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Research paper

Prophylactic and therapeutic functions of T-type calcium blockers against noise-induced hearing loss

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Abstract

Cochlear noise injury is the second most frequent cause of sensorineural hearing loss, after aging. Because calcium dysregulation is a widely recognized contributor to noise injury, we examined the potential of calcium channel blockers to reduce noise-induced hearing loss (NIHL) in mice. We focused on two T-type calcium blockers, trimethadione and ethosuximide, which are anti-epileptics approved by the Food and Drug Administration. Young C57BL/6 mice of either gender were divided into three groups: a 'prevention' group receiving the blocker via drinking water before noise exposure; a 'treatment' group receiving the blocker via drinking water after noise exposure; and controls receiving noise alone. Trimethadione significantly reduced NIHL when applied before noise exposure, as determined by auditory brainstem recording. Both ethosuximide and trimethadione were effective in reducing NIHL when applied after noise exposure. Results were influenced by gender, with males generally receiving greater benefit than females. Quantitation of hair cell and neuronal density suggested that preservation of outer hair cells could account for the observed protection. Immunocytochemistry and RT-PCR suggested that this protection involves direct action of T-type blockers on α1 subunits comprising one or more Ca_v3 calcium channel types in the cochlea. Our findings provide a basis for clinical studies testing T-type calcium blockers both to prevent and treat NIHL.

Keywords: Noise-induced hearing loss; Mouse; T-type calcium channel; Trimethadione; Ethosuximide

1. Introduction

Excessive noise is the predominant cause of permanent sensorineural hearing loss. At least 30 million people in the United States encounter hazardous levels of noise at work, particularly in jobs such as construction, mining, agriculture, manufacturing, transportation, and in the military (Le Prell et al., this volume; Bohnker et al., 2002; Henderson and Salvi, 1998, 2003; Seixas et al., 2005). The incidence of noise-induced hearing loss (NIHL) continues to grow, moreover, partly due to growing popularity of portable music players with highly efficient headphones (Fligor and Cox, 2004; Serra et al., 2005). Although several promising approaches have been identified for reducing NIHL (LeFebvre et al., 2002; Niu and Canlon, 2002; Kopke et al., 2005; Lynch and Kil, 2005; Le Prell et al., this volume; Gagnon et al., this volume), there are currently no pharmacologic agents approved by the Food and Drug Administration (FDA) for this purpose.

Typically there are two phases of hearing loss after noise, a temporary threshold shift (TTS) that is most prominent in the first 24 h, but may extend for 1–2 weeks, and permanent threshold shift (PTS) (Clark, 1991; Quaranta et al., 1998; Nordmann et al., 2000). Previous studies have

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suggested that TTS and PTS are two distinct phenomena with different cellular pathological changes. TTS may reflect reversible buckling of the pillar cell bodies (Nordmann et al., 2000), temporary strial edema and reduction of the endocochlear potential (Hirose and Liberman, 2003), or excitotoxic damage to afferent fibers (Pujol and Puel, 1999). Histopathologic correlates of PTS include permanent stereocilia damage, hair cell loss, and degeneration of afferent fibers in the organ of Corti (Slepecky, 1986; Saunders et al., 1991).

Molecular mechanisms underlying NIHL are only partly known, but probably include dysregulation of calcium (LeFebvre et al., 2002; Le Prell et al., this volume; Henderson et al., 2006). Elevated intracellular calcium levels may impair mitochondrial function and compromise a host of cellular functions (Luer et al., 1996). Calcium elevation affects cell membranes by activation of phospholipase A_2 and C, resulting in hydrolysis of membrane phospholipids, release of free fatty acids (Farooqui et al., 2004) and lipid peroxidation (Sullivan et al., 2004). Disturbance in calcium homeostasis contributes to trauma-induced neuronal injury and age-related neuronal loss (Zipfel et al., 2000; Toescu et al., 2004). In the noise-exposed cochlea, calcium may participate in both hair cell and neuronal damage (Minami et al., 2004; LeFebvre et al., 2002; Pujol and Puel, 1999). Calcium homeostasis in hair cells and spiral ganglion neurons (SGNs) is maintained by regulatory proteins such as calmodulin and calbindin (e.g., Hansen et al., 2003; Hackney et al., 2005), and by several types of calcium channels (Niedzielski et al., 1997; Morley et al., 1998; Parks, 2000; Lopez et al., 2003). Voltage-gated calcium channels (VGCCs) play a key role in calcium entry into neurons and control various calcium-dependent functions, including intracellular signaling and gene expression (Snutch and Reiner, 1992; Fuchs, 1996; Kochegarov, 2003; Errington et al., 2005). VGCCs can be divided into two groups: low-voltage activated calcium channels that respond to small (~10 mV) changes in the resting membrane potential, and high-voltage activated calcium channels that require stronger (~30 mV) depolarization to open (Perez-Reyes, 1998; Lacinova et al., 2000; Yunker and McEnery, 2003; Layton et al., 2005; Triggle, 2006). Diltiazem, a blocker of L-type calcium channels (high-voltage activated channels) was reported to attenuate NIHL (Heinrich et al., 1997). However, other studies (Boettcher, 1996; Ison et al., 1997; Boettcher et al., 1998) have not supported any protective effect of blocking L-type calcium channels. Recently, blockers of T-type calcium channels (low-voltage activated channels) were found to prevent cisplatin-induced death of cochlear cells in vitro (So et al., 2005). Whether these agents are able to prevent or treat NIHL has not been tested. Here we test the protective actions against NIHL of T-type calcium blockers trimethadione and ethosuximide, anti-epileptic drugs approved by the FDA, when administered in drinking water to inbred C57BL/6 mice. We show that both agents can reduce NIHL, whether applied before or after noise exposure, although efficacy may depend on gender. The primary action of T-type calcium antagonists may be to promote hair cell survival.

2. Methods

2.1. Animals

All animal procedures were approved by the Animal Studies Committee at Washington University in St. Louis. The study included 44 male and female C57BL/6J mice aged 2–3 months, purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed three to five per cage in a noise-controlled environment on a 12 h light/dark cycle with light onset at 6:00 a.m.

2.2. Drug application

Animals were subject to one of two protocols, a 'prevention' protocol under which drugs were administered prior to a single noise exposure, and a 'treatment' protocol wherein drugs were administered only after noise. Trimethadione and ethosuximide were obtained from Sigma Chemical Co. (St. Louis, MO). Pilot experiments led us to select dosages of 200 mg/day/kg body weight for trimethadione and 1.5 g/day/kg body weight for ethosuximide. Dosages were kept constant throughout the experimental period by monitoring the amount of water uptake and body weight measured every three days. Drinking water containing the drug was kept in dark bottles and changed every three days. Animals in the prevention group received trimethadione in their drinking water for three weeks prior to noise. Animals in the therapy group received trimethadione or ethosuximide beginning immediately after noise exposure in drinking water for two weeks.

2.3. Noise exposure

As described previously (e.g., Ohlemiller et al., 2000), noise exposures were performed in a foam-lined, double-walled soundproof room (Industrial Acoustics). The noise exposure apparatus consisted of a $21 \times 21 \times 11$ cm wire cage mounted on a pedestal inserted into turntable. The cage was rotated at 1 revolution/80 s. A Motorola KSN1020A piezo ceramic speaker (four total) was attached to each side of a metal frame surrounding the cage. Opposing speakers were driven by independent channels of a Crown D150A power amplifier. Noise was generated by two General Radio 1310 generators and filtered to 4.0–45.0 kHz by Krohn-Hite 3550 filters. The overall noise level was measured at the center of the cage using a B&K 4135 1/4 in. microphone in a combination with a B&K 2231 sound level meter set to broadband (0.2 Hz–70 kHz). Mice were exposed in pairs at 110 dB SPL for 30 min.