Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hearing Research 201 (2005) 132–144
HEARING RESEARCH
www.elsevier.com/locate/heares

Changes in transient receptor potential cation channel superfamily V (TRPV) mRNA expression in the mouse inner ear ganglia after kanamycin challenge

Tadashi Kitahara [a], Ha-Sheng Li [a], Carey D. Balaban [a,b,c,*]

[a] *Department of Otolaryngology, University of Pittsburgh School of Medicine, 107 Eye and Ear Institute, Room 153, 203 Lothrop Street, Pittsburgh, PA 15213, USA*
[b] *Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA, USA*
[c] *Department of Communication Sciences, University of Pittsburgh, Pittsburgh, PA, USA*

Received 6 August 2004; accepted 10 September 2004

Abstract

The transient receptor potential cation channel subfamily V (TRPV) is a non-specific cation ion channel receptor family that is gated by heat, protons, low extracellular osmolarity and arachidonic acid derivatives. Since some of these endogenous agonists of TRPV receptors are reactive oxygen intermediates produced by lipoxygenases, it has been hypothesized that some members of the TRPV family may respond to challenges by reactive oxygen species. This study used real-time PCR to quantitatively track changes in TRPV1-4 mRNA expression in the spiral, vestibular, and trigeminal ganglia and the kidney from kanamycin (KM)-treated mice. TRPV1, TRPV2, TRPV3 and TRPV4 mRNAs were expressed in spiral and vestibular ganglia, and TRPV2 and TRPV1 mRNAs were most predominant in control mice. After KM (700 mg/kg s.c. b.i.d., 14 days), TRPV1 mRNA and protein expression were significantly up-regulated both in the spiral and vestibular ganglia, but expression was unaffected in the trigeminal ganglion and kidney. Real-time PCR also demonstrated a significant down-regulation in TRPV4 mRNA expression in the inner ear ganglia and kidney after KM treatment. All these mRNA and protein expression changes were eliminated by simultaneous administration of dihydroxybenzoate (300 mg/kg s.c. b.i.d., 14 days), an anti-oxidant that blocks KM ototoxicity. It is proposed that up-regulated TRPV1 expression during KM exposure may promote ganglion cell survival by contributing to neuronal depolarization, with KM-induced tinnitus and dizziness as consequences.

Keywords: Transient receptor potential cation channel subfamily V (TRPV); Vanilloid receptors; Kanamycin; Dihydroxybenzoate; Inner ear; Real-time PCR

1. Introduction

The transient receptor potential cation channel subfamily V (TRPV) is a non-specific cation ion channel receptor family with six identified members (Montell et al., 2002; Benham et al., 2003; Nilius et al., 2004). The best-known member of the family is TRPV1, also called vanilloid receptor type 1 (VR-1). TRPV1 is located predominantly in dorsal root ganglia (DRG) and

Abbreviations: CT, cycle threshold; Cx, cerebral cortex; DHB, 2,3-dihydroxybenzoate; KM, kanamycin; KY, kidney; LIR, like immunoreactivity; PCR, polymerase chain reaction; SG, spiral ganglion; TG, trigeminal ganglion; TRPV, transient receptor potential cation channel subfamily V; VG, vestibular ganglion

* Corresponding author. Tel.: +1 412 647 8050/2298; fax: +1 412 647 0108.
E-mail address: cbalaban@pitt.edu (C.D. Balaban).

0378-5955/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2004.09.007

trigeminal ganglia, and it is responsive to noxious heat (>43 °C) and protons (pH < 5.9) (Caterina et al., 1997). Endogenous capsaicin-like ligands include arachidonic acid products such as hydroxyperoxyeicosatetraenoic acid (HPETE), hydroxyeicosatetraenoic acid (HETE) (Hwang et al., 2000) and arachidonyl-dopamine (Huang et al., 2002; Toth et al., 2003). Both TRPV1 and 5-lipoxygenase (the enzyme that produces 5-HPETE and 5-HETE) are expressed by spiral and vestibular ganglion cells in the inner ear (Balaban et al., 2003). TRPV2, also called vanilloid receptor like protein-1 (VRL-1), is expressed most heavily in the DRG, trigeminal ganglia, spleen and intestines (Caterina et al., 1999) and TRPV4, also called vanilloid receptor-like protein-2 (VRL-2), is expressed preferentially in kidney, trachea, spleen and trigeminal ganglia (Liedtke et al., 2000; Wissenbach et al., 2000). Although TRPV2 is sensitive only to noxious heat >53 °C (Caterina et al., 1999), TRPV4 (also called VRL-2, VR-OAC, TRP12 or OTRPC4) expression confers sensitivity to both a physiological range of temperature changes (Guler et al., 2002) and low extracellular osmolarity (Liedtke et al., 2000). TRPV5 and TRPV6 are both highly calcium selective channels and are activated by low intracellular calcium and hyperpolarization (Montell et al., 2002).

It has been proposed that some members of the TRPV family serve an important role in nociception and hyperalgesia (Stucky et al., 1998; Shin et al., 2002). However, other studies suggest that TRPV1 expression is regulated dynamically as a component of responses to potentially injurious challenges. For example, TRPV1 was up-regulated in dorsal root ganglia after local inflammation was induced in the periphery by either capsaicin (Ha et al., 2000) or Freund's complete adjuvant injections (Amaya et al., 2003). Veldhuis et al.'s (2003) recent report that activation of TRPV1 affords neuroprotection against excitotoxic insult suggests that TRPV1 up-regulation may be an endogenous neuroprotective mechanism to promote cell survival.

The hypothesis that TRPV1 up-regulation occurs in response to challenges that are potentially injurious was tested in a factorial design in CBA mice using kanamycin (KM) administration as a reactive oxygen species-generating challenge and 2,3-dihydroxybenzoate (DHB) administration as an antioxidant countermeasure (Wu et al., 2001). Treatment with this KM protocol is known to produce degeneration of cochlear outer hair cells and some vestibular hair cells; these effects are blocked by co-administration of DHB (Wu et al., 2001). Since the combined direct effects of aminoglycosides and secondary effects of hair cell degeneration appear to be factors in eliciting degeneration of spiral and vestibular ganglion cells (Kellerhals et al., 1967; Spoendlin, 1975; Webster and Webster, 1981; Bichler et al., 1983; Hinojosa and Lerner, 1987; Sera et al., 1987; Harada et al., 1991; Zimmermann et al., 1995; Dodson, 1997; Sone et al., 1998), this protocol concurrently provides multiple challenges to survival of inner ear ganglion cells. By contrast, the nephrotoxic effects provide an example of responses to the direct cellular effects of KM alone.

2. Materials and methods

Experimental procedures involving animals reported in this study were performed according to NIH guidelines and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC).

2.1. Experimental animals and drug administration

A two-by-two factorial design was used to assess effects of (1) KM (700 mg/kg) treatment (saline vehicle versus KM dissolved in saline), (2) antidoxidant treatment with 300 mg/kg DHB ($NaHCO_3$ vehicle versus DHB dissolved in $NaHCO_3$). The doses and frequency of treatment (twice daily for 14 days) followed a model established by Wu et al. (2001), who demonstrated convincingly that both the histopathological and physiological evidence of ototoxicity from this KM dose are blocked by the selected DHB dose.

Four-week-old CBA/J (CBA) mice were divided into four groups ($n = 10$ in each) as follows: a saline and $NaHCO_3$ vehicle injected control (CONT) group, a KM and $NaHCO_3$ vehicle injected (KM) group, a KM and dihydroxybenzoate-injected (KM/DHB) group and a saline and DHB-injected (DHB) group. Kanamycin sulfate (USB Corporation, Cleveland, OH, USA) was dissolved in physiological saline at a concentration of 35 mg KM base/ml; a dose of 700 mg of KM base/kg body was achieved by injecting 0.02 ml/g body weight. DHB (Aldrich Chemical Corporation, Milwaukee, WI, USA) was dissolved in 2.5% $NaHCO_3$ at a concentration of 15 mg/ml (pH between 7.0 and 8.0) so that a subcutaneous injection of 0.02 ml/g body weight produced a final dose of 300 mg/kg. On the 15th day of the protocol, the mice were euthanized according to the methods described below.

2.2. Real-time PCR (TaqMan PCR)

2.2.1. Animals and tissue preparation

Three adult male mice (out of 10) in each group were euthanized with a pentobarbital overdose (100 mg/kg, i.p.). The left and right inner ear tissues were dissected from the osseous labyrinth in cold PBS (pH 7.4) (Biofluids, Rockville, MD) under a stereomicroscope. The bony wall of the cochlea was removed with fine forceps and probes. The organ of Corti, the tectorial membrane and the basilar membrane were peeled en masse from