Neuroscience 228 (2013) 23–35

ALPHA2-ADRENERGIC RECEPTORS IN SPIRAL GANGLION NEURONS MAY MEDIATE PROTECTIVE EFFECTS OF BRIMONIDINE AND YOHIMBINE AGAINST GLUTAMATE AND HYDROGEN PEROXIDE TOXICITY

J. CAI,[a,b] J. LI,[b,c] W. LIU,[b,c] Y. HAN[a] AND H. WANG[a,b,c]*

[a] *Otolaryngology-Head and Neck Surgery, Provincial Hospital Affiliated to Shandong University, Jinan, PR China*

[b] *Shandong Provincial Key Laboratory of Otology, Jinan, PR China*

[c] *Institute of Eye and ENT, Provincial Hospital Affiliated to Shandong University, Jinan, PR China*

Abstract—Brimonidine, an alpha2-adrenergic receptor (α_2-AR) agonist, is thought to be neuroprotective in some types of neurons via the activation of α_2-AR. However, it is still unknown whether the α_2-ARs exist in cochlear spiral ganglion neurons (SGNs). The authors aimed to demonstrate the presence and localization of α_2-ARs in rat-cultured SGNs and to investigate the effect of brimonidine on glutamate- and hydrogen peroxide (H_2O_2)-induced damage in the primary-cultured rat SGNs. The expression of α_2-ARs was determined by reverse transcription-polymerase chain reaction, Western blot analysis and immunofluorescence. Then SGNs were exposed to glutamate or H_2O_2 respectively with or without brimonidine. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. Apoptosis was determined by acridine orange and Hoechst 33342/propidium iodide double staining. The protein expressions of α_2-ARs, Bax, Bcl-2, Caspase-9, Caspase-3, p-ERK1/2, iNOS, and artemin were determined by Western blot respectively. The cell viability was markedly reduced after exposure of glutamate (1 mM) or H_2O_2 (300 μM) to SGNs. Treatment with brimonidine protected SGNs against glutamate- or H_2O_2-induced cell damage, enhanced SGNs survival, decreased the elevation of Bax, Caspase-9, Caspase-3, p-ERK1/2, and artemin triggered by glutamate or H_2O_2, and altered the expressions of Bcl-2 and iNOS. These protective effects of brimonidine can be reversed by yohimbine. Overall, the study describes the localization of α_2-ARs in rat-cultured SGNs and indicates that brimonidine, which may work directly via interaction with α_2-ARs, attenuates glutamate- and H_2O_2-induced damage in SGNs by Caspase-dependent modes as well as Caspase-independent modes. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: α_2-adrenergic receptors, spiral ganglion neurons, brimonidine, glutamate, hydrogen peroxide, yohimbine.

INTRODUCTION

The alpha2-adrenergic receptors (α_2-ARs), which mediate physiological responses to nor-adrenaline and adrenaline, consist of three subtypes: α_{2a}, α_{2b}, α_{2c} (Bylund et al., 1992; Regan and Cotecchia, 1992). These receptors are encoded by three different genes but all couple to the G_i/G_o subfamily of G proteins. They are involved in regulation of analgesia, sedation/hypnosis, behavior, endocrine, cardiovascular and metabolic functions (Link et al., 1996; MacMillan et al., 1996; MacDonald et al., 1997; Kable et al., 2000). Recent studies show that the activation of α_2-ARs has been implicated in enhanced neuronal survival *in vivo* (Knels et al., 2008) and *in vitro* (Wheeler et al., 1999; Donello et al., 2001; WoldeMussie et al., 2001). Hence, α_2-AR agonists form one of the most studied neuroprotective agents (Wheeler et al., 1999; Vidal-Sanz et al., 2001b; Lai et al., 2002; Dong et al., 2008). Brimonidine, also known as UK-14304, a high selective α_2-AR agonist, has been shown to protect retinal ganglion cells in experimental models of optic nerve degeneration (Yoles et al., 1999), chronic ocular hypertension (WoldeMussie et al., 2001), transient ischemia (Vidal-Sanz et al., 2001a; Avilés-Trigueros et al., 2003; Goldenberg-Cohen et al., 2009), and photoreceptor degeneration (Wen et al., 1996).

Although the mechanisms for these protections are not completely understood, available data show that brimonidine can decrease adenyl cyclase (Dong et al., 2008), inhibit voltage-gated Ca^{2+} channels (Han and Wu, 2002), activate receptor-operated K^+ channels (DeBock et al., 2003), and up-regulate anti-apoptotic genes such as bcl-2, bcl-xl, and several growth factors (Lai et al., 2002; Lönngren et al., 2006). Most importantly, brimonidine can alleviate glutamate-induced neurotoxicity and prevent an ischemia-induced rise in extracellular glutamate (Donello et al., 2001). On the other hand, studies have shown that brimonidine

*Correspondence to: H. Wang, Otolaryngology-Head and Neck Surgery, Provincial Hospital Affiliated to Shandong University, Jinan, PR China. Tel: +86-531-85187583; fax: +86-531-87980304.

E-mail address: wang.hb7585@hotmail.com (H. Wang).

Abbreviations: α_2-ARs, alpha2-adrenergic receptors; AO, acridine orange; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; H_2O_2, hydrogen peroxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NSE, neutron specific enolase; PBS, phosphate-buffered saline; PI, propidium iodide; RT-PCR, reverse transcription-polymerase chain reaction; SGNs, spiral ganglion neurons; TBST, Tris-buffered Saline-Tween; UV, ultraviolet.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.10.004

completely abolished reactive oxygen species generation, and attenuated cellular impairment induced by hydrogen peroxide (H_2O_2), thereby preventing cell from oxidative stress-induced apoptosis (Knels et al., 2008; Ozdemir et al., 2009).

Excitotoxicity injury and oxidative damage to spiral ganglion neurons (SGNs) are basic mechanisms for many types of sensorineural hearing disorders, such as noise-induced hearing loss, age-related hearing loss, cochlear ischemia–reperfusion injury, etc. (Huang et al., 2000). As we know, glutamate is a major excitatory neurotransmitter at the synapse between the cochlear inner hair cells and the dendrites of the SGNs (Puel, 1995; Hakuba et al., 2000). Excessively released glutamate and the failure of its removal from synaptic clefts can lead to the excitotoxicity of cochlear afferent neurons, resulting in a swelling and destruction of the afferent nerve terminals as well as a reduction in the number of neurons (Pujol et al., 1993; LePrell et al., 2001). Traumatic noise, cochlear ischemia, aminoglycoside antibiotics, and so forth can cause such an excessive release of glutamate from the inner hair cell into the synaptic cleft (Dodson, 1997; Hakuba et al., 1997, 2000; Jäger et al., 1998; Matsubara et al., 1998). The application of glutamate or glutamate agonists results in functional deficits and swelling of auditory neurons equivalent to those observed after noise exposure (Puel et al., 1998; Yamasoba et al., 2005). A study directly shows that apoptosis is induced by the neurotoxic effect of high concentrations of glutamate on neurites and SGNs (Steinbach and Lutz, 2007).

It is well known that free radicals, which include reactive oxygen species and reactive nitrogen species, are essential for cellular life processes. However, in excess, they damage cellular lipids, proteins, and DNA, and trigger apoptotic pathways (Figueiredo-Pereira et al., 1998; Chen et al., 2008). In fact, oxidative stress is central to current theories of aging- and noise-induced hearing loss (Someya and Prolla, 2010). H_2O_2 has been widely used as an inducer of oxidative stress in a wide range of cell types including neurites and spiral ganglion cells (Satoh et al., 1996).

As brimonidine has been shown to protect retinal ganglion cells in various experimental models, we hypothesize that brimonidine can protect cochlear SGNs from glutamate and H_2O_2 injury, which may represent a novel strategy for the treatment of sensorineural hearing disorders. The prerequisite for this hypothesis is the existence of α_2-ARs in SGNs. While a study found that noradrenaline, perhaps acting through α_2-ARs, attenuated the inward current evoked by GABA in cultured SGNs, causing an increased excitability of the SGNs (Zha et al., 2007), the precise location of α_2-ARs in SGNs, still remains unclear and need a comprehensive histological research to address the distribution of α_2-ARs in SGNs. Therefore, the present study was designed to determine whether the α_2-ARs exist in SGNs as well as whether brimonidine possesses a neuroprotective action against glutamate and H_2O_2 on primary-cultured SGNs via the activation of α_2-ARs and, if so, the possible mechanisms underlying the actions of such stresses.

EXPERIMENTAL PROCEDURES

Materials

Dulbecco's modified Eagle's medium (DMEM) with high glucose and fetal calf serum was obtained from GIBC (USA). Brimonidine (UK-14,304), yohimbine hydrochloride (Y-3125), poly-L-lysine, and L-glutamine were purchased from Sigma (St. Louis, MO). Primary antibodies used in this work included anti-α_{2a}-AR antibody (Thermo savant, Holbrook, NY, USA), anti-α_{2b}-AR antibody (Abnova, Taiwan, China), anti-α_{2c}-AR antibody, antineutron specific enolase (NSE) antibody, Caspase-9, iNOS (Abcam, Cambridge, MA, USA), Artemin (Abbiotec, San Diego, CA), Caspase-3, Bcl-2, Bax, p-ERK1/2, and β-actin (Santa Cruz Biotechnology, CA, USA). BCA protein assay kit was a product from Shenergy Biocolor Bioscience & Technology Company (Shanghai, China). RevertAid First Strand cDNA synthesis kit was obtained from Fermentas (Burlington, Ontario, Canada). All other agents used in this experiment were purchased from Sigma (St. Louis, MO, USA).

Primary cultures of rat SGNs

Rats at age less than 5 postnatal days (majority at day 3) were used to obtain cultured SGNs. After anesthesia with pentobarbital sodium, animals were decapitated at the base of the foramen magnum, the cranium was bisected along the sagittal suture and the brain halves were removed, then the temporal bone was put in phosphate-buffered solution (PBS). After the bulla of the temporal bone was opened under the microscope, the cochlea was exposed. The capsule of the inner ear as well as the spiral ligament and the organ of corti were removed. Rosenthal's cannal was isolated and placed into Ca^{2+}–Mg^{2+}-free Hank's balanced salt solution (HBSS) containing 0.125% trypsinase for digestion (15 min, 37 °C), then the tissue was eluted with the plating medium DMEM intermingled with 10% fetal bovine serum and 100 U/ml penicillin. The cells were collected by centrifugation at 1000 rpm for 5 min, resuspended and plated in poly-L-lysine-coated 6, 24 or 96 well plates (Corning, USA) at a density of 1.0×10^5 cells/ml for culture under normoxic conditions (20% O_2, 5% CO_2, 75% N_2 at 37 °C).

Identification of SGNs

Primary SGNs were inoculated in a poly-L-lysine-coated 24-well plate for 48 h, then fixed in 4% paraformaldehyde for 15 min, and permeabilized in PBS containing 0.3% Triton X-100 6 min and blocked by 10% goat serum 30 min at 37 °C in a humid atmosphere. Subsequently, the cells were incubated with primary anti-NSE antibody for 16–18 h at 4 °C at a concentration of 1:400 diluted, and then stained with secondary goat-anti-rabbit Cy3 antibody (Liu et al., 2011). The images were obtained using inverted phase contrast and fluorescence microscope.

Detection of α_2-ARs in SGNs

To determine the types and subunits of α_2-ARs in SGNs, we performed analyses of α_2-ARs gene expressions and protein expressions in SGNs, together with an immunocytochemistry study.

mRNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated from primary SGNs using Trizol (Invitrogen, USA). The mRNA expressions of α_{2a}-AR, α_{2b}-AR and α_{2c}-AR genes were examined using RT-PCR with M-MuL V reverse transcriptase in the presence of

Download English Version:

https://daneshyari.com/en/article/6275275

Download Persian Version:

https://daneshyari.com/article/6275275

Daneshyari.com