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Research paper

The potential use of low-frequency tones to locate regions of outer hair cell loss

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ABSTRACT

Current methods used to diagnose cochlear hearing loss are limited in their ability to determine the location and extent of anatomical damage to various cochlear structures. In previous experiments, we have used the electrical potential recorded at the round window —the cochlear response (CR) —to predict the location of damage to outer hair cells in the gerbil. In a follow-up experiment, we applied 10 mM ouabain to the round window niche to reduce neural activity in order to quantify the neural contribution to the CR. We concluded that a significant proportion of the CR to a 762 Hz tone originated from phase-locking activity of basal auditory nerve fibers, which could have contaminated our conclusions regarding outer hair cell health. However, at such high concentrations, ouabain may have also affected the responses from outer hair cells, exaggerating the effect we attributed to the auditory nerve. In this study, we lowered the concentration of ouabain to 1 mM and determined the physiologic effects on outer hair cells using distortion-product otoacoustic emissions. As well as quantifying the effects of 1 mM ouabain on the auditory nerve and outer hair cells, we attempted to reduce the neural contribution to the CR by using near-infrasonic stimulus frequencies of 45 and 85 Hz, and hypothesized that these low-frequency stimuli would generate a cumulative amplitude function (CAF) that could reflect damage to hair cells in the apex more accurately than the 762 stimuli. One hour after application of 1 mM ouabain, CR amplitudes significantly increased, but remained unchanged in the presence of high-pass filtered noise conditions, suggesting that basal auditory nerve fibers have a limited contribution to the CR at such low frequencies.

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1. Introduction

In the audiology clinic, the diagnosis of hearing loss relies heavily on the audiogram. While certain audiologic patterns differentiate sensorineural from conductive hearing loss, the audiogram is unable to discern the underlying anatomical damage in the cochlea; damage to different structures of the inner ear can manifest as similar patterns of hearing loss on an audiogram (Suzuka and Schuknecht, 1988). Thus, specific sites-of-lesion for many sensorineural hearing losses go unidentified, leaving the audiologist or physician to speculate on treatment and prognosis. As treatment for hearing disorders advance —both technologically in terms of hearing aids and cochlear implants, as well as medically with the promise of hair cell regeneration —so does the need for more sensitive and specific diagnostic measures.

The cochlear microphonic (CM) is an electrophysiologic response to acoustic stimulation that results from current flow through ion channels in hair cell membranes, and therefore is a strong candidate to measure the health of outer hair cells (Withnell, 2001; Cheatham et al., 2011). The CM, as picked up by an electrode placed at the round window (RW), contains the responses from hair cells across the entirety of the cochlea, weighted by their distance to the electrode. This poses two potential problems: a high-frequency stimulus causes maximal displacement of the basilar membrane and thus large phase incoherence at the base of the cochlea, proximal to the electrode site, altering the amplitude of the response and risking a false diagnosis of damage (Whitfield and Ross, 1965; Laszlo et al., 1972; Patuzzi, 1987). This can be solved

Abbreviations: CR, cochlear response; CM, cochlear microphonic; CAF, cumulative amplitude function; AP, artificial perilymph; CAP, compound action potential; DPOAE, distortion-product otoacoustic emissions

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by using a low-frequency stimulus so that the majority of the basilar membrane remains in-phase. The response, however, is still dominated by basal hair cells near the electrode, which can confound an attempt to measure outer hair cell (OHC) health at the apex of the cochlea (Dallos, 1969; Patuzzi et al., 1989). To solve this problem, Chertoff et al. (2012, 2014) implemented a filtered noise paradigm in which 733 & 762 Hz tone stimuli were embedded in high-pass filtered noise with seventeen consecutively increasing cutoff frequencies, allowing them to measure a cumulative response from seventeen corresponding regions along the length of the cochlear partition. Taking into account the electric field decay and geometric distance from each point of measurement to the electrode, they modeled a growth function of the CM amplitude as a function of distance along the length of the cochlear partition. This growth function was deemed the cumulative amplitude function (CAF), and they showed that damage to OHCs will alter the growth of this function (Chertoff et al., 2012, 2014).

Although the CAF proved a fairly accurate predictor of the location of onset of anatomical damage to OHCs ($r = 0.734$, $p = 0.001$), there were two outstanding obstacles to the predictive power of the 762 Hz CAF. The first is that the literature suggests that a significant proportion of the RW response is generated from auditory nerve potentials in addition to OHCs, especially for low-frequency stimuli (Henry, 1995; Patuzzi et al., 1989; He et al., 2012; Lichtenhan et al., 2014). In order to quantify the proportion of neural and OHC potentials present in the RW response, or cochlear response (CR), Chertoff et al. (2014) damaged the auditory nerve with 10 mM of an ototoxic drug, ouabain, and recorded the CR before and after an acute application to the RW niche. Chertoff et al. (2014) reported reductions in CR amplitude to a 762 Hz tone of up to 70% (at low signal levels) after the application of 10 mM ouabain on the RW for 30 min. They attributed the effect primarily to the loss of basal auditory nerve fibers; however, they also found a small decrease in distortion-product otoacoustic emission (DPOAE) amplitudes to a 24 kHz primary tone, indicating some OHC damage.

Ouabain is Na^+-K^+-ATPase pump inhibitor and has a high affinity for the α3-receptor subunit isoform (O'Brien et al., 1994; Pierre et al., 2008) which, in the cochlea, is expressed by auditory nerve fibers (McLean et al., 2009). Several studies found that the sensitivity to ouabain is dependent on nerve type: type I neurons are more sensitive than type II (Lang et al., 2005), and low spontaneous rate fibers are more sensitive than high spontaneous rate fibers (Bourien et al., 2014). Ouabain can be used to selectively damage the auditory nerve because of its low affinity for the α1-receptor isoform found in the epithelial cells of the cochlea, including hair cells (McGuirt and Schultea, 1994). Several studies argue over the effect of ouabain on OHCs, which seems to be dependent on species, dose, time, and method of application. Bourien et al. (2014) measured the physiologic effects of ouabain on OHCs by recording DPOAEs after an acute application of 100 μM ouabain on the gerbil RW for 30 min. They found that ouabain did not affect the DPOAE amplitudes in the f_2 range of 0.5–20 kHz at 60 and 55 dB SPL signal levels for f_2 and f_1, respectively. Schmiedt et al. (2001) showed similar results for 1 mM ouabain, but found that 10 mM significantly reduced DPOAE amplitude after 3 h. In spite of the apparent hair cells' resistance to low concentrations of ouabain, Fu et al. (2012, 2013) reported detrimental anatomical effects of ouabain on rat cochlear hair cells; application of 1 mM ouabain to a dissected Organ of Corti for 24 h resulted in an 80% loss of inner hair cells and 50% loss of OHCs. Chertoff et al. (2014) found an effect on DPOAEs at high frequencies with 10 mM ouabain. In Experiment 1 of this study, we aimed to quantify the physiologic effects, via DPOAEs, of a smaller dose (1 mM) on OHCs in order to confirm the conclusion that the large drop in CR amplitude found by Chertoff et al. (2014) was indeed a consequence of auditory nerve fiber loss and not OHC dysfunction.

In addition to the 762 Hz response containing large amounts of activity originating from the auditory nerve, another issue in using the 762 Hz CAF to predict location of OHC loss was the shape of the growth curve itself. The CAF resulting from CR recordings at each high-pass filtered noise condition to 762 Hz creates a sigmoidal curve that has a shallow, if non-existent, slope at low cutoff frequencies, which could make any anatomical changes in the apical regions of the cochlear partition difficult to diagnose. Fig. 1a shows a schematized typical 762 Hz CAF curve (solid line) and how the curve would change due to damage in apical regions (dashed line). Since the normal curve has little growth in the apex, damage to this region could be missed because the effect on the resulting CAF is not pronounced. In Experiment II, we hypothesized that a stimulus lower in frequency than 762 Hz would result in a steeper growth in the apical regions of the CAF. Fig. 1b shows the hypothesized low-frequency CAF with a steeper growth in amplitude from the apical region of the cochlear partition (solid line), which, when damaged, would result in a more dramatic change to the CAF curve (dashed line). With this in mind, we recorded the CR to near-infrasonic stimuli (45 & 85 Hz) with the intent of both circumventing phase-locking activity arising from the low-frequency tails of basal fiber tuning curves —resulting in a CR representing hair cell activity —as well as produce a CAF that would grow in a manner

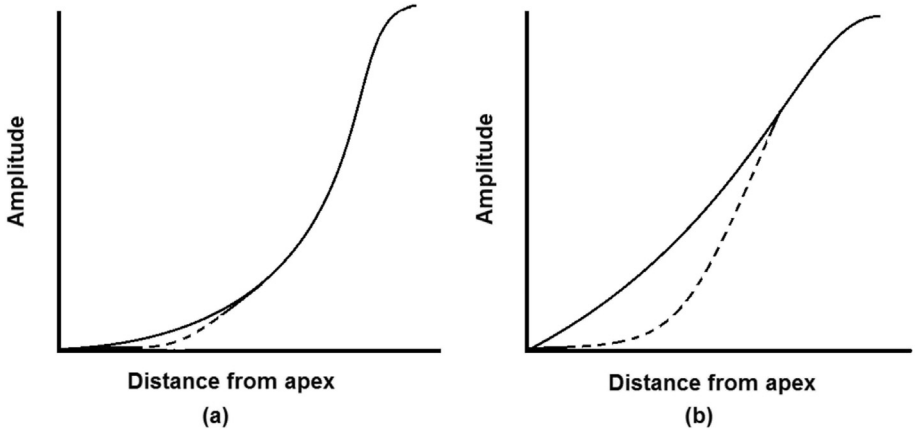

Fig. 1. (a) Schematic portrayal of typical CAF curve (solid line) and CAF curve post damage to apex (dotted line). (b) Schematic portrayal of hypothesized CAF curve with steeper growth in apical responses (solid line) which would more potentially show a more dramatic change when damaged (dotted line).