Auris Nasus Larynx xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Stress hormonal changes in the brain and plasma after acute noise exposure in mice

Sang Gyun Jin, Min Jung Kim, So Young Park, Shi Nae Park *

Department of Otorhinolaryngology–Head and Neck Surgery, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Republic of Korea

ARTICLE INFO

Article history:
Received 14 May 2016
Accepted 20 July 2016
Available online xxx

Keywords:
5-HIAA
Cochlea
Noise-induced hearing loss
Norepinephrine
Organ of Corti
Serotonin
Stress hormone

ABSTRACT

Objective: To investigate the effects of acute noise stress on two amine stress hormones, norepinephrine (NE) and 5-hydroxyindoleacetic acid (5-HIAA) in the brain and plasma of mice after noise exposure.

Methods: Mice were grouped into the control and noise groups. Mice in the noise group were exposed to white noise of 110 dB sound pressure level for 60 min. Auditory brainstem response thresholds, distortion product otoacoustic emissions, the organ of Corti grading scores, western blots of NE/5-HIAA in the whole brain and hippocampus, and the plasma levels of NE/5-HIAA were compared between the two groups.

Results: Significant hearing loss and cochlear damage were demonstrated in the noise group. NE and 5-HIAA in the hippocampus were elevated in the noise group ($p = 0.019/0.022$ for NE/5-HIAA vs. the control). Plasma levels of NE and 5-HIAA were not statistically different between the groups ($p = 0.052/0.671$ for NE/5-HIAA).

Conclusion: Hearing loss with outer hair cell dysfunction and morphological changes of the organ of Corti after noise exposure in C57BL/6 mice proved the reliability of our animal model as an acute noise stress model. NE and 5-HIAA are suggested to be the potential biomarkers for acute noise stress in the hippocampus.

1. Introduction

Noise is a well-known stressor and has been documented to influence various monoamine levels in different brain regions as well as causes noise-induced hearing loss (NIHL) [1]. Previous study showed that the brain is the key organ to alter the biogenic amines after noise exposure [2]. Studies have also documented the responsiveness of the endocrine system toward the stress of auditory stimuli such as excessive noise and the interactions between the stress and brain including the auditory system [3–5]. Excessive noise damages the cochlear hair cells and nerve synapses [6]. White-noise overstimulation is a widely known stress inducer and higher intensities of 90 and 105 dB can induce hormonal changes [7] by stimulation of two major hormonal systems of the body, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenomedullary system [8,9].

Various studies have shown a clinical significance of stress-related hormones such as cortisol, epinephrine, norepinephrine (NE), metabolites of serotonin (5-hydroxyindoleacetic acid, 5-HIAA), and a metabolic end product of 5-hydroxytryptamine (5-HT) in stress conditions although the results are not conclusive yet [9,10]. Among the stress hormonal changes, NE increases in the plasma as well as in the brain such as the

* Corresponding author. Fax: +82 2 595 1354.
E-mail address: snparkmd@catholic.ac.kr (S.N. Park).

http://dx.doi.org/10.1016/j.anl.2016.07.013
0385-8146/

hippocampus and hypothalamic paraventricular nucleus region [10–13]. Under stressful circumstances, serotonin (5-HT) has been found to be synthesized and released in the brain areas, and it plays a role in the activation of the HPA axis [14,15]. High performance liquid chromatography (HPLC) has been employed in the clinical area to determine the neurotransmitters of stress hormones in biological fluids including 5-HT, dopamine (DA), NE, and 5-HIAA [16].

Among the animal models, C57BL/6 mice is particularly vulnerable to noise exposure. Broadband noise of 110 dB SPL for 60 min sustains significant noise-induced hearing loss [17]. The susceptibility of C57BL/6 mice to noise has also been demonstrated at the age of 1 mo with ABR threshold shifts at all frequencies and decreased DPOAEs [18]. While there have been clinical and basic researches that suggest the interactions between noise and stress hormones, few studies showed the stress hormonal changes after acute noise stress in the brain and plasma simultaneously. In this study, we investigated the effects of acute noise stress on two amine stress hormones (NE and 5-HIAA) in the brain and plasma of mice with NIHL to determine a potential biomarker for noise stress in the body.

2. Materials and methods

2.1. Animals and noise exposure

Sixteen male C57BL/6 mice aged 1 mo were purchased from the Orient Bio (Sungnam, Korea). The animals were kept in the animal facility (4 mice/cage, 23 °C, 50% relative humidity, 12-h light–dark cycle) and fed with regular rodent chow and distilled water ad libitum. All animal procedures followed the international ethical guidelines and relevant laws. The experimental protocol was approved by the Animal Care and Use Committee of the Catholic University of Korea College of Medicine. The mice were divided into two groups: the control and noise groups ($n = 8$ in each group). Noise exposure and auditory brainstem response (ABR) testing were performed in a radiofrequency-shielding soundproof room according to our guaranteed protocols [18]. The device for noise exposure consisted of an acrylic frame and a speaker on the top with sign random generators (B&K Type 1027) which generates white noise centered at 10 kHz. Overall noise level was measured at the center of the cage using B&K 2144 frequency analyzer/sound level meter (Type 2690, 2669, 4231) and Tektronix AM700 audio measurement set to broad band (0.2–70 kHz). Mice in the noise groups were exposed to white noise of 110 dB SPL for 60 min in a pie-shaped wire cage with eight compartments to avoid an inappropriate exposure to noise by gathering together and hiding their heads.

2.2. Hearing tests

ABR and distortion product otoacoustic emissions (DPOAEs) were performed to measure the hearing level before and immediately after noise exposure. Mice were anesthetized with intraperitoneal injection of a mixture of zolazepam-tiletamine (5 mg/kg) and xylazine (5 mg/kg). The ABR thresholds were measured using Intelligent Hearing System (IHS) Smart EP System, running high-frequency software (ver. 2.33) and high-frequency transducers (HFT9911-20-0035, IHS, Miami, FL). The responses were recorded by subdermal stainless steel needle electrodes at the vertex, below the pinna of the left ear (reference), and below the contralateral ear (ground). Acoustic stimuli, click (100-μs duration; cos shaping; 21 Hz) and tone bursts using an exact Blackman envelope, were presented directly to the ear canal through an insert earphone in a decreasing intensity series in 10-dB steps. Evoked potentials were amplified (×200,000), band pass filtered (100–3000 Hz), and averaged over 1024 sweeps. Recording epochs comprised the following 12 ms. Thresholds were obtained for a broadband click and for 8-, 16-, and 32-kHz tone bursts. DPOAE measurement was conducted for F_2 primary tones from 6 to 32 kHz using IHS Smart OAE 4.26 system (IHS, Miami, FL, USA). L_1 amplitude was set to 65 dB SPL and L_2 was set to 55 dB SPL with an F_2/F_1 ratio of 1.22. A probe, Etymotic ER-10B+, was inserted into the external ear canal and used in conjunction with two different types of transducers depending on the range of the stimulation frequencies: Etymotic ER-2 stimulator for frequencies from 6 to 16 kHz, and IHS high-frequency transducer for frequencies from 16 to 32 kHz. A 16-bit D/A converter sampled stimulus response signals at a rate of 128 kHz. Mice were sacrificed immediately after the hearing tests, and the blood, brain, and cochlea were collected rapidly.

2.3. High-performance liquid chromatography

High-performance liquid chromatography-electron capture detector (HPLC-ECD) was applied to measure the concentrations of plasma NE and 5-HIAA. We used a commercially available assay from Chromsystems Instruments & Chemicals. To measure NE level in the plasma, extraction buffers were added to the collected plasma sample and shaken briefly. The plasma (1.5 ml) and internal standard (50 μl) were mixed for 10 min. The supernatant was discarded by centrifugation and the effluent was discarded. After repeating the process three times, wash buffer was added and shaken briefly, and then centrifuged to discard the effluent. Elution buffer was added and stood for 5 min, followed by the vortex for 30 s. After centrifuge at 2000 rpm for 1 min, the eluate (20–50 μl) was collected and injected into the HPLC system. To measure 5-HIAA level, internal standard (1 ml) was added to the plasma sample and drawn through by centrifugation to discard the effluent. Wash buffer I and II were added to the sample, and the effluents were discarded. The elution buffer and the finisher were added to collect the eluate (10–20 μl) injected into the HPLC system.

2.4. Western blot assay

Both whole brains and isolated hippocampus were dissected and homogenized in a lysis buffer (T-PER Tissue Protein Extraction Reagent, Thermo science, IL, USA), centrifuged at 13,000 rpm at 4 °C for 15 min, and stored at −20 °C. The protein concentration in the supernatant was determined by the BCA protein assay. Samples were

Please cite this article in press as: Jin SG, et al. Stress hormonal changes in the brain and plasma after acute noise exposure in mice. Auris Nasus Larynx (2016), http://dx.doi.org/10.1016/j.anl.2016.07.013