Hearing Research 347 (2017) 28–40

Contents lists available at ScienceDirect

Hearing Research

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

Research Paper

Plastic changes along auditory pathway during salicylate-induced ototoxicity: Hyperactivity and CF shifts

Chen Jiang [a, b], Bin Luo [a, b], Senthilvelan Manohar [b], Guang-Di Chen [b, *], Richard Salvi [b]

[a] Department of Neurosurgery, Anhui Provincial Hospital, 17 Lujiang Road, Hefei, Anhui 230001, China
[b] Center for Hearing and Deafness, State University of New York at Buffalo, 137 Cary Hall, 3435 Main Street, Buffalo, NY 14214, USA

ARTICLE INFO

Article history:
Received 31 May 2016
Received in revised form
30 September 2016
Accepted 26 October 2016
Available online 27 October 2016

ABSTRACT

High dose of salicylate, the active ingredient in aspirin, has long been known to induce transient hearing loss, tinnitus and hyperacusis making it a powerful experimental tool. These salicylate-induced perceptual disturbances are associated with a massive reduction in the neural output of the cochlea. Paradoxically, the diminished neural output of the cochlea is accompanied by a dramatic increase in sound-evoked activity in the auditory cortex (AC) and several other parts of the central nervous system. Exactly where the increase in neural activity begins and builds up along the central auditory pathway are not fully understood. To address this issue, we measured sound-evoked neural activity in the cochlea, cochlear nucleus (CN), inferior colliculus (IC), and AC before and after administering a high dose of sodium salicylate (SS, 300 mg/kg). The SS-treatment abolished low-level sound-evoked responses along the auditory pathway resulting in a 20–30 dB threshold shift. While the neural output of the cochlea was substantially reduced at high intensities, the neural responses in the CN were only slightly reduced; those in the IC were nearly normal or slightly enhanced while those in the AC considerably enhanced, indicative of a progress increase in central gain. The SS-induced increase in central response in the IC and AC was frequency-dependent with the greatest increase occurring in the mid-frequency range the putative pitch of SS-induced tinnitus. This frequency-dependent hyperactivity appeared to result from shifts in the frequency receptive fields (FRF) such that the response areas of many FRF shifted/expanded toward the mid-frequencies. Our results suggest that the SS-induced threshold shift originates in the cochlea. In contrast, enhanced central gain is not localized to one region, but progressively builds up at successively higher stage of the auditory pathway either through a loss of inhibition and/or increased excitation.

1. Introduction

Aspirin, a potent anti-inflammatory drug first synthesized by Bayer in 1897, was the mainstay treatment for rheumatoid arthritis during much of the twentieth century. Effective treatment of arthritic pain and inflammation required titration of the dose; a common approach involved increasing the aspirin dose until the patient experienced ringing in the ears, and then lowering it slightly until the tinnitus disappeared (Mongan et al., 1973). While aspirin is seldom used nowadays to treat arthritis, it's active ingredient, salicylate continue to be used in auditory research to investigate temporary ototoxic hearing loss and the perceptual and neural mechanisms underlying tinnitus (Bauer et al., 1999; Brennan and Jastreboff, 1991; Cazals, 2000; Chen et al., 2014a; Jastreboff and Sasaki, 1994; Jastreboff et al., 1988; Kizawa et al., 2010; Lobarinas et al., 2004; Ruttiger et al., 2003; Yang et al., 2007). Excessive sodium salicylate (SS) is thought to induce tonal tinnitus with a pitch around 10–20 kHz (Brennan and Jastreboff, 1991; Kizawa et al., 2010; Lobarinas et al., 2004; Yang et al., 2007) and recent behavioral studies indicate that salicylate also induces hyperacusis, a condition in which moderate intensity sounds are perceived as intolerably loud (Chen et al., 2014a; Zhang et al., 2014).

Abbreviations: AC, auditory cortex; AP, anterior-posterior; CAP, compound action potential; CF, characteristic frequency; CIC, central nucleus of inferior colliculus; CN, cochlear nucleus; FRF, frequency receptive field; IC, inferior colliculus; I/O, input/output; LFP, local field potential; ML, medial-lateral; N1, first negative peak of CAP; OHC, outer hair cell; PSTH, peristimulus time histogram; pvCN, posterior ventral cochlear nucleus; RMS, root mean square; SS, sodium salicylate

* Corresponding author. Center for Hearing and Deafness, State University of New York at Buffalo, 137 Cary Hall, 3435 Main Street, Buffalo, NY 14214, USA.
E-mail address: gchen7@buffalo.edu (G.-D. Chen).

http://dx.doi.org/10.1016/j.heares.2016.10.021
0378-5955/

As with most ototoxic drugs, high-dose SS causes a cochlear hearing loss on the order of 25 dB, presumably due to its binding to prestin in the outer hair cell (OHC), which is the OHC motor protein responsible for OHC electromotility and the cochlear amplification (Kakehata and Santos-Sacchi, 1996; Rybalchenko and Santos-Sacchi, 2003). SS also greatly reduces the neural output of the cochlea as reflected in the compound action potential (CAP) which arises from the synchronized onset response of type I auditory nerve fibers (Chen et al., 2010, 2013; Stolzberg et al., 2011b; Stypulkowski, 1990; Sun et al., 2009). While the threshold shifts in the AC are comparable to the CAP suprathreshold sound-evoked responses in the AC are paradoxically much larger than normal (Chen et al., 2012, 2014a, 2013; Lu et al., 2011; Sun et al., 2009). In addition to the AC, sound-evoked hyperexcitability has also been observed in several other central structures such as the medial geniculate body and amygdala after drug or noise-induced damage to the cochlea (Chen et al., 2013, 2016; Stolzberg et al., 2011a). The hyperexcitability provides evidence for homeostatic plasticity in which the central auditory structures overcompensates for the reduced neural output of the cochlea or enhanced central gain (Auerbach et al., 2014; Norena, 2011b). Excessive central gain, resulting from diminished inhibition or increased excitation (Brummett, 1995; Gong et al., 2008; Lu et al., 2011; Sun et al., 2009; Takahashi et al., 2015; Wang et al., 2006; Xu et al., 2005), is believed to contribute to hyperacusis, loudness recruitment and subjective tinnitus (Chen et al., 2014a; Norena, 2011a). Many neurons in the AC and other auditory regions of the central nervous system also shift their frequency receptive fields (FRFs) to the mid-frequency region following SS treatment (Chen et al., 2012, 2014a; Stolzberg et al., 2011b). This imbalance and over representation of FRF may be linked to the tinnitus pitch (Chen et al., 2012). While there is clear evidence for hyperexcitability and tonotopic reorganization in central auditory structures, it is unclear whether these functional changes suddenly emerge in these regions if they are inherited in whole or in part from the lower areas of the midbrain or brainstem. To address this issues, sound-evoked responses were obtained from the cochlea, the cochlear nucleus (CN), inferior colliculus (IC) and the AC of the rat before and after administering a high dose of SS known to produce behavioral evidence of tinnitus and hyperacusis (Chen et al., 2014a; Stolzberg et al., 2012).

2. Experimental methods

2.1. Subjects

The 23 male Sprague–Dawley rats (300–500 g, Charles River Laboratories Inc.) used in this study (8 for CAP, 8 for CN, 4 for IC, and 3 for AC) were housed in the Laboratory Animal Facility (LAF) at the University at Buffalo, given free access to food and water and maintained at 22 °C on a 12-h light-dark cycle. All procedures regarding the use and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University at Buffalo.

2.2. Sodium salicylate dosing

SS (Sigma-Aldrich, # S3007) was dissolved in sterile saline (25 mg/ml) and intraperitoneally (i.p.) injected at a dose of 300 mg/kg. Control rats received a similar volume of saline. This dose of SS has been shown to produce behavioral evidence of tinnitus and hyperacusis in rats (Chen et al., 2014a; Lobarinas et al., 2004).

2.3. Electrodes

A ring-electrode (~250 μm in diameter) made of Teflon-coated silver wire (76.2 μm in diameter, A-M systems) was placed on the round window to record cochlear compound action potential. A 16-channel linear silicon microelectrode array (A-1 × 16-10mm 100–177, NeuroNexus Technologies) was used to record sound-evoked local field potentials and multiunit clusters in CN, IC, and AC. The distance between adjacent recording electrodes on the array is 100 μm and the diameter of each electrode is 15 μm. The electrode was coated with DiI prior to insertion and the DiI label was used to confirm the location of the recording electrode from cryostat sections of CN, IC and AC as described in detail in earlier publications (Chen et al., 2013).

2.4. CAP

Tone-bursts (10 ms duration, 1 ms of rise/fall time, cosine^2-gated, 6, 8, 12, 16, 20, 24, 30 and 40 kHz) were generated by a TDT RP2.1 real-time processor (100 kHz sampling rate) and presented at 20/s. Stimuli were fed to a programmable attenuator (TDT PA5), power amplifier and high frequency transducer assembly (ACO half-inch microphone) inserted in the ear canal in front of the tympanic membrane. The transducer was calibrated in a cavity that approximated the volume of the ear canal using a microphone preamplifier (Larson Davis, model 2221) and half-inch microphone (Larson Davis, model 2540).

Rats were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.), placed on a homoeothermic blanket (Harvard Apparatus) to maintain body temperature at 37 °C and held with a custom head holder. The right bulla was surgically exposed through a ventrolateral approach, a small hole was made on the bulla to expose the round window and the electrode was placed on the round window. A silver chloride electrode was inserted into the neck muscles as a reference. The cochlear responses to tone bursts (0–90 dB SPL, 10-dB steps) were amplified (1000X) and filtered (0.1 Hz-50 kHz) using a Grass AC preamplifier (Model P15); the output of the amplifier was digitized (TDT RP2.1 real-time processor, 10.24 μs/sample), averaged 50 times and stored on a personal computer for offline analysis. The CAP response was low-pass filtered offline (1 kHz below the tone frequency using custom MATLAB software) and the CAP amplitude was calculated as the mean value of N1-P1 and N1-P2 (see Fig. 1A).

2.5. CN, IC and AC recordings

Broadband noise bursts and tone bursts (50 ms duration, 1 ms rise/fall time, cosine^2-gating, 1–42 kHz) were generated using a TDT RX6 multifunction processor (100 kHz sampling rate) and presented with an interstimulus interval of 300 ms. The stimuli were delivered through a loudspeaker (FT28D, Fostex) located 10 cm in front of the right ear. Sound levels at the location of the animal's ear were measured with microphone preamplifier (Larson Davis, model 2221) and one-quarter inch microphone (Larson Davis, model 2520).

Rats were anesthetized with ketamine and xylazine (50 mg/kg and 6 mg/kg, i.p., respectively) and then fixed in a stereotaxic apparatus using a rat head holder and two blunted ear bars. Body temperature was maintained at 37 °C using a homoeothermic heating blanket (Harvard Apparatus). A stable plane of anesthesia was maintained with supplement doses (0.1 ml) of the ketamine-xylazine mixture (10:1 ratio) every hour. The dorsal surface of the skull was exposed and a head bar was firmly attached to the right parietal bone by a screw and dental cement. Afterwards, the right ear bar was removed permitting free-field acoustic stimulation of the right ear. An opening was made on the skull at the appropriate location to gain access to the right (ipsilateral) CN, the left (contralateral) IC, or the left (contralateral) AC. The dura was