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Evaluation of inner hair cell and nerve fiber loss as sufficient pathologies underlying auditory neuropathy

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ABSTRACT

Auditory neuropathy is a hearing disorder characterized by normal function of outer hair cells, evidenced by intact cochlear microphonic (CM) potentials and otoacoustic emissions (OAEs), with absent or severely dys-synchronized auditory brainstem responses (ABRs). To determine if selective lesions of inner hair cells (IHCs) and auditory nerve fibers (ANFs) can account for these primary clinical features of auditory neuropathy, we measured physiological responses from chinchillas with large lesions of ANFs (about 85%) and IHCs (45% loss in the apical half of the cochlea; 73% in the basal half). Distortion product OAEs and CM potentials were significantly enhanced, whereas summating potentials and compound action potentials (CAPs) were significantly reduced. CAP threshold was elevated by 7.5 dB, but response synchrony was well preserved down to threshold levels of stimulation. Similarly, ABR threshold was elevated by 5.6 dB, but all waves were present and well synchronized down to threshold levels in all animals. Thus, large lesions of IHCs and ANFs reduced response amplitudes but did not abolish or severely dys-synchronize CAPs or ABRs. Pathologies other than or in addition to ANF and IHC loss are likely to account for the evoked potential dys-synchrony that is a clinical hallmark of auditory neuropathy in humans.

1. Introduction

Auditory neuropathy (AN) is a hearing disorder characterized by severely abnormal or absent auditory brainstem responses (ABRs) in the presence of normal outer hair cell (OHC) function as shown from intact cochlear microphonic (CM) potentials or otoacoustic emissions (OAEs) (Starr et al., 1996). Auditory thresholds may be normal, or hearing loss ranging from mild to severe may be present. However, synchronous firing of neurons at stimulus onset, a necessary condition for generating an ABR, is always impaired, and this is believed to account for the difficulties individuals with AN invariably have in understanding speech (Kraus et al., 2000).

AN appears to be much more common than was initially thought. Among infants and children with permanent sensorineural hearing loss, prevalence of AN has been reported to range from 5% to 24%, with 10–14% being the most frequently reported prevalence (Berg et al., 2005; Foerst et al., 2006; Ngo et al., 2006). Management of AN is challenging for audiologists and speech therapists. The vast majority of infants and children with AN do not develop speech and language normally, even if they are fitted properly with hearing aids (Berlin et al., 1995, 2003; Sininger, 2002; Sininger and Oba, 2001; Starr et al., 2000). Cochlear implantation has been successful for some but not all children with AN, and it is far from being an accepted method for management (Mason et al., 2003; Peterson et al., 2003; Shallop et al., 2001). Understanding the pathology underlying AN may help in managing and treating the disorder.

Based on the clinical manifestations of AN, it is likely that the condition has multiple causes. Among postulated pathologies are lesions of inner hair cells (IHCs) or auditory nerve fibers (ANFs) (Harrison, 1998; Salvi et al., 1999; Starr, 2001; Starr et al., 1996) or demyelination of the auditory nerve (El-Badry and McFadden, 2007; El-Badry et al., 2007). Whether IHC or ANF lesions alone can produce the clinical manifestations that are hallmarks of AN is the focus of the current study.

Chinchillas injected systemically with carboplatin, a widely used platinum-based chemotherapy agent, provide a model system for determining if selective IHC and ANF lesions can account for AN. Numerous studies have shown that carboplatin rapidly destroys both IHCs and ANFs in the chinchilla cochlea, while leaving OHCs intact and functioning (Ding et al., 1999; Takeno et al., 1994; Trautwein et al., 1996; Wake et al., 1994, 1996; Wang et al., 2003). We used carboplatin-treated chinchillas to determine if selective

Abbreviations: ABR, auditory brainstem response; AN, auditory neuropathy; ANF, auditory nerve fiber; ANOVA, analysis of variance; CAP, compound action potential; CM, cochlear microphonic; DPOAE, distortion product otoacoustic emission; IHC, inner hair cell; OAE, otoacoustic emission; OHC, outer hair cell; SP, summating potential

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IHC and ANF lesions could produce physiological abnormalities paralleling the clinical features of AN in humans. Distortion product OAEs (DPOAEs), summating potentials (SPs), compound action potentials (CAPs), and ABRs were recorded in eight chinchillas before and 2 weeks after carboplatin injection (i.e., before and after IHC and ANF lesions). These physiological measures were chosen because they reflect the function of the cochlea, auditory nerve and auditory brainstem, and they represent most of the diagnostic criteria of AN. We were particularly interested in determining if IHC and ANF loss would result in absent or dys-synchronous ABRs in the presence of intact CMs and OAEs, since ABR abnormalities are invariably present in humans with AN.

2. Materials and methods

2.1. Subjects and electrode implantation surgery

Eight adult chinchillas (*Chinchilla laniger*) obtained from a vendor licensed by the US Department of Agriculture (Jarr Chinchilla Inc., Hubbard, OH) underwent surgery for implantation of recording electrodes. Animals were anesthetized with an intramuscular injection of ketamine (50 mg/kg) and acepromazine (0.3 mg/kg), and placed on a Deltaphase® Isothermal Pad (Braintree Scientific, Inc., Braintree, MA), with the head secured in a stereotaxic apparatus. A postauricular incision was made to expose the right bulla and a small hole was made in the dorsal portion for insertion of a silver-ball electrode. The electrode was placed against the round window niche and fixed to the bulla with dental cement. The dorsal cranium was then exposed and a small hole was drilled in the rostral cranium for implantation of a ground electrode, which was fixed to the skull using dental cement. Buprenorphine (0.05 mg/kg) was administered subcutaneously for 3 days after surgery, and animals were allowed to recover for at least 14 days before electrophysiological testing was initiated.

2.2. Electrophysiology, DPOAE recording, and carboplatin injection

Electrical activity and DPOAEs were recorded in response to stimuli (4 kHz tone bursts for CM, SP, CAP and DPOAE; clicks for ABR) presented to the right ears of the animals through ER-2 insert earphones (Etymotic Research, Inc., Elk Grove Village, IL). CMs, SPs and CAPs were recorded from the round window electrode. ABRs were recorded from subdermal needle electrodes inserted at the scalp midline (noninverting), posterior to the stimulated right ear (inverting), and at the midline of the back (common).

CM, SP, CAP, and ABR recording utilized commercial hardware and software (Tucker Davis Technologies, Inc., Alachua, FL). Stimuli were generated digitally using an array processor (AP2), and a 16-bit digital-to-analog (D/A) converter (DA 3–4). The signals were attenuated with a programmable attenuator (PA5), then routed through a headphone buffer (HB7) and delivered to the animal's right ear. Repetition rates were 6.1/s for the CM, 21/s for the SP, and 2.1/s for the CAP. Stimulus duration was 50 ms for the CM, 15 ms for the SP, and 5 ms for the CAP. Rise/fall time was shaped with a $\cos^2$ window of two cycles of the 4 kHz stimulus. The stimuli were presented with an alternating polarity, starting at a level of 80 dB SPL and decreasing in 5 dB steps. ABRs were recorded in response to alternating polarity clicks of 100-μs duration, presented at a repetition rate of 20.1/s, at levels starting at 90 dB SPL and decrementing in 5 dB steps.

All testing was conducted in a single-walled sound booth. For CM, SP, CAP, and DPOAE tests, awake animals were placed in a custom-designed animal restraint (Snyder and Salvi, 1994). For ABR recordings, animals were anesthetized with an intramuscular injection of ketamine (30 mg/kg) and acepromazine (0.1 mg/kg) and placed on a heating pad in the sound booth. Responses were band-pass filtered (100–15,000 Hz for the CM; 5–300 Hz for the SP; 100–3000 Hz for the CAP and ABR), amplified (10,000× for the CAP, CM, and SP and 50,000× for the ABR) using a bioamplifier (DB4), and digitized using an analog-to-digital (A/D) converter (AD1). The responses were recorded over an epoch of 20 ms for the SP and CAP, and 60 ms for the CM, and 100 sweeps were averaged at each stimulus level. For the ABR, responses were recorded over a 12.5 ms epoch and 1000 sweeps were averaged at each stimulus level.

Input–output functions for the DPOAEs were obtained using two primary tones (f_1 and f_2, where $f_2/f_1 = 1.2$ and f_1 frequency was 4 kHz) generated using two D/A converters (16 bits, 100 kHz) on two separate signal-processing boards in a computer. The output of each D/A converter was low-pass filtered (roll-off 90 dB between 20 and 24 kHz) and routed to a computer controlled attenuator and buffer amplifier, and then to the insert earphones, which were coupled to a low-noise ER10B microphone (Etymotic Research, Inc., Elk Grove Village, IL) through a narrow tube. Stimulus level was incremented in 5 dB steps from 0 to 80 dB SPL. The output of the microphone was delivered to an A/D converter (16 bits) on a signal-processing board and sampled for 500 ms at a sampling rate of 31 kHz (15,500 samples at each stimulus level).

After baseline measures were obtained, each animal received a single intraperitoneal injection of carboplatin (LKT Laboratories, Inc., St. Paul, MN; 75 mg/kg). Animals were retested 2 weeks after injection, then euthanized for histology.

2.3. Histology

Chinchillas were anesthetized with an intramuscular injection of ketamine (30 mg/kg) and acepromazine (0.1 mg/kg) and perfused intracardially with phosphate buffered saline (PBS, pH 7.4) followed by 2.5% glutaraldehyde in PBS. The animals were then decapitated and the right bulla was quickly removed. The round window and oval window were opened and the cochlea was immersed in fixative (2.5% glutaraldehyde in PBS) for at least 24 h at 4 °C.

Histological procedures have been described in detail previously (Ding et al., 2001a,b). Briefly, the basilar membrane was carefully dissected from the right cochlea for surface preparations and stained with hematoxylin. Missing hair cells across the entire basilar membrane were counted and cochleograms, showing the percent of IHC and OHC loss as a function of percent distance from the apex, were constructed for all right ears. Position in the cochlea was translated to frequency using Greenwood's generalized cochlear frequency-place map (Greenwood, 1990). The right cochleas (minus the organ of Corti) were then decalcified (Decal, Baxter Scientific Products, Deerfield, IL, USA) and embedded in Epon 812 for counts of myelinated nerve fibers in the habenula perforatae. After polymerization, sections were cut parallel to the modiolar axis at a thickness of 1.5 μm, mounted on glass slides and stained with 0.5% toluidine blue. Myelinated ANFs were counted in three habenula perforatae located in the second turn of each cochlea. The average number of myelinated ANFs in cochleas of the eight carboplatin-treated chinchillas was compared to ANF counts from cochleas of normal chinchillas used in a previous study (Ding et al., 2001a).

All procedures regarding the use and care of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at the University at Buffalo.

3. Results

Fig. 1 shows IHC and OHC losses caused by carboplatin, averaged across all eight chinchillas. OHC loss was negligible, averaging