Review Article

Communication pathways to and from the inner ear and their contributions to drug delivery

Alec N. Salt*, Keiko Hirose
Department of Otolaryngology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO, USA

ARTICLE INFO

Article history:
Received 17 September 2017
Received in revised form 8 November 2017
Accepted 5 December 2017
Available online 19 December 2017

Keywords:
Round window
Intratympanic
Pharmacokinetics
Cochlea
Perilymph
Cerebrospinal fluid

ABSTRACT

The environment of the inner ear is highly regulated in a manner that some solutes are permitted to enter while others are excluded or transported out. Drug therapies targeting the sensory and supporting cells of the auditory and vestibular systems require the agent to gain entry to the fluid spaces of the inner ear, perilymph or endolymph, which surround the sensory organs. Access to the inner ear fluids from the vasculature is limited by the blood-labyrinth barriers, which include the blood-perilymph and blood-strial barriers. Intratympanic applications provide an alternative approach in which drugs are applied locally. Drug from the applied solution enters perilymph through the round window membrane, through the stapes, and under some circumstances, through thin bone in the otic capsule. The amount of drug applied to the middle ear is always substantially more than the amount entering perilymph. As a result, significant amounts of the applied drug can pass to the digestive system, to the vasculature, and to the brain. Drugs in perilymph pass to the vasculature and to cerebrospinal fluid via the cochlear aqueduct. Conversely, drugs applied to cerebrospinal fluid, including those given intrathecally, can enter perilymph through the cochlear aqueduct. Other possible routes in or out of the ear include passage by neuronal pathways, passage via endolymph and the endolymphatic sac, and possibly via lymphatic pathways. A better understanding of the pathways for drug movements in and out of the ear will enable better intervention strategies.

© 2017 Elsevier B.V. All rights reserved.
1. Introduction

The bony otic capsule represents a major anatomic and physiological barrier to the inner ear, isolating labyrinthine fluids and tissues from adjacent structures, such as the middle ear and the brain. The bony encasement of the inner ear, present in the early evolution of vertebrates (Carey and Amin, 2006), is an adaptation to optimize effective transduction of sound and head movements into electrical signals. The inner ear can be regarded as a membrane-bound tube containing endolymph suspended within a rigid-walled compartment containing perilymph, with compliant openings at the round window membrane and stapes. This arrangement enables responses to mechanical stimuli, including sound waves delivered by the stapes and head motions, while attenuating body-generated pulsations, such as those associated with respiration, heartbeat, coughing and sneezing.

A second type of barrier separating the inner ear from other organs, is created by specialized cell layers. The lipid membranes of either epithelial or endothelial cell layers, combined with tight junctions between adjacent cells of the layer restrict solute movement (Giepmans and van IJzendoorn, 2009). The blood vessels of the inner ear allow exchange of gases, O2 and CO2, while restricting the passage of even small molecules and cells (Hirose et al., 2014). The vessels have properties that are dynamic and are influenced by temperature, pH, muscle tone, O2 and CO2 content as well as cytokines/chemokines and inflammatory molecules. They form the blood-labyrinth barrier which consists of barriers of at least two distinct types. The blood-perilymph barrier separates blood from perilymph. The blood-rial barrier separates the blood from the cochlear intrastrial space. Epithelial cells which line the endolymph compartment provide a tight barrier between endolymph and perilymph, limiting solute movements between the two fluids. A respiratory pseudostatified columnar epithelium and non-keratinizing epithelium line the middle ear. These epithelia cover the middle ear spaces and contribute to the barriers between the middle ear and perilymph at the round window membrane and stapes. Passage of molecules across cellular barriers depends on many factors, including the lipophilicity, polarity and size of the molecule, and whether they pass the barrier membranes by active or passive transport processes.

The major communication pathways in and out of the ear are summarized in Table 1. Pathways between the ear and adjacent spaces include the cochlear aqueduct the endolymphatic duct and sac, the internal auditory canal through which the cochlear, vestibular and facial nerves pass. Under some conditions, passage through the dense petrous bone of the otic capsule can play a role. Knowledge of entry and exit pathways and whether they have substance-specific characteristics is important to understanding pharmacokinetics of the ear with local and/or systemic drug delivery. Communication pathways of the ear have been reviewed elsewhere from a clinical perspective (Ciuman, 2009).

2. Perilymph, endolymph, and tissues of the inner ear

The sensory hair cells of the cochlea are located in the organ of Corti and lie on the boundary between the perilymph and endolymph. In early anatomic studies, the basilar membrane, on which the organ of Corti rests, was first recognized as a prominent anatomic boundary and became defined as the boundary between scala tympani and scala media. The basilar membrane comprises layers of collagen fibers (Liu et al., 2015), with a cellular, tymphonic covering layer lacking tight junctions, thereby allowing it to be permeable to small molecules. The basilar membrane does not provide a barrier to the movement of fluid or small molecules. It provides an example of an anatomic boundary that is structurally identifiable with light microscopy but does not create a barrier for perilymph. In the organ of Corti, the main physiologic barrier is instead formed by the reticular lamina, comprising those cells contacting endolymph, including hair cells, Deiter cells, Claudius and Hensen cells and the cells lining the inner sulcus, which are coupled together at their apical membranes by tight junctions (Jahnke, 1975), forming a tight boundary there. The same is true for other structures bounding endolymph, which in the cochlea includes Reissner’s membrane, marginal cells of stria vascularis and outer sulcus cells. Reissner’s membrane forms the boundary between scala vestibuli and scala media and comprises of two cuboidal cell layers separated by a basal lamina. The cell layers facing endolymph has tight junctions between the apical membranes of the cells, while the layer facing perilymph contains larger cells only loosely joined together. While physiologists initially ascribed Reissner’s membrane a permeable role involved in radial fluid flow, subsequent studies have shown its permeability properties are comparable with the organ of Corti, with circulating current flows of similar magnitude through both structures (Zidanic and Brownell, 1990). It is therefore important to recognize that for many structures, permeability does not correlate with the number of cell layers or thickness of the structure. Rather, it depends more on the properties of the cellular boundaries and adhesion molecules between cells. For all cells bordering endolymph, the primary boundary is the apical membrane in contact with endolymph, in conjunction with the tight junctions between cells at their apical surfaces.

Perilymph is ionically comparable to other extracellular fluids, having high Na+, and low K+ content, but differs slightly in composition between scala tympani and scala vestibuli, with scala vestibuli having slightly higher K+ content (Wangemann and Marcus, 2017). Solutes in perilymph readily equilibrate with the extracellular spaces of most of the tissues of the inner ear, including the spiral ligament, spiral ganglion, organ of Corti and the canalculi in the bony walls of the ear.

2.1. Perilymph

Perilymph homeostasis is affected by a vast number of processes. Perilymph is not secreted through an exocrine gland, such as
lacrimal fluid or saliva. Perilymph is also not an ultrafiltrate of blood, as was suggested in previous literature (Schnieder, 1974) as it contains an enrichment of some proteins and reduction of others compared to blood (Thalman et al., 1992). The substantial (0.5–2 μL/min) efflux of fluid observed when the otic capsule is perforated in small experimental animals (guinea-pigs, mice and other rodents) does not result from a physiological volume production within the perilymph compartment. Rather, it occurs because intracranial pressure is transmitted to perilymph through the cochlear aqueduct (Thalen et al., 2001). When the hydrostatic pressure of perilymph is released by opening the otic capsule, cerebrospinal fluid (CSF) pressure forces a perilymph efflux, dominated by entry of CSF into the basal turn of scala tympani through the cochlear aqueduct. Perilymph flow results from intracranial pressure driving CSF through the cochlear aqueduct, starting as soon as perilymph pressure falls. There is no evidence that CSF enters the ear in appreciable volume at any other sites, such as through the auditory nerve, even though a boundary between the nerve and perilymph is lacking (Tinling and Chole, 1994). In experiments where hydrostatic pressure of the cochlea was released by perforating the round window membrane, there was no basally-directed flow down scala tympani toward the site of perforation (Salt et al., 1991a), demonstrating the cochlear aqueduct is the predominant site of CSF entry. The displacement of perilymph by CSF has been a major cause of contamination of perilymph samples collected from the basal turn of scala tympani (Salt et al., 2003). In recent years, the phenomenon has been used to collect pure perilymph samples from regions distant from the cochlear aqueduct, as discussed in more detail below. When the otic capsule is intact, marker and drug distribution studies show that longitudinal flow of perilymph is almost non-existent (Chen et al., 2005; Salt and Ma, 2001). Some studies in guinea pigs suggest there may be a very low rate of apically-directed flow in scala tympani, consistent with an ongoing entry of CSF through the cochlear aqueduct at a rate of approximately 30 nL/min (Salt et al., 2015). While this is an extremely low rate, its cumulative effect on drug distribution could be significant as it amounts to almost 2 μL per hour, which is approximately half of scala tympani volume. In summary, perilymph can be considered as a fixed volume of fluid, the composition of which is influenced by local processes involving any of the many cell types that contact it. The kinetics of any substance in perilymph depends on the rate of local processes and whether they occur rapidly or slowly. K⁺ ions in perilymph, for example, show fast kinetics as they enter scala tympani perilymph as part of the transduction current through the hair cells, to be taken up by cells of the spiral ligament and organ of Corti and “recycled” back to endolymph. When scala tympani perilymph K⁺ was elevated by perfusion, elimination occurred rapidly with a half time averaging 9 min (Salt and Stopp, 1979). At the other extreme, some substances are lost only slowly from perilymph, such as gentamicin or high molecular weight dextran, both of which have elimination half-lives from vestibular perilymph of over 200 min (Salt et al., 2015, 2016). In these studies, their elimination from scala tympani perilymph was instead dominated exchange between CSF and perilymph across the cochlear aqueduct. The degree to which such fluid exchange influences other substances with more rapid local homeostasis is uncertain, but it has undoubtedly distorted prior estimates of substance elimination from scala tympani perilymph. While local homeostatic processes maintaining perilymph by exchange across the blood-labyrinth barrier are likely to be highly substance-specific, their quantification for scala tympani perilymph in experimental animals is fraught with technical difficulties due to the cochlear aqueduct’s involvement in physiologic fluid exchange and in non-physiologic sample contamination.

It must also be considered that the measured kinetics for a substance depends not only on the rate of solute flux in or out of the compartment but also on the volume, or in the cochlea the cross-sectional area, of the fluid compartment into which the flux occurs. The same transport process occurring in the basal turn where cross-sectional area is greater, will result in slower kinetics than in the apical turn, where the cross-sectional area of the fluid space is smaller. With perilymph homeostasis dominated by local mechanisms, this raises the possibility that perilymph kinetics would vary by location, with faster kinetics in apical cochlear regions. Passage of the marker trimethylphenylammonium (TMPA) between scala tympani and scala vestibuli demonstrates that solutes rapidly equilibrate between the two compartments. Faster exchange was found in apical turns compared to the basal turn to a degree that was completely attributable to area differences of the scala (Salt et al., 1991b). It was also found that gentamicin entry kinetics following systemic delivery occurred more rapidly into perilymph of the apical turns than in the basal turn (Hahn et al., 2013). Longitudinal variations in tissue volumes, vascularity, metabolic rate and activity kinetics could also contribute to kinetic differences between locations. However, given the major influence of CSF on perilymph kinetics in the basal turn, it has not been possible to demonstrate conclusively whether kinetic differences in elimination exist from base to apex in the cochlea.

Water flow between plasma and perilymph can be driven by osmotic gradients across the cellular boundaries (Juhn and Rybak, 1981). However, manipulations of systemic osmolality by administration of glycerol or urea (the basis of the now rarely-used

---

**Table 1**

<table>
<thead>
<tr>
<th>System</th>
<th>Internal Compartment (Inner ear)</th>
<th>External Compartment</th>
<th>Boundary Cell Type or Structure</th>
<th>Influential Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood-perilymph barrier</td>
<td>Perilymph (all structures bathed in perilymph)</td>
<td>Blood</td>
<td>Endothelial cells, pericytes</td>
<td>Inflammation/infection, structural damage of vessels</td>
</tr>
<tr>
<td>Blood-strial barrier</td>
<td>Intralial fluid</td>
<td>Blood</td>
<td>Endothelial cells, pericytes, intermediate cells</td>
<td>Inflammation, structural integrity of vessels</td>
</tr>
<tr>
<td>Endolymphatic sac</td>
<td>Endolymph</td>
<td>Blood, CSF</td>
<td>Lining of the endolymphatic sac, dura of the posterior fossa</td>
<td>Inflammation/infection, endolymph volume, perilymph pressure</td>
</tr>
<tr>
<td>Cochlear aqueduct</td>
<td>Perilymph</td>
<td>CSF</td>
<td>Variable: in humans, patency is variable, in other mammals, widely open</td>
<td>CSF pressure-induced fluid oscillations.</td>
</tr>
<tr>
<td>Round window membrane</td>
<td>Perilymph of scala tympani</td>
<td>Middle Ear</td>
<td>Middle ear mucosa, round window</td>
<td>Middle ear environment, occupants</td>
</tr>
<tr>
<td>Stapes footplate</td>
<td>Perilymph of the vestibule</td>
<td>Middle Ear</td>
<td>Middle ear mucosa, stapes footplate</td>
<td>Middle ear environment, middle ear, occupants</td>
</tr>
<tr>
<td>Bony otic capsule</td>
<td>Perilymph</td>
<td>Middle Ear</td>
<td>Middle ear mucosa, Otic capsule bone</td>
<td>Trauma, cochlear implantation</td>
</tr>
<tr>
<td>Bony otic capsule</td>
<td>Perilymph</td>
<td>Bone marrow, blood</td>
<td>Otic capsule bone</td>
<td>Trauma</td>
</tr>
</tbody>
</table>

---
clinical tests for endolymphatic hydrops) increases perilymph osmolarity through a combination of osmolyte entry and perilymph water loss (Juhn et al., 1982), preventing an accurate quantification of hydraulic conductivity. Induced perilymph volume changes within the fixed volume of the ear also result in hydrostatic pressures sufficient to drive a compensatory volume changes across the cochlear aqueduct.

2.2. Endolymph

Endolymph is separated from perilymph by cellular boundaries and tight junctions between cells (Jahnke, 1975). In contrast to perilymph, there is no open communication between endolymph and any other adjacent extracellular fluid. Endolymph composition similarly varies by location in the inner ear (Wangemann and Marcus, 2017). Throughout most of the ear the predominant cation in endolymph is K⁺, but both endolymph K⁺ (about 155 mM) and endolymph osmolarity (about 316 mOsm/L) are higher at the base of the cochlea than at the apex (Sziklai et al., 1992). These differences in endolymph composition are related to gradients in ion transport and permeability along the length of the cochlea. The gradients also result in a higher endocochlear potential (EP) in the basal turn, declining towards the apex (Salt et al., 1989). Endolymph K⁺ content is higher in the cochlea than in vestibular structures, as is endolymph Ca²⁺ content which varies with an even greater extent with location. Very low Ca²⁺ concentration (about 20 μM) is found in endolymph at the base of the cochlea, with concentration increasing towards the apex largely accounted for by the EP decrease towards the apex (Salt et al., 1989). Ca²⁺ is notably higher (over 1 mM) in endolymphatic spaces of the vestibular system, as a consequence of their lower electrical potentials. Composition in the endolymphatic sac differs greatly from the rest of the endolymphatic system, with Na⁺ the primary cation and a K⁺ content less than 20 mM (Mori et al., 1987). The inhomogeneity of endolymph throughout the ear is consistent with its regulation in each region by local mechanisms dominated by local ion recycling (Steel, 1999). The ion transport mechanisms underlying endolymph homeostasis have been recently reviewed by Wangemann and Marcus (2017).

Marker measurements show that endolymph does not flow along the scala at an appreciable rate in the normal cochlea (Salt and Thalmann, 1989). Basally-directed volume flow is induced by local volume injections into the endolymphatic space (Salt and DeMott, 1997; Brown et al., 2016). This basally-directed flow is a consequence of vestibular endolymphatic structures, primarily the boundary membrane of the sacculus, being more mechanically compliant than those in the cochlea (Wit et al., 2000). Analogous to a “weak spot” on a balloon, fluid flows from the cochlea into the region that expands most easily, which is the sacculus. The induction of flow by injections accounts for early reports that first suggested a physiologic basally-directed flow of endolymph existed, as they were based on experiments in which marker solutions were injected into endolymph (Guld, 1927).

The kinetics of drugs and other solutes in cochlear endolymph depends largely on ion transport properties. Charged substances can also be highly influenced by the EP. As a result of the EP, anions such as the marker AsF₆⁻ are drawn in (Salt and DeMott, 1995) while cationic markers (TPMA⁺) and ions such as Na⁺ are driven out through the non-specific cation channels in the apical membranes of hair cells (Kros et al., 1992) and of outer sulcus cells (Kim and Marcus, 2011). Additional efflux of Na⁺ occurs through epithelial sodium channels in the apical membranes of cells in Reissner’s membrane (Kim and Marcus, 2011). As a result of the EP both driving efflux and opposing passive entry, small cations do not build up in cochlear endolymph unless, as in the case of K⁺ and Ca²⁺, they are actively transported in. For the vestibular endolymph, the influence of polarization is substantially lower and charge influences on distribution will differ considerably from those in the cochlea.

Endolymph was originally regarded as a “compartment within a compartment”, suggesting that solutes such as K⁺ or systemically-applied drugs entered endolymph only indirectly from perilymph, rather than directly from blood (Konishi et al., 1978; Sterkers et al., 1982). This may be appropriate for some substances, such as K⁺ which is recycled between endolymph and perilymph, but is certainly not a general principle. For example, the concept that drugs reach endolymph only via perilymph does not appear to apply to gentamicin, which is also a cation. Fluorescent gentamicin has been shown to enter the ear via the vessels of the stria vascularis, reaching the hair cells predominantly by an endolymphatic route (Li and Steyger, 2011). Kinetic models thus cannot be simplified representing endolymph as a deep compartment for all substances. Passage from strial capillaries directly into endolymph, through the blood-strial barrier is considered further below.

Water moves readily between endolymph and perilymph, in part mediated by aquaporins (Eckhard et al., 2014). No pressure changes or volume movements across the cochlear aqueduct are associated with such water movements as the volume change in perilymph will be equal and opposite to that in endolymph. As a result of the circulating K⁺ current through endolymph, endolymph K⁺ content is remarkably stable and is only altered by severe pathology. It is rare for another cation to build up in pathology unless the endolymph-perilymph barrier is compromised, allowing the EP to fall. When EP is near-normal, cationic substances will be driven out. Changes of K⁺ transport into endolymph or of K⁺ efflux out of endolymph are unlikely to change endolymph K⁺ concentration as the accompanying EP change opposes the concentration change. For example, with increased K⁺ secretion by stria, the resulting EP increase would drive a greater efflux. Even if the amount of K⁺ was decreased or increased by a manipulation, the resulting osmotic pressure change would drive water movements compensating the change. As a result, endolymph volume could increase or decrease, but K⁺ concentration changes would be small. The only situation where endolymphatic K⁺ can change appreciably in an ear with a near-normal EP, is when perilymph osmolarity is altered, resulting in water movements in or out of endolymph and thereby altering the concentration. Therefore, if EP is near normal, it can generally be assumed that endolymph cation (K⁺) content is normal. As soon as endolymph cation content changes from being predominantly K⁺, it is catastrophic for the hair cells due to the high influx of cations other than K⁺. An example is provided by the gradual loss of EP associated with the development of chronic endolymphatic hydrops. As EP becomes reduced, cations such as Ca²⁺ become elevated to a degree correlated with the EP decline (Ninoyu & Meyer zum Gottesberge, 1986), subsequently causing dysfunction and morphologic changes of the hair cells (Rydmarker and Horner, 1991).

2.3. Tissue compartments of the ear

Consideration of homeostasis or pharmacokinetics of the inner ear cannot be based only on the open fluid spaces of endolymph and perilymph, but must include the tissue spaces of the ear. From 3D reconstruction of the mouse cochlea it was calculated that the soft tissue volume amounted to 28% of the total volume of the inner ear (Hirose et al., 2014). A similar analysis of the guinea pig inner ear determined that soft tissues made up 24% of the total volume. These estimate included the tissues of the lateral wall including stria vascularis, the organ of Corti and sensory structures of the vestibular system, the spiral ganglion and the auditory and vestibular nerves. These volumes are further subdivided into...
extracellular spaces, that readily equilibrate with perilymph, and intracellular spaces. In drug delivery studies, the extracellular spaces represent an additional volume, or sink, into which drugs can distribute, lowering the concentration in perilymph. Pharmacokinetic models of the inner ear fluids need to take these additional spaces into account. Access to intracellular spaces is much more complex and highly substance-specific, varying from exclusion to avid accumulation. In most cases it is extremely difficult to quantitatively as historically measures may be distorted by tissue processing and therefore not accurately represent the in vivo situation. Nevertheless, the intracellular compartment is a major factor influencing pharmacokinetic studies, especially for small lipophilic molecules that either pass the cell membrane or bind to the lipid phase of the membrane and accumulate there. The inner ear is no different from other body systems in this respect, except that the ratio of tissue to fluid volumes is lower in the ear than most other organ systems due to the fluid filled scala. It is also important to recognize that while some drugs act through receptors or channels on the extracellular membranes, most act by influencing intracellular processes. In these cases, drug entry into cells and intracellular drug concentrations are of greater importance than endolymph or perilymph concentrations. For the inner ear, this is important as we try and reconcile pharmacokinetics (drug distribution in the ear) with pharmacodynamics (the effects of drugs on the ear).

3. The blood-labyrinth barrier

Every arteriole, capillary and venule in the ear can contribute to the exchange of solutes and fluid between the ear and the blood. The “blood-labyrinth barrier” is a term that refers to the boundary between the vascular compartment and the inner ear fluid spaces which include both perilymph-containing and endolymph-containing spaces. Some have attributed the term blood-labyrinth barrier to refer specifically to the boundary between the endovascular space and the intrastrial space of the stria vascularis. For clarity, we will refer to the “blood-perilymph barrier” and the “blood-strial barrier” as separate entities, with potentially different physiological properties, and the “blood-labyrinth barrier” as a term that encompasses both entities. Under this terminology, use of the term “blood-labyrinth barrier” to refer to only the boundary in stria vascularis would be incorrect.

3.1. The blood-perilymph barrier

In the cochlea, arterioles follow the bony wall of scala vestibuli to the capillary beds of stria vascularis and the lateral wall, draining via venules which follow the wall of scala tympani (Axelsson, 1988). The arterioles and venules do not run in the scala or directly contact perilymph, but rather run in channels within the bone around the walls of scala vestibuli and scala tympani. It is unknown whether this anatomy restricts the contribution of the arterioles and venules to blood-perilymph exchange. Thin bone can be permeable to small molecules and the perivascular fluids within these channels will, in the absence of a tight cellular barrier, equilibrate with perilymph. The major capillary beds of the cochlea are located in the lateral wall (stria vascularis and spiral ligament), the spiral ganglion, the spiral limbus and to a lesser degree at the osseous spiral lamina and basilar membrane. The spiral ganglion and modiolus are well vascularized and their fluid spaces have open communication with the perilymph of scala tympani (Shepherd and Colreavy, 2004; Rask-Andersen et al., 2006). Therefore, exchange between blood and perilymph undoubtedly involves numerous sites but is likely dominated by the major capillary beds of the spiral ganglion and the spiral ligament. When the ear is damaged, such as by noise exposure, marrow-derived macrophages appear to enter the ear from the vasculature and are most concentrated in the spiral ligament and spiral limbus which are areas that are known to be susceptible to acoustic injury (Hirose et al., 2005).

The blood labyrinth barriers are often compared to the blood-brain barrier, which has been studied far more extensively. The blood-brain barrier is created by the endothelial cells of capillaries which are surrounded by basement membrane, pericytes and astrocyte endfeet, as shown in Fig. 1A (Takeshita and Ransohoff, 2012). The endfoot processes of the astrocytes form the glia limitans, which reinforces the endothelial cells and create a physical barrier. The blood-labyrinth barrier is similar in concept to the blood-brain barrier, but differs in structure. The capillaries in tissues contacting perilymph, as shown in Fig. 1B, do not have a structure analogous to the glia limitans. The capillary wall comprises endothelial cells sealed together by tight junctions (Jahnke, 1980). Around the endothelial cell is a region often referred to as the perivascular space. Tight junctions that are present at all transitions between endothelial cells result in limited solute movement between the blood vessel lumen and the perivascular space unless specific cues are present to allow changes in barrier permeability to cells and small molecules. In the brain, pericytes that lie on the abluminal surface of the endothelial cell reinforce the barrier between the vessel lumen and the perivascular space. It has been shown the density of pericytes surrounding endothelial cells corresponds to the strength of the barrier in that a higher density of pericytes corresponds to a lower permeability of the blood brain barrier (Daneman et al., 2010). To what degree the inner ear vessels form a perivascular space, what contribution pericytes make to the blood perilymph barrier and whether there is a structure analogous to the glia limitans in the inner ear remains uncertain.

As mentioned above, older literature (Schnieder, 1974) suggested that perilymph could be considered an ultrafiltrate of blood, which at the time was thought to be consistent with studies suggesting the presence of fenestrated capillaries in the ear (Jahnke, 1980). However, biochemical studies have consistently shown the content and the concentration of small molecules in perilymph to be distinctly different from those in the CSF and in the serum, calling into question the presence of such fenestrated capillaries (Schiebe and Haupt, 1985; Hara et al., 1989). Furthermore, animal experiments demonstrate that small molecules injected intravenously do not readily enter the perilymph. Juhn and Rybak (1981) compared the entry of similar molecules into perilymph and CSF, including urea, sugars, aminoglycoside antibiotics and mannitol. After 1 h, they found perilymph levels to be substantially lower than serum levels, with entry into perilymph decreasing as molecular weight increased. The data supported the concept of a barrier between the blood and perilymph, comparable or less permeable than the blood-CSF barrier. Similarly, Sterkers et al. (1987) compared the entry of small molecules into the perilymph after intravenous administration, specifically, Na⁺, urea, glycerol, mannitol and sucrose. They found that these reagents, originating from the serum, entered slowly into the perilymph, comparable to entry into the CSF, and they concluded that a blood labyrinth barrier, or more accurately, a blood-perilymph barrier, restricted the movement of small hydrophilic solutes. A tight barrier between the vascular space and the perilymph has also been demonstrated with TMA⁺ marker (Inamura and Salt, 1992) and more recently with fluorescein (Hirose et al., 2014).

While the blood-perilymph barrier is often regarded as comparable with the blood-brain and blood-CSF barriers, this provides only a limited analogy as there are substantive differences between entry of solutes into the ear fluids when compared to the central nervous system. There are numerous entry and exit pathways for the cerebrospinal fluid (Tarasoff-Conway et al., 2015), many of
which are not present in the inner ear. Active transport across the blood-brain barrier involves many proteins in the solute carrier (SLC: 52 families, almost 400 genes) and ATP-binding cassette (ABC) families (Brzica et al., 2017), many of which may be similarly utilized in the blood-labyrinth barrier. Efflux of small molecules (including corticosteroids) from the brain has been shown to be mediated by p-glycoproteins of the ATP-binding cassette (ABC) transporter family (Loscher and Potschka, 2005; Strazielle and Gherzi-Egea, 2015). ABC transporters have been demonstrated in tissues of the cochlea (Saito et al., 2001; Manohar et al., 2016) but whether these transporters serve a similar role in the ear and the brain is not yet known.

The blood-perilymph barrier has been shown to be altered by osmotic manipulations (Juhn and Rybak, 1981), by epinephrine associated with rise in blood pressure (Inamura and Salt, 1992) and by systemic lipopolysaccharide (Hirose et al., 2014). In addition, mechanical damage to capillaries, resulting in plasma leakage into perilymph, profoundly increased the rate of local entry of marker (Inamura and Salt, 1992). These data demonstrate that the barrier is vulnerable and is prone to breakdown reflected by passage of small molecules at its weakest and most leaky region. These studies that use methods to perturb the barrier demonstrate that isolated focal lesions are sufficient to compromise the separation of fluid compartments and to impair the barrier functionally.

Although it has been presumed that an intact blood-perilymph barrier was essential for the normal function of the ear, recent data show that hearing is not necessarily changed by compromise of the blood-perilymph barrier. When lipopolysaccharide (LPS) is administered systemically, there is a significant increase in vessel permeability when measured by solute entry into the perilymph. A four-fold increase in solute permeability is observed after systemic LPS administration (1 mg/kg per day x 2 days). However, this change in permeability is not associated with change in ABR threshold. Furthermore, the EP is maintained despite a three-fold increase in fluorescein entry from the serum into the perilymph as measured by fluorescent intensity. Given that normal sound transduction can occur in the presence of a leaky blood-perilymph barrier, one might query the purpose of such a barrier. The separation of ions, molecules, cells and fluids are in part to maintain normal physiology, but the barrier also exists to separate pathogens such as bacteria and virus that may circulate in the blood stream and threaten to infect the inner ear. The barrier also limits the entry of leukocytes that enter the inner ear and provide immunological surveillance or activation. The dynamic nature of this boundary is what renders the ear vulnerable to infection, but also allows the immune system to enter this space when and where it is needed.

3.2. The blood-strial barrier

The capillaries of stria vascularis differ anatomically from those in contact with perilymph. As shown in Fig. 1C, these vessels pass through the intrastrial space surrounded by basal, intermediate and marginal cells. The space is polarized by the ion channels generating the EP, generating an environment that is highly positively charged with a DC potential of 90—120 mV as shown in the figure. The high electrical potential produced by stria vascularis has been shown to be generated across potassium channels (KCNJ10) in the membranes of intermediate cells (Wangemann and Marcus, 2017). A consequence is that the strial capillaries pass through a highly electrically-polarized space. The voltage gradient could influence the passage of charged substances across the barrier in a manner that is distinctly different from the vessels of the spiral ligament and spiral ganglion. The degree of influence critically depends on which boundary is influenced by the voltage gradient. Initially, based on dye-coupling studies in gerbils, it was reported that the endothelial cells of the strial vessels were in gap-junction continuity with intermediate cells, pericytes, and basal cells (Takeuchi and Ando, 1998). In this case, the intracellular potential of the endothelial cells would be similar to that of the basal and intermediate cells, and the large electrical potential gradient would exist across the endothelial cell membrane contacting the intrastrial space, as it does in the intermediate cells. The positive charge across the capillary boundary would resist the passage of cationic molecules and facilitate the entry of anionic molecules between the vascular system and the intrastrial space. This would make the blood-strial barrier distinctly different from the blood-perilymph barrier. However, a subsequent dye-coupling study in mice found that endothelial cells were not coupled to the basal and
intermediate cells (Cohen-Salmon et al., 2007). In this situation, the electrical potential of the endothelial cell and capillary lumen becomes more uncertain and it is possible that the entire capillary, including the lumen, could become positively polarized to some degree. In this case, there would be no major potential gradient influencing the passage of charged substances between blood and the intrastrial space.

Various substances are pharmacologically active in the intrastrial space and many are used in experimental studies. Furosemide and other loop diuretics inhibit the Na/K/2Cl cotransporter, reducing EP and inducing expansion and retention of fluid in the intrastrial space probably due to solute accumulation from the ongoing efflux from marginal cells (Santos and Nadol, 2017). In addition to expansion of the intrastrial space, we observe synergistic changes in the toxicity of aminoglycosides with inhibition of the cotransporter through loop diuretics, leading to the speculation that aminoglycosides concentrate within the intrastrial space and in the endolymph as a result of dysregulated strial ion and solute circulation. As discussed above, it remains uncertain whether aminoglycoside entry from the vascular space to the intrastrial space is partially opposed by the highly positively charged fluid environment.

Noise has long been suspected of inducing breakdown of the blood labyrinth barrier. Studies to date have included dye extravasation studies using fluorescently tagged immunoglobulin (Yang et al., 2011) and intravascular lanthanum (Wu et al., 2014). These studies suggest that noise causes these reagents to leak from strial vessels although the technique used in dye extravasation studies is not a rigorously quantitative technique.

4. The cochlear aqueduct

The cochlear aqueduct is a bony channel that projects from the posterior fossa of the cranium to the basal end of scala tympani in the cochlea. It is lined with connective tissue which makes up the periotic duct that connects the CSF of the subarachnoid space to the perilymph of scala tympani. It enters scala tympani adjacent to the round window membrane near the basal hook region of the basilar membrane. In rodents, the duct is patent to an extent that perforating the bony otic capsule or round window of a guinea pig, mouse, rat, or chinchilla results in a substantial rate of fluid flow. From within the cochlear aqueduct, the fluid is withdrawn from the cochlea.

Similarly, the tip of a pipette inserted through the round window membrane is located close to the cochlear aqueduct outlet so sampling through the round window membrane results in contaminated samples. Based on marker sampling data it was shown that even small fluid samples taken through the round window were highly contaminated with CSF, drawn in as the fluid was sampled. Samples of 1 μL were estimated to contain 20–40% CSF while samples larger than 5 μL were estimated to contain over 80% CSF (Salt et al., 2003). This artefactual contamination of samples, thought to represent perilymph but often mostly CSF, has led to a number of erroneous perceptions of perilymph. Some concluded that perilymph had similar origins or homeostatic processes to CSF (e.g. Sterkers et al., 1987). Others, taking repeated samples from perforated ears, have concluded drugs are rapidly eliminated from perilymph when the elimination was dominated by the non-physiological CSF flow passing through the scala (Parnes et al., 1999). In local drug application studies, it would generally be expected that sample contamination with CSF would cause perilymph drug concentrations to be underestimated and rates of elimination to be overestimated by lack of consideration of this major artifact.

In 2005, we demonstrated that perilymph samples of higher purity (i.e. less contamination with CSF) could be obtained by perforating the otic capsule and collecting fluid at a greater distance from the cochlear aqueduct, specifically from the cochlear apex (Salt et al., 2006). Perforation of the apex causes the fluid contents of scala tympani to be driven out of the perforation by CSF entering through the cochlear aqueduct. Using appropriate procedures, the emerging fluid can be collected without loss or contamination. This method was subsequently adapted to what has been termed the “sequential sampling” method, in which multiple small samples are collected from a site distant from the aqueduct, either the cochlear apex or from one of the semi-circular canals. The method has allowed the distribution of drugs along the perilymphatic spaces to be quantified in guinea pigs (Salt et al., 2011; Salt et al., 2012, 2015, 2016) and in mice (Hirose et al., 2014).

In the unperforated, physiologic state, CSF volume entry though the aqueduct is thought to be low, as discussed earlier in the section on perilymph. This is consistent with the finding that surgical obstruction of the cochlear aqueduct (avoiding the nearby vein of the cochlear aqueduct) did not affect cochlear morphology (Kimura et al., 1974). However, another form of perilymph-CSF communication was implicated by experiments in which the round window membrane was altered mechanically (Salt et al., 2015; Plontke et al., 2016). In these studies, FITC-dextran, or fluorescein markers were injected into the basal turn of scala tympani and perilymph subsequently sampled from the cochlear apex. When the round window membrane was occluded with cyanoacrylate glue prior to marker injection, the concentration in samples from the basal region of scala tympani was found to be higher than in the normal state. In all cases, there was no fluid in the round window niche during the experiment, excluding the possibility of marker loss to the middle ear in the unoccluded state. It was concluded that by mechanically stiffening the round window membrane, small volume oscillations across the cochlear aqueduct driven by respiratory pressure fluctuations would be attenuated, reducing solute exchange between perilymph and CSF. Fluid oscillation across the cochlear aqueduct would permit drugs or other substances to move in both directions across the aqueduct, including from perilymph to CSF, even in the presence of a sustained low rate of CSF entry into perilymph.

The movement of substances from perilymph to CSF across the aqueduct, whether driven by volume injections into perilymph or by fluid oscillations across the aqueduct, leads to the possibility that drugs applied to one ear may reach the CSF, the brain, and the
The spread of a drug applied to one ear affecting the contralateral ear has been described as the “Schreiner effect” (Schreiner, 1999). This has been clearly demonstrated for transfections mediated by injected adenoviruses (Stöver et al., 2000). For small molecules, it would be expected that the concentration reaching the opposite ear would be far lower than the treated ear, but the difference between ears has yet to be quantified. If the treated ear is dosed at a therapeutic concentration, the dose in the opposite ear should be well below a therapeutic concentration. On the other hand, if the treated ear is dosed at well-above a therapeutic concentration, an influence on the opposite ear may be possible. This effect is of considerable concern because in order to reduce the animal numbers required by drug delivery experiments, investigators are encouraged to use the opposite, untreated ear as a control (e.g. Wise et al., 2016 and many others). This is a potentially serious problem, decreasing the statistical significance of a therapy’s influence on the treated ear if drug reaches the opposite ear at therapeutic concentration.

In primates, including man, the aqueduct is longer and narrower than in rodents (Copen et al., 1997). Perforating the human cochlea typically does not result in fluid efflux to the extent seen in experimental animals. However, the degree of patency of the human aqueduct is in most cases sufficient to permit pressure equilibration between the inner ear and CSF. Using an indirect measure of intracochlear pressure changes during postural changes, the aqueduct was assessed as functionally patent in 89% of young adults and in 70% of older adults (Phillips and Marchbanks, 1989; Wagner and Walsted, 2000). Although the aqueduct may allow pressure equilibration in most younger adults, the degree of fluid communication, specifically the passage of drugs between perilymph and CSF in the intact human, remains uncertain. As the fluid compartments of the human ear are larger than in most research animals, the influence of perilymph-CSF communications would be expected to be less. There are instances of patients undergoing spinal anesthesia where hearing loss follows the procedure, as a result of the transient reduction of CSF pressure (Cosar et al., 2004). A patient with Nieman-Pick C1 disorder treated with intrathecal 2-Hydroxypropyl-Beta-Cyclodextrin subsequently developed bilateral high-frequency hearing loss (Maapur et al., 2015) comparable to that seen in similarly-treated animals (Crumling et al., 2012; Cronin et al., 2015). 2-Hydroxypropyl-beta-Cyclodextrin is toxic to outer hair cells (Lichtenhan et al., 2017) due to its ability to bind cholesterol. These studies suggest that drug movements between CSF and perilymph across the cochlear aqueduct may not always be insignificant in humans.

5. Endolymphatic duct and sac

Although the endolymphatic duct and sac represent an additional potential fluid connection between the inner ear and the cranial, the physiology of this pathway differs markedly from that of the cochlear aqueduct. There is no open fluid connection between the fluids of the ear and CSF through the endolymphatic sac. The endolymphatic duct is an open, tissue-lined duct extending from the sacculus to the endolymphatic sac, but the endolymphatic sac itself is bounded by tissue boundaries, specifically the dura mater and wall of the sigmoid sinus. The pathway is therefore completely endolymphatic and there is no perilymphatic communication across the endolymphatic duct. Although an increase of hydrostatic pressure of the inner ear would be expected to drive endolymph into the endolymphatic sac, pressure measurements demonstrate that positive pressure pulses are not transmitted to the sac, while negative pressure pulses are (Salt and Rask-Andersen, 2004). The explanation is that the endolymphatic sinus, a small bulging of the sacculus membrane near the endolymphatic duct opening, acts as a one-way valve, closing against the bony wall when perilymphatic pressure is elevated.

Therefore, although endolymphatic sac tissues represent potential communication sites between endolymph and blood or CSF, the limited fluid communication across the vestibular aqueduct has to be factored in. Endolymphatic sac injections have been used in animals to be a way to introduce drugs directly into the inner ear while minimizing the risk of hearing damage by the procedure (Yamasoba et al., 1999). Limited studies, involving injection of gadolinium and/or dexamethasone into the endolymphatic sac of humans, have also been performed (Colletti et al., 2010; Mandalà et al., 2010). They confirmed that gadolinium solution injected into the endolymphatic sac did distribute to the endolymphatic spaces of the vestibule and cochlea.

6. Nerves between the inner ear and the cranium

It is well established that markers such as HRP applied intracellulary to afferent fibers of the cochlea distribute along the fibers, allowing them to be traced (e.g. Liberman, 1982). This raises the possibility that drugs applied to perilymph could be taken up by afferent fibers and transported to central brain nuclei. Zhang et al. (2012) found that in animals treated with intratympanic gentamicin, gentamicin-positive cells (determined immunohistochemically) were found in vestibular and auditory nuclei from 1 to 14 days after the application, with amounts peaking at 3–7 days. The locations of staining were thought to be consistent with retrograde axonal transport by vestibular efferent neurons and to the superior olivary complex and anterioregade transport to the ipsilateral cochlear nucleus. The specific staining pattern of auditory and vestibular nuclei would not have been expected if gentamicin reached the brainstem from perilymph via the cochlear aqueduct and CSF. Other studies that have documented the passage of intratympanically-applied drugs to the brain (Lee et al., 2012; Dean et al., 2012) have not considered potential pathways involved. Nevertheless, as the range of drugs applied tympanically increases, the possibility that drug may reach, and have unexpected influences on the brain requires more detailed consideration.

7. Evidence for a glial/lymphatic system in the ear

In the brain, the lymphatic system plays a part in circulating metabolites and cells from the CSF. CSF from the perivascular spaces is cleared to the lymphatic system via the cervical lymph nodes (Tarassof-Conway et al., 2015). A lymphatic system has also been documented from the middle ear with macromolecular markers traced to the retroauricular and jugular lymph nodes (Lim and Hussl, 1975). There is very limited evidence that an analogous drainage system may exist from the inner ear. Histologic studies suggest lymphatic vessels are present in the connective tissue layer of the round window membrane (Goycoolea, 2001). Following injection of keyhole limpet hemocyanin (KLH) antigen into perilymph, the antigen was found to be present in both the parotid and superficial ventral cervical lymph nodes. These data were interpreted as suggesting the inner ear was directly connected to a lymphatic drainage system (Yitmee et al., 2001). While this is certainly possible, an alternate explanation is that the pressure injection of KLH into perilymph of the basal turn of scala tympani drove an efflux of KLH solution into CSF through the cochlear aqueduct, thereby allowing the KLH to reach the cervical lymph nodes. The anatomic basis, route and physiologic contribution of lymphatic drainage from the inner ear therefore still remains highly uncertain. If present, lymphatic drainage could contribute to volume movements and to non-specific elimination of solutes from perilymph.
8. Perilymph — bone vascular communication

It has been reported that substantial communication exists between the fluid spaces of the bone of the otic capsule and perilymph. Chole and Tinling (1994) first reported that the usual bone lining cells, that are present on all other bones of the body, were patchy and discontinuous in the temporal bone, allowing perilymph to be in direct contact with bone matrix. Shepherd and Colleavy (2004) described functionally patent micropores (canaliculi perforantes) through the bone between the perilymph of scala tympani and the fluid spaces of both the organ of Corti and of the spiral ganglion in Rosenthal’s canal. Zehnder et al. (2005) showed an extensive system of interconnected canaliculi within in the bone of the otic capsule that directly opened into the perilymphatic spaces. Horseradish peroxidase, when applied to perilymph, was found to readily diffuse throughout this lacuno-canalicul system. Wang and Stegger (2009) reported that although systemically-applied texas-red-labeled gentamicin (GTR) was found in stria vascularis, it was also distinctly present in the fluid spaces of the bony cochlear capsule. The vasculature and fluid spaces of the otic capsule therefore need to be considered as a potential communication site for the inner ear. This may be especially relevant in humans, where the temporal bone is thicker and vascularized.

9. Perilymph — middle ear communications

9.1. Round window membrane

The primary route of communication between the middle ear and perilymph has been widely assumed to be the round window membrane, as it forms the most obvious anatomic opening between the two compartments in both animals and humans. Anatomically, the round window membrane consists of three layers. The outer epithelium facing the middle ear consists of a single layer of cuboidal cells interconnected by tight junctions (Franke, 1977). The middle, connective tissue layer is made of collagen and elastic fibers and includes fibroblasts, blood vessels and lymphatics. The scala tympani surface is lined with a squamous epithelium. Although these cells exhibit both tight junctions and gap junctions (Franke, 1977) large extracellular spaces also exist to an extent that portions of the connective tissue layer are in direct contact with perilymph (Goycoolea, 2001). Goycoolea noted that the “passage of substances through the membrane is by different pathways, the nature of which is seemingly decided at the outer epithelium of the membrane”. This reinforces the concept that the physiologic boundary is not formed by the connective tissue matrix of the membrane but rather by the properties of the outer epithelium. Comparisons between round window thickness between animals and humans and between different pathologic conditions, where the round window matrix becomes thickened, would be expected to have minimal influence on permeability properties and drug entry. Goycoolea (2001) also noted that macromolecules (ferritin), latex spheres, and vesicles readily diffused through cytoplasm and extracellular spaces of the round window membrane, indicating that small molecules would do similarly.

Round window membrane permeability has been shown to be highly sensitive to manipulations. Mikulec et al. (2008) showed that round window membrane permeability was increased by nearby suction (pulling dry air over the membrane) or elevating solution osmolarity. In studies conducted in guinea pigs, opening the bulla, allowing release of the humid air was found to increase round window membrane permeability over time. Other treatments that increase round window membrane permeability include treatments with endotoxins and exotoxins (Ikeda and Morizono, 1988) and by benzyl alcohol (Mikulec et al., 2008). None of these treatments caused overt damage of the round window membrane structure, supporting the view that permeability is not restricted by the connective tissue component, but rather by the outer epithelial layer. Microperforations have been shown to increase round window membrane permeability in vitro (Kelso et al., 2015), but even small perforations were found to result in perilymph efflux in vivo, which has a profound influence on perilymph kinetics (Plonkte et al., 2016). Otitis media has been shown to cause expression of cytokines, tight junction claudins and gap junction proteins in the inner ear (MacArthur et al., 2013). If similar changes occur at the round window membrane epithelium, an influence on permeability might occur. Changing the pH of the round window environment was found to alter the toxicity of locally-applied cisplatin (Tanaka et al., 2003). This was not attributed to an influence of pH on round window membrane permeability to cisplatin as toxicity to systemic cisplatin was similarly influenced (Tanaka et al., 2004).

9.2. Oval window/stapes

The oval window is not open, but is covered by the footplate of the stapes. As a result, with the exception of a few studies it was largely ignored as possible route of drug entry into the inner ear. The stapes is attached to the perimeter of the oval window by an annular ligament, composed of an open meshwork of elastic and collagen fibers (Ohashi et al., 2006). In similarity with other structures of the ear with communicating extracellular spaces, such as the basilar membrane and spiral ligament, the structure of the annular ligament is not likely to impede diffusion of small molecules. A mucosal epithelium overlies the stapes area on the middle ear side, comparable to that over the round window membrane (King et al., 2013). The passage of substances from the middle ear into the perilymph of the vestibule is therefore as feasible at the stapes as it is via the round window membrane. Although solute entry is likely to be limited in a similar manner by the outer epithelial membrane, this remains to be confirmed. In studies using horseradish peroxidase (HRP) as a tracer, Sajio and Kimura (1984) noted that the oval window was a secondary route of passage of HRP from the middle ear cavity to the inner ear. Nevertheless, the degree of entry at the stapes was not fully appreciated until King et al. (2011) showed with MRI that gadolinium levels in the vestibule were commonly far higher than in scala tympani following middle ear applications. Quantitative interpretation of the data indicated that majority of the gadolinium (~90%) entered the ear at the stapes. Based on perilymph measurements, the amount of entry at the stapes was estimated to be 35% for the marker TMPA (Salt et al., 2012) and 35% for gentamicin (Salt et al., 2016). For gentamicin, the amount entering at the stapes results in higher perilymph concentrations of the scala vestibuli and vestibule than in scala tympani, due to slower elimination there, and better accounts for the observed cochleotoxicity than the gentamicin in scala tympani (King et al., 2013; Salt et al., 2016).

9.3. Areas of thin bone of the otic capsule

In studies in guinea pigs when the middle ear space was completely filled with drug solution (immersing the entire cochlea), high drug levels were found in perilymph near the apex, not consistent with entry occurring only through the windows in the basal turn (Mikulec et al., 2008). Similar, high apical levels of drug were found in guinea pigs dosed with a suspension of micronized dexamethasone in poloxamer gel for 24 h (Salt et al., 2011). These findings are accounted for by the thin bony capsule of the guinea pig in the apical region, and its covering mucosa, allowing drugs to pass. Although the permeability of bone to small molecules is
assumed to be low, the otic capsules of both humans and mice have minute fluid filled channels (a lacunocanalicular system) passing through them that is in open communication with perilymph (Zehnder et al., 2006). Thus, under some conditions, the bony capsule cannot be assumed to be impermeable. Although entry through the bone from the middle ear to perilymph occurs in experimental animals, it is unlikely to occur in humans in significant amounts as the bone of the otic capsule of humans is far thicker.

10. Intratympanic drug applications

Some of the pathways relevant to intratympanic drug delivery are summarized in Fig. 2. Drug solution applied to the middle ear enters the middle ear at multiple sites and from there distributes throughout the fluid and tissue spaces of the inner ear. Drug levels and distribution throughout the inner ear are influenced by losses from the middle and inner ears to the vascular system, and by interactions with CSF through the cochlear aqueduct.

10.1. Middle ear kinetics

The physiology of the middle ear exerts a prominent influence on drug entry to the inner ear following intratympanic injections. In the normal state, there are multiple process by which drug can be removed, including volume loss to the pharynx by the Eustachian tube and drug loss to the vasculature of the middle ear mucosa. The middle ear also has the capacity to secrete and/or resorb fluid volume. In pathologic states, middle ear physiology for injected fluids can be markedly altered by negative pressure, serous effusion and inflammation. The middle ear is normally gas-filled with gas composition dominated by venous gas partial pressures (Ostfeld and Silberberg, 1991). Composition is thus dominated by exchange with the vasculature with middle ear pressure changes balanced by intermittent opening of the Eustachian tube. In the normal state, fluid exchange between perilymph and the gas-filled middle ear would be minimal and the exchange occurring with intratympanic injections represents an abnormal state. In mammals, the epithelium of the ventral middle ear is of endodermal origin and is densely ciliated while the epithelium of the dorsal middle ear is derived from neural crest and lacks cilia (Thompson and Tucker, 2013). Cilia on the ventral epithelium drive fluids and foreign substances towards the Eustachian tube through which they are eliminated to the pharynx. Both particles and fluids drain from the middle ear to the lymphatic system and to the vasculature of the middle ear. When applied to the middle ear, the markers Evans blue and horseradish peroxidase were subsequently found to accumulate in the retroauricular and junctional lymph nodes (Lim and Huxl, 1975). The middle ear epithelium is well vascularized and is capable of secreting fluid in the presence of infections, exposure to inflammatory mediators or with cold air in the external ear canal (Goldie and Hellström, 1988; Goldie et al., 1989). The mucosa also resorbs fluid from the middle ear cavity, using transport processes dependent on the sodium and chloride content of the solution (Li et al., 2005). Many of these processes could contribute to the dilution or elimination of applied drug solution in the middle ear space. Following application to the round window niche, TMPA marker was found to decline with a half-time of ~36 min (Mikulec et al., 2008) and generally a halflife of ~70 min (Salt et al., 2016) in anesthetized, recumbent guinea pigs. A single application therefore results in only a brief application of drug to perilymph. Elimination from the middle ear occurs more rapidly in the normal, upright position, when the Eustachian tube combined with the ciliated ventral surface of the middle ear and the swallowing reflex work in concert to remove fluid.

The stabilization of volume by applying drug solutions in the form of a gel has been shown to improve retention in the middle ear (Wang et al., 2009, 2011). The gel acts primarily by keeping the applied volume in the middle ear rather than being cleared away by cilia to the Eustachian tube. Maintaining the volume, however, does not mean that drug is retained in the middle ear space. Elimination of drug from the solution still proceeds so that with time the gel volume may not be reduced but the concentration of drug in the gel may have markedly decreased. For solutions of drug delivered in gel, concentration of the drug will typically decline before the gel is lost. The declining middle ear drug concentration with time can be overcome by applying the drug as a suspension or encased as a nanoparticle in the gel, representing a form of sustained release of the drug. For example, in the OTO-104 formulation micronized dexamethasone is given as a suspension in Poloxamer 407 gel (Wang et al., 2009; Salt et al., 2011). With this formulation, the gel is lost from the middle ear over a few days but undissolved drug remains dispersed around the middle ear and in contact with the mucosa. Therapeutic levels in perilymph have been maintained for 30–40 days with this approach (Wang et al., 2011). In this case, the elimination of the undissolved dexamethasone component occurs far more slowly than the elimination of the gel. Gels other than Poloxamer have been compared (Berglin et al., 2015). In contrast to Poloxamer 407 that is injected as a liquid and polymerizes to become solid at body temperature, viscous gels injected into the middle ear may subject the ear to mechanical stresses. In their experiments with drugs in hyaluronate gel, there was concern that some injections could cause rupture of the round window membrane, with associated compromise of cochlear function.

There are now numerous investigations using encapsulated or other forms of timed-release drug to prolong residence time of drug in the middle ear. This approach potentially increases the efficiency of drug delivery and is therefore capable of generating higher, more uniform drug concentrations in perilymph.

Fig. 2. Schematic of the major processes influencing inner ear drug distribution following intratympanic application (purple). Purple arrows indicate drug movements. Red arrows indicate elimination to the vascular system. Cyan arrow: indicates losses due to perilymph-CSF mixing across the cochlear aqueduct. Abbreviations are: CSF: cerebrospinal fluid; ES: endolymphatic space; ESac: endolymphatic sac; ET: Eustachian tube; RW: round window; S: saccus; ST: scala tympani; SV: scala vestibule; SS: sigmoid sinus; UT: utricle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
10.2. Pathways by which intratympanically-applied drugs could reach the brain

Recently, investigators have found that intratympanic delivery of some drugs could result in those agents reaching the brain and spinal fluid in experimental animals, including guinea pigs (Zhang et al., 2012), rats (Lee et al., 2012) and mice (Dean et al., 2012). There is concern that some active agents applied to the ear, such as neurotrophins or proliferative agents to encourage hair cell regeneration could represent potential risks if they were to reach the central nervous system. The pathways by which different drugs distribute from the middle ear to the brain have not been studied in detail. In the studies cited above, the authors concluded that gentamicin (Zhang et al., 2012), manganese (Lee et al., 2012) and fluororgold (Dean et al., 2012) moved to the brain via neural pathways. Lee et al. (2012) provided support for this interpretation, showing that less Mn⁺² reached the brain in animals deafened by locally-applied gentamicin. There are also other pathways that may be implicated. Fluid communication across the cochlear aqueduct, allowing intermixture of perilymph and CSF will drive significant amounts of drug from perilymph to CSF. Analysis of pharmacokinetic measurements for gentamicin (Salt et al., 2016) indicate that 53% of the gentamicin entering the inner ear is subsequently lost to CSF.

Another potential pathway for intratympanic reagents to reach the brain is via cranial nerves passing through the middle ear. The main trunk of the facial nerve passes through the mastoid and middle ear and enters the calvarium through the internal auditory canal. The facial nerve also has branches, the chorda tympani nerve and the nerve to the stapedius muscle which provide some potential absorptive surface area. The mandibular division of the trigeminal nerve innervates the tensor tympani and travels to the brainstem as well. With intratympanic application, drugs applied to the middle ear, to which these nerves and muscles are exposed, may be 1000 times greater than they are in perilymph. Thus, it is possible that these pathways carry some reagent to the brain in addition to the trace amount transmitted through the perilymph via the cochlear aqueduct or eighth nerve.

Although the losses of drug from the middle and inner ears to blood could elevate plasma concentration, this is likely to be substantially lower than when the drug is applied systemically, and in both cases the drug must traverse the blood-brain barrier. In most cases, therefore, the vascular pathway from the middle ear to the brain may be limited. Nevertheless, quantification of the pathways for drug movements between the middle ear and the brain is an area that needs further investigation.

11. Conclusions and challenges

One of the major challenges for studies of the ear has been to deal with the issue of limited accessibility due to the encasement of the inner ear in the temporal bone. As a result, there are a limited number of pathways in and out of the ear. Characterizing these pathways will allow us to optimize manipulations of the inner ear fluids and tissues. Although fluid sampling from the inner ear was previously plagued by artifacts and contamination with CSF, we now have available new techniques to collect perilymph in a manner that allows drug concentrations and distribution to be accurately defined. Assay systems that can accommodate the small sample volumes from the ear, fluorescent or radiotracer markers, and novel techniques of imaging the entire cochlea, are providing increasingly sensitive quantitative analyses of drug distribution in the ear. Instead of binary terms such as whether a duct is “open” or “closed”, whether a barrier is “intact” or “permeable”, whether a fluid “flows” or is “stationary”, we can now, with the aid of computer modeling, quantify flow rates, permeability coefficients and elimination rates. We are now in a position to reconcile measurements from the ear with other fluid systems of the body that have been studied more extensively, such as the eye and the brain. Our goal should be to develop a robust understanding of the ear that can provide scientific insight into disease processes and help develop drug therapies that treat them effectively.

Acknowledgement

This research was supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health under Award Numbers R01 DC001368 (AS) and R01 DC011315 (KH and AS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heares.2017.12.010.

References


Frank, K., 1977. Freeze fracture aspects of the junctional complexes in the round


interlabyrinthine connection. Laryngorhinootologie 78, 387–393 [Article in German].