

# Quantification of solute entry into cochlear perilymph through the round window membrane

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Received 19 September 2000; accepted 19 December 2000

## Abstract

The administration of drugs to the inner ear via the round window membrane is becoming more widely used for both clinical and experimental purposes. The actual drug levels achieved in different regions of the inner ear by this method have not been established. The present study has made use of simulations of solute movements in the cochlear fluids to describe the distribution of a marker solute in the guinea pig cochlear fluid spaces. Simulation parameters were derived from experimental measurements using a marker ion, trimethylphenylammonium (TMPA). The distribution of this ion in the cochlea was monitored without volume disturbance using TMPA-selective microelectrodes sealed into the first and second turns of scala tympani (ST). TMPA was applied to perilymph by irrigation of the intact round window membrane with 2 mM solution. At the end of a 90 min application period, TMPA in the first turn, 1.4 mm from the base of ST, reached an average concentration of 330  $\mu\text{M}$  (standard deviation (S.D.) 147  $\mu\text{M}$ ,  $n=8$ ). TMPA in the second turn, 7.5 mm from the base of ST reached a concentration of 15  $\mu\text{M}$  (S.D. 33  $\mu\text{M}$ ,  $n=5$ ). The measured time courses of TMPA concentration change were interpreted using the Washington University Cochlear Fluids Simulator (V 1.4), a public-domain program available on the internet at <http://oto.wustl.edu/cochlea/>. Simulations with parameters producing concentration time courses comparable to those measured were: (1) round window permeability:  $1.9 \times 10^{-8}$  cm/s; (2) ST clearance half-time: 60 min; (3) longitudinal perilymph flow rate: 4.4 nl/min, directed from base to apex. Solute concentrations in apical regions of the cochlea were found to be determined primarily by the rate at which the solute diffuses, balanced by the rate of clearance of the solute from perilymph. Longitudinal perilymph flow was not an important factor in solute distribution unless the bony otic capsule was perforated, which rapidly caused substantial changes to solute distribution. This study demonstrates the basic processes by which substances are distributed in the cochlea and provides a foundation to understand how other applied substances will be distributed in the ear. © 2001 Elsevier Science B.V. All rights reserved.

*Key words:* Cochlea; Perilymph; Round window membrane

## 1. Introduction

Clinically, there is increasing interest in intratympanic therapy, that is the local application of drugs directly to the inner ear across the intact round window membrane. This method permits the fluids and tissues

of the ear to be manipulated without systemic disturbance and avoids problems associated with the entry of drugs being blocked by the blood–labyrinth barrier. In cases of Meniere's disease, the instillation of gentamicin or streptomycin solutions into the middle ear has been widely used as a method of suppressing vestibular function in the affected ear (Blakley, 1997). This approach avoids the risk of damaging the non-affected ear, as would occur with systemic treatments. Experimental studies are developing uses for a wide variety of agents, including steroids, local anesthetics, anti-oxidants, glutamate antagonists, neurotrophins and vectors for gene therapy, delivered on or through the round window, as treatments for various cochlear pathologies (Blakley, 1997; Coles et al., 1992; Seidman, 1998; Kopke et al.,

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*Abbreviations:* TMPA, trimethylphenylammonium; ST, scala tympani; SV, scala vestibuli; ELS, endolymphatic space; S.D., standard deviation

1995; Stover et al., 1999; Yage et al., 1999). A number of groups have also developed specific delivery systems for applying drugs to the inner ear, including a round window microcatheter (Durect Inc., Cupertino, CA, USA), the MicroWick (Silverstein, 1999), and an implantable delivery system (Lehner et al., 1997).

The amount and distribution of applied substances within the ear have received remarkably little attention due to the considerable technical difficulties associated with making such measurements. As the fluid spaces of the inner ear are regarded as extremely small, it is sometimes assumed that a drug applied to the round window will be widely and uniformly distributed throughout the ear. This perception has been supported by the predominance of vestibulotoxic effects over auditory damage following gentamicin administration to the round window. Using horseradish peroxidase (HRP) as a marker, Saijo and Kimura (1984) found that marker entering the cochlea from the middle ear was largely confined to the basal regions of the cochlea and to the vestibular system and was absent from higher turns of the cochlea. Substances are believed to reach the vestibular system by extracellular communication between the perilymphatic scalae across the spiral ligament (Salt et al., 1991a,b) rather than via the helicotrema.

The goal of the present study was to document where substances spread in the cochlear fluids when they are applied to the intact round window membrane and to quantify the biophysical processes involved in their movements.

## 2. Materials and methods

The experimental portion of this study was based on measurements made in eight guinea pigs. Animals were anesthetized with 100 mg/kg Inactin (Research Biochemicals International, MA, USA) and their right cochlea exposed by a ventrolateral approach. A cannula was placed in the left jugular vein to allow anesthetic supplements to be given and to administer a muscle relaxant (pancuronium bromide: to effect). The trachea was cannulated and the animals were artificially ventilated. The respirator was adjusted to maintain an end-tidal CO<sub>2</sub> concentration near 5%.

The entry of a marker ion, trimethylphenylammonium (TMPA), into perilymph of scala tympani (ST) was monitored by sealing TMPA-selective electrodes into the perilymphatic space. Access to perilymph was gained by making a small (20–30 µm diameter) fenestrae in the bone overlying the scala, in which the electrodes were inserted tightly. In three experiments a TMPA-selective electrode was located in the basal turn of ST. In five experiments, two TMPA-selective

electrodes were sealed into ST, one in the basal turn and one in the second turn. Basal turn electrodes were placed an average of 1.1 mm (standard deviation (S.D.) 0.1) from the bony lip of the round window niche. Electrodes were sealed in place by drying the bone around the fenestra with desiccated air and then applying a drop of thin cyanoacrylate adhesive. This technique prevents perilymph leakage from around the electrodes which would permit longitudinal perilymph movements.

Ion electrodes were manufactured and calibrated using methods identical to those in earlier studies (Salt et al., 1991a). One barrel of a double-barreled electrode was silanized with dimethyldichlorosilane, following which the tip was broken to 2–5 µm diameter. The silanized ion barrel was filled with 500 mM KCl and the non-silanized potential barrel was filled with 500 mM NaCl. A small column of ion-exchanger was drawn into the ion barrel by suction. The ion-exchanger was made as a solution of 5% potassium tetrakis (4-chlorophenyl) borate in 2-nitrophenyloctylether which was pre-equilibrated with aqueous TMPA solution prior to use. Electrodes were calibrated before and after use in solutions at 37°C containing 0, 1, 10, 100 and 1000 M TMPA made up in an artificial perilymph solution. Electrodes were highly sensitive to low concentrations of TMPA, with a detection limit of 3.9 µM (S.D. 2.9,  $n=13$ ) and a response slope of 62.9 mV/decade (S.D. 8.8,  $n=13$ ). The detection limit is defined as the lowest concentration measurable if a linear electrode response is assumed. Using a non-linear function that describes the electrode response down to zero concentration (Salt and Vora, 1991), concentrations well below the detection limit were accurately resolved. Potentials were sampled and stored digitally under computer control, with readings stored at 20 s intervals throughout the experiment. Concentrations were derived from potential data off-line, based on the calibrations performed before and after the recording period.

TMPA was applied to the round window in the form of a 2 mM solution in a background of artificial perilymph that was irrigated across the membrane. Solution flowed from a glass pipette connected to a Harvard HPD2000 infusion pump which maintained a constant flow rate of 5 µl/min. A small tube connected to a vacuum line, continually removed accumulating solution from the bulla. Care was taken to ensure that the irrigation pipette did not make contact with the round window membrane to ensure that the membrane remained intact.

Experimental protocols were reviewed and approved by the Animal Studies Committee of Washington University, approval number 1990029.

### 3. Results

#### 3.1. Experimental measurement of solute entry through the round window

The concentrations of TMPA measured in the first and second turns of ST following application of solution containing 2 mM TMPA to the round window membrane are summarized in Fig. 1. In the basal turn, there was a brief delay before the TMPA began to increase following a non-linear curve. TMPA concentration reached an average of 330.0  $\mu\text{M}$  (S.D. 147.1,  $n=8$ ) after 90 min. In the second turn, virtually no TMPA was detected for approximately 60 min, followed by a small increase in some animals. The average concentration measured after 90 min was 15.5  $\mu\text{M}$  (S.D. 33.5,  $n=5$ ). In qualitative terms, these measurements demonstrate that the marker TMPA does pass through the round window membrane in appreciable quantities, reaching a concentration in the basal turn which at 90 min is 16.5% of that in contact with the round window. In this time period however, extremely little TMPA reaches the second turn, with an average amount of only 0.8% of that in the middle ear, demonstrating that substances entering the perilymph only move slowly along the perilymphatic spaces.

#### 3.2. Quantitative analysis of entry through the round window

In order to interpret the experimental findings quantitatively, there are a number of basic processes that must be taken into account. We have considered the

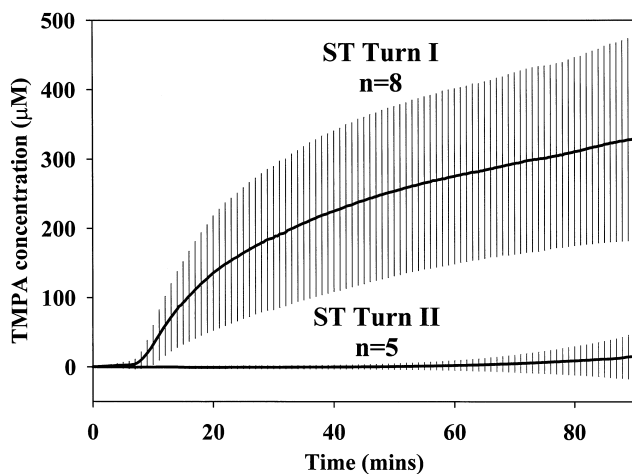


Fig. 1. Mean TMPA concentration measured in the basal (I) and second (II) turns of the guinea pig cochlea during sustained irrigation of solution containing 2 mM TMPA across the round window membrane. Bars indicate the S.D. of the number of experiments indicated.

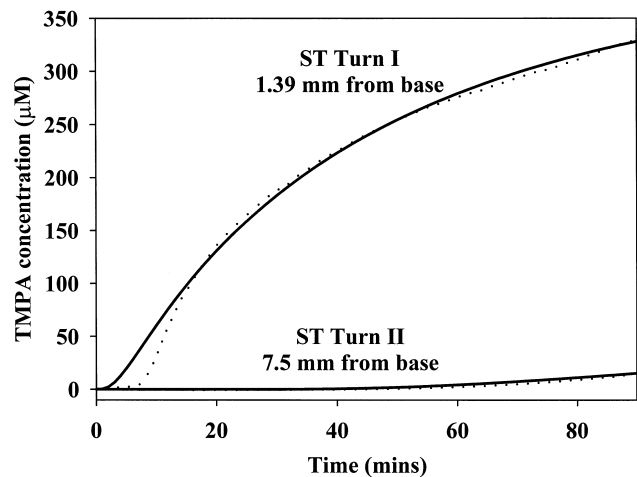


Fig. 2. Solid lines show calculated changes of concentration with time in the basal (I) and second (II) cochlear turns using the simulation model with parameters set as listed in Table 1. The mean concentrations recorded as a function of time in vivo taken from Fig. 1 are shown as dotted lines for comparison.

contribution of the following factors in the observed dispersal of TMPA in the cochlea.

1. The volumes of the cochlear fluid spaces and the variation of cross-sectional area as a function of distance along the cochlea.
2. The area of the round window membrane and its geometric relationship to ST.
3. The permeability of the round window membrane.
4. The rate at which TMPA spreads by diffusion along the fluid in ST.
5. The rate of longitudinal perilymph flow in ST.
6. The rate of clearance or leak of TMPA from ST to other fluid spaces of the cochlea and to the rest of the body via the vasculature.

The above factors have been incorporated into a simulation computer program which was developed by our group and is available for download from our web site at <http://oto.wustl.edu/cochlea/>. This model allows relevant parameters to be varied and the time course of concentration change for any specific location to be predicted for the specified set of conditions. Simulations take into account the volume and cross-sectional areas of the scalae in the guinea pig, which were derived from three-dimensional (3-D) magnetic resonance microscopy images (Salt et al., 1995; Thorne et al., 1999). They also incorporate the area of the round window and its geometric relationship to the basal turn of ST, which was also derived by analysis of 3-D magnetic resonance images of the guinea pig cochlea (Ghiz et al., 2000).

To demonstrate how the measured concentration time courses are interpreted, output from the model using parameters which best approximate the recorded

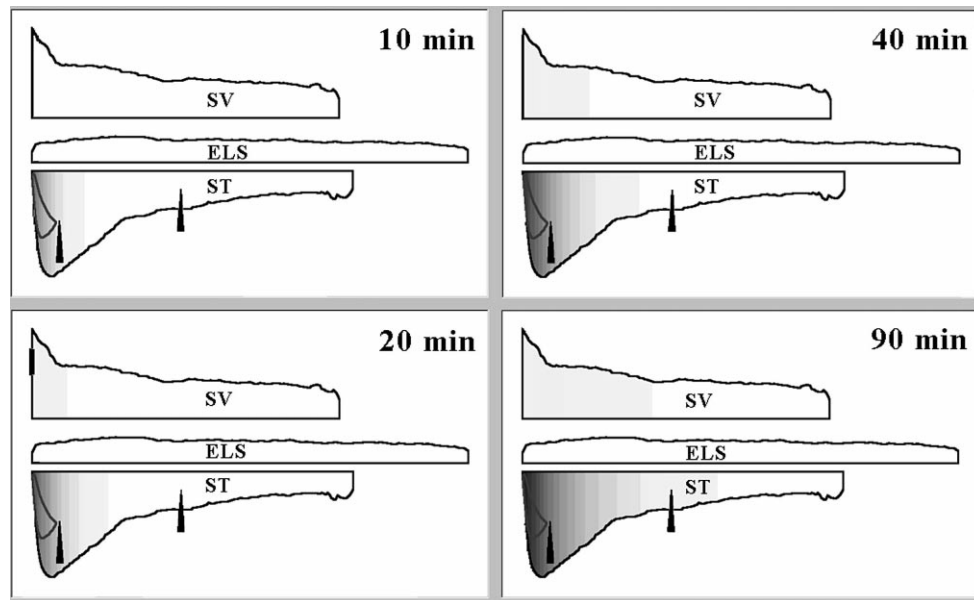


Fig. 3. Schematic of the guinea pig cochlear fluid spaces showing distribution of TMPA as a function of time following its application to the round window membrane. The lengths and cross-sectional areas of scalae in the guinea pig cochlea are based on measurements from specimens imaged in 3-D by magnetic resonance microscopy (Thorne et al., 1999). Scala length was measured along the geometric mid-point of each scala, which results in the ELS being longer than the two perilymphatic spaces as its mid-point follows a wider spiral. The abbreviations for the fluid spaces are: SV: scala vestibuli; ELS: endolymphatic space; ST: scala tympani. TMPA concentration is shown by grayscale, with darker shading representing areas of higher TMPA concentration. The basal and second turn recording sites are shown as black triangles. Note that the spread of marker along ST occurs slowly, primarily driven by passive diffusion.

curves is first presented. This analysis is followed by demonstration of the influence of varying each of the parameters on calculated result. Fig. 2 shows the calculated concentrations as a function of time for two locations corresponding to the basal and second turn recording sites. Distances are those measured from the base of ST along the mid-point of the scala. The fit of the calculated curves was achieved by varying the three parameters shown in Table 1. Variation of each parameter resulted in unique changes in the calculated curves, as detailed below, which were used to optimize the fit. Changes of round window permeability primar-

Table 1  
Calculation parameters used to simulate recorded data

$19.4 \times 10^{-9}$ cm/s	Parameters varied to fit experimental data Permeability of round window membrane to TMPA
4.4 nl/min	Perilymph flow rate (from base of ST to apex, to base of SV)
60 min	Half-time of clearance from ST
1.164 mm <sup>2</sup>	Parameters held constant for all simulations Round window membrane area
$1.01 \times 10^{-5}$ cm <sup>2</sup> /s	Diffusion coefficient for TMPA
2.0 mM	TMPA concentration applied to the round window membrane
100 min	Half-time of clearance from SV
25 min	Radial cross-communication half-time for ST–SV (figure represents the entry rate into a 0.1 mm <sup>2</sup> CSA segment)

ily resulted in amplitude changes of the curves at both first and second turn recording sites. Increasing an apically-directed perilymph flow resulted in an increase in marker concentration in the second turn and a decrease in concentration in the basal turn. Increasing the rate of clearance of marker from ST changed the time course of concentration change in the basal turn, with higher clearance rates resulting in more rapid kinetics. It was therefore possible to derive a unique solution, with one set of parameters providing a close fit to the measured curves. The simulation permits the characteristics of solute distribution within the cochlea to be described, as shown in Fig. 3. In this figure, the cochlea is shown schematically with areas of high TMPA concentration indicated by dark shading. Prior studies have shown that TMPA does not enter the endolymphatic space (ELS), primarily because entry is opposed by the endocochlear potential (Salt et al., 1991a). The spread of TMPA apically in ST occurs primarily by passive diffusion, with longitudinal perilymph flow playing only a minor role, as detailed below. After simulation of 90 min irrigation, low tracer concentrations are reaching the second turn, in agreement with the concentration time course recorded there (Fig. 1). Opposing the apical movements of TMPA are clearance processes, in which solute is removed from the scala. Based on the fitted curves, we estimate that the half-time of TMPA clearance from ST is near 60 min. In the absence of significant longitudinal flow, the rates of clearance and diffu-

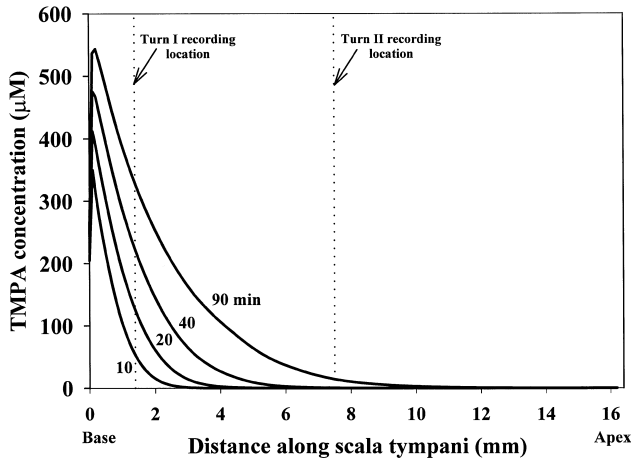


Fig. 4. Calculated TMPA concentration as a function of distance along the cochlea for four time intervals during irrigation of the round window membrane with 2 mM TMPA. Although concentrations in the basal region increase with time, spread of TMPA to apical regions of the cochlea progresses extremely slowly. Basal and second turn recording sites are indicated by dotted lines.

sion establish how far the substance will diffuse along the cochlea before a steady-state is established. When a steady-state is reached, the substance in each region is cleared at the same rate as it enters by diffusion and may never reach the apical regions of the cochlea. Also incorporated in the simulation is the known communication between ST and scala vestibuli (SV) across the spiral ligament. This communication, which allows TMPA to reach the vestibular perilymph without passing through the helicotrema, will be discussed further below.

The spread of TMPA as a function of distance along ST is shown more quantitatively in Fig. 4. The profile for TMPA concentration as a function of distance along ST is shown at four time periods. The locations of the two recording locations in turn I and turn II of the cochlea are indicated by dotted lines. It is apparent that over the time period shown, the marker ion increases in concentration in the most basal region but spread of the marker to the higher turns proceeds very slowly with marker reaching less than 10 mm along the cochlea within the 90 min period.

The influence of clearance processes on the calculated curves for ST is summarized in Fig. 5. The upper curve in each group shows the situation for no clearance at all from any compartment of the cochlea. The lines labeled ST–SV show the situation when radial cross-communication between ST and SV is incorporated together with a clearance from SV with half-time 100 min. Both of these processes have been quantified in prior studies (Salt et al., 1991a,b). For this curve there has been no clearance from ST included. These combined processes result in some loss of TMPA from ST, although the

contribution is small relative to the amount of clearance from ST subsequently required to fit the data. It should be noted that all clearances could have been grouped into one parameter but this would have slightly reduced the numeric value of the estimated ST half-time. The lower lines show concentration functions calculated with the above ST–SV communication and TMPA clearance from ST with half-times of 90 min (ST 90), 60 min (ST 60) and 30 min (ST 30), respectively. Increasing clearance results in a more rapid time constant in the basal turn, which results in a more rapidly achieved steady-state condition. Furthermore, higher rates of clearance result in disproportionately lower concentrations of TMPA reaching the higher cochlear turns. Thus the combination of the relatively slow rate at which diffusion occurs and clearance from ST result in this marker remaining substantially confined to the basal regions.

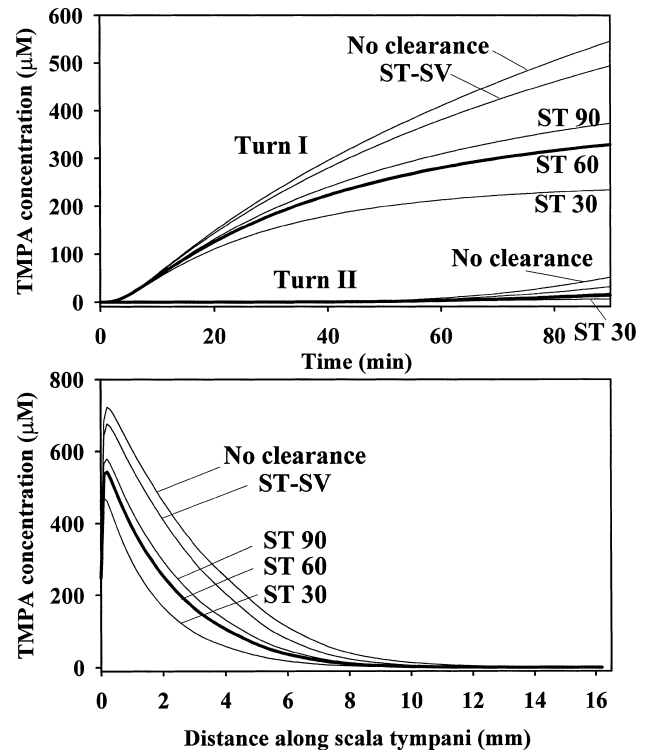


Fig. 5. Influence of clearance rates on TMPA concentrations calculated for 90 min irrigation of the round window. Upper panel: concentration at basal (I) and second (II) turn locations as a function of time. Lower panel: concentration as a function of distance along the cochlea after 90 min. Both graphs show curves calculated for the following clearance conditions. No clearance: no loss at all from ST. ST–SV: TMPA loss from ST to SV through radial communication pathways. ST 90: clearance from ST with half-time of 90 min in addition to radial loss to SV. ST 60: clearance from ST with half-time of 60 min in addition to radial loss to SV. ST 30: clearance from ST with half-time of 30 min in addition to radial loss to SV. Heavy lines show the curves which best fit the recorded data.

The influence of changes in round window permeability on calculated curves is shown in Fig. 6. Changes in permeability influence the amplitude of the calculated curves but have negligible influence on time constants or spread characteristics with distance.

Longitudinal perilymph flow produces more complex effects, as illustrated in Fig. 7. Although flow has relatively little influence on the concentration time course recorded in the basal turn, it had a marked influence on the amount of tracer reaching the second turn. It can be seen that in the absence of flow, marker concentration is high at the base and low in the second turn (Fig. 7; lower panel). As apically-directed flow is increased, this reduces the concentration at the base and increases the concentration in the second turn, thereby reducing the base to apex concentration gradient. Thus, the rate of flow can be accurately assessed by comparing the ratio of tracer concentrations at the basal and second turn recording sites. The estimated rate of 4.4 nl/min is still an extremely low rate and would, in the absence of diffusion, take over 18 h to displace the 4.76  $\mu$ l volume of ST. Nevertheless, this low rate does skew the rate of

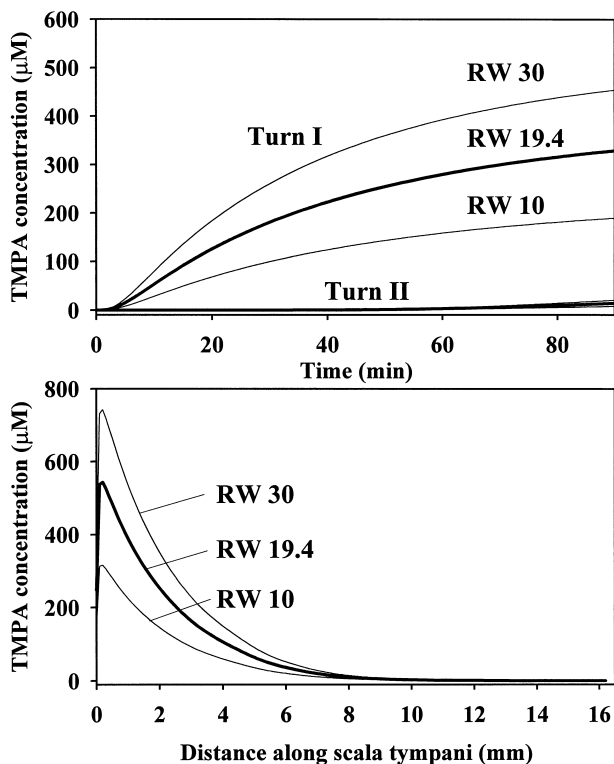


Fig. 6. Effect of varying round window permeability on TMPA concentrations calculated for 90 min irrigation of the round window. Upper panel: concentration at basal (I) and second turn (II) locations as a function of time. Lower panel: concentration as a function of distance along ST after 90 min irrigation of the round window. Heavy lines show the curves which best fit the recorded data. RW 30: round window permeability  $30.0 \times 10^{-9}$  cm/s; RW 19.4: round window permeability  $19.4 \times 10^{-9}$  cm/s; RW 10: round window permeability  $10 \times 10^{-9}$  cm/s.

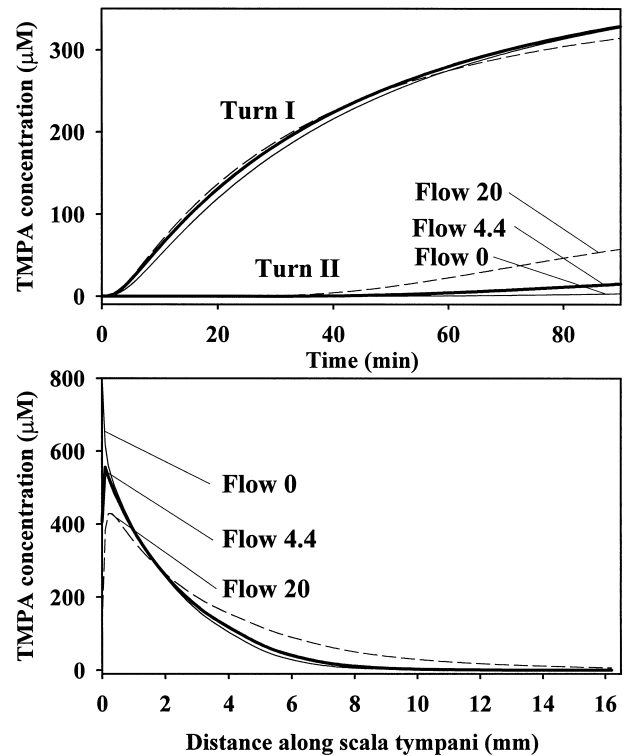


Fig. 7. Influence of longitudinal perilymph flow rate on TMPA concentrations calculated for 90 min irrigation of the round window. Upper panel: concentration at basal (I) and second turn (II) locations as a function of time. Lower panel: concentration as a function of distance along the cochlea after 90 min irrigation of the round window. Heavy lines show the curves which best fit the recorded data. The following rates of flow were used: Flow 0: no longitudinal flow in ST. Flow 4.4: apically-directed perilymph flow in ST at a rate of 4.4 nl/min. Flow 20: apically-directed perilymph flow in ST at a rate of 20 nl/min. Note that apically-directed flow at low rates increases the TMPA concentration in apical regions and decreases concentration at the base of the scala.

dispersion of a substance along the scala slightly and does need to be taken into account when dispersion into higher turns is considered.

### 3.3. Extrapolation of calculated data to longer periods

The simulation, based on an experimental period of 90 min, can be used to estimate what solute distribution is likely to result from longer application periods, which is more relevant to clinical treatments of the ear or chronic application of drugs. Fig. 8 summarizes the concentration as a function of distance and time simulated for a 24 h application to the round window. It is apparent that, with the calculation parameters used, a steady-state is established within 4–6 h, so that sustained application of the agent will not change the subsequent distribution profile. A pronounced longitudinal gradient remains along the length of ST with concentration at the apex a factor of 40 times lower than that

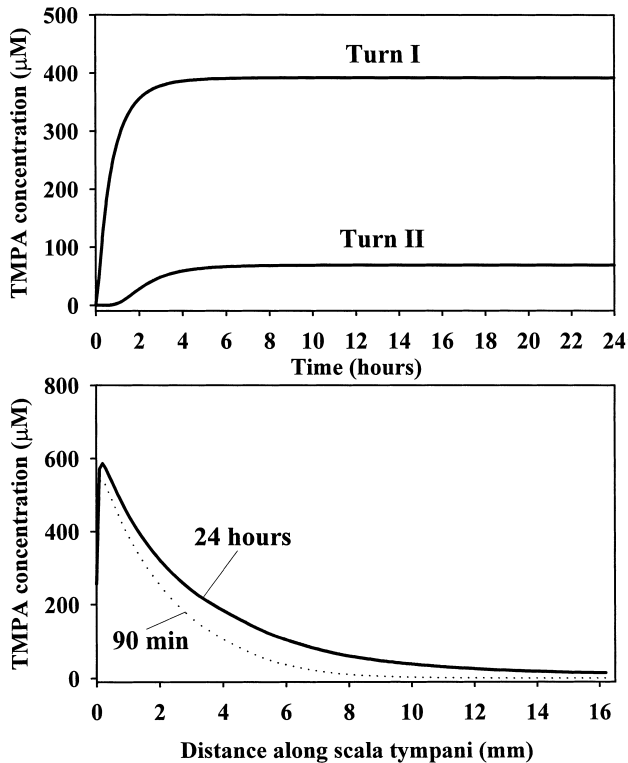


Fig. 8. Upper panel: calculated concentration time courses for a simulated 24 h application of TMPA to the round window membrane, based on the parameters established in 90 min experiments. Within a few hours a steady-state concentration is established in both the basal and second turns. Lower panel: calculated concentration as a function of distance along ST after 24 h irrigation of the round window compared to that calculated at 90 min. In the steady-state seen after 24 h, a marked concentration gradient remains along ST, with substantially lower concentrations in higher turns compared to the base. An even distribution of the solute along the length of ST cannot be achieved by prolonging the application duration.

at the base. This is reflected in the concentration time courses estimated for the first and second turns, in which the concentration at the second turn never exceeds 18% of that measured in the basal turn. Thus, application for a longer period will not result in an even distribution in the inner ear ever being achieved, even when the solute concentration at the round window is maintained constant. The measured and calculated longitudinal concentration gradients result from the combined influences of solute diffusion and volume flow, which are acting to move solute apically, which are counteracted by solute clearance processes, acting to remove solute from the scala.

### 3.4. Perforation of the otic capsule

The prior results are highly dependent on the cochlea being in the normal, sealed state. Any perforation of the bony otic capsule induces profound changes in sol-

ute distribution, as shown in Fig. 9. In this experiment, TMPA was monitored simultaneously in the first and second turns of ST for 90 min (data from which were included in the prior summaries) at which time the apex of the cochlea was perforated. In turn 1, concentration showed a sharp spike within a minute of the perforation, before falling. In turn 2, concentration increased dramatically, but more slowly, reaching a peak at approximately 3 min after the perforation, before again declining. The time courses are consistent with the ST contents being displaced in an apical direction by CSF entering the base of the ST through the cochlear aqueduct. If the concentration profile as a function of distance at 90 min, depicted in Fig. 3 (lower right panel) and Fig. 4, is displaced in an apical direction then curves similar to those measured would be anticipated. The curves resulting from perforation are therefore in accordance with our calculated profiles as a function of

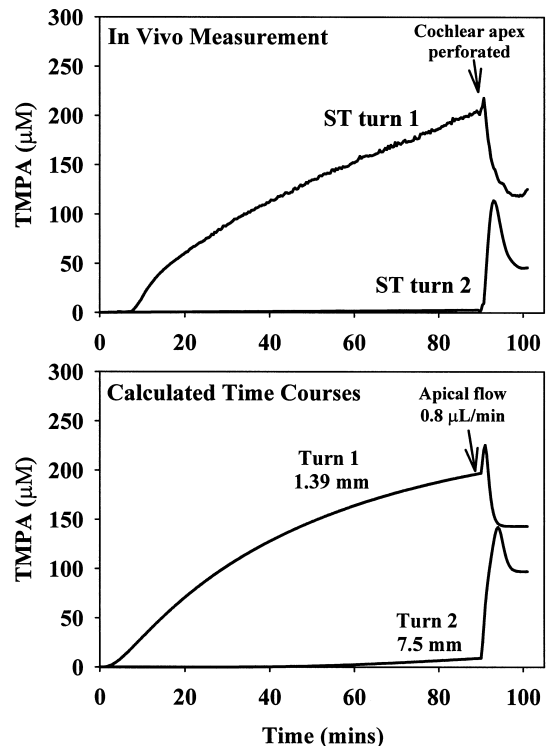


Fig. 9. Upper panel: experiment in which TMPA was monitored simultaneously in the first and second turns of ST for 90 min irrigation of the round window, at which time the apex of the cochlea was perforated. The basal turn shows a rapid, transient increase followed by a decline. The second turn shows a delayed, substantial increase, followed by a decline. These results are consistent with an entry of CSF into the basal turn of ST through the cochlear aqueduct, that induces an apically-directed flow in ST directed towards the outlet at the apex. Lower panel: calculated time courses for the situation where an apically-directed flow in ST at a rate of 0.8 μL/min is added to the simulation at 90 min. See text for details of other calculation parameters used. The model confirms that the measured time courses result from longitudinal flow of CSF through ST.

distance. The lower panel of Fig. 9 shows concentration time courses calculated by the simulator for this experiment. The parameters used for the initial 90 min are identical to those in Table 1, except the round window permeability which was set to  $11.0 \times 10^{-9}$  cm/s in order to better approximate the measured TMPA concentrations in this experiment. At 90 min, an additional flow from the base to apex of ST was superimposed at a rate of 0.8  $\mu$ l/min. In addition, we increased round window permeability and ST clearance each by an order of magnitude to more closely represent the TMPA levels remaining after perforation. We have not tried to fit the post-perforation data more closely because we realize there are many processes involved that are not incorporated in our model, such as turbulent or laminar flow at higher flow rates and the possible stirring of ST contents and resulting loss of unstirred layers, especially in the vicinity of the round window membrane. Nevertheless, the findings do confirm that the measured curves are consistent with a substantial longitudinal flow rate, and demonstrate the disturbance of solute gradients following perforation of the otic capsule. The degree of disturbance of perilymph by CSF entry will depend on the location of the perforation site, on CSF pressure, on the volume of the inner ear fluid spaces and on the patency of the cochlear aqueduct.

#### 4. Discussion

This study demonstrates that while it is possible to introduce an exogenous solute into the cochlear fluids by applying it to the round window membrane, there are inherent limitations to the method which must be recognized. Our measurements and analysis demonstrate that by applying our marker (TMPA) in the guinea pig it was not possible to establish a uniform concentration of the agent throughout the perilymphatic space. Instead, the concentration near the round window remained substantially higher than that of apical turns. Calculations indicate that delivery of the solute for a prolonged period of time does not change the situation substantially and longitudinal concentration gradients persist even after many hours of application. Depending on the intended goal of the solute application, the gradients in concentration may be a potential problem or in other cases may be an unintended benefit of the method. In the case of clinical application of gentamicin to the middle ear as a method for chemically ablating the vestibular system, it may be advantageous for the drug to be substantially confined to the basal cochlear turn to limit potential cochleotoxic effects. Similarly, if comparison between high-dose basal regions and low-dose apical regions is valuable then round window delivery would be appropriate. On the

other hand, if the purpose of the study is to compare sensitivity of apical and basal hair cells to specific drug concentration, which is assumed to be constant throughout the cochlea, then the round window application method may not be appropriate, as markedly different drug doses are delivered to different regions.

TMPA was chosen as a marker for this study as it exhibited a number of desirable characteristics. It can be detected in extremely low concentrations by ion-selective microelectrodes, permitting the entire concentration time course to be monitored during the application. TMPA does cross the round window but does not enter endolymph (Salt et al., 1991a) which facilitates analysis. Although TMPA is a cation, longitudinal voltage gradients in perilymph are low, so that longitudinal spread is not influenced by charge effects. In addition, ion electrodes sealed into the cochlear scalae permit measurements to be made without disturbance by artifactual perilymph leaks or from fluid movements associated with aspirating samples. Our simulation program allowed TMPA distribution as a function of distance and time to be modeled by varying only three parameters. Although knowledge of the characteristics of spread of TMPA has limited practical value, many of the physical principles relevant to this solute apply to all solutes and the simulation is readily modified to represent specific drugs or other substances that may be applied to the ear. One of the derived parameters, the rate of flow of perilymph in ST, is independent of the solute characteristics and the figure determined in this study is applicable to all other solutes. The generally close fit of the present simulation to the experimental data also excludes a number of potential other dispersal mechanisms, such as an oscillation or stirring of perilymph. The discrepancies in the initial minutes of application may arise from a number of factors, including infusion pump delays and possible limitations of the 'tube' diffusion model in calculating the initial entry into scalae of wide cross-section. In order to simulate the dispersal characteristics of other substances the major determining factors are the permeability characteristics of the round window membrane to the substance, and the rate of clearance of the substance from ST. This latter component may include a number of components and may include a combination of clearance to blood, clearance to any other scala, uptake or binding by cells, or metabolism by any of the cochlear tissues. It is also necessary to take into account the diffusion coefficient for the substance, but this can be reasonably estimated from the molecular size or weight. The most even distribution of drug would be achieved with a small, rapidly diffusing solute that is cleared very slowly from ST. In contrast, large, slowly diffusing substances, which are metabolized or otherwise cleared rapidly from the scala, would result in concentration gradients with distance

along the cochlea more pronounced than those shown here. Simulations also permit solute distribution to be approximated in the cochleae of other species, taking into account different scala volumes and lengths. In the human cochlea, in which ST is approximately 28.5 mm long, drugs will reach the apical turns in lower concentration than shown here for the guinea pig. The opposite is true for the mouse cochlea in which ST is approximately 4.5 mm long. In mice, drugs will quickly spread the entire length of the cochlea although longitudinal concentration gradients will still persist depending on the clearance rate for the substance.

This conclusion that substances applied to the round window primarily affect the basal regions of the cochlea is supported by studies with a variety of different drugs or markers that have been applied. Brummett et al. (1976) applied the ototoxic antibiotics neomycin and polymyxin B topically to the middle ear space three times a day for 2–4 weeks. They found widespread morphological damage but noted that the earliest drug effects were on high frequency function which corresponded to loss of hair cells at the basal end of the cochlea. Saijo and Kimura (1984) performed three HRP applications to the middle ear over a 48 h period and found reaction products predominantly in the basal turn. Stover et al. (1999) inoculated cochleae with an adenovirus carrying the reporter gene *lacZ*. After 5 days they found basal to apical gradients of transfected cells, with highest density in the basal turn and few transfected cells in apical turns. The above studies are generally in keeping with basal to apical gradients of the agent applied to the round window even after a period of days.

Our results represent the situation for a small ion which is not transported and which does not readily pass through cell membranes. With regard to interactions with biologic tissues, each substance may vary. Physical factors affecting movement of the drug or solute, such as the diffusion coefficient, the dilution effect related to the size of the cochlear fluid spaces and the influence of longitudinal perilymph flow, can be readily incorporated into calculations for all substances. Other potential communication pathways for some drugs may exist, including the vasculature and the ELSs, although none of these pathways is likely to increase apical concentrations significantly.

Our findings confirm that perforation of otic capsule immediately permits substantial flow to occur from base of ST to the site of the outlet, as reported previously (Moscovitch et al., 1973; Salt and Stopp, 1979; Salt et al., 1991c). This flow originates as CSF entering the base of ST through the cochlear aqueduct which rapidly washes out the native perilymph. The measured TMPA concentration profile is immediately affected by such perforations. This provides a graphic demonstra-

tion of the technical problems associated with sampling fluids to determine drug levels. Experimental procedures must obtain fluid samples before the perilymph has been displaced by CSF influx in order to be meaningful.

We conclude from this study that round window application provides an excellent technique to introduce drugs into the cochlea, but a uniform distribution of drug along the length of the cochlea cannot be assumed. The most even distribution would be for a small, rapidly diffusing solute, which is not cleared, absorbed or metabolized. In practice, most drugs will diffuse more slowly than TMPA, which is a relatively small ion, so the distribution of the drug will depend on the rate at which it is cleared from perilymph. Estimation of round window entry and perilymph clearance rates will permit the distribution of any drug to be simulated with reasonable accuracy. Data are recently becoming available, such as the measured kinetics of gentamicin following round window application in the chinchilla (Balough et al., 1998; Hoffer et al., 1999) from which entry and clearance rates may be derived. The long-term goal of this work is to be able to simulate drug levels throughout both auditory and vestibular portions of the labyrinth which will aid in the interpretation of clinical and scientific findings.

#### Acknowledgements

This work was supported by research Grant RO1 DC01368 from the National Institute on Deafness and Other Communication Disorders (NIDCD), National Institutes of Health.

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