Cochlear anatomy, function and pathology III

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Aims and objectives of this lecture

• Focus (3) on the cochlear lateral wall and Reissner’s membrane:
  – The spiral ligament
  – The *stria vascularis*
  – The endolymphatic potential and potassium recycling
  – Reissner’s membrane
Homeostatic mechanisms generate a battery called the endocochlear (endolymphatic) potential which drives the sensitive transduction process.
Fluid segregation

• The three chambers contain different fluids
• Endolymph, high in potassium, in *scala media*
• Perilymph, high in sodium, in *scala vestibuli* and *scala tympani*
Transduction by inner hair cells

- The driving voltage of endocochlear potential and cell membrane potential rapidly depolarises the cell which produces neurotransmitter from the base.
Transduction to motion - the endocochlear potential powers the outer hair cell movement
Vulnerability of BM tuning to furosemide which inhibits generation of the EP

From: Robles and Ruggero, Physiological Reviews 81, No. 3, 2001
Structure of the lateral wall

- Stria vascularis
- Spiral ligament
- Spiral prominence
- Basilar crest
The *stria vascularis*

- Consists of three layers of cells: basal, intermediate and marginal
- Contains numerous blood vessels and melanocytes in pigmented animals
- Expresses proteins involved in potassium recycling such as potassium channels and sodium/potassium ATPases
- Basal and intermediate cells are connected together by gap junctions, and basal cells to adjacent cells of the spiral ligament (fibrocytes) also by gap junctions
Structure of the *stria vascularis*

- Basal cells
- Intermediate cells
- Marginal cells
- Basal cell
- bv
- m
- i
- f
Structure of the *stria vascularis*

- Marginal cells
- Intermediate cells
- Basal cells
- Fibrocytes
The spiral ligament

• The ligament is a much neglected, yet important part of the cochlea

• It consists of extracellular matrix with several cell types in it and has a complex cytoarchitecture.
  – The majority of cell types are called fibrocytes, of which there are five types
  – There are also endothelial cells surrounding blood vessels/capillaries
  – There are also root cells
Fibrocyte types

Type I
Type II
Fibrocytes

Type III
Fibrocytes – five types

Type IV
Fibrocytes – five types

Type V
Sodium/potassium ATPase is localised to membrane processes

- Type II and type V cells possess many processes
- These strongly express the sodium/potassium ATPase and increase the surface area to increase the ability to exchange sodium and potassium
Fibrocytes express a range of proteins - some associated with homeostasis

- Sodium/potassium ATPase
- Aquaporin
- Glutamate transporter
- Potassium channels
- Gap junctions
- Calcium binding proteins
- Connective tissue growth factors
Fibrocyte protein expression is complex

- Each of these proteins has a different distribution
Fibrocyte structural features

- Membranes of all types are thrown into folds, but especially type II and type V which also express the most sodium/potassium ATPase
- Type III fibrocytes have a honeycomb membrane surface and are the only types to express aquaporin
- However, they also possess thick actin cables and are considered ‘tension’ fibrocytes, maintaining BM tension
- Type IV fibrocytes are similar in appearance to type III but lack the ATPase, aquaporin and actin cables
- Type I fibrocytes have few membrane folds and possess some ATPase
Fibrocyte membrane morphology is designed to increase surface area – type II
Type III fibrocytes also have increased surface area and contain actin cables attaching to ecm and the bony wall.
Root cells also contribute to homeostasis

- Root cells connect the outer sulcal cells to the ligament cells.
- They possess potassium recycling proteins and are thought to assist in the recycling of potassium via the epithelial cell gap junction network.

The extracellular matrix of the ligament

- Dominated by type II and type IX collagen but also contains type IV and type V
Ultrastructure of ligament ecm

Organised into thick and thin fibres and plaques of type II collagen connected by type IX collagen fibrils
Ligament and *stria* work together to generate EP and recycle potassium

- Potassium recycling is necessary for maintenance of:
  - The endocochlear potential
  - Transduction by the hair cells
  - Amplification by the OHCs

- Potassium ions follow at least two routes, both ending up passing through fibrocytes and then into the *stria vascularis*

- This is a highly energy-dependent, metabolic process
Connexins

- Connexins play a vital role in the function of the lateral wall
- Connexin 26 and 30 are the dominant types and are localised to both stria vascularis and spiral ligament
Distribution of connexins 26 and 31

From: Jagger and Forge, 2015, Cell and Tissue Research 360, 633-644
Two potassium recycling routes
Possible mechanism of potassium recycling and generation of the endocochlear potential

Fibrocyte culture – one of the few cochlear cell types that can be grown long term *in vitro*

- Fibrocytes are mesenchymal in origin
- This means they retain a capacity to divide, unlike hair cells and spiral ganglion neurones
- A bank of fibrocyte cultures has several uses:
  - Study the electrophysiology and their role in generating the EP
  - Develop stem cell/replacement cell therapy
  - Investigate growth promoting factors to stimulate cell survival and proliferation *in vivo*
- Fibrocytes grown on well plates in 2D look very flat
- They resemble fibroblasts
- They do not have an endogenous fibrocyte phenotype
Fibrocyte cultures grown on hydrogels

- Have a more fibrocytic appearance than when grown flat
- Express characteristic proteins
- Appear to form networks, perhaps with gap junctions between them
Reissner’s membrane – the third border of the *scala media*

Left: Mahendrasingam et al., 2011, JARO; Right Hackney and Furness, Noise and its Pathophysiology (eds Luxon and Prasher, 2007, Wiley)
Reissner’s membrane

• Reissner’s membrane is required to separate endolymph from perilymph and so maintain the endocochlear potential

• Other functional roles are uncertain, but it appears to express epithelial sodium channels and chloride channels

• It is likely to be involved in homeostasis of sodium chloride between the scala media and the scala vestibuli/tympani
Structure of Reissner’s membrane

- Scala vestibuli surface, mesothelial
- Scala media surface, endothelial
- Tight junctions
Summary

- The cochlear lateral wall plays a major role in cochlear function
- The cells present in the outer sulcus, spiral ligament and *stria vascularis* work together to recycle potassium and regenerate the endocochlear potential on a continuous basis
- The EP powers both transduction and amplification to maintain the sensitivity and frequency selectivity of the cochlea