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Research paper

Changes in cochlear function during acute endolymphatic hydrops development in guinea pigs

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ABSTRACT

Previous studies have injected artificial endolymph into scala media in anaesthetized guinea pigs as an acute model of endolymphatic hydrops. Here, we have injected artificial endolymph into scala media in guinea pigs at rates of 40–80 nl/min, whilst monitoring Compound Action Potential (CAP) thresholds, the Summating Potential (SP)/CAP ratio, Cochlear Microphonic (CM) distortion, low-frequency modulated Distortion Product Otoacoustic Emissions (DPOAEs), and the Endocochlear Potential (EP). We found that abrupt recovery of CAP thresholds, SP/CAP ratio, and CM and DPOAE asymmetric distortion could occur several times during a single injection of less than 3 μl, suggesting that endolymph pressure could periodically decrease while the injection was ongoing. Larger volumes are thought to produce a rupture of the membranous labyrinth, however, our results suggest that multiple injections, each larger than 3 μl and within 40 min of each other, cause multiple pressure-related changes, which are difficult to be explained on the basis of a simple labyrinth rupture. We have also examined the morphological changes of the temporal bones *ex vivo* using X-ray micro-tomography. Both the functional changes and the micro-CT images suggest ruptures of the membranous labyrinth may not always be responsible for abrupt changes in inner ear function. Our results provide a new insight into the changes in cochlear function occurring during acute hydrops development, which compares well to the clinical findings observed in Ménière's Disease. We suggest that hydrops development may be a continual process, yet cause discontinuous functional changes due to mechanisms other than a simple rupture of the membranous labyrinth.

1. Introduction

Despite numerous theories on Ménière's Disease (MD) and the effects of endolymphatic hydrops on hearing and balance function, it is still not known how or even if hydrops causes hearing fluctuation, tinnitus, or, in particular, sudden attacks of vertigo. Schuknecht (1963) first suggested that the vertigo attacks in MD might be due to a rupture of the membranous labyrinth due to an excessive endolymph volume. A sudden rupture was thought to allow mixing of perilymph and endolymph, causing a potassium toxicity of the inner ear hair cells or neurones, resulting in a sudden hearing loss and vertigo attack.

However, the "rupture theory" has been disputed on the basis of several functional changes observed in experimental guinea pigs, which do not mimic symptomatic changes in MD patients. First, experimentally increasing perilymph potassium concentration leads to a nystagmus that switches from an initial irritative to a paralytic direction (Dohlmann, 1976; Brown et al., 1988), however it has been suggested that MD patients actually demonstrate a nystagmus during a vertigo attack which switches from a paralytic to an irritative direction (McClure et al., 1981, 1982). Second, increasing perilymph potassium in guinea pigs results in a significant hearing loss (Tasaki and Fernadez, 1952; Marcon and Patuzzi, 2008), however recent clinical evidence suggests there is little or no hearing loss around the time of the attack (McNeill et al., 2009a). Third, increased perilymph potassium results in an abrupt loss of vestibular function (Kingma and Wit, 2010), but recent clinical studies have demonstrated that in early MD sufferers there is often an enhanced utricular and horizontal canal sensitivity, but not saccular sensitivity (Manzari et al., 2010, 2011). It is difficult to envisage how ionic changes resulting from mixing of perilymph

Abbreviations: DPOAE, distortion product otoacoustic emissions; ECochG, electrocochleography; OP, operating point; CAP, compound action potential; SP, summating potential; SNHL, sensorineural hearing loss.

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and endolymph could affect one otolith macula and not the other, or how it could lead to vertigo but not a hearing loss, or indeed how potassium toxicity could enhance utricular responses.

To investigate if, and how hydrops alters cochlear and vestibular sensitivity, researchers have acutely injected artificial endolymph into the membranous labyrinth of anaesthetised animals as a simple model of endolymphatic hydrops (Salt and Plontke, 2010). Endolymph injection of volumes up to 1–2 μl results in changes of cochlear function suggestive of a displacement of the basilar membrane towards scala tympani due to an increased endolymph pressure relative to perilymph (Sirjani et al., 2004; Valk et al., 2006). Additionally, when 3–4 μl of endolymph is rapidly injected into scala media, perilymph potassium concentration in scala vestibuli increases substantially (Kakigi et al., 2010), which seems to result in an abrupt recovery of the organ of Corti displacement (Valk et al., 2004, 2006), and rapid decline in vestibular sensitivity that can take hours to recover (Kingma and Wit, 2010). It has been suggested that these abrupt changes are due to a rupture of the membranous labyrinth, and that this represents an animal model of the vertigo attacks in MD (Valk et al., 2004, 2006; Kingma and Wit, 2010). However, previous artificial endolymph injection studies have used relatively high rates of injection (500–3000 nl/min), which might have induced non-physiological changes in the inner ear, and which may have masked some subtle, short-term changes in inner ear function, which may mimic the mechanisms underlying MD symptoms.

Here, we further investigate the functional changes induced by injecting artificial endolymph at relatively slow rates (40 nl/min) into scala media of the basal turn of the cochlea via a glass micropipette. We continuously monitored cochlear function before, during, and after the injection, with a relatively high sampling rate. Our measurements are intended to provide a simple estimation of the time-course of changes in cochlear function as endolymph volume increases slowly.

2. Methods

2.1. Animal preparation & surgery

Experiments were performed on 34 normal adult guinea pigs (Cavia porcellus) of either sex with body weights between 250 and 450 g. All animal preparation, surgery and protocol were approved by The University of Sydney's Animal Ethics Committee. Animals were anaesthetised in a gas anaesthesia chamber with 4% isoflurane, and also given a once-off 0.05 ml intraperitoneal injection of Temgesic (Buprenorpherine; Reckitt Benckiser, Auckland NZ) and 0.1 ml of Atrosine (0.6 mg/ml atropine sulphate; Apex Laboratories, NSW, Australia). Once sedate and the foot-withdrawal reflex was absent, animals were moved to the experimental setup in the sound-isolation booth, tracheotomized, and thereafter artificially ventilated with a mixture of carbogen (95% O_2, 5% CO_2) and isoflurane (1–2%) by an artificial respirator pump. The animal's core temperature was regulated to 38 °C (±2 °C) by a rectal temperature probe and an electric heating pad, and the sound-isolated room was heated with an infrared heating lamp. After the main surgery, to suppress middle ear reflexes and overall EMG activity animals were given a 0.1 ml intramuscular injection of the neuromuscular blocker Pauvulon (2 mg/ml pancuronium bromide; Astra Pharmaceuticals, L.P.). To expose the cochlea, a small opening about 6 mm in diameter was made in the acoustic bulla via a dorso-lateral approach, providing a direct view of the round window and the basal cochlear turn. A thin tissue wick was placed in the bulla to minimize any acoustical, functional or electrical changes due to fluid build-up in the bulla. In experiments requiring access to scala media via the lateral wall, the guinea pig was placed in a supine position, and a ventral approach to the bulla was surgically made, exposing a larger view of the cochlea.

In all experiments animals were placed in a custom-designed ear bar, which housed a low-noise microphone (ER10B+, Etymotic Inc., IL, USA), and the ends of 3 silastic tubes (20 cm long) which were connected to 3 separate headphone speakers, forming a closed acoustic sound-field in the ear canal.

2.2. Physiological measures

To measure cochlear responses, a 0.2 mm hole was made in the first turn of the cochlea, through which the exposed tip of a teflon-coated AgCl recording wire was placed, and then sealed-in using superglue. To inject artificial endolymph and to record the endocochlear potential, a 5–10 μ tip diameter, 5–10 MΩ glass micropipette was inserted into scala media, typically via the round window and basilar membrane. In this study, the composition of artificial endolymph consisted of (in mM): KCl, 126; NaCl, 1; $KHCO_3$, 25; $MgCl_2$, 0.025; $CaCl_2$, 0.025; K_2HPO_4, 1.4; mannitol, 25; and had a pH of 7.4 and an osmolarity of 310–320 mOsmol, as previously described by Marcus et al. (1983).

On several occasions, the micropipette was inserted into scala media of the basal turn through a small hole made in the lateral wall of the cochlea, directly accessing scala media through stria vascularis. To prevent fluid leakage from the lateral wall injection location, prior to making a hole through the lateral wall, the bone was shaved slightly, and a thin layer of a hydrophilic glue was applied to the bone ("Gorilla Glue", Cincinnati, Ohio). This ensured that the glue applied later had a solid, dry surface to adhere to (Salt et al., 2006). The hole was then made through the dried layer of glue and bone, and the pipette tip was inserted into the hole under micromanipulation control. Excess fluid was then removed from the insertion point with a tissue wick, and then sealed in place with superglue (Selleys Plastic Glue, DuluxGroupPty Ltd., Australia) to prevent fluid leakage from the hole.

For both round window and lateral wall approach injections, the glass pipette was connected to a 10 μl glass syringe, attached to an UltramicroPump III perfusion pump (WPI, Sarasota, FL). Prior to inserting the glass pipette into the cochlea, the tip of the pipette was visually inspected while injecting into air to confirm an immediate injection of fluid, and to confirm there was no "lag" in the injection onset. Scala tympani and scala media potentials were amplified using a custom designed low-noise AC and high-impedance DC amplifier respectively. Amplified signals were digitized using a battery powered Wi-Fi data acquisition device (NI 9205, National Instruments, TX), and wirelessly transmitted to PC. The 3 speakers and 1 low-noise microphone signals were controlled and measured via an external USB soundcard (UltraLite-mk3 Hybrid, MOTU Cambridge, MA). All recordings and stimulus generation were performed with custom designed LabVIEW programs (National Instruments, TX).

From the scala tympani wire, the Compound Action Potential (CAP) and Summating Potential (SP) response to a train of high-frequency acoustic tone-bursts were measured. This tone-burst train consisted of 10 ms duration, 1 ms cosine-tapered, 15, 11, 6, 4 and 2 kHz tone-bursts, separated by 20 ms of silence. CAP thresholds were determined programmatically using a software algorithm that adjusted the sound level of each tone-burst independently, until such time that the correlation between successive averaged responses (with 8 averages for each response) was close to 0.4. This process is similar to adjusting the level of a tone-burst until the response is just visually noticeable above the noise floor of the recording.

After determining CAP threshold for each frequency, CAPs and SPs could be evoked by tone-bursts 40 dB louder than the threshold