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Physiopathology of the Cochlear Microcirculation

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Abstract

Normal blood supply to the cochlea is critically important for establishing the endocochlear potential and sustaining production of endolymph. Abnormal cochlear microcirculation has long been considered an etiologic factor in noise-induced hearing loss, age-related hearing loss (presbycusis), sudden hearing loss or vestibular function, and Meniere's disease. Knowledge of the mechanisms underlying the pathophysiology of cochlear microcirculation is of fundamental clinical importance. A better understanding of cochlear blood flow (CoBF) will enable more effective management of hearing disorders resulting from aberrant blood flow. This review focuses on recent discoveries and findings related to the physiopathology of the cochlear microvasculature.

Keywords

Cochlear microvessels; hearing loss; diseases; measurements

Introduction

Normally functioning cochlear microcirculation is critically important for maintaining ion and fluid balance in the inner ear, as sensory hair cells are strikingly vulnerable to ischemia (Nuttall, 1999b; Wangemann, 2002b). The inability to measure cochlear blood flow (CoBF) in humans has limited the investigation in human subjects, but numerous studies using different animal models have aptly demonstrated physiological changes with the alteration of CoBF, including changes in leukocyte dynamics. Vascular permeability and deformation have been shown to be contributing factors in various hearing disorders including presbycusis, noise-induced hearing loss, and ear hydrops (Brown et al., 1995; Chen et al., 2005a; Gratton et al., 1996a; Gratton et al., 1997; Hawkins, 1971; Kellerhals, 1972; Lamm et al., 1998; Mazurek et al., 2006; Miller et al., 2003; Nuttall, 1999a; Ohlemiller, 2009; Prazma et al., 1990; Seidman et al., 1999a; Shi et al., 2003). In humans, compelling clinical evidence has associated blood risk factors and myocardial disease with hearing and vestibular abnormalities (Aimoni et al., 2010; Mitchell et al., 2009). Capillary and stria vascularis degeneration have also been shown in presbycusis patients (Nelson et al., 2006; Wagenaar et al., 2000). In addition, the incidence of hearing loss in patients with various

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systemic autoimmune diseases is quite high, reported to be between 15% – 75% (Barkhuizen et al., 2006; Mouadeb et al., 2005). One mechanism for hearing loss is disruption of the vascular barrier in the stria vascularis (Cadoni et al., 2002; Fattori et al., 2001; Naarendorp et al., 1998; Ottaviani et al., 1999), with subsequent loss of endocochlear potential (Lin et al., 1997; Ruckenstein et al., 1999). Study of the vascular system in the inner ear has a long and rich history, which has been well-documented in previous reviews (Axelsson, 1988; Axelsson et al., 1986; Kimura, 1986; Lawrence, 1980; Miller et al., 1988; Miller et al., 1995a; Nakashima et al., 2003; Nuttall, 1988; Seidman et al., 1999b; Sillman et al., 1989; Wangemann, 2002a). Animal models of cochlear microcirculation have provided a good understanding of cell-mediated CoBF homeostasis, and further studies will extend this basic understanding to clinical studies, which directly address vascular-related hearing disorders. This review focuses on the microvasculature, and in particular on recent findings that show CoBF regulation at the microvessel level. The review introduces a new view of the blood-labyrinth barrier (BLB), which has ramifications for treatment of clinical hearing disorders such as noise-induced hearing loss, presbycusis, and sudden hearing loss, or ear hydrops associated with the dysfunction of cochlear blood supply. The microvasculature is a key component of tissue and organ health (Klijin et al., 2008; Lockhart et al., 2009), and understanding the role of the microvasculature in the BLB and CoBF is the foundation for preventing, diagnosing, and treating many hearing disorders.

1. Features of cochlear microcirculation

1.1. Capillary networks of the cochlear lateral wall are distinctively layered in a parallel arrangement and anatomically distant from sensory hair cells in the cochlea

The main blood supply to the cochlea is the terminating spiral modiolar artery (SMA), a branch of the anterior inferior cerebellar artery (AICA) (see Figure 1). As shown in Figure 1A, the SMA branches from the AICA centrifugally and radiates over the scala vestibuli and across the spiral lamina. The spiral modiolar artery has radial branches to the lateral cochlear wall which form the two major capillary systems in the spiral ligament and stria vascularis. The two capillary systems form four distinct networks that are arranged in parallel in the cochlear lateral wall (Illustrated in Figure 1 B) (Axelsson, 1968). The networks are: (1) *The supra-strial capillary network (arteriole system) of the spiral ligament*. These microvessels, located above the attachment of Reissner's membrane and just below the perilymphatic surface, are surrounded by a generous number of pre-capillaries. Location and arrangement is suggestive of a plasma filter for the perilymph. The perilymph may also originate in this network. (2) *The post-strial capillary network (venous system) of the spiral ligament*. Capillary branches from radiating arterioles passing down behind the stria turn longitudinally in the body of the prominence, and descend beneath the outer sulcus and insertion of the basilar membrance to join the collecting venules of the tympanic portion of the spiral ligament. (3) *The ad-strial capillary network (true capillary system*) *of the spiral ligament*. Most of the capillaries in the middle part of the spiral ligament that pass behind the stria run a more or less straight downward course in the spiral ligament until they reach the floor of the outer sulcus. A majority of the vessels turn longitudinally to join the venules in the wall of the scala tympani. (4) *The capillaries of the stria vascularis*. The largest branches of the radiating arterioles enter the stria vascularis just below the attachment of Reissner's membrane, where they divide to form the strial network with its multiple anastomoses. The volume of cochlear blood flow is extremely small, with CoBF estimated on the order of 1/10 000 of total cardiac output in rodents such as guinea pigs or rats, and on the order of 1/1 000 000 of total cardiac output in humans (Nakashima et al., 2003). Strial capillaries are usually of larger diameter (12–16 micron) than spiral ligament capillaries (9–12 micron) (Miller et al., 1988). The strial capillaries are tightly packed with red blood cells. The flow is non-pulsatile and anatomically distant (> 100 micrometers) from sensory hair cells (Fig 1A and B), minimizing the acoustic perturbation of blood flow on hair cell

transduction (Axelsson et al., 1990). The velocity of blood flow in the strial network is much slower than it is in the vessels of the spiral ligament. Themean velocity of blood flow in spiral ligament vessels was measured at 0.12 mm/s, while strial flow measured 0.08 mm/s. In a typical animal, blood velocity in various vessels of the ligament ranged between 0.09–0.18 mm/s, but only ranged 0.03–0.10 mm/s in strial vessels (Nuttall, 1987). The strial network is widest and most complex near the basal end and becomes narrower and simpler towards the apex.

External wall vessels only form a single-layer capillary network at birth, but subsequently divide into two layers, constituting the microvessels of the stria vascularis and spiral ligament. This process occurs progressively from the basal turn toward the apical turn between days 5 and 8 in mice (Iwagaki et al., 2000). The cochlear vasculature tends to mature from the basal turn towards the apex (Iwagaki et al., 2000). In guinea pigs, the main stem of the inner ear vessel is formed by day 30 of fetal life, however, the peripheral capillary nets remain immature in form and vessel density is low (Nakai et al., 1986). It is also reported that BLB permeability is much greater before 14 days of birth in rats (Suzuki et al., 1998).

Pre-capillary and post-capillary vessels of the spiral ligament have vessel walls with smooth muscle cells, and regulation of lateral wall blood flow is largely considered to be a function of this network (Wangemann et al., 1996). In contrast, capillaries of the stria vascularis, formed in polygonal loops, are highly specialized vascular epithelia (Axelsson, 1968). Strial capillaries have a minor role in blood flow regulation, but a crucial one in maintaining the endocochlear potential, ion transport, and endolymphatic fluid balance essential for the ear's sensitivity (Spicer et al., 2002a; Wangemann, 2002a).

1.2. The cochlear capillary network is densely populated with pericytes

The capillary networks of the cochlear lateral wall include a rich population of pericytes (Shi et al., 2008). Pericytes are smooth-muscle-like cells and also are considered as pluripotential progenitor cells. Pericytes are generally situated on microvessels, such as arterioles and venules, and particularly on the smallest capillaries, where there is little or no smooth muscle (Gerhardt et al., 2003; Hirschi et al., 1996; Thomas, 1999). Pericytes typically have a prominent nucleus and relatively little cytoplasm, and display nearby short processes as well as several long processes which embrace the abluminal endothelium wall (Diaz-Flores et al., 1991). The long cytoplasmic processes of pericytes, often in contact with numerous endothelial cells, serve to integrate signals along the length of the vessel (Bergers et al., 2005) (Figure 2 showing cochlear pericytes).

Pericytes show considerable morphological heterogeneity in the capillary beds of different tissues as well as wide differences in distribution density. For example, the ratio of pericytes to endothelial cells varies from 1:1 in retina, 1:5 in brain, 1:10 in lung, to 1:100 in skeletal muscle (Frank et al., 1987; Shepro et al., 1993). In the cochlea, the ratio of pericytes to endothelial cells in the stria vascularis and spiral ligament are approximately 1:2 (Shi et al., 2008), similar to that in the retina. The cochlear capillary system has a relatively high population of pericytes. The morphology of pericytes also differs depending on where they are found. The majority of cochlear pericytes on true capillaries have a polygonal cell body and long, slender processes (see Figure 3, Panels A–C), while most pericytes in the pre-capillary areas have prominent soma and band-like processes which completely encircle the vessel (see Figure 3, Panels D–F). Most pericytes in the post-capillary venule areas have flattened cell bodies and, likewise, circumferential band-like vessel-enshrouding processes (see Figure 3, Panels G–I). Pericytes on branch points have spindle-cell bodies and long processes distributed over the two branches (see Figure 3, Panels J–L). Pericytes on the vessels of the spiral ligament express contractile proteins, including α-SMA, desmin, F-

actin, and tropomyosin (Shi et al., 2008), and exhibit vasocontractility (Dai et al., 2009). In contrast, pericytes on the vessels of the stria vascularis, lacking expression of α-SMA or tropomyosin, express abundant desmin structural proteins.

Pericytes in the kidney, retina, liver, and lung not only play a role in regulating capillary blood flow, but also in blood vessel formation, immune response, and regulation of endothelial activity via different cell factors and signaling agents, including neuromodulators, vasoactive peptides, metabolic factors, growth factors, and cytokines (Allt et al., 2001; Betsholtz et al., 2005; del Zoppo et al., 2006; Donoghue et al., 2006; Kim et al., 2006; Nehls et al., 1993; Pallone et al., 2001; von Tell et al., 2006; Yamagishi et al., 2005). In addition, pericytes exhibit multipotent stem cell activity and can differentiate into a variety of different cell types, including macrophages and phagocytes, fibroblasts, and smooth muscle cells (Dore-Duffy et al., 2006; Sims, 2000). Moreover, various vascular diseases have been found to be associated with pericyte pathology (von Tell et al., 2006). Pericytes are receiving increased attention in microcirculation studies. However, cochlear pericytes have traditionally received little attention and the specific role of pericytes in cochlear homeostasis is largely unknown.

1.3 Autoregulation

Another feature of cochlear microcirculation is its strong autoregulation (Brechtelsbauer et al., 1995; Brown et al., 1994; Laurikainen et al., 1993; Miller et al., 1995a; Nakashima, 1999; Nakashima et al., 2003). A significant decrease in systemic blood pressure only causes a slight change in CoBF (Albera et al., 2003; Degoute et al., 1997; Tono et al., 1998). The rapid recovery of CoBF that occurs during occlusion of the anterior inferior cerebellar artery, the main blood supply to the ear, is a further indication of the autoregulation (Nakashima, 1999; Ren et al., 1993). Moreover, Suzuki et al. (1993) found that when cerebrospinal fluid pressure is increased, CoBF is not correspondingly decreased by the elevation in fluid pressure.

2. Regulation of CoBF in the inner ear

Sound stimulation of the inner ear imposes an energy demand that requires efficacious delivery of oxygen and glucose. A well-regulated cochlear blood flow (CBF) is needed to meet these requirements while also effectively clearing away metabolites. Regulation of CoBF, under the prevailing model, is hypothesized to include both local auto-regulatory and central control through neural pathways. The model incorporates neural- and autocrine/paracrine-based regulation of vasoconstriction and dilation at the level of artery and arterioles, as well as at the level of capillaries.

2.1 Regulation of CoBF by smooth muscle cells

Contraction of the smooth muscle cells in the spiral modiolar artery is hypothesized to be tightly regulated to meet the demand of cochlear tissues (Wangemann, 2002b). Contraction of the smooth muscle cells of the vascular wall reduces its luman diameter with the effect of decreasing blood flow, while relaxation of the smooth muscle cells increases blood flow. Smooth muscle cell contractility is signaled both with central neural and local metabolic signals. Sympathetic (peptidergic and adrenergic) nerve fibers have been found in the spiral modiolar artery of the gerbil and guinea pig (Brechtelsbauer et al., 1990; Carlisle et al., 1990; Rauchegger et al., 1981). Norepinephrine-induced vasoconstriction in the spiral modiolar artery is mediated by α1A-adrenergic receptors (Gruber et al., 1998). Stimulation applied in the sympathetic ganglia, stellate ganglion, or superior cervical chain in the guinea pig has been shown to alter CoBF in situ (Laurikainen et al., 1994; McLaren et al., 1993; Ren et al., 1993). In addition, distribution of vasoactive intestinal peptide (VIP),

neuropeptide Y (NPY), substance P (SP), and calcitonin gene-related peptide (CGRP) are also found in the spiral modiolar artery (Carlisle et al., 1990; Qiu et al., 2001). These findings support a hypothesis that CoBF is controlled by neuronal signals at the level of the artery (Gruber et al., 1998; Herzog et al., 2002; Sadanaga et al., 1997; Scherer et al., 2005; Wangemann, 2002b; Wangemann et al., 1998; Wonneberger et al., 2000).

2.2 Regulation of CoBF by pericytes

Capillary-mediated local control of perfusion was first reported by (Wangemann et al., 1996). Recent findings on the vascular capillaries in the brain and retina highlight the role of pericytes in controlling capillary blood flow and maintaining microvascular homeostasis (Peppiatt et al., 2006). Microvessels in the spiral ligament contain a high density of pericytes, spaced approximately 2–25 μm apart as compared to 100 μm on true capillaries (Shi et al., 2008). The pericytes express contractile proteins, including α-smooth muscle actin and tropomyosin, and exhibit vasocontractility under both *in vivo* and *in vitro* conditions (Dai et al., 2009). The contractility of pericytes could affect flow resistance of the vascular network, and may profoundly alter overall blood flow. In particular, cochlear pericyte long processes span considerable distances (~ 60 μm) within the microcirculatory network and touch each other on the surface of microvessels, which may set the stage for signal integration. Pericytes may be functionally linked to form a “pumping system” to regulate blood flow.

2.3 Regulation of CoBF by fibrocytes

Recent experiments have also shown that CoBF is modulated by lateral fibrocyte input (Dai and Shi, 2011, an illustration in Figure 4). Fibrocytes in the cochlear lateral wall are divided into five types (I–V) based on morphological appearance, immunostaining pattern, and general location (Spicer et al., 1991; Suko et al., 2000). Fibrocytes have long been regarded as simple supporting cells; however, recent evidence suggests other functional roles under both physiological and pathological conditions (Adams, 2009; Doherty et al., 2004; Hirose et al., 2003; Moon et al., 2006; Nakashima, 1999; Nakashima et al., 2003; Qu et al., 2007; Spicer et al., 1991; Spicer et al., 2002b; Trowe et al., 2008; Wangemann, 2002c; Wu et al., 2003). In particular, fibrocytes participate in ion transport. They facilitate generation of the endocochlear potential by recycling K^+ from hair cell transduction, through gap junctions to strial intermediate cells and marginal cells, into the endolymph.

Normal hearing requires tight control over the supply of oxygen and glucose. In the brain and retina, “neuro-vascular units” (NVUs) provide direct and fast control of local blood flow. Activation of smooth muscle cells and pericytes, mediated by brain astrocytes and retinal glial cells, enable these tissues to accommodate the metabolic demand. Type V fibrocytes resemble astrocytes and glial cells. The fibrocytes are in morphological association with pre-capillaries of the spiral ligament through “end-feet” structures similar to astrocyte/pericyte junctions in NVUs (See Figure 5) (Dai et al., 2011). Fibrocyte activation significantly affects capillary diameter and blood flow velocity by initiating COX-1 activity and release of several vasoactive metabolites of arachidonic acid (Dai et al., 2011). The mechanism is analogous to the NVU for regulation of blood flow in brain.

2.4 CoBF regulation by local metabolites

Multiple metabolic factors, including ATP, NO, lactate, PGE and K^+, are involved in local blood perfusion.

2.4.1 Nitric oxide (NO)—NO is a potent vasodilator and regulator of vascular tone, and thus is an agent controlling organ blood flow (Brechtelsbauer et al., 1994; Feletou et al., 1996; Nelson et al., 1995). Nitric oxide synthase (NOS) has been found in a variety of

cochlear cell types in several animal models including the mouse and guinea pig (Chen et al., 2005b; Konishi et al., 1998; Ruan, 2002; Shi et al., 2002). Direct NO production is found in cochlear vascular and smooth muscle cells (Chen et al., 2005b; Ruan, 2002; Shi et al., 2002; Shi et al., 2001). The NO causes smooth muscle and pericyte relaxation by activating cGMP and affecting its downstream target, protein kinase G (PKG) (Haefliger et al., 1994; Tian et al., 1999). NO also directly inhibits voltage-gated calcium channels, to the effect of relaxing smooth muscle cells (Sakagami et al., 2001), and activates ATP-sensitive K^+ channels in endothelial and smooth muscle cells of the spiral modiolar artery, causing hyperpolarization and smooth muscle relaxation (Jiang et al., 2004; Si et al., 2002). Through one of these several pathways, pharmacological intervention of NO production offers a viable strategy for modulating regional blood flow in the cochlea.

2.4.2 Prostaglandin—Prostaglandin E (PGE), a major arachidonic acid metabolite in a wide variety of tissues, has a complex and diverse pathophysiology in blood flow regulation (Yang, 2007). PGE signaling, mediated by four distinct E-prostanoid receptors (EP1–4), has been demonstrated in the stria vascularis, spiral ligament, and organ of Corti (Nakagawa, 2011). The EPs also have significant roles in blood flow regulation in other tissues (Gordon et al., 2007). EP2 and EP4 have been shown to mediate vasodilatation in several organs, with EP1 and EP3 shown to mediate vasoconstriction (Legler et al., 2010; Nakagawa, 2011). CoBF in animals, measured by laser Doppler anenometry, was increased by local administration of prostaglandin E1 (PGE1) (Sone et al., 2003; Tominaga et al., 2006). PGE2 also induces a dose-dependent increase in inner ear blood flow (Rhee et al., 1999; Umemura et al., 1997). The prostaglandins are generally shown to enhance autoregulation of the inner ear vessels (Nagahara et al., 1988). Indeed, PGE has been used to treat idiopathic sudden sensorineural hearing loss (Nishimura et al., 2002). However, further studies are needed to delineate the distinct regulatory roles of PGE signaling on CoBF.

2.4.3 Adenosine 5'-triphosphate (ATP)—ATP also plays a role in CoBF regulation. Extracellular ATP applied to vessels has been shown to produce a dose-dependent increase in CoBF (Munoz et al., 1999). ATP transiently increases intracellular Ca^{2+} in ECs. 1 mM ATP caused a 10% dilation in spiral ligament capillaries *in vivo* (Wu et al., 2010). The ATP-induced effect on CoBF involves P2X- and P2Y-subtype purinoceptors (Ren et al., 1997; Takago et al., 2001). Inhibition of P2X4 receptor significantly blocks ATP-induced vessel dilation (Wu et al., 2010). Humoral adenosine 5'-triphosphate (ATP), adenosine, and uridine 5'-triphosphate (UTP) have also been shown to have a role in controlling local blood flow in the stria vascularis (Munoz et al., 1999). Manipulations of the adenosine signaling system hold significant promise for therapeutic management of dysfunctional CoBF.

2.4.4 Lactate—Lactate, a major by-product of metabolism, is involved in the regulation of local blood flow in many tissues (Gordon et al., 2008; Lombard, 2006; Mendrinos et al., 2008). Cochlear perilymph has a three times higher concentration of lactate than blood and cerebrospinal fluid. This suggests the perilymph lactate is of intracochlear origin (Scheibe et al., 1976) and may rise to effective levels with sound stimulation. Different concentrations of extracellular lactate serve as dynamic signals for pericyte relaxation and contraction, which cause perturbations in intracellular Ca^{2+} by inhibiting the Na^+/Ca^{2+} exchanger in the retinal capillary system (Lombard, 2006). Recently, we found that lactate also has a significant effect on regulation of CoBF. The effect on capillary diameter is mediated by an NO signaled coupling with fibrocytes (Dai et al., 2010). Few experiments have been done on lactate-based regulation of CoBF, and further study is needed, particularly considering the high concentration of lactate in the cochlea. Lactate may be an essential signal in the control of CoBF.

2.4.5 Potassium (K^+)—Elevating the K^+ concentration from 3.6 to 150 mM by superfusion of the spiral modiolar artery *in vitro* caused transient vasoconstriction (Wangemann et al., 1998). Pericytes can also "detect" extracellular K^+ signals (Matsushita et al., 2006). Under both *in vitro* and *in vivo* conditions, we found that an extracellular K^+ concentration of 10 mM induces pericyte contraction (Dai et al., 2009). Endolymphatic K^+ recycling through sensory hair cells and non-sensory supporting cells has recently been shown to have an important role in maintaining normal hearing function (Fujimura et al., 2005; Marcus et al., 2002; Mistrik et al., 2009; Rickheit et al., 2008; Wangemann, 2002a). Although cochlear blood flow is anatomically distant from sensory hair cells, the cells are morphologically coupled to intermediate cells and fibrocytes by gap junctions (Ando et al., 1998; Takeuchi et al., 2001). K^+ movement through gap junctions between hair cells and lateral wall supporting cells could produce a variable K^+ concentration in the interstrial space. K^+ cycling through the cochlear lateral wall may be regulate pericyte function. The outward ERG channel found in intermediate cells (Nie et al., 2005) is consistent with this regulation. The channel produces a marked K^+ extrusion into the interstitial fluid which affects extracellular K^+ concentration (Nie et al., 2005). Retinal pericytes, hyperpolarized or depolarized depending on the concentration of extracellular K^+, cause pericyte relaxation or contraction (Cao et al., 2006; Matsushita et al., 2006; Quignard et al., 2003). Whether cochlear K^+ recycling regulates pericyte function to control capillary diameter has not been determined.

3. Blood-labyrinth barrier in the stria vascularis

3.1. The physical structure of the blood-labyrinth barrier

The capillary bed in the stria vascularis is essential for solute homeostasis and preventing the influx of toxic substances into the inner ear (Juhn et al., 1981; Juhn et al., 2001). In the classic view, the BLB is composed of endothelial cells and an underlying basement membrane (Sakagami et al., 1982; Sakagami et al., 1987). Endothelial cells connect to each other by tight junctions (Sakagami et al., 1982; Takeuchi et al., 2001) and form a diffusion barrier which selectively excludes most blood-borne substances from entering the ear, protecting it from systemic influences (Juhn, 1988; Juhn et al., 1981). In a recent study, the BLB was discovered to include, in addition to endothelial cells and basement membrane, a large number of pericytes (Shi, 2009; Shi et al., 2008; Takeuchi et al., 2001) and perivascular resident macrophages (Shi, 2010) (Figure 6). The perivascular resident macrophages, with foot processes strikingly rich in mitochondria and vesicles, are highly invested on the abluminal surface of capillaries. They are positive for several macrophage surface molecules, including F4/80, CD68, and CD11b (Shi, 2010). They are also similar to astrocytes in the brain and glial cells in the retina, both of which are known to have an essential role in regulating barrier integrity (Abbott, 2002; Abbott et al., 2006; Cardoso et al., 2010; Prat et al., 2001). In the absence of astrocytes and glial cells, BBB and BRB lose tight junction proteins and become leaky to large molecules (Abbott, 2002; Abbott et al., 2006; Haseloff et al., 2005; Willis, 2011). Presence of perivascular resident macrophages in the BLB may suggest an analogous regulatory mechanism in the cochlea. Pericytes in the BLB are rich in the structural protein desmin which gives mechanical strength to the capillary and enhances general integrity of the network. Blood vessels deficient of pericytes are abnormally large and leaky (Hellstrom et al., 2001). Perivascular resident macrophages and pericytes are new classes of cells in the BLB, and their function is largely uncharacterized.

3.2. Molecular composition of blood-labyrinth barrier

The BLB contains an array of enzymes and transporters, which together maintain the necessary extracellular environment of the cochlear system (Saito et al., 1997; Yang et al.,

2011). Using a mass-spectrometry, shotgun-proteomics approach, combined with a novel "sandwich-dissociation" method of isolating capillaries from the stria vascularis, more than 600 strial capillary proteins have been identified (Yang et al., 2011) (Figure 7). Strikingly, a high number of identified proteins are involved in metabolism and transport. For example, the most abundant protein identified in the blood-labyrinth barrier is the ion transporter subunit, Na^+/K^+-ATPase α1. In addition, a large number of proteins are metabolic enzymes, including glutathione S-transferase (GST), prosaposin, leukotriene A4 hydrolase, and glutamate oxaloacetate transaminase. Prosaposin, synthesized and secreted by the stria vascularis, is pivotal to maintaining homeostasis in the auditory system (Terashita et al., 2007). LTB4 is a vasoconstrictor which can cause hearing loss by down-regulating cochlear blood flow (Rhee et al., 1999). The large number of transporters and metabolic enzymes in the blood-labyrinth barrier is indicative of a high level of energy and transport activity. Moreover, stria vascularis capillaries are rich in tight junction and cell adhesion proteins, which is consistent with blood-labyrinth barrier function.

3.3. Regulation of blood-labyrinth barrier permeability

The inner ear has an endothelial blood-tissue barrier in the stria vascularis that is as tight as the blood brain barrier. However, the mechanisms that control stria vascularis endothelial blood-barrier permeability remain largely unknown. Information on regulation of the BLB is sparse. Early studies showed that BLB permeability is more robust in developing rat cochlea (Suzuki et al., 1998). Recently, protein kinase C eta (PKCη) was found to regulate barrier permeability by directly interacting with Na^+/K^+-ATPase α1 and mediating tight junction protein status, for example, by affecting the phosphorylation status of occludin.

Highly regulated transport of ions in and out of the BLB maintains the fluid composition in the inner ear essential for auditory function. A better understanding of how the BLB is regulated would enable development of therapies for restoring the barrier in BLB related hearing disorders.

4. Hearing loss associated with impaired microvasculature

4.1. Noise-induced hearing loss

The cause of noise-induced hearing loss remains unclear, despite years of investigation. Insufficient blood supply is one mechanism which accounts for temporary or permanent noise-induced threshold shifts. For example, several histological and physiological studies have demonstrated signs of reduced circulation (vessel contraction and cochlear hypoxia) and inflammation, including leukocyte infiltration and up-regulation of adhesive molecules, in the cochlea after noise exposure (Hillerdal et al., 1987; Lamm et al., 1999; Quirk et al., 1992; Scheibe et al., 1993; Seidman et al., 1999b; Shi et al., 2007; Yamane et al., 1991). A recent study by Arpornchayanon et al. (2011) shows that noise exposure reduces red blood cell velocity compared to stable control measurements. In addition to noise-induced disruption of endothelial cells, noise also causes upregulation of vascular endothelial growth factor (VEGF), a potent inducer of vascular breakdown (Nag et al., 2011; Selivanova et al., 2007). Furthermore, noise exposure causes down-regulation of COX enzymes (Heinrich et al., 2010; Heinrich et al., 2006), which can decrease endogenous PGE_2 (a vasodilator) levels in the cochlea. Down-regulation of PGE_2, particularly EP2 and EP4, could be the cause of noise induced cochlear ischemia. In addition, structural and molecular changes in the cochlear endothelium are involved in the BLB breakdown from noise-exposure. We have observed that pericytes, hypothesized to provide structural support in the BLB, lose their tight association with endothelial cells following loud sound damage (Shi, 2009) (Figure 8).

4.2. Endolymphatic hydrops

Endolymphatic hydrops is a condition in which too much endolymph is present (Pirodda et al., 2010; Semaan et al., 2010). Meniere's disease is characterized by fluctuating hearing loss, episodic vertigo, and tinnitus, and by endolymphatic hydrops found on examination post-mortem (Semaan et al., 2005). The cause of Meniere's disease remains unclear. Numerous factors play a role in the development of hydrops and in the pathogenesis of related cochleovestibular dysfunction. However, the evidence from research on animal models suggests the pathophysiology in Meniere's disease is closely associated with dysfunctional CoBF. For example, Miller and his co-worker demonstrated that the magnitude of evoked CoBF response was reduced by approximately one third in hydropic ears compared to normal ears (Miller et al., 1995b; Vass et al., 1995; Yazawa et al., 1998). Brechtelsbauer et al. (Brechtelsbauer et al., 1995) reported reduced autoregulation of CoBF in guinea pigs with endolymphatic hydrops. Significantly higher levels of plasma norepinephrine and vasopressin have been reported in patients with Meniere's disease (Juhn et al., 1999). However, the evidence is not consistent. Others have not found endolymphatic hydrops associated with blood flow. For example, Larsen et al. (Larsen et al., 1988) found no change in regional or total cochlear blood flow in hydropic ears. CoBF measurement in patients with Ménière's disease and control groups showed no statistically significant difference with respect to CoBF amplitudes (Selmani et al., 2001). Resolving the issue of whether microcirculation and ear hydrops are correlated needs to wait on development of better means to measure blood flow in the cochlea.

4.3. Presbycusis

Age-related hearing loss is the major form of hearing loss and the predominant neurodegenerative disease of aging (Frisina, 2009; Lang et al., 2010; Ohlemiller et al., 2008; Ohlemiller et al., 2010; Schacht et al., 2005). Insufficient CoBF and decline in the endocochlear potential (EP) (strial presbycusis) are considered responsible for hair cell damage and hearing loss in the elderly (Gacek, 1969; Harkins, 1981; Seidman et al., 1999b). For example, Prazma et al. (Prazma et al., 1990) reported that CoBF in old gerbils was less than in young animals. Gratton et al (Gratton et al., 1996b) reported that age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. Brown et al. (Brown et al., 1995) found that cochlear vascular reactivity to topical application of nitroprusside, a vasodilating agent, was less in old mice than in young mice. Suzuki et al. (Suzuki et al., 1998) demonstrated that autoregulation was significantly reduced in the aged group. Using a microsphere technique to quantify blood flow, they found diminished flow in morphologically normal-appearing basal turn capillaries. Changes in whole blood viscosity and red-cell rigidity have also been correlated with high-frequency hearing loss in elderly human subjects (Gatehouse et al., 1990). Furthermore, in a series of *in vivo* experiments using intravital microscopy of the cochlear microvasculature, Seidman et al. (Seidman et al., 1996) demonstrated age-dependent, statistically significant reductions in mean red blood cell velocity accompanied by increases in capillary permeability. Increased immunoglobulin and laminin deposits were observed in thickened basement membranes of aged strial capillaries (Sakaguchi et al., 1997a; Sakaguchi et al., 1997b). In humans, an age-related, gradual loss of capillaries in the spiral ligament of the scala vestibuli was observed. For example, in a human temporal bone study, presbycusis patients showed loss of hair cells and neurons and atrophy of the stria vascularis (Sprinzl et al., 2010).

However, the literature is also inconsistent. Hillerdal and co-workers (Hillerdal et al., 1987) reported no difference in CoBF in young and aged normotensive rats. The conflicting results may reflect a difference in the species studied or age at which animals were selected for investigation. The association of age-related pathological changes with disturbance of CoBF

is not clear at present. A better understanding of cochlear homeostasis requires a way to measure CoBF in humans, as animal models currently provide our only means to study CoBF.

4.4. Sudden deafness

The pathogenesis of idiopathic hearing loss remains unknown, but vascular involvement is one hypothesis (Mosnier et al., 2011). Observational clinical studies have shown that patients with sudden idiopathic sensorineural hearing loss often present with systemic arterial hypertension, *diabetes mellitus, dyslipidemias,* alone or associated with systemic sclerosis, and thromboembolic risk (Nagaoka et al., 2010). The sudden deafness patient often presents with high precontrast signals in the inner-ear fluid space and an increased concentration of protein passing through blood vessels, indicating a breakdown of the blood-labyrinth barrier (Sone et al., 2009; Sugiura et al., 2006; Yoshida et al., 2008).

4.5. Genetic Hearing loss

The endocochlear potential (EP) is essential to hearing, because it provides approximately half of the driving force for the mechanoelectrical transduction current in auditory hair cells (Salt et al., 1987; Smith et al., 1954; Tasaki et al., 1958; Tawackoli et al., 2001; Wangemann, 2002a). The EP is produced in the stria vascularis (SV) (Ferrary et al., 1998; Marcus et al., 1983; Offner et al., 1987; Salt, 2001; Salt et al., 1987; Tran Ba Huy et al., 1986; Wangemann, 2002a; Wangemann, 2002b). Disruption of the endothelial barrier in the stria vascularis leads to loss of EP in genetic hearing loss (Cohen-Salmon et al., 2007). For example, connexin30 deficiency results in severe congenital hearing impairment with disruption of the BLB (Cohen-Salmon et al., 2007). In addition, hearing loss resulting from Nr3b2(−/−) mutation is associated with reduction of the density of the cochlear strial capillaries (Chen et al., 2007).

5. Measurement of CoBF

Direct measurement of CBF is difficult and techniques for assessing blood flow are still under development. Various techniques are used for evaluation of cochlear blood flow, including laser-doppler anemometry (LDA), magnetic resonance imaging (MRI), fluorescence intravital microscopy (FIVM), microendoscopy (FME), as well as approaches based on injection of radioactive or labeled microspheres into the boodstream. Here, I discuss two recent methods for measurement of cochlear blood flow.

Fluorescence microendoscopy (FME)

The fluorescence microendoscope, consisting of a flexible imaging fiber, coupled to a system for detection of fluorescence, enables study of cochlear blood flow on a scale of microns. Blood flow velocity is determined by analysis of video sequences (Monfared et al., 2006). The small size of the instrument makes it versatile and suitable for relatively non-invasive imaging of regional blood flow. In 2006, Monfared et al. observed single red blood cells passing through individual capillaries in several cochlear structures, including through the round window membrane, spiral ligament, osseous spiral lamina, and basilar membrane. They determined blood flow velocity using this technique by analyzing the acquired video sequences. Fluorescence microendoscopy has several advantages: (1) The endoscope probe can be placed at the round window without disturbing the membrane; (2) The vasculature of the round window membrane itself, as well as the most proximal portion of the osseous spiral lamina and basilar membrane, can be imaged; (3) With resection of the round window membrane, the endoscope probe can easily be introduced into the scala tympani to image blood flow in the osseous spiral lamina, spiral ligament, and basilar membrane. Disadvantages include disruption of the delicate homeostatic balance in the cochlea.

Optical microangiography (OMAG)

Optical microangiography (OMAG) is a recently developed technique which enables the imaging of circulation patterns at capillary level resolution in tissue beds up to 2 mm thick (Wang, 2008a; Wang, 2008b). Endogenous light scattering from moving blood cells provides the image contrast, and no exogenous contrast agents are necessary. The technique is sensitive enough to image very slow blood flow velocities, such as those found in capillary networks of the cochlea, without opening a window in the cochlear lateral wall. Volumetric reconstruction of microvascular flow in the cochlea with this technique has been demonstrated (see Figure 9). The collection to the left displays OMAG images of the otic capsule, stria vascularis of the apical (SVa) and middle (SVm) turns, and radiating arterioles that emanate from the modiolus (M) in an intact cochlea (Choudhury et al., 2009). Further improvements to the resolution of the OMAG imaging system, for example with a higher numerical aperture (NA) objective, would enable visualization of individual capillaries in the stria vascularis, as well as measurement of blood flow in the intact cochlea of living animals, without need of compromising the lateral wall.

Conclusion

Normal blood supply to the cochlea and BLB are essential for sustaining endocochlear potential, ion transport, and endolymphatic fluid balance, and for preventing toxic substances from entering the cochlea. Many of these functions are well-documented in previous reviews (Axelsson, 1988; Axelsson et al., 1987; Kimura, 1986; Lawrence, 1980; Miller et al., 1988; Miller et al., 1995a; Nakashima et al., 2003; Nuttall, 1988; Seidman et al., 1999b; Sillman et al., 1989; Wangemann, 2002b). This review has focused on regulation of blood flow in the microvasculature, as dysfunction of cochlear blood flow and disruption of the cochlear BLB are shown to result in hearing impairment in animal models. Recent research has shown breakthroughs in explaining some of the underlying mechanisms. Progress in understanding the cellular and molecular structure of the blood-labyrinth barrier is accelerating, and new experimental methods are providing opportunities to study the physiology of the inner ear microvasculature more deeply. Future directions for cochlear microcirculation research include (i) developing novel CoBF measurement tools for diagnosis of vascular dysfunction related hearing loss; (ii) investigating the role of the blood-labyrinth-barrier in generating the endolymphatic potential; (iii) investigating the role of CoBF in cochlear homeostasis; and (iv) defining the pathological mechanisms in clinical diseases which involve flow dysregulation and barrier disruption.

> Cochlear microcirculation is essential for normal hearing function. > A better understanding of cochlear microcirculation will benefit clinic treatment. > Progress in this field is accelerating due to new methods and technologies. > This review focuses on recent discoveries on cochlear microcirculation.

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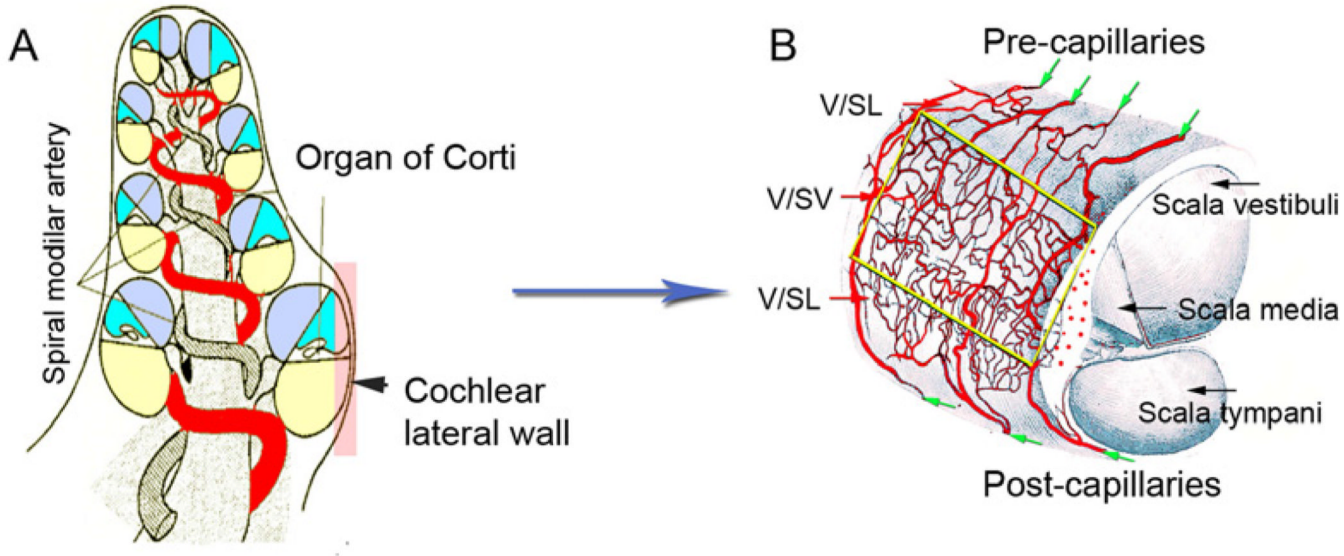

Figure 1. Schematic view of CoBF supply

A, The SMA, a major artery, supplies blood to the cochlea image from (Axelsson, 1968)]. **B,** A characterization of the vascular pattern on the outer wall of the cochlea is shown. Radiating arterioles arching over the roof of the scala vestibuli run in bony channels, branching as they emerge from the upper margin of the spiral ligament. Two distinct capillary networks in the spiral ligament and stria vascularis are apparent in the lateral wall. The networks parallel each other without cross connections [image adapted from (Mudry et al., 2009)]. **V/SL**: vessels of the spiral ligament; **V/SV**: vessels of the stria vascularis.

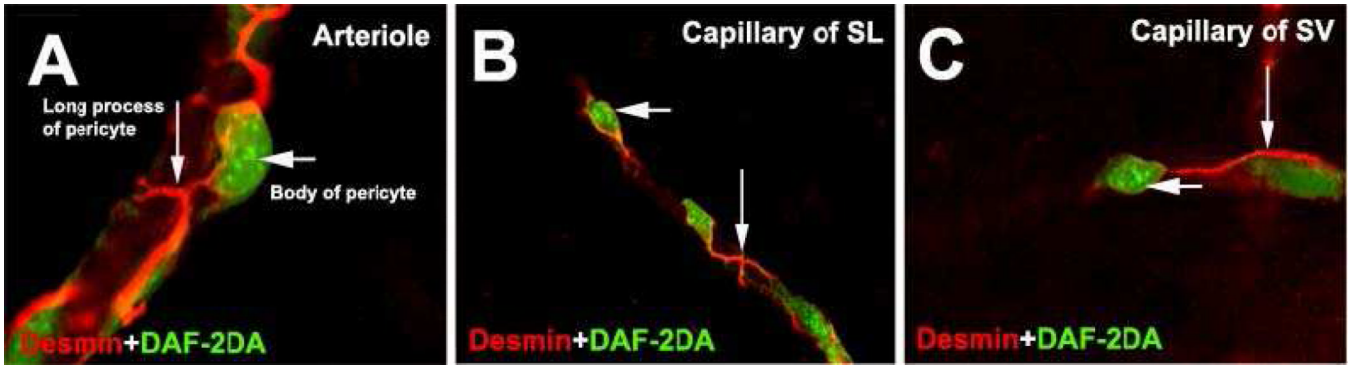

Figure 2. Cochlear pericytes on cochlear microvessels in adult guinea pig

Pericytes are idenified with double-staining for desmin (red), a pericyte marker protein, and nitric oxide (DAF-2DA, green). A: an arteriole; B: a capillary of the spiral ligament (SL); **C:** a capillary of the stria vascularis (SV). Pericytes have a body (short arrows) and many primary processes (long arrows) which tightly embrace the endothelial tube. Pericytes on the outer wall of vessels have a characteristic “bump on a log” shape.

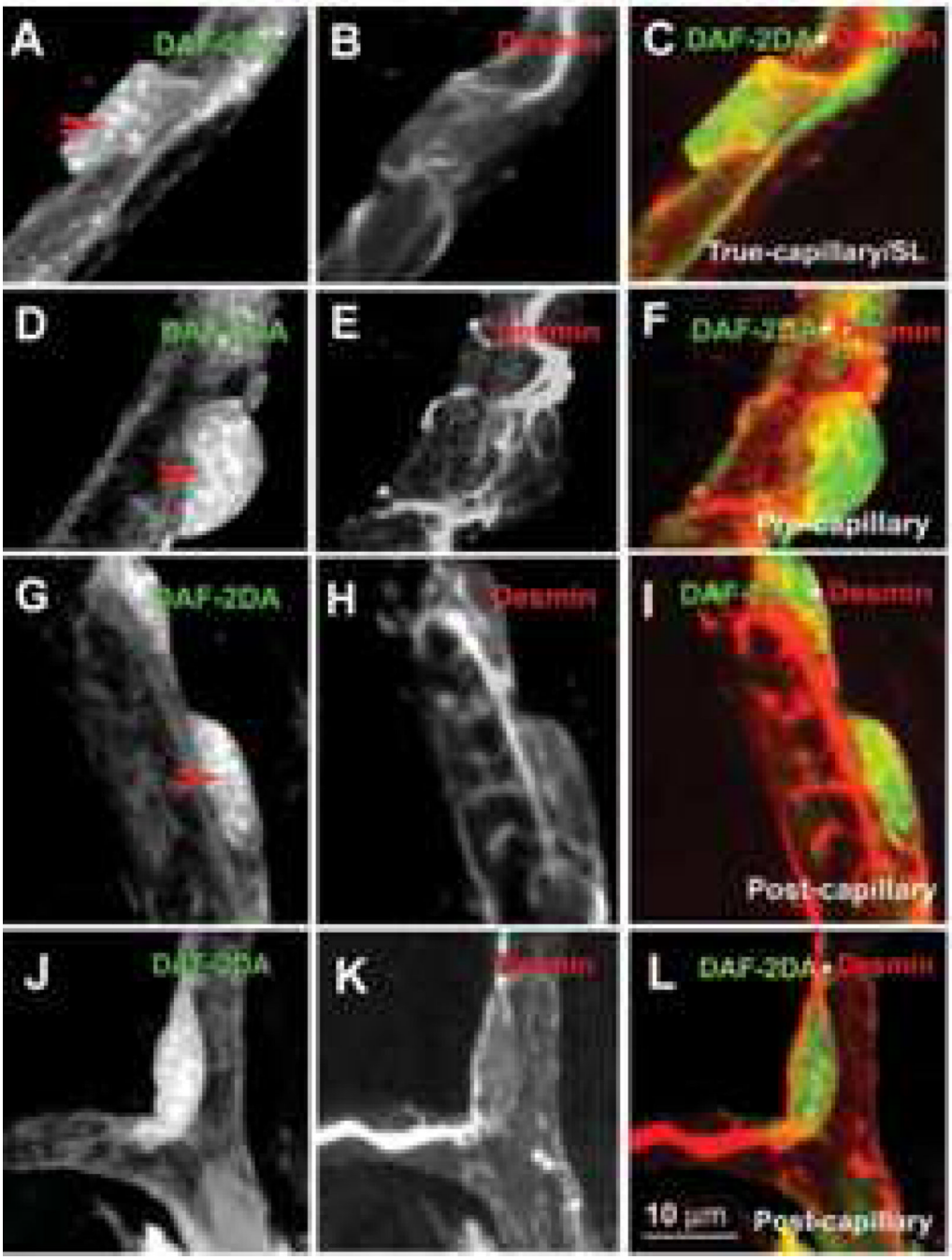

Figure 3. Shapes of pericytes on different cochlear microvessels

The pericytes were double-labeled with a pericyte marker protein: desmin (red), combined with fluorescent indicator for intracellular nitric oxide DAF-2DA (green). Panels A–C show the morphology of a pericyte on a true capillary. The pericyte has a polygonal-shaped cell body (Panel A, 10 sections; interval: 1 μm), relatively few long longitudinal processes, and short, fine circumferential projections (Panel B, 10 sections; interval: 1 μm). Panel C is a merged image of Panels A and B. Panels D–F show the morphology of a pericyte on a precapillary. The pericyte has a “bump-shaped” soma (Panel D, 11 sections; interval: 1 μm) and relatively large processes that encircle the capillary (Panel E, 10 sections; interval: 1 μm). Panel F is a merged image of Panels D and E. Panels G–I show the morphology of a

pericyte on a postcapillary. These pericytes have a flattened cell body (Panel G, 11 sections; interval: 1 μm) and short processes encircling the vessel (Panel H, 11 sections; interval: 1 μm). Panel I is a merged image of Panels G and H. Panels J–L show the morphology of a pericyte on a branch point of the postcapillary. The pericyte has a spindle-shaped cell body (Panel J, 10 sections; interval: 1 μm) and long processes distributed over the two branches (Panel K, 10 sections; interval: 1 μm). Panel L is a merged image of Panels J and K

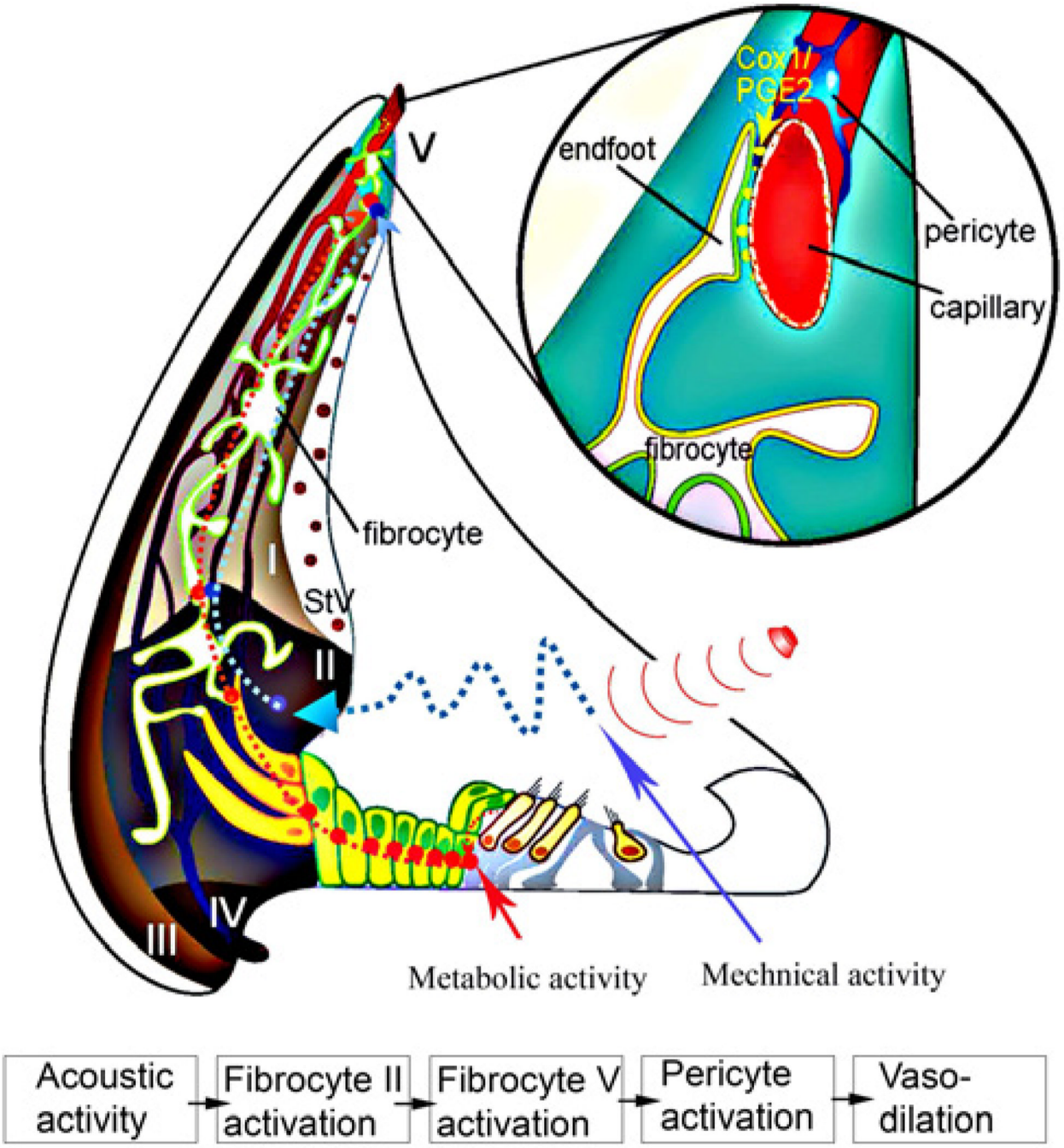

Figure 4. Morphological details of fibro-vascular coupling is shown in confocal and TEM images

(*A*) Type V fibrocytes positive for S100 (green) abut capillary walls labeled by isolectin IB4 (blue). (*B*) Type V fibrocytes are positive for Na^+/K^+ ATPase β1 (red). (*C*) Type V fibrocytes also contain high levels of NO, as detected with the intracellular NO indicator, DAF-2DA (gray). (*D*) Magnification of panel B shows foot processes in contact with a capillary. (*E*) A multiple-foot process of a fibrocyte abuts capillary wall. (*F*) A high magnification image shows a fibrocyte end-foot structure at the soma of a pericyte. The soma of pericytes were labeled by an antibody for NG2, (red), and processes were labeled with an antibody for the structural protein, desmin (blue). Capillary walls are labeled by phalloidin (green). (*G*) and (*H*) Fibrocytes contact capillaries with enlarged endings. (*I*) The

endings display electron-dense membrane regions rich in mitochondria. Abbreviations: FC, fibrocyte; EC, endothelial cells; PC, pericyte; Mt, mitochondria. Calibration bars in *H* and *I* are 500 nm.

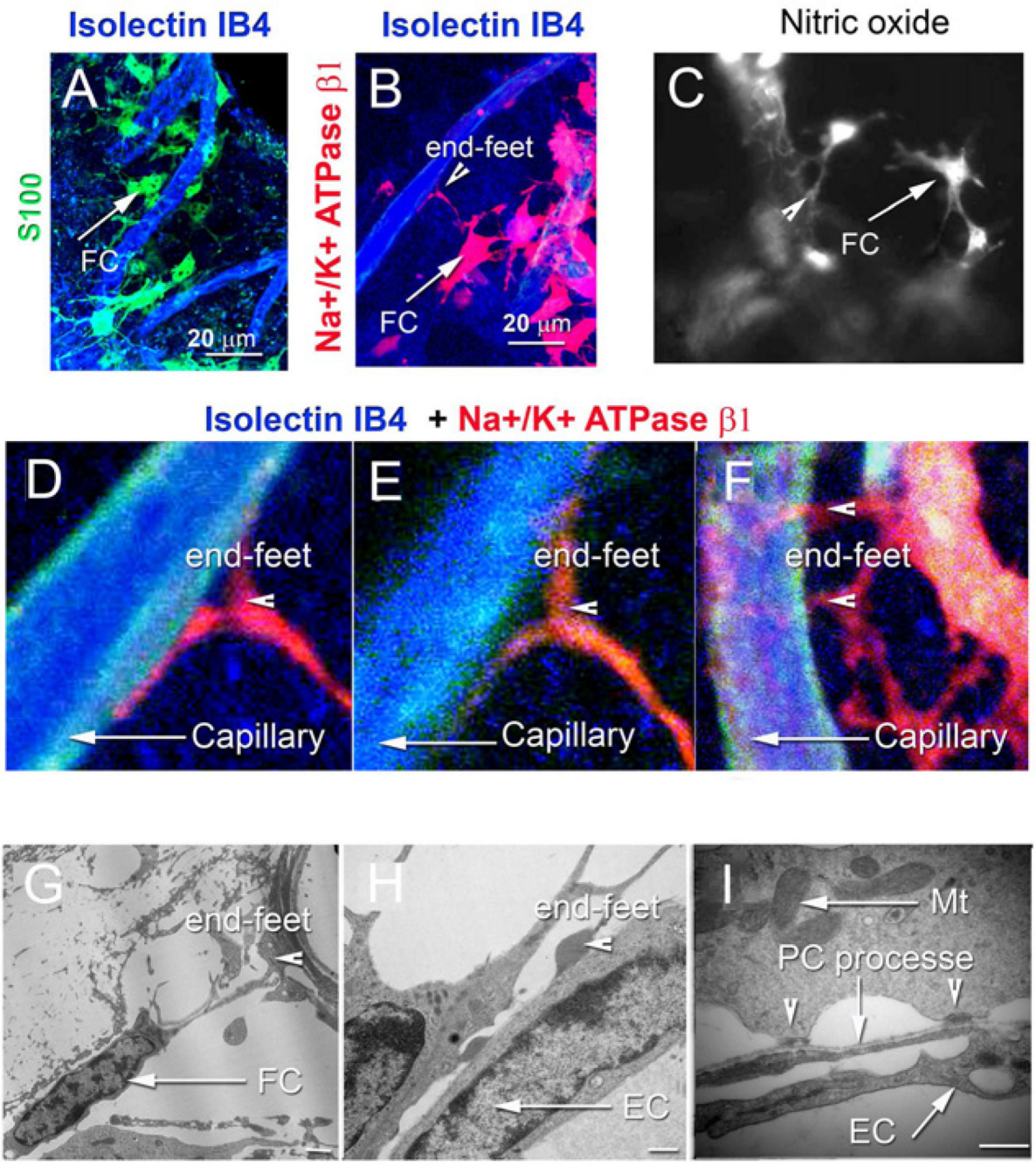

Figure 5. A working model of fibro-vascular coupled signaling in the inner ear

Cochlear blood flow is anatomically distant from sensory hair cells, but the cells are morphologically coupled to supporting and fibrocytes by gap junctions. Mechanical activity (red line) or metabolic activity (red dotted line) increases COX-1 enzymatic activity in type V fibrocytes, but the exact pathway is unknown. Activation of COX-1 may result in conversion of arachidonic acid into metabolic intermediates such as PGE2. The PGE2 diffuses into the perivascular space and elicits vasodilatation through the mediation of fibrocyte-coupled pericyte activity.

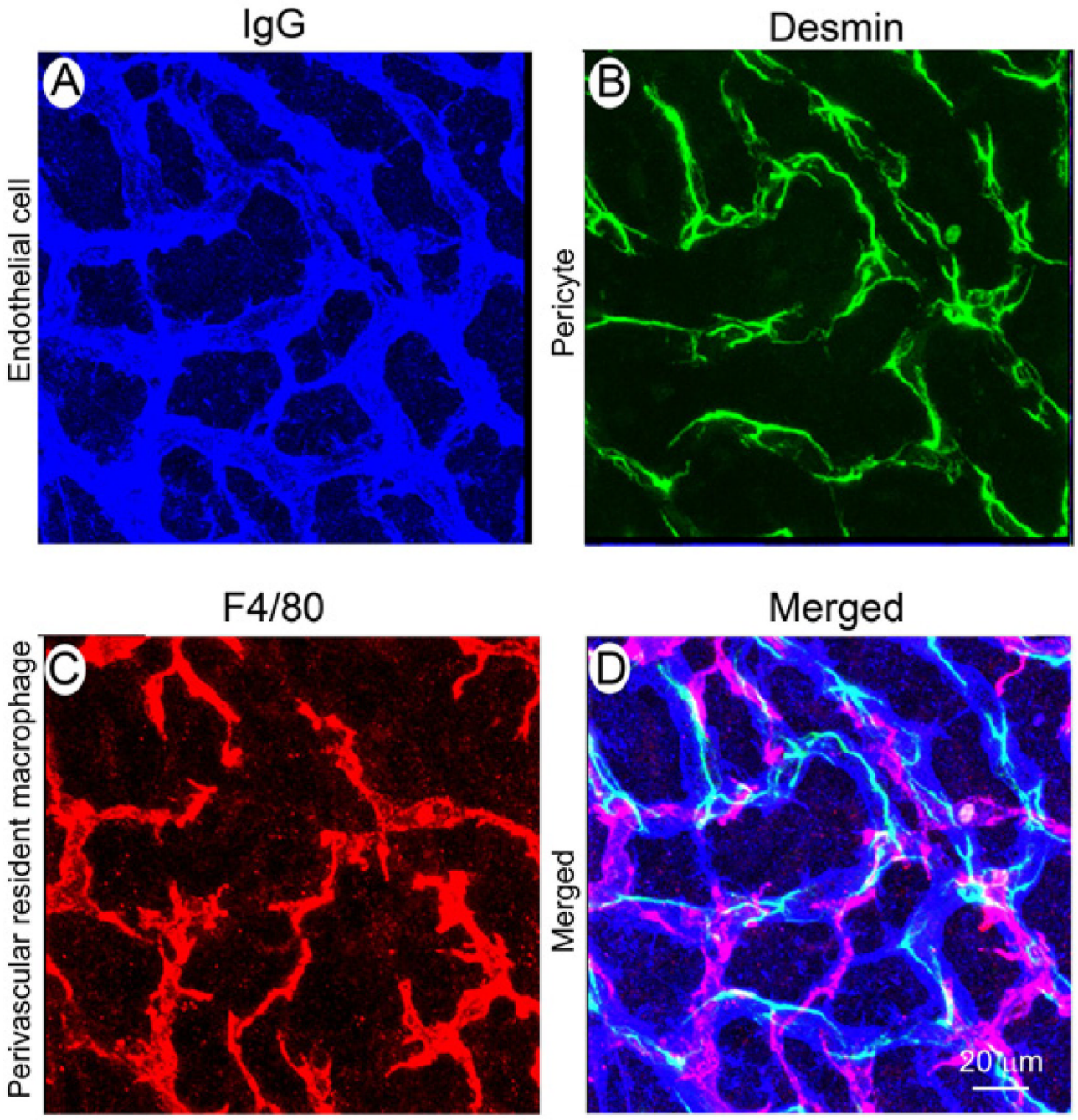

Figure 6. Cellular structure of the blood-labyrinth barrier

Endothelial cells in normal BLB are identified with an antibody for mouse endothelial IgG (A, blue), pericytes with an antibody for desmin (B, green), and macrophages with an antibody for F4/80 (C, red). The merged image (D) shows the complexity of the blood-labyrinth-barrier.

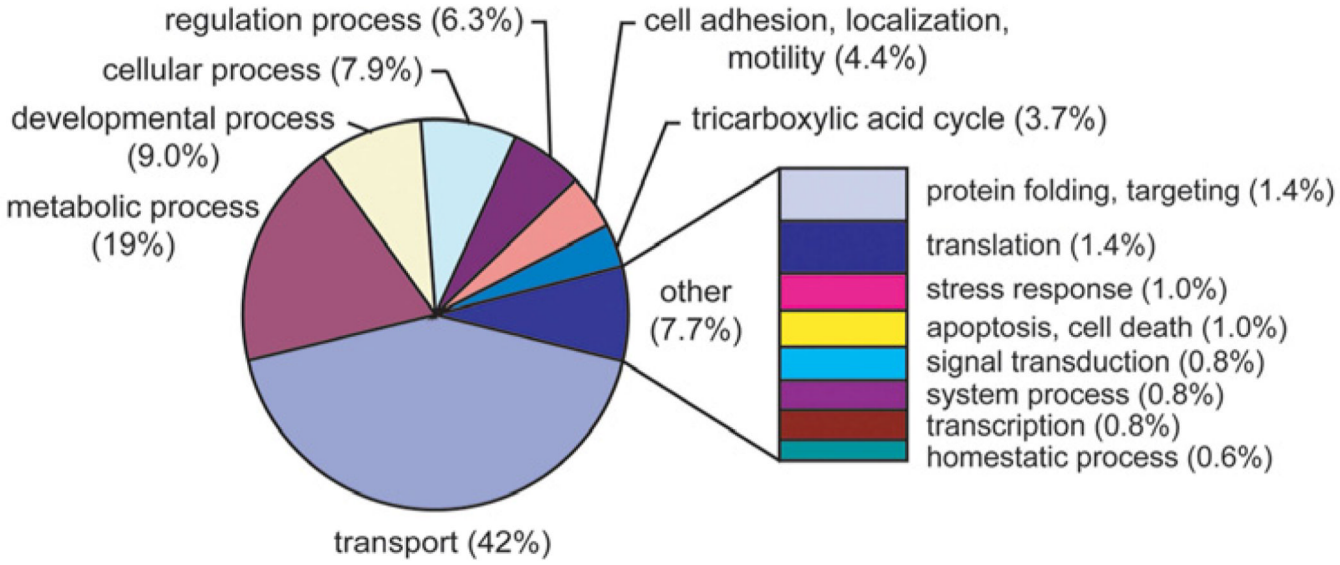

Figure 7. Noise induces breakdown of the blood-labyrinth-barrier and causes irregularities in pericyte coverage

A & C, Serum protein IgG is confined to blood plasma (IgG/arrow) in vessels of the stria vascularis in normal mice (A) and guinea pigs (C). B and D, Serum protein IgG leaks from vessels (arrow/IgG) in noise-exposed mice (B) and guinea pigs (D). Arrowheads indicate sites of vascular leakage. GP: guinea pig. Pericytes containing desmin filaments are evenly distributed on the vessel walls of the stria vascularis in both guinea pigs (E) and mice (F). Pericytes are labeled with an antibody for desmin (green), and vessels with an antibody for isolectin IB4 (red). G and H: Confocal fluorescent images from noise-exposed guinea pigs and mice show abnormal pericyte morphology and increased pericyte coverage. Arrows point to irregular pericyte foot processes turning away from the vessel wall (G) and detached from it (H). I and J: Drawings illustrate the pattern of pericyte distribution on vessel walls in normal and noised-exposed animals. V/SV, vessel of the stria vascularis; NE, noise exposure; GP, guinea pig; MS, mouse.

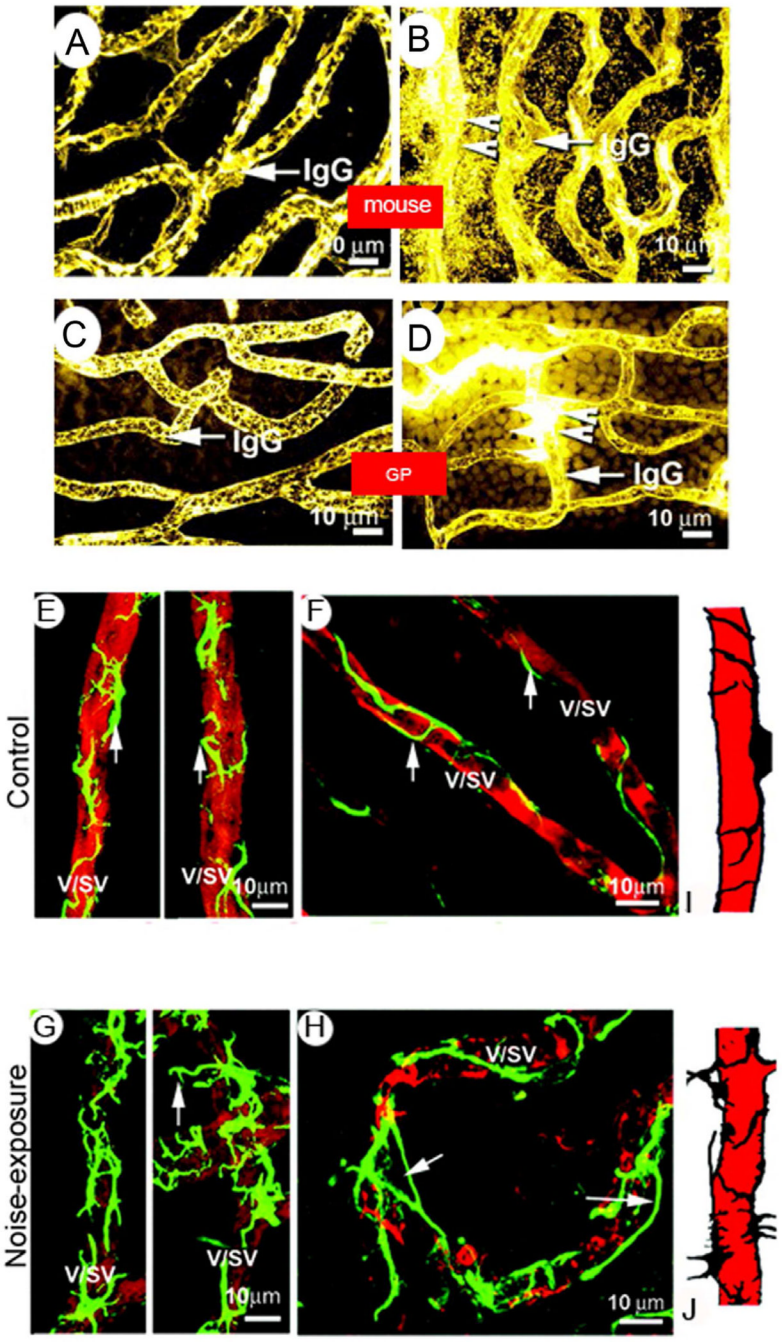

Figure 8. Classification of isolated stria vascularis capillary proteins identified ATP1A1 as the most abundant protein in the blood-labyrinth barrier

The pie graph shows a spectral count-weighted tabulation of the GO annotation by biological process. Proteins involved in transport (42%) and metabolism (19%) are highly expressed in the blood-labyrinth barrier

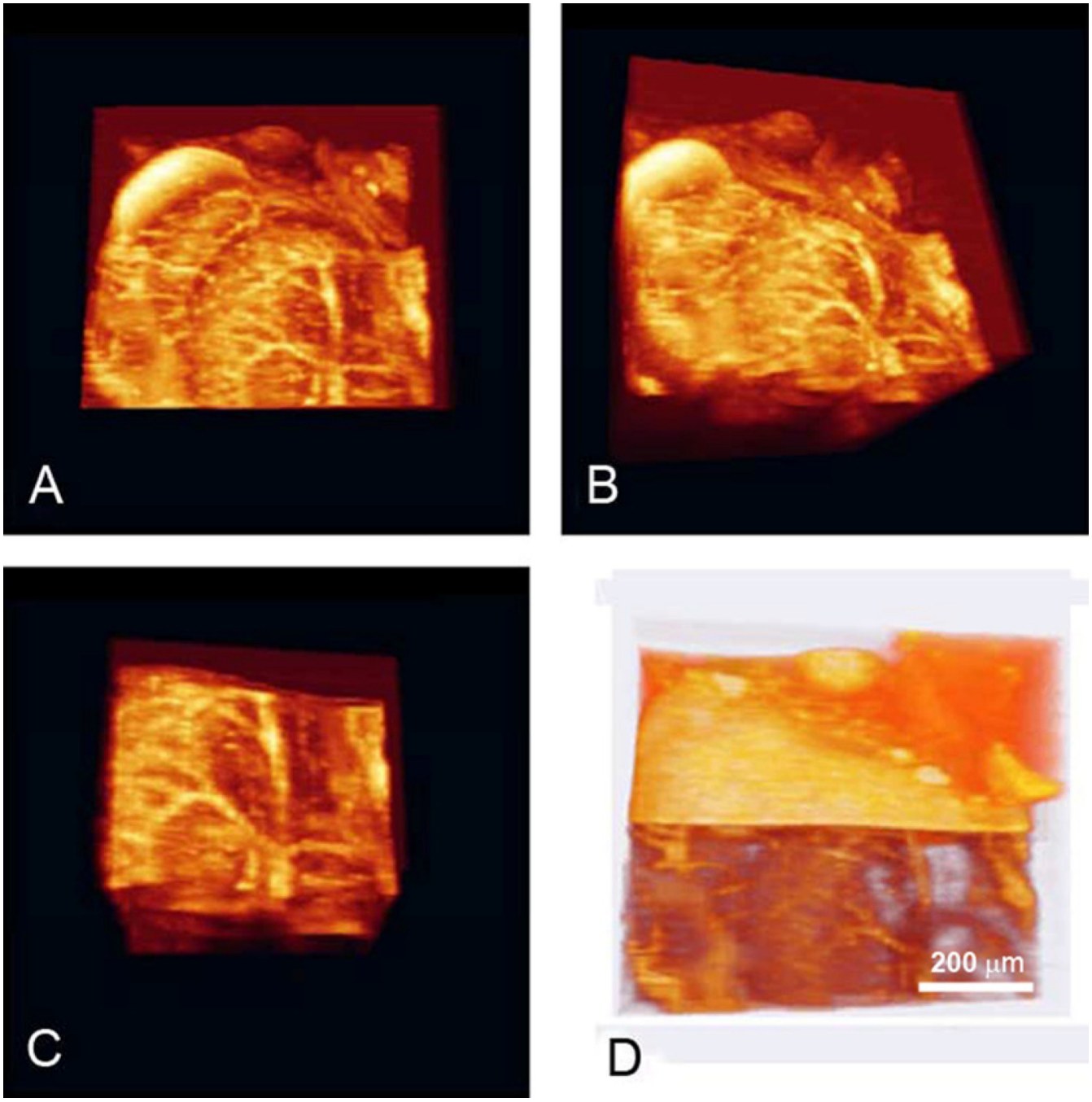

Figure 9. The 3D volume rendering of mouse cochlea is segmented and displayed in four different orientations to provide a detailed view of the cochlear microvasculature

A & B show a 3D volumetric perfusion image of the entire cochlea (Media3 & Media4). C is a segmented 3D volumetric microvascular perfusion at the Modiolus (Media5), and D a 3D volumetric reconstruction of the microvascular perfusion together with cochlear structures (Media6).