Contamination of perilymph sampled from the basal cochlear turn with cerebrospinal fluid

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Abstract

Our understanding of the perilymph kinetics of drugs depends largely on data obtained by the analysis of perilymph samples. Although a number of studies have demonstrated qualitatively that perilymph samples may be contaminated by cerebrospinal fluid (CSF), and some investigations adopt specific methods to minimize CSF contamination of their samples, many other studies fail to consider the influence of this potential artifact on their measurements. In the present study we have attempted to quantify the degree of CSF contamination of perilymph samples taken from the basal turn of the guinea pig cochlea using the ionic marker trimethylphenylammonium (TMPA). TMPA solution was irrigated across the round window membrane while a TMPA-selective electrode sealed into the perilymphatic space continuously monitored perilymph TMPA concentration. After a period of TMPA loading, a perilymph sample was aspirated and its TMPA content determined. Differences between the sample concentration and the measured TMPA time course during perilymph loading and sampling were analyzed using a finite element computer model for simulation of solute movements in the inner ear fluids. The experimental results were consistent with the aspirated fluid sample from the cochlea being replaced by CSF drawn into the perilymphatic space through the cochlear aqueduct. The dependence of perilymph sample purity on the location of sampling and on the volume withdrawn was quantified. These relationships are of value in the design and interpretation of experiments that utilize perilymph sampling.

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1. Introduction

The local delivery of drugs to the cochlea by their application to the round window (RW) membrane is widely used in both experimental research (Brummett et al., 1976; Rybak et al., 1984; Li et al., 2001) and clinical medicine (Lange, 1981; Nedzelski et al., 1992; Silverstein, 1999; Silverstein et al., 1999; Thomsen et al., 2000). One of the limitations of the method lies in the uncertain concentration of drug achieved in the perilymph for each specific delivery protocol. To address this issue, a number of experimental studies have applied drugs to the RW membrane and measured the drug levels in samples of perilymph taken from the inner ear (Balough et al., 1998; Hoffer et al., 1999; Parnes et al., 1999). Obtaining pure samples of perilymph is fraught with technical difficulty. The available perilymph volume is small. The scala tympani (ST) in the guinea pig contains 4.5-4.7 μl (Shinomori et al., 2001; Thorne et al., 1999) and in the mouse only 0.32 μl (Thorne et al., 1999). Perilymph is also maintained under a positive pressure by communication with the cerebrospinal fluid (CSF) space through the cochlear aqueduct. In guinea pigs intracochlear pressure is approximately 3-4 mm Hg (Yoshida and Lowry, 1984; Long and Morizono, 1987). The potential for artifacts arises when the otic
capsule is perforated in order to take a perilymph sample. Immediately after intracochlear pressure is released, perilymph leaks from the opening, driven by CSF entering the perilymphatic space through the cochlear aqueduct. In guinea pigs, the leakage rate can be up to 1 μl/min (Moscovitch et al., 1973; Salt and Stopp, 1979) so that the normal ST contents can be washed away within a few minutes. Fluid samples taken from ST after this time will be predominantly CSF. Contamination of the sample with CSF is insidious as perilymph and CSF have similar ionic composition, so that contamination of the sample is not readily detected. Haras et al. (1989) made use of glycine difference between CSF and ST perilymph to show that samples became increasing contaminated by CSF as the sample volume increased.

The goal of the present study was to quantify the purity of perilymph samples taken from the basal turn of ST in the guinea pig in a form that could be generalized and applied to future investigations. The experiments utilized the ionic marker trimethylphenylammonium (TMPA) that is detectable at non-toxic, micromolar levels by ion-selective microelectrodes. TMPA was applied to the RW membrane and the perilymph concentration monitored continuously by an ion-selective electrode sealed into the perilymphatic space. After a period during which the perilymph was loaded with TMPA, a perilymph sample was withdrawn. The perilymph TMPA concentration was monitored during and after the sample was taken. In addition, the TMPA content of the aspirated perilymph sample was measured. Experimental results were interpreted using the Cochlear Fluids Simulation Program, a finite element model developed by our group and made available to the public at http://oto.wustl.edu/cochlea/. The simulations take into account detailed anatomic data of the guinea pig cochlea, including scala area as a function of distance (Thorne et al., 1999), RW membrane area and its relationship to the basal region of ST (Ghiz et al., 2001), and the entry location of the cochlear aqueduct into ST (Ghiz et al., 2001).

2. Methods

Experiments utilized 16 pigmented, NIH strain guinea pigs, which were anesthetized with 100 mg/kg sodium thiobutabarbital (Inactin). An intravenous line was placed in the external jugular vein for the administration of anesthetic supplements. The trachea was cannulated and the animal was artificially respirated, maintaining the end-tidal CO₂ level close to 39 mm Hg (5%). Body temperature was maintained at 38°C by a thermistor-controlled DC-powered heating blanket. The animal was mounted in a head-holder and the cochlea exposed by the ventrolateral approach. Prior to the placement of electrodes animals were given pancuronium bromide as a muscle relaxant.

Ion electrodes were made according to methods described previously. Briefly, double-barreled glass pipettes were pulled and stored in a humidity cabinet at 40°C, 70% humidity overnight. One barrel of the electrode was silanized by exposure to dimethyldichlorosilane vapor followed by baking at 140°C for 1 h. Electrodes were beveled to produce tip diameters of 2–4 μm. The ion barrel was filled with 500 mM KCl and the reference barrel was filled with 500 mM NaCl. A small column of TMPA-selective ion exchanger was drawn into the tip by suction. The ion exchanger consisted of 5% potassium tetrakis(4-chlorophenyl)borate in 2-nitrophenyloctylether which was pre-equilibrated with aqueous TMPA solution prior to use. For in vivo measurements, electrodes were calibrated in standards containing 0, 2, 20, 200 and 2000 μM TMPA in a background of artificial perilymph (see below) at 37°C. To measure the TMPA content of samples aspirated from the perilymphatic space, electrodes were calibrated at room temperature at 40 μl of each standard held in wells in a Teflon block. Glass pipettes used to aspirate perilymph samples were beveled to give tip diameters of 20–30 μm. They were connected by a polyethylene tubing to a syringe and the pipette and tubing was filled with mineral oil. Sample volume was determined from the length of the sample and the internal diameter of the pipette. Since the sample volumes used in the study averaged 4.4 μl (corresponding to a sample length of 16 mm in the pipette) measured sample lengths were based on the distance between the oil meniscus and the pipette shoulder. After the ion electrode was calibrated, the sample was expelled into a well in the Teflon block and its concentration measured immediately. Based on repeated measurements over a period of 1 h we determined that evaporative losses from samples in the wells were negligible over the few minutes required to establish the sample concentration.

The experimental sequence consisted of sealing an ion-selective electrode, and a sample pipette as necessary, into the perilymphatic space. This was achieved by thinning the bone overlying ST with a flat knife (Mueller AU13400) and making a small 30–50 μm fenestra with a fine 30° pick (Storz N170580). After insertion, the electrode was sealed by blowing desiccated air over the insertion site while thin cyanacrylate glue was applied. In later experiments, a thin layer of two part silicone adhesive (WPI Kwik-Cast silicone elastomer sealant) was applied to the dry, thinned bone before the fenestra was made, to facilitate sealing the pipettes in place. When electrodes were in place, perilymph TMPA concentration was monitored as the RW membrane was irrigated with an artificial perilymph containing 2 mM TMPA. The artificial perilymph contained (in
mM): NaCl 125; KCl 3.5; NaHCO$_3$ 25; MgCl$_2$ 1.2; CaCl$_2$ 1.3; NaH$_2$PO$_4$ 0.75; C$_6$H$_{12}$O$_6$ 5.0. After a 30–40 min TMPA loading period a perilymph sample was aspirated into an oil-filled micropipette. In four experiments perilymph was sampled through the RW. RW irrigation was stopped prior to sampling and the bulla was dried, rinsed with artificial perilymph and dried again before the sample was taken. In four experiments perilymph was sampled through the scala wall into a pipette that had been sealed in place prior to the irrigation commencing. In three additional experiments perilymph was sampled through the scala wall while irrigation of the RW with 2 mM TMPA was maintained. For each experiment the sample volume was determined by the product of the fluid length in the collection pipette, measured by a calibrated operating microscope, and the cross-section of the pipette. The times of the start and end of sampling were recorded, from which the total sampling duration, and hence the rate of sampling, was derived in each case. Data collection was performed with Tucker Davis System 3 hardware controlled by a custom Visual Basic Program. Potentials from the ion electrodes were sampled and graphed at 10 s intervals. The data from five experiments were not usable for technical reasons, including the inability to aspirate a sample or because of fluid leakage at one of the electrode insertion sites.

The data from each experiment were interpreted by simulation of the precise experimental conditions, including the electrode locations and the timing of events. This was performed with a finite element cochlear fluids simulation program, version 1.6e, developed by our group and made available at http://oto.wustl.edu/cochlea/. The program incorporates a number of anatomic features appropriate for the guinea pig. The diffusion coefficient used for TMPA was 1.01 × 10$^{-9}$ m$^2$/s. The initial loading of ST was accomplished by simulating TMPA entry across the RW membrane and by varying the numerical values for RW permeability and clearance from ST until the calculated curve best fit the measured time course, as detailed in Salt and Ma (2001). The procedure of fluid sampling was incorporated into the model by simulating a volume flow towards the sampling site at an appropriate rate and duration, with summation of the total solute and total volume removed at this site from which a sample concentration was calculated. Parameters for the sampling procedure were established for each individual experiment, as described above. The location of sampling and ion-selective electrodes in the basal turn of ST was established by measurement along the outer wall of the scala from the ‘lip’ of the RW. A correction of 0.13 mm was added to distance measurements along the bony wall, to allow for the region of ST basal to the RW lip.

Experimental studies were performed under protocols approved by the Animal Studies Committee of Washington University, protocol numbers 19990029 and 20020010.

3. Results

In four experiments, perilymph was sampled through the RW membrane. The results of a typical experiment and its simulation by the computer model are shown in Fig. 1. In this experiment, the TMPA-selective recording electrode was sealed into ST 1.1 mm from the RW. The TMPA concentration at the electrode location increased during irrigation of the RW with solution containing 2 mM TMPA. During the period when the RW was dried, rinsed and dried again, the recording was noisy due to the movements of the experimenter influencing the high resistance ion electrode, but a small decline of concentration was apparent. As a 2.3 μl sample was withdrawn, TMPA at the recording site rapidly declined, but partially recovered after withdrawal of the sample ceased. When the samplepipette was withdrawn, leaving a small perforation of the RW membrane, the TMPA showed a small decline. Interpretation of this experiment was provided by simulation of the processes occurring in the cochlear fluids, as summarized in the lower left panel of Fig. 1. The rate of TMPA entry was established using an approach similar to that used previously (Salt and Ma, 2001). The best fit for this experiment was provided by a RW permeability of 0.03 × 10$^{-6}$ cm/s and a ST clearance half-time of 70 min. Other parameters for the model (scala vestibuli clearance and radial cross-communication) were set to the same values as those established in the prior study. The graph (right column) representing time point A shows the concentration profile along the basal turn of ST as determined by the simulator. Concentration is highest in the basal region, adjacent to the RW, and declines in an apical direction. As a result of the gradient, TMPA is diffusing in the apical direction, which is partially balanced by clearance from the scala. In the simulator the drying procedure was implemented by disabling the entry of TMPA through the RW. This resulted in a small decline of tracer at the measurement location. The concentration profile along ST at time point B shows a large decline in the basal region and a resulting reduction in the longitudinal gradient along the scala. The aspiration of the sample between time points B and C is implemented in the model by moving solute and volume according to simple physical principles, with 2.3 μl of solution leaving ST at the sample aspiration site, in this case 0.3 mm from the base, with volume being replaced by CSF entering ST through the cochlear aqueduct, entering ST 1.1 mm
Fig. 1. Analysis of an experiment in which 2.3 μl perilymph was sampled through the RW membrane. Upper left: TMPA concentration recorded from an ion-selective electrode sealed into ST during loading, drying of the RW, and then aspiration of the perilymph sample. Lower left: Marker time course derived by simulation of the experiment. For each of the five time points (A–E) the estimated profile of TMPA concentration along the basal portion of ST is shown in the right column.

Fig. 2. Analysis of an experiment in which 5.4 μl perilymph was sampled through a fenestra in the bony wall of ST 1.9 mm from the lip of the RW. Upper left: Concentration recorded from an ion-selective electrode sealed into ST at the sampling site during loading, drying of the RW, and then aspiration of the perilymph sample. Lower left: Marker time course derived by simulation of the experiment. For each of the four time points (A–D) the estimated profile of TMPA concentration along the basal portion of ST is shown in the right column.
from the base. The duration of sampling in this experiment was 60 s. The substantial fall in concentration at the ion electrode recording site represents the entry of CSF, with low TMPA concentration, into the scala at the site of the cochlear aqueduct (1.1 mm) and flowing through the scala to partially contribute to the aspirated sample. The influence on the concentration profile at time point C is highly apparent, with low TMPA concentration between the aqueduct and the sample aspiration site. When the sampling stops, and CSF no longer enters the scala, the solute in those regions basal to the sampling site and apical to the aqueduct diffuse into the regions of lower concentration caused by the sampling, and give rise to the rebound between time points C and D. Finally, to simulate the situation with the sample pipette withdrawal, we incorporated a small, 0.05 μl/min volume leak from the sampling site at 0.3 mm, with fluid being replaced by entry through the aqueduct at 1.1 mm and resulting flow between the two sites. It is apparent that even such small leaks have a marked influence on the perilymph concentration of solutes near the site of leakage. The measured TMPA concentration of the 2.3 μl sample taken from this animal was 206 μM while the concentration derived from the simulation of sampling, incorporating perilymph flows and CSF entry, was 174.5 μM. This compares to the estimated TMPA concentration at the RW sampling site prior to taking the sample of 349 μM.

A second series of four experiments sampled perilymph through a fenestra made in the bony wall of the basal turn, for which an example time course and analysis is given in Fig. 2. In this experiment the concentration time course was lower and delayed compared to that in Fig. 1, due to the greater distance from the RW to the ion electrode. This is apparent in the distance profile for this experiment shown in Fig. 2A, which is quite similar to that found in Fig. 1. Similarly, the decline during the period when the RW is rinsed and dried is not as apparent in either the experimental or the simulated time courses, even though the simulated decline at the extreme basal region of ST is considerable (Fig. 2B). The time course during sampling at this location goes through an initial increase, followed by a pronounced decrease comparable to that seen with sampling through the RW. The origin of the transient increase is apparent from the concentration profile in panel B. As the sample is aspirated and perilymph moves apically from the site of cochlear aqueduct entry to the sample location, perilymph with higher TMPA content passes by the recording electrode, subsequently to be replaced by CSF with lower concentration (Fig. 2C). The subsequent rebound of the concentration time course when sampling ceases again results from tracer diffusion from regions less disturbed by CSF influx, although the model does not replicate the animal data very closely in this respect. The concentration of the 5.4 μl sample withdrawn over a 1.3 min period in this experiment was measured to be 63.1 μM, which compared to the 57.4 μM derived from simulation of the sample aspiration. Estimated concentration at the sampling site prior to sample aspiration was 132 μM.

In three early experiments in which perilymph was sampled through a fenestra in the basal turn of ST, the sample was aspirated while the irrigation of 2 mM TMPA was maintained across the RW membrane. An example of such an experiment is shown in Fig. 3. With this protocol, the TMPA concentration recorded in ST showed a substantial rise as the sample was taken, a rise that could not be accounted for by any of the physical processes incorporated into the simulations. The only way to account for such a large increase was for high concentration solution from the middle ear to be drawn into ST as the sample was aspirated. Since the electrodes were all sealed in place, the site of entry is presumed to be through the RW membrane. To avoid this artifact, the aspiration of samples in all subsequent experiments (as shown previously) was performed with the bulla dried and with air in contact with the RW membrane.

![Fig. 3. Analysis of an experiment in which perilymph was sampled through a fenestra in the bony wall of ST 3.0 mm from the lip of the RW, similar to the experiment shown in Fig. 2 except that RW irrigation of 2 mM TMPA was maintained throughout the sampling period.](HEARES 4702 18-8-03)
A summary of the measured concentrations of perilymph samples compared with the sample concentrations predicted by simulation of the specific conditions for each experiment is given in Fig. 4. For those experiments in which the RW was rinsed and dried before sampling and samples were taken through the RW or through a fenestra in the bone, the mean difference between predicted and observed sample concentrations was 4.21 μM. This difference was not significant (paired t-test, \( P = 0.67 \)) confirming that the simulations can provide a reasonable estimate of the sample concentration without untoward bias towards overestimating or underestimating the concentration. In all these experiments the sample concentration obtained (average 95.4 μM, S.D. 69.3, \( n = 8 \)) was well below that determined to be present at the sampling site prior to taking the sample (average 280.8 μM, S.D. 186.5, \( n = 8 \)), suggesting considerable contamination of the samples with CSF. In contrast, for those experiments in which samples were taken while RW irrigation of the 2 mM TMPA solution was maintained, the perilymph samples showed substantially higher concentrations than those predicted by the model (Fig. 4). The mean difference was 88.4 μM, which was statistically significant (paired t-test, \( P = 0.032 \)). This confirms that without considering alternative mechanisms of solute entry into the cochlea during sampling, the model cannot accurately predict the concentration when the sample is aspirated while drug remains on the RW membrane.

The determination of the proportions of each sample contributed by perilymph and CSF was complicated by the fact that following RW delivery, perilymph concentration is not uniform along the cochlea. In order to predict the concentration of an ideal ‘pure perilymph’ sample we therefore summed, in the model, the total solute and volume between the site of sampling and the cochlear aqueduct at the time prior to sampling, from which the overall concentration of the region was determined. Since the sample volumes were larger than the volume in this region, it is assumed that the balance of the volume sampled consists of CSF. The concentration summed across this region thus represents the concentration if the sample volume were small enough to consist completely of perilymph. Subsequent dilution of this ‘pure perilymph’ by CSF as more sample volume is taken reduces the final concentration relative to this value. Thus for any given sample, the amount of perilymph in the sample can be determined from the sample concentration relative to the ‘pure perilymph’ concentration for the region. The proportion of perilymph for both real and simulated samples is summarized in Fig. 5, and varies according to the volume of sample taken and the location from which the sample is taken. In general, the proportion consisting of peri-

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**Fig. 4.** Comparison of simulated sample concentrations (black bars) and actual sample concentrations (gray bars). Each pair of bars represents the results of a single experiment. Experiments are grouped according to the three conditions used in the study as indicated. When the RW niche was dried prior to sampling, the difference between the simulated and observed sample concentrations averaged 4.2 μM, which was not statistically significant. In experiments where TMPA solution remained in the RW niche during sampling, the aspirated samples had significantly higher concentrations than those predicted by the simulations.
lymph decreases with larger samples taken and decreases at sites closest to the site of cochlear aqueduct entry.

It is apparent that for all experiments, the samples taken from basal turn locations cannot be assumed to be pure perilymph. The estimated amount of perilymph in the sample varies from 13% to 61%, depending on the location and volume of sampling. The mean difference between the purity of the real samples (average 32.2% perilymph) was not significantly different from that for the simulated samples (average 34.7% perilymph) (paired t-test, $P=0.48$), again confirming that the simulations are a reasonable representation of the degree of perilymph contamination with CSF.

The above studies (Figs. 4 and 5) provide validation that the many processes associated with drug delivery and perilymph sampling are appropriately represented in the model. With the model validated in this manner, the purity of perilymph samples of different volumes and taken from different locations along ST have been derived and are summarized in Fig. 6 in two formats. When sample purity is considered with respect to

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![Fig. 5. Perilymph content of simulated (black) and actual (gray) samples of varying volume taken at different locations from the basal turn of the cochlea. Each pair of bars represents the results derived from a single experiment.](image1)

![Fig. 6. Dependence of sample perilymph content on the volume of sample aspirated and on the location of sampling in ST. Samples of highest purity are obtained when small volumes are aspirated at sites far from the cochlear aqueduct. Large samples taken from the RW or other basal location are highly contaminated with CSF.](image2)
the location where the sample is taken (Fig. 6, left panel), it is apparent that even for sample volumes as small as 1 µl, there may be some contamination of the sample with CSF for all basal turn sampling locations. Less contaminated samples could be obtained by making the sampling site further from the cochlear aqueduct, such as from the higher turns of the cochlea (Fig. 6, right panel), although the purity of the sample rapidly decreases as larger volumes are aspirated. In addition, since drug concentrations may vary from base to apex, as with local delivery to the RW, perilymph sampled from the apex may not be representative of perilymph sampled from the base. It is, however, important to note that large samples taken from the basal turn may contain less than 10% perilymph.

In all the simulations presented above, it was assumed that the fluid volume aspirated was replaced by CSF entering ST through the cochlear aqueduct. This is based on the prior documentation that volume flow occurs from the aqueduct to a site of cochlear perforation (Salt et al., 1991) and on the fact that efflux from the perforated cochlea is influenced by CSF pressure (Salt and Stopp, 1979). To test whether this assumption was reasonable for sampling fluid from the cochlea, we compared simulations of the tracer time courses during sampling with different proportions of fluid entering from the aqueduct and from apical regions of ST. Fig. 7 shows the predicted time courses for the two example experiments in which fluid was sampled from the RW (left column) or through a fenestra in the bone of the basal turn (right column). Simulations are shown for all the fluid replaced from the aqueduct or with increasing proportions arising from basally directed perilymph flow originating at a more apical, or even vestibular, site. For both sampling locations, it is apparent that the recovery of concentration after sampling only occurs when the proportion of perilymph drawn from the apical regions is low. Since higher proportions drawn from the apex reduce the marker levels in regions apical to the recording site (Figs. 1C and 2C), this results in a smaller recovery. For a proportion of more than 20% from the apex, there is no ‘bounce back’ or recovery of concentration at all. The experimental data in fact show amounts of recovery after sampling greater than we see in the simulations. Thus, while we cannot exclude the possibility that a small proportion of the sample volume may be replaced by sources other than CSF, there is no indication from the time course data that this occurs to a significant extent.

4. Discussion

This study shows that the procedure of aspirating samples from the basal turn of ST has a pronounced influence on perilymph composition. Although the initial portion of the aspirate may contain pure perilymph, the sample becomes increasingly contaminated with CSF as the aspirated volume increases. This finding is in agreement with a number of prior studies in which the issue of sample contamination with CSF was raised (Scheibe et al., 1984; Hara et al., 1989). Based on our simulations, the degree of contamination has been quantified for a range of sampling locations and sample volumes, as summarized in Fig. 6. The demonstrated relationships have major practical implications for any study that utilizes perilymph sampling and should allow the purity of perilymph samples to be approximated. In some cases, they may allow measurements of samples to be corrected to more accurately represent the perilymph status.

A major conclusion from this study is that a fluid sample aspirated from the basal turn of ST is not ‘pure perilymph’, but instead is a mixture of perilymph and CSF. It cannot be assumed that a drug level measured in an aspirated sample is the ‘perilymph concen-
tration’ of the drug. By realizing that the sample contains components that do not originate in the perilymphatic space, the mis-interpretation of sample concentrations in terms of perilymph concentrations can be avoided. However, the unavoidable difficulties associated with obtaining pure samples of perilymph do not mean that studies based on sampling methods cannot be interpreted. For drugs that cannot be monitored continuously with ion-selective electrodes, sampling of the cochlear fluids is the only way in which the perilymph kinetics can be quantified. Rather, the present study confirms the need to consider the degree of contamination in the interpretation of the data. With the aid of detailed simulations, the sampled fluid mixture can be interpreted with a greater degree of confidence. Such an analysis of gentamicin concentration data from perilymph samples published by Hoffer et al. (1997), Balough et al. (1998) and Hoffer et al. (1999) has recently been performed by Plontke et al. (2002). From the reported time course data it was possible to establish the basic processes contributing to gentamicin kinetics following its application to the RW membrane. One conclusion drawn was that gentamicin reached the perilymph of the scala vestibuli and vestibule primarily by local communication processes in the basal turn, rather than diffusing along the scalae and through the helicotrema. The analysis showed that a remarkably quantitative interpretation can be derived from data obtained through perilymph sampling. On the other hand, for large samples taken from the basal turn with a greater degree of contamination, errors in interpretation are likely to be greater. Although there are undoubtedly errors involved in simulating such procedures, they are far smaller than the error made in assuming the sample concentration represents a true perilymph concentration. Detailed simulations of the specific conditions of an experiment make it possible to correct for the sample volume taken at the specific location used and to extrapolate back to the perilymph levels that existed prior to taking the sample.

Another issue relevant to the RW application of drugs to the cochlea is that there may not be a meaningful ‘perilymph drug concentration’, that is a specific concentration that represents the drug level in perilymph. Drug concentrations will be highest for perilymph close to the RW membrane and will decline with distance along ST. The rate of decline varies with many factors, including the duration of application, the size of the drug and its diffusion rate, the rate of clearance of the drug, and the animal species involved, specifically the cochlear length and dimensions (Salt and Ma, 2001). As a result, the adoption of a strategy of taking small samples from apical cochlear regions also presents difficulties in interpretation, even if the sample consists of virtually pure perilymph. The fact that perilymph in apical cochlear regions may contain markedly lower drug levels than in the basal turn after RW application means that small perilymph samples taken from the ST apex may not be representative of the perilymph in other cochlear regions. Both the present and a priori study (Salt and Ma, 2001) have shown that the assumption of a homogeneous distribution of drug throughout the perilymph is not valid. Instead, the distribution of drugs is dominated by diffusion, which occurs extremely slowly over distances corresponding to the lengths of most cochlear scalae.

In the present study, the similarity of simulated and measured concentration time courses at the location of the ion-selective electrode provides validation that the primary processes involved during sampling, such as volume flow and diffusion, are represented accurately in the model. Nevertheless, the model has some limitations and differences between the simulations and the experiments exist, especially for the period associated with the fluid sampling. Differences arise from the existence of processes that are not included in the simulation, such as the likely turbulence or stirring of the scala contents during the sampling procedure, or variations in the rate at which the sample was aspirated. Thus, while the simulations demonstrate the primary components associated with local delivery of drugs to the cochlea, they do not include all possible processes and therefore may differ from the experimental data under some circumstances.

An important observation in the present study was the substantial effects on sample concentrations of drug that remained in contact with the RW membrane during fluid sampling. The measured perilymph concentration increased markedly as the sample was aspirated, consistent with a higher rate of entry through the RW membrane. This raises the question whether the reduced intracochlear pressure during sample aspiration is sufficient to influence RW permeability. The pressure decrease may act either by permitting the marker to diffuse across the RW membrane more rapidly, or by permitting fluid from the middle ear to be drawn into ST. For the purposes of collecting fluid samples, this type of entry can be avoided by rinsing and drying the RW membrane prior to sample aspiration. The possibility, however, that RW permeability varies according to the pressure differential across it, thereby depending on intracochlear and middle ear pressures, may merit further investigation.

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References


Silverstein, H., 1999. Use of a new device, the Microwick, to deliver medication to the inner ear. Ear Nose Throat J. 78, 595–598.


