3′, β-actin Reverse primer 5′-AAG CAA TGC TGT CAC CTT CCC-3′ with amplicon length 98 bp and TRPV1 Forward primer; 5′-TACTATCGGCCTGTGGAAGG-3′, TRPV1 Reverse primer; 5′-ATTGAATCCCTCGGAAGAAGAAG-3′ with amplicon length 127 bp. Reactions were prepared in a total volume of 20 μl containing: 5 μl template, 0.5 μl of each 10 μM primer (Macrogen, South Korea), 4 μl of 5× HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia) and 10 μl RNase/DNase-free sterile water (Sigma, Germany). The cycle conditions were set as follows: initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 30 s, primer annealing at 63 °C for 30 s and elongation at 72 °C for 30 s. This step was followed by a melting curve analysis (range: 65–95 °C, temperature increased by steps of 0.5 °C every 5 s). In order to minimize the number of rats for molecular studies, from two hippocampi removed from each brain one was randomly assigned for qRT-PCR and the other for western blotting. We used the $2^{-\Delta\Delta Ct}$ method to analyze the relative changes in gene expression from qRT-PCR experiments (Livak and Schmittgen, 2001). The Cts of target genes were normalized to the levels of β-actins as an endogenous control in each group.

Western blot

Hippocampi were removed from epileptic rats after 3 months of SE onset. Control group was age-matched with epileptic rats. The samples were homogenized in lysis buffer (pH 7.4, containing 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonylfluoride, 1 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 μg/ml leupeptin). The proteins were quantified using a Bradford test. For western blot analysis, protein samples were transferred to polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany) for 30 min. Then membranes were incubated overnight at 4 °C with rabbit polyclonal anti-rat TRPV1 primary antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, Germany) (Saffarzadeh et al., 2015) and mouse monoclonal antibody to label β-actin rabbit monoclonal antibody (1:1000, Sigma, St.