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Drug delivery into the cochlear apex: Improved control to sequentially affect finely spaced regions along the entire length of the cochlear spiral

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Abstract

Background—Administering pharmaceuticals to the scala tympani of the inner ear is a common approach to study cochlear physiology and mechanics. We present here a novel method for in vivo drug delivery in a controlled manner to sealed ears.

New method—Injections of ototoxic solutions were applied from a pipette sealed into a fenestra in the cochlear apex, progressively driving solutions along the length of scala tympani toward the cochlear aqueduct at the base. Drugs can be delivered rapidly or slowly. In this report we focus on slow delivery in which the injection rate is automatically adjusted to account for varying cross sectional area of the scala tympani, therefore driving a solution front at uniform rate.

Results—Objective measurements originating from finely spaced, low- to high-characteristic cochlear frequency places were sequentially affected. Comparison with existing methods(s): Controlled administration of pharmaceuticals into the cochlear apex overcomes a number of serious limitations of previously established methods such as cochlear perfusions with an injection pipette in the cochlear base: The drug concentration achieved is more precisely controlled, drug concentrations remain in scala tympani and are not rapidly washed out by cerebrospinal fluid flow, and the entire length of the cochlear spiral can be treated quickly or slowly with time.

Conclusions—Controlled administration of solutions into the cochlear apex can be a powerful approach to sequentially effect objective measurements originating from finely spaced cochlear regions and allows, for the first time, the spatial origin of CAPs to be objectively defined.

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Keywords

cochlear action potential; Auditory Nerve Overlapped Waveform; cochlear fluid; kainic acid; cochlear perfusion

INTRODUCTION

Administering solutions into the inner-ear fluids spaces to affect mechanical and physiological responses is a classic approach used to address research questions related to hearing and vestibular function. More recently, there has been additional interest in directly administering therapeutics to the ear to bypass the blood-labyrinth barrier. However, drugs applied intratympanically or by injections directly into the basal cochlear turn that is accessible in an office procedure spread slowly along the scala only by passive diffusion, limiting treatment concentrations to the basal-most portions of the cochlea that are not tuned to most speech frequencies. The dramatic gradation in cross sectional area of scala tympani – a variable that is currently unaccounted for in cochlear drug administration methods – also needs to be considered for controlled delivery. In guinea pig, scala tympani cross sectional area varies from 1.28 mm² in the base to 0.54 mm² in the cochlear apex, a factor of 24.

We developed a technique to experimentally affect the entire cochlear length by administering pharmaceuticals from a microsyringe sealed into a fenestration made in the cochlear apex. This novel approach overcomes the limited distance of treatments applied to, for example, the round window niche, or through fenestrations made in low- and mid-cochlear turns. Solutions injected from a micropipette sealed into the cochlear apex were driven basally along the length of the scala tympani towards the cochlear aqueduct, which acts as the outlet for volume flow. Solution flow into scala tympani must be negligible because there is no outlet for vestibule perilymph. Solutions in scala tympani readily reach the sensory cells because there is no tight-junction barrier between scala tympani and the interior cells of the organ of Corti. Apically injected solutions can be delivered quickly or slowly depending on requirements of the study and are influenced by substance-dependent properties such as the rate of concentration losses to blood, passive diffusion, communication with adjacent cochlear ducts and tissues, and mixture with sustained cerebrospinal fluid entry across the cochlear aqueduct (see Salt & Plontke 2009 for a review). Our original development of the apical injection technique compensated for the graded cross sectional area of scala tympani by manually increasing the rate of injection several times over the course of the injection procedure (Lichtenhan et al. 2014). Initially, this was adequate to identify the origins of responses in the apical and basal halves of the cochlea (Lichtenhan et al. 2014). However, a more precise control to produce a uniform rate of drug progression along the graded cochlea allows the method to be used to study the origins of physiological measurements from finely spaced cochlear regions. Here we describe an advance of the apical injection technique in which scala tympani cross sectional area measurements from a three dimensional reconstruction of a guinea pig cochlea are used to inform a computer-controlled microsyringe pump, allowing it to drive solution at a constant rate of 0.5 mm/min along scala tympani.

2. METHODS

2.1. Animal Preparation

NIH-strain pigmented guinea pigs of either sex weighing between 400 and 600 grams were used for these experiments. Intraperitoneal injection of 100 mg/kg of sodium thiobutabarbital was used for initial anesthetic. Fur from the head and neck was removed and a tracheotomy was performed. The animal was artificially ventilated with isofluorane (~1 % in oxygen) with respiratory volume adjusted to maintain 5% end-tidal CO₂. Heart rate, O₂ saturation, and expired CO₂ level was monitored with a pulseoximeter/CO₂ analyzer. A ventral approach was used to access the right cochlea. Non-bony portions of the right ear canal were removed and the head was mounted with hollow ear bars allowing acoustic stimuli delivery. Pancuronium bromide (0.06 mg/kg) was administered through a cannula in the left jugular vein before making objective measurements from the ear to eliminate middle-ear muscle contractions. Body temperature was maintained at 38°C with a dc-powered heating blanket and rectal thermometer system. Experimental protocols for this study were approved by the Animal Studies Committee of Washington University (protocol numbers 20120113 and 20130069).

2.2. The Apical Injection Method

Kainic acid (2.16 mM in artificial perilymph) was injected from a pipette that was sealed into the fourth cochlear turn scala tympani (schematized in Fig. 1). Kainate receptor agonists reduce CAP amplitudes and elevate CAP thresholds without affecting otoacoustic emissions, suggesting that kainate receptors contribute to the neural response from transmitter release from inner hair cells during in the presence of sound (Peppi et al. 2012). Artificial perilymph composition (in mM) was NaCl (127.5), KCl (3.5), NaHCO₃ (25), CaCl₂ (1.3), MgCl₂ (1.2), NaH₂PO₄ (0.75), and Glucose (11) (Lichtenhan et al. 2014). To insert the injection pipette, the mucosal covering at the cochlear apex was removed with a moist, cotton wrapped applicator or cellulose wipe. A thin layer of cyanoacrylate glue was applied to the dried area followed by a layer two-part silicone elastomer to make a cup-shaped hydrophobic surface. A fenestration was made through the adhesives and underlying bone, which is thinner in the apex than the base. A fenestration was made with a 30°, 1/3mm oval window pick by repeatedly resting the pick in place and lifting the pick off the surface, each time in a different asterisk-like direction. The surgeon did not apply a force to the pick, and effectively guided the weight of the pick make the fenestration. The size of the fenestration was just large enough to insert the pipette with a 20–30 µm diameter tip that was pulled from 100 mm × 0.58 mm inner-diameter glass tubing. The diameter of the injection pipette was achieved by making a flush break, not by beveling. The injection pipette was sealed in place by wicking perilymph from the hydrophobic surface and then applying additional cyanoacrylate glue to form a fluid-tight seal between the glass and the hydrophobic, circular levee. Solutions in the injection pipette were driven by a 50 or 100 µL gas-tight syringe (1710TLL Hamilton), glued to a plexiglass coupler (MPH6S10 World Precision Instruments, Sarasota, FL), mounted on a computer-controlled WPI Ultrapump (World Precision Instruments, Sarasota, FL).

A three dimensional reconstruction of a guinea pig inner ear was used to quantify the gradation in the cross sectional area of scala tympani (Fig. 2). In Fig. 2, the lateral wall is green and scala vestibuli is dark orange. The same cochlea is shown in Fig. 2b, but cut at the midline to reveal the scala media (purple), scala tympani (pale orange), spiral ganglion (gray), and auditory nerve (dark green). *To achieve a constant flow of solution at 0.5 mm/min along the scala tympani that has a cross sectional area that varies by a factor of 24, from 0.054mm² in the apex to 1.28mm² in the base (Fig. 2c), the rate of apical injection was varied from 25 nl/min to 626 nl/min for a total of 5.916 μ l over 37 minutes.* Constant-rate injections, which were not used, would have yielded reduced flow rate as scala tympani cross section widens. Dilution of solution concentration from mixture with cerebrospinal fluid (CSF) that enters through cochlear aqueduct is one of several reasons why the common procedure of round window niche applications cannot treat the entire cochlear length (Salt et al. 2015). In contrast to drug delivery at the cochlear base, apical injections avoid dilutions even at the lower range of injection rates (i.e., 25 nl/min) which are close to the measured sustained volume of cerebrospinal fluid entry of ~30 nl/min through the cochlear aqueduct (cf. Salt & DeMott 1998, Salt et al. 2015). Thus, from the start of the injection thereafter, the entry of cerebrospinal fluid at the base will be suppressed.

We used a simulation of drug delivery described by Salt et al. (2015) to understand how the injection rates into the apex of scala tympani influence drug concentration levels. Concentrations of fluorescein isothiocyanate-dextran (FITC) were measured, and the underlying kinetics characterized (Salt et al. 2015). We simulated injection of 1 mM FITC-dextran solution into the cochlear apex at either a constant rate (Fig. 3A) or a variable rate informed by gradations in scala tympani cross sectional area from Fig. 2 (Fig. 3B). The ideal results would show concentration to start increasing along the distance of scala tympani in a manner that is a function of time. Administration with a constant pump rate (Fig. 3A) yielded 50% of the maximum, applied concentration by ~3 minutes at the most apical region. The simulation shows that a uniform concentration along the distance of scala tympani was not achieved with the constant pump rate, as the rate is too fast during the early injection times and too slow during the later injection times (i.e., the three-dimensionally expressed concentrations from the constant rate injection are highly “bowed” near the middle of the injection experiment – Fig. 3A). In contrast, administration with a varying pump rate (Fig. 3B) yielded 50% of the maximum 1 mM applied concentration within ~3 minutes at the most apical region, and sequentially reached 0.5 mM with a far more linear time sequence and distance along scala tympani. This simulation shows that concentration gradients along the length of scala tympani are far more linear, or more ideal, for the varied pump rate as compared to the constant pump rate¹.

2.3. Acoustic Stimuli and Electrophysiologic Recordings

Electrophysiologic measurements were made with Tucker-Davis System 3 hardware and a personal computer with custom-written software (Visual Basic, Microsoft). Stimulus generation was done in TD-RP2 modules, passed through TD-PA5 attenuators, and TD-HB7

¹To the extent that the pharmacokinetics of FITC-dextran and kainic acid are similar, comparing these simulation results to empirical results presented later suggest that the effective concentration of kainic acid is ~60% of the injected concentration.

headphone amplifiers. Probe tones were delivered in a closed sound system consisting of an Etymotic ER-10C connected to the hollow ear bar. In-the-ear calibrations were completed in individual ears by tracking 70 dB SPL tones from 125 Hz to 26 kHz in ¼ octave steps. Cochlear response measurements were made differentially between an Ag/AgCl ball-tipped electrode in the round window niche and a platinum-needle electrode in the vertex. Measurements were made with an optically-coupled amplifier (TD HB7, 1000× gain, 0.005 – 15 kHz bandpass filter), routed to TD-RP2 modules for digitization (48.8 kHz) and averaging. Guinea pigs were electrically grounded with an Ag/AgCl pellet electrode connected by a fluid bridge in exposed soft tissue of the neck.

3. RESULTS

Slow and controlled injections of kainic acid into the cochlear apex can abolish a CAP from a given probe frequency and level (Fig. 4). Agonists for synapses between the inner hair cells and the primary afferent fibers reduces CAP amplitudes and elevates auditory neural thresholds without affecting distortion product otoacoustic emission thresholds (i.e., producing an “auditory neuropathy”, Peppi et al. 2012; Fujikawa et al. 2014). Response waveforms to 2 kHz tone bursts recorded from a round window electrode during an injection are displayed in Fig. 4a. Response waveforms from the start of the injection are plotted at the bottom of Fig. 4a, and measurements made progressively throughout the duration the injection are sequentially plotted vertically. Similarly, CAP amplitude measurements made over the course of the injection are plotted from left to right in Fig. 4b. CAP waveform peaks are deflected downward in Fig. 4a and occur ~2.5 ms. The origins of waveform peaks remaining after CAP abolition are unknown, but presumably include responses such as the “summing potential”. *CAP amplitudes from this 85 dB SPL 2 kHz probe tone rapidly declined between 5 – 20 minutes after the start of injection and were abolished by 32 minutes.*

Apical injection of kainic acid can be used to understand the spatial origin of CAPs evoked from different probe levels (Fig. 5). CAP amplitudes increased when the level of the 2 kHz probe level was increased over a 24 dB range in 6 dB steps, as expected (e.g., Fig. 5A, time zero). Reductions of CAP amplitudes from low to high probe levels occurred sequentially during ~10 minutes of apical injection: abolition of CAP amplitude to 50 dB SPL occurred at ~15 minutes after the start of apical injection and abolition to 74 dB SPL occurred at ~25 minutes (Fig. 5A). Differences in successive CAP amplitudes identify the time when amplitude reduction rate was greatest (Fig. 5B).

The relative differences in CAP amplitudes between probe levels were maintained during CAP amplitude reductions from apical injections. Maximal CAP amplitude reduction rate from low to high levels occurred sequentially during ~6 minutes: maximal rate of CAP amplitude reduction occurred at ~8 minutes after the start of injection to 50 dB SPL, and ~14 minutes to 74 dB SPL. *Increased abolition times, and maximal CAP amplitude reduction rates, for increasing probe tone levels were consistent with origins of CAP from more basal cochlear locations as probe levels increased.* This technique allows, for the first time, the spatial sites of origin of the CAP to be objectively defined. Slow and controlled injection of ototoxic solutions into the cochlear apex can sequentially ablate CAPs evoked

from different cochlear frequency places (Fig. 6). It is well known that CAPs originate from the peak of the traveling wave (see Lichtenhan 2012 for a review). Tone-burst frequencies were chosen such that they evoked CAPs in a peak of threshold fine structure, and tone-burst amplitudes were chosen to produce a ~40 μ V response. A battery of measurements was repeated in 90 second intervals during an apical injection. The time course of CAP amplitude reductions demonstrate that the apical injection technique can sequentially effect CAPs evoked from half-octave spaced tone bursts (Fig. 6a) as well as finely spaced tone bursts (~16 per octave; Fig. 6b).

Controlled injections into the cochlear apex can treat the apical half of the cochlear spiral, as demonstrated with Auditory Nerve Overlapped Waveform (ANOW) amplitude measurements evoked from 50 dB SPL 500 & 707 Hz tone bursts (Fig. 7). Previously we found that ANOW can adequately quantify low-frequency auditory thresholds that no other objective measure can, the ANOW originates from afferent auditory nerve fibers in the cochlear apex, and that physiology of the cochlear apex is affected by increasing pump rates that account for the widening cross sectional area of scala tympani in the basal half (Lichtenhan et al. 2013; 2014). As kainic acid was driven from injection pipette sealed into a fenestra in the cochlear apex, the ANOW measurements were ablated before, and more gradually than, the traditional CAPs evoked from 3 to 6 kHz. Ablation of ANOW measurements before CAP measurements supports our previous finding that the ANOW originates in the apical half of the cochlear spiral (Lichtenhan et al. 2014). The more gradual ablation of ANOW found from our advancement of the apical injection method is consistent with broader tuning in the cochlear regions tuned to low-frequencies (Furman et al. 2013). These data show that the apical injection method for a sealed and intact cochlea can treat cochlear regions tuned to low-frequencies, which round window application of pharmaceuticals cannot.

The apical injection procedure used to abolish neural function did not cause apparent disruption of outer hair cell mechanics assessed with distortion product otoacoustic emissions (DPOAEs; Fig. 8). DPOAE amplitude fine structure was maintained after an apical injection procedure (Fig. 8a). While the step size of our probe frequencies was coarser than that used for studies of otoacoustic emission amplitude fine structures, the > 10 dB depth of fine structure at ~2 kHz shown here is consistent with that found in guinea pig (Shera et al. 2000) and human (Henin et al. 2011; Abdala & Dhar 2012). DPOAE amplitude was not markedly affected during an apical injection procedure (Fig. 8b). These results are consistent with Lichtenhan et al. (2014) demonstrating that the apical injection method with neurotoxic solutions does not disrupt cochlear mechanics.

4. DISCUSSION

In this report we demonstrated a method for highly controlled administration of microliter volumes of drug-containing solutions into the cochlear apex. This apical injection method can be a powerful approach to sequentially effect or abolish objective measurements of cochlear responses and thus identify the spatial origin of any physiologic response so long as cell-specific treatments are used. A constant rate of injection would yield a flow that slows as scala tympani cross sectional area widens toward the base. In our initial attempt to

achieve a more uniform longitudinal flow along the cochlear spiral, we began injections at a rate of 50 nl/min, increasing to 100 nl/min after 10 min, and to 200 nl/min after 30 min (Lichtenhan et al. 2014). This combination of flow rates provided a more uniform rate of spread down the cochlea, adequate to differentiate the sites of origin of objective measurements from the cochlear apex and base. Here we report an advance of this method by varying the injection rate progressively, based on empirical cross sectional area measurements of scala tympani. This allowed far more precise manipulation of finely separated cochlear frequency places than we had anticipated.

4.1. Comparison with existing methods

Direct administration of pharmaceuticals to the inner ear is essential to bypass the blood-labyrinth barrier. One approach to directly administer solutions to the inner-ear fluids of intact ears is administration to the cochlear base, such as round window niche. Solutions in scala tympani influence the sensory cells or neurons either by passing through the acellular basilar membrane or by entering the spiral ganglion (Shepherd & Colreavy 2004). However, limitations of passive diffusion and losses of drug concentrations out of the ear restrict treatment effects to the basal regions of cochleae that are tuned to high frequencies (e.g., Salt & Ma 2001; Le Prell et al. 2014; Chertoff et al. 2015; Kang et al. 2016). Indeed, administration of gentamicin to the human round window is commonly used by otologists because hearing loss in response to speech frequencies is rarely induced but partially ablates vestibular function and relieves symptoms such as vertigo. Advancements to this classic round-window administration approach have been to surgically implant osmotic and controlled pumps, but even sustained administration to intact ears with pumps in chronic experiments does little, if any, to overcome the limitation of restricted treatment to the basal region of the cochlea (e.g., Chen et al. 2005).

One drug delivery technique in particular *perfuses* solutions throughout the cochlear length by injecting at relatively high rates (2 – 5 uL/min) into the basal turn of scala tympani with an outlet, or fenestration, for flow made at the apex or in the basal turn of scala vestibuli (e.g., Konishi & Kelsey 1968; Kujawa et al. 1995; Bobbin & Salt 2005; Wang & Olson 2016). There are several problems that arise from patent fenestrations used with cochlear drug perfusion. Sustained flow of CSF through the ear is 0.5 – 1.5 uL/min in the presence of a fenestration, a marked increase compared to the ~30 nL/min in a normal state (cf. Salt & Stopp 1979; Salt et al. 2015). CSF flow dilutes applied drug concentrations and rapidly washes drugs out when application ceases, as we demonstrate in Fig. 9. Perilymph differs in chemical composition from CSF and scala tympani perilymph composition differs from that of scala vestibuli, causing greater chemical disturbance in the presence of an inner-ear fenestration. A consequence is that larger volumes of drug-containing solution must be injected, potentially causing greater disruption of temperature and, by disturbance of CO₂ levels, intracochlear pH.

Inner-ear fenestration used with the cochlear perfusion method compromises low-frequency function (e.g., Dong & Cooper 2006; Lichtenhan et al. 2015; Niesten et al. 2015). The cochlear perfusion method thus cannot be reliably used to address research questions related to cochlear frequency places tuned to the low-frequency speech vowels and background

noise. Fluid accumulation near an electrode placed outside the cochlea is an additional problem with the cochlear perfusion method. Solutions perfused through the cochlear base pass through the ear and exit through the fenestration. An accumulation of fluid near an electrode creates a medium that vectorally smears electrical responses originating from throughout the ear, thus challenging the integrity of objective assessment made with these electrical measurements. Empirical concentration and flow measurements were used to inform simulations designed by Salt et al. (2015) to quantify as a function of time drug distribution along the length of scala tympani for a cochlear perfusion that uses a patent fenestra (Panel C) and an injection from a pipette sealed into the cochlear apex (Panel D). Results from the simulation show that, for cochlear perfusions into the cochlear base, ~50% of the applied concentration is sustained throughout scala tympani for the duration of the perfusion. In this experimental preparation, CSF entry through the cochlear aqueduct in the base of scala tympani dilutes drug concentrations. In contrast, for the apical injection, drug levels closer to ~100% of the applied concentration are achieved and maintained for a substantial time, except at the extreme base of scala tympani which is considerably affected by interactions with CSF.

Applying drugs to scala tympani is certainly not the only approach that has been used to sequentially affect regions along the cochlear length to study cochlear mechanics and physiology. Acoustic over exposure was recently used to sequentially affect cochlear regions (Charaziak & Siegel 2015). Controlled administration of solutions into the cochlear apex produces less variable results across individual ears as compared to the well-known, and sometimes desired, variability of acoustic over exposure in outbred animals (cf. Liberman 1984; Wang et al. 2002; Chertoff et al. 2003; Siegel et al. 2005; Lichtenhan & Chertoff 2008; Choudhury et al. 2011; Lichtenhan et al. 2014). As compared to apical injections, acoustic over exposure cannot sequentially effect cochlear regions that are as finely spaced as what we showed here (cf. Fig. 4 of Charaziak & Siegel 2015) and always produces damage extending toward the cochlear basal away from the intended lesion area (e.g., Wang et al. 2002; Chertoff et al. 2003). Acoustic over exposure does not ablate the sensory cells of the cochlear apex, thus limiting the useful effects of sound trauma to studying the basal, high frequency half of the cochlea (Harding et al. 2007). Thus, our approach to studying the origins of cochlear responses is more accurate throughout the cochlear length than the use of acoustic over exposure.

Our new method of controlled injections of solutions with a pipette sealed into the cochlear apex overcomes many of the limitations of applying solutions to the round window niche or perfusion into the cochlear base, and the use of sequential noise exposure. Apically injected solutions sequentially effect cochlear regions and avoid problems associated with cerebrospinal fluid entry by causing an outward flow at the cochlear aqueduct. The apical injection method for *in vivo* drug delivery to sealed cochleae allows intra-cochlear pressure to be maintained. A result of these innovations is that physiologic measurements can be sequentially effected with much greater control of drug levels at different locations and times than had previously been possible (Fig. 6b).

4.2. Potential Uses of the Apical Injection Method

Objective measurements from the ear are routinely used in the clinic and laboratory. If their spatial and cellular origins could be better understood, clinicians and investigators could be more informed on how to differentially diagnose diseased ears. In this report we described an approach that can be used to help identify origins of objective measurements from the cochlea by sequentially manipulating finely spaced regions. A similar approach is to sequentially vary the cut off frequency of high-pass masking to study the origin(s) of objective measurements that can be influenced by different stimulus rise times and sound levels (Earl & Chertoff 2012). However, both the stimuli and noise masker can excite unintended, adjacent cochlear frequency places. Using the apical injection method to better understand results from sequential high-pass masking would be a worthwhile effort to translate the correct use of sequential high-pass masking to study human ears.

Sensorineural hearing loss in humans and animals is often described with auditory thresholds. But, it is not clear that auditory thresholds can precisely identify the damaged, diseased, and normal cochlear cell types that influence the generation of objective measurements (e.g., Probst et al. 1987; Canlon et al. 1993, Subramaniam et al. 1995; Bian & Chertoff 2001, Harding et al. 2002; Lichtenhan et al. 2005; Moleti et al. 2014; Landegger et al. 2016). One likely reason for this conundrum is that probe levels have to be increased to quantify auditory threshold in ears with sensorineural hearing loss, thus stimulating unintended cochlear regions away from the cochlear frequency place that is tuned best to the probe frequency. It is likely that using single probe levels and the apical injection method is a simpler approach to studying the origins of objective measurements.

5. CONCLUSIONS

A method to pharmacologically effect electrical responses along the length of the cochlear spiral was described. Slow and controlled administration of solutions was performed through a fenestra made in the cochlear apex. Solutions were driven from the injection site to the cochlear aqueduct in the base of scala tympani, overcoming losses of drug concentrations that limit the use of other administration methods. A three-dimensional reconstruction of a guinea pig ear was used to quantify gradations in scala tympani cross sectional area and inform the rate of injection needed to achieve a constant flow. Slow and controlled injections of solutions into the cochlear apex can sequentially affect finely spaced cochlear regions.

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HIGHLIGHTS

- A new method to pharmacologically affect the cochlea is described.
- Solutions are administered from a pipette sealed into the cochlear apex.
- Controlled injections drive solutions towards the cochlear aqueduct in the base.
- The method better controls drug concentrations than conventional cochlear perfusion.
- Responses from finely spaced stimulus frequencies (~16 per octave) were affected.¹

Fig. 1.

A schematic of the injection method into the cochlear apex of the inner ear. The endolymphatic spaces are shown blue and the perilymph-containing scala vestibuli is shown in yellow. Drug-containing solution injected through a pipette sealed into the cochlear apex fills the length of scala tympani (orange) as fluid is driven out through the cochlear aqueduct into the cerebrospinal fluid space (cyan).

Fig. 2.

A three dimensional reconstruction of a guinea pig cochlea was used to quantify the variation in scala tympani cross sectional area. The outer most structures are shown in Panel A, and the midline structures of the same cochlea are shown in Panel B. The thin arrows associate the three dimensional reconstructed scala tympani with cross sectional area measurements in Panel C. The variations of scala tympani cross sectional area were used to inform the rate of apical injection. Panel D: The rate of apical injection was set each minute to achieve a consistent flow of solution along scala tympani.

Fig. 3.

Calculated concentration-time profiles for apical drug delivery. 1 mM FITC-dextran was injected at a constant rate (Panel A) or a variable rate informed by gradations in scala tympani anatomy of Fig. 2C (Panel B). Constant pump rate caused the solution to rapidly spread along apical regions and subsequently very slowly in more basal regions. In contrast, varying pump rate improved the linearity of drug concentration progress. The result is that varying the pump rate to account for gradations in scala tympani cross sectional area provides a more uniform concentration along the length of the cochlea.

Fig. 4.

Cochlear action potentials (CAPs) were abolished as a neurotoxic solution was injected into the cochlear apex. In this example, response waveforms from an 85 dB SPL 2 kHz tone burst were made with a round-window electrode (Panel A). CAP amplitude was measured as the negative deflection at ~2.5 ms from each response (Panel B). CAP amplitudes from this 2 kHz probe tone rapidly declined between 5 – 20 minutes after the start of injection, and was abolished by 32 minutes.

Fig. 5.

CAP amplitudes from varying levels of 2 kHz tone bursts were sequentially abolished during apical injection of kainic acid in an individual ear. CAP amplitudes from 50 dB SPL tone bursts were fully ablated by ~15 minutes after the start of injection, while CAP amplitudes from 74 dB SPL were ablated by ~25 minutes (Panel A). The associated difference in successive CAP amplitudes showed that the highest rate of CAP amplitude reduction was sequential for tone bursts that varied from low to high stimulus levels (Panel B). Later times

for maximal CAP amplitude reductions to higher level stimuli are consistent with origins from more basal cochlear regions.

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Fig. 6.

The time course of 40 µV CAP amplitude reductions from two different ears: half-octave spaced tone bursts (Panel A) and finely spaced (~16 per octave) tone bursts (Panel B). Responses to higher frequency tone bursts are reduced later in time, corresponding to their more basal origins

Fig. 7.

Apical injections can be used to affect measurements that originate from the apical half of the cochlea. ANOWs were evoked from 50 dB SPL 500 and 707 Hz tone bursts and traditional CAPs were evoked from 3–6 kHz tone bursts with sound pressure levels that yielded 40 μ V amplitudes at the start of injection. As the kainic acid solution was driven from the cochlear apex to base, ANOW measurements were ablated before CAP measurements. These data demonstrate that drug delivery into the cochlear apex can treat the cochlear apex.

Fig. 8. DPOAE amplitude measurements made from two different experiments, one made before and after injecting a neurotoxin (Panel A) and another made *during* the injection (Panel B). $f_1=1.4757$ kHz, $f_2=2.5708$ for Panel B. $L_1=65$ dB SPL and $L_2=55$ dB SPL for both Panels A & B. The apical injection method with neurotoxic solutions did not disrupt DPOAE amplitude measurements.

Fig. 9.

The cochlear perfusion method that uses a patent fenestra has a unique problem that the apical injection method for sealed cochleae does not have: augmented flow of cerebral spinal fluid. A 10 min perfusion FITC-dextran 1 mM solution of scala tympani was used for this experiment because its concentration and volume flow rate can be readily measured. Fluid accumulated in a hydrophobic dike at the cochlear apex made of a thin layer of cyanoacrylate glue applied to the dried cochlear bone followed by a layer two-part silicone elastomer. Samples of 1–3 μL were collected from the dike at sequential times, allowing concentration to be measured (Panel A) and flow rate calculated (Panel B). FITC-dextran reached a maximum concentration of 0.56 mM during perfusion and declined rapidly after perfusion. The ~1.8 μL/min baseline flow rate is increased to ~4 μL/min during perfusion.