Hearing Research 302 (2013) 113–120

Contents lists available at SciVerse ScienceDirect

Hearing Research

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

Research paper

Insensitivity of the audiogram to carboplatin induced inner hair cell loss in chinchillas

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ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form
18 March 2013
Accepted 22 March 2013
Available online 6 April 2013

ABSTRACT

Noise trauma, aging, and ototoxicity preferentially damage the outer hair cells of the inner ear, leading to increased hearing thresholds and poorer frequency resolution.

Whereas outer hair cells make synaptic connections with less than 10% of afferent auditory nerve fibers (type-II), inner hair cells make connections with over 90% of afferents (type-I). Despite these extensive connections, little is known about how selective inner hair cell loss impacts hearing. In chinchillas, moderate to high doses of the anticancer compound carboplatin produce selective inner hair cell and type-I afferent loss with little to no effect on outer hair cells. To determine the effects of carboplatin-induced inner hair cell loss on the most widely used clinical measure of hearing, the audiogram, pure-tone thresholds were determined behaviorally before and after 75 mg/kg carboplatin. Following carboplatin treatment, small effects on audiometric thresholds were observed even with extensive inner hair cell losses that exceed 80%. These results suggest that conventional audiometry is insensitive to inner hair cell loss and that only small populations of inner hair cells appear to be necessary for detecting tonal stimuli in a quiet background.

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1. Introduction

The inner ear of mammals contains two distinct sensory cell types. Inner hair cells (IHC) are believed to convey nearly all of the acoustic input to the brain through extensive synaptic connections with over 90% of afferent auditory nerve fibers (type-I) whereas outer hair cells (OHC) make connections with the remaining auditory nerve afferent fibers (type-II, less than 10% of total ANF population). However, OHCs are important to hearing as these cells play a key role in cochlear non-linear amplification (Brownell et al., 1985) and frequency selectivity. Hearing impairment as a result of aging, noise exposure, or ototoxicity, typically begins with OHC loss or dysfunction, followed by a progressive loss of IHC and spiral ganglion cells (Felder and Schrott-Fischer, 1995; Harding and Bohne, 2004; McFadden et al., 2001; Stebbins et al., 1979).

The effects of OHC loss on hearing have been well described in several species across a number of studies (Ahroon et al., 1993; Davis et al., 1989; Moore et al., 1999; Smith et al., 1987b). Primarily, OHC loss results in increased hearing thresholds at frequencies associated with OHC damage and a loss of frequency selectivity, rendering affected ears more susceptible to the masking effects of background noise (Davis et al., 1989).

In contrast to the effects of OHC loss, few models describe the effects of selective IHC loss on hearing, in part, because of the inherent difficulty in damaging IHCs in the absence of significant OHC damage. This limitation was overcome when the first model of selective IHC ototoxicity was developed in chinchillas using moderate to high doses of the anticancer drug, carboplatin (Takeno et al., 1994). This selective ototoxic effect produced IHC losses of more than 50% but spared OHCs and had no effect on distortion product otoacoustic emissions (DPOAE) and cochlear microphonics (CM); functional measures of OHC integrity (Hofstetter et al., 1997b; Salvi et al., 2000b).

Subsequent physiological studies have shown that carboplatin-induced IHC loss reduces the compound action potential (a measure of cochlear output) commensurate with the extent of the IHC loss. However, responses to the same acoustic stimuli measured at the inferior colliculus (IC) showed little if any change (McFadden et al., 1998) and near-field responses in the auditory cortex (AC)

Abbreviations: ABR, auditory brainstem response; AC, auditory cortex; CAP, compound action potential; CM, cochlear microphonic; dB, decibel; DPOAE, distortion product otoacoustic emissions; IC, inferior colliculus; IHC, inner hair cell; OHC, outer hair cell; SD, standard deviation; SEM, standard error of the mean; SPL, sound pressure level; SDH, Succinate dehydrogenase

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0378-5955/$ – see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.heares.2013.03.012

were sometimes enhanced (Qiu et al., 2000; Salvi et al., 2000b). Consistent with these findings, a more recent report showed that whereas carboplatin-induced IHC loss (20–50%) reduced the amplitude of the compound action potential (CAP) and increased the CAP threshold, DPOAE and CM were unaffected and thresholds obtained from the IC were relatively unchanged (El-Badry and McFadden, 2007). The outcomes of these studies suggest increased central gain following loss of IHC, and appear to indicate that threshold estimates beyond the periphery may be inadequate for detecting IHC loss.

Additional physiological studies measuring the auditory brainstem response (ABR), a clinical far-field potential for determining hearing thresholds, have shown mixed results. Two studies in chinchillas showed that ABR thresholds either did not significantly change after large carboplatin-induced IHC loss (Jock et al., 1996) or had only modest changes in threshold (El-Badry and McFadden, 2009). More importantly, the morphology, latency, repeatability, and amplitude of the ABR waveforms were relatively maintained despite large IHC losses (El-Badry and McFadden, 2009). Similar ABR results were reported in mice with selective IHC loss (70%); thresholds did not change despite significant IHC loss (Schrott et al., 1989). The results of these experiments suggest that the ABR, a commonly used objective measure of hearing, may underestimate IHC loss even when the loss is extensive. In contrast to these results, two earlier studies reported significant ABR threshold increases in carboplatin treated chinchillas (Harrison, 1998; Wake et al., 1993). However, because these studies did not specify which waveforms were used to determine threshold, a direct comparison to the more recent reports (El-Badry and McFadden, 2009) is not possible. In addition, it is also possible that functional differences in surviving IHC or varying extents of afferent type-I loss could explain the lack of agreement between the two sets of studies.

More recent published reports have begun to explore the effects of afferent fiber loss on ABR metrics using a noise-induced progressive type-I afferent degeneration model that does not produce hair cell loss. The results showed no changes in ABR thresholds despite significant loss of afferent fibers in both mice and guinea pigs (Kujawa and Liberman, 2009; Lin et al., 2011). Additionally, in guinea pigs treated with the glutamate agonist AMPA, prolonged swelling of auditory nerve fibers had no long term effects on behavioral measures of hearing or ABR thresholds/amplitude, despite histological evidence of vacuoles still evident in synaptic regions (Le Prell et al., 2004). In other words, both ABR and behaviorally derived pure-tone thresholds shifted only during the most severe swelling, with complete threshold recovery despite incomplete synaptic repair. Collectively, the results of the majority of these studies appear to suggest that moderate to potentially severe losses of IHC or damage to afferent fibers have little effect on thresholds.

Whereas previous studies have evaluated the effects of both IHC and afferent auditory nerve fiber loss on physiological measures of hearing, how IHC loss impacts behaviorally derived measures of auditory performance is relatively unknown. To address this fundamental question, we trained chinchillas to respond to sound using a conditioned shock avoidance paradigm previously established to assess hearing changes associated with noise exposure or aminoglycoside ototoxicity (Giraudi-Perry et al., 1982; Giraudi et al., 1980; Graf et al., 1992; Salvi et al., 1982). Based on the aforementioned physiological threshold measures following carboplatin induced IHC loss, our working hypothesis was that behaviorally derived pure-tone thresholds would likely shift only after large IHC loss and that the rate of threshold shift would increase rapidly thereafter. Further, we sought to determine the relationship between any changes observed audiometrically and the extent of the IHC lesion. To our knowledge, this is the first report that evaluates audiometric threshold changes following carboplatin-induced IHC loss, and the first to correlate the extent of IHC loss with these changes.

2. Methods

2.1. Subjects

Nine, healthy, adult, 1-2-year-old male chinchillas were used (400–600 g). Thresholds to pure-tones (250–11,300 Hz) were assessed before and after treatment with 75 mg/kg of carboplatin (i.p.), a dose known to produce moderate to severe IHC loss (Hofstetter et al., 1997a). Subjects were housed in custom individual wire mesh cages in a temperature controlled room with a 12 h light/dark cycle. Animals had free access to food and water. All procedures were approved by the University at Buffalo's Institutional Animal Care and Use Committee (IACUC).

2.2. Equipment and psychophysical methods

Audiometric thresholds in quiet were obtained using a shock avoidance conditioning procedure similar to that described in earlier reports (Blakeslee et al., 1978; Giraudi-Perry et al., 1982; Giraudi et al., 1980; Salvi et al., 1978). During testing, subjects were placed in a restraining yoke that held the subject in a fixed, standing position in a calibrated sound field within a single walled sound booth (Inside dimensions: L × W × H 91.4 × 101.6 × 193 cm, Industrial Acoustics Company Inc. Bronx, NY) lined with 3-inch acoustic foam. A micro-switch mounted on the restraining yoke was used to record the animal's behavioral response. This response consisted of a 1-cm upward movement on the restraining yoke that closed a micro-switch generating a +5 V pulse that was delivered to a TTL input module (TDT Smartport PI2) and recorded by custom psychophysical software. For shock delivery, two silver disk electrodes covered with conductive paste (Synapse Conductive Electrode Cream, SYN 1505) were taped onto the shaved tail of the chinchilla; electrodes were placed on either side of the tail near the base. Brief current pulses (1–5 mA, 500 ms on, 500 ms off) could be delivered to the electrodes using a constant current generator (Coulbourn Instruments, Precision Regulated Animal Shocker E13-14) that was controlled by the output of a TTL module (TDT Smartport PI2) with custom software running on a personal computer. A calibrated speaker (Realistic Minimus-7 40–2030 A, 40w 50–20,000 Hz) was located at the level of the subjects' head approximately 50 cm from the left ear (270° azimuth). A software controlled safety light (40-watt light bulb) was mounted on the wall of the sound booth in front of the subject (0° azimuth) at a distance of 55 cm. A small piezoelectric buzzer that was paired with shock delivery was mounted on the restraining yoke. Both the light and the buzzer were used to provide response feedback during the experimental sessions. Presentation of the light provided feedback for correct responses whereas the buzzer was paired with brief shock presentation to indicate an incorrect response.

2.3. Tone threshold

To determine thresholds as a function of frequency (audiogram), tone bursts (500 ms duration, 5 ms rise/fall time) were presented at 0.25, 0.5, 1, 2, 4, 8, and 11.3 kHz. Tone bursts were compiled using a signal generator [Tucker Davis Technology (TDT) AP2] running custom software (Borland C, TDT RPVDS). The output of the signal generator was fed to a D/A converter (TDT DA3), then through a headphone amplifier (TDT HB7) and a programmable attenuator (TDT PA4) before being sent to the loudspeaker. The output of the loudspeaker was calibrated using a 0.5 inch microphone (Larson