Different rates of endocytic activity and vesicle transport from the apical and synaptic poles of the outer hair cell

Hair cells in the sensory epithelium of the cochlea are vulnerable and do not regenerate spontaneously. Therefore, ideally, the cells must function for the entire lifetime of the mammal. However, there is still little known about the membrane- and protein-recycling machinery of hair cells. The present study investigates endocytosis and vesicle trafficking of outer hair cells using fluorescent membrane markers. Understanding of the underlying mechanisms is a prerequisite for the future design of cell-specific medication for hair-cell rehabilitation or regeneration.

Introduction

Outer hair cells (OHCs)—the electromotile elements of the organ of Corti—are responsible for the high-frequency selectivity as well as low-threshold and broad dynamic range of hearing [2, 6, 20]. In response to a change of transmembrane potential, they produce an electromechanical force of up to at least 50 kHz [10], which acts against frictional forces [8], resulting in the intensity-dependent frequency tuning and amplitude gain of the vibration response of the organ of Corti. The apical pole of the OHC is the site of mechanoelectrical transduction, where hair-bundle deflection causes ion influx accompanied by a change of the transmembrane potential [9]. The basal pole is the place of synaptic communication with nerve fibers from the efferent and afferent systems. The (medial) efferents modulate the electromechanical response of the OHC via calcium-activated changes of the transmembrane potential and cell stiffness [17], which result in a conformation change of the electromotile protein, prestin [35], expressed in high density in the lateral plasma membrane (PM) of the OHC [19], as well as modulation of the electromechanical force produced by the cell. The (type II) afferents are the source of a feedback signal to the soma of the medial efferents in the brainstem [11].

Although cochlear hair cells are functionally vulnerable and not capable of spontaneous regeneration and, hence, required for the entire lifetime of the mammal, there is still little known about the membrane- and protein-recycling machinery driven by endo- and exocytosis. Different types of vesicles have been demonstrated at the apical and basal poles of OHCs [21, 27]. Investigations of the dynamics of membrane recycling using PM fluorescent labeling and confocal microscopy revealed rapid apical endocytic activity and intracellular vesicle trafficking in OHCs [16, 25]. Endocytosis has been proposed to be involved in gentamicin uptake by hair cells and accessory epithelia of the organ of Corti [7]. The development of cell-specific medication to treat sensorineural hearing loss will rely not only on investigations of drug delivery to specific targets within the cochlea [29], but also on an understanding of fundamental cellular internalization processes such as endocytic activity and vesicle trafficking.

Here, we investigate the spatial and temporal properties of endocytic internalization and transport of fluorescent membrane markers applied to OHCs isolated from the guinea pig cochlea. Using a newly developed double-barrel perfusor to apply the markers separately to the basal and apical halves of the OHC [18], we demonstrate greater endocytic uptake in the synaptic region and faster vesicle trafficking in the basoapical direction.

Methods

Cell isolation

Outer hair cells were isolated and handled as previously described [18]. Adult pigmented guinea pigs with a weight of 350–900 g (N = 21) were used for the study. The data derive from 37 cells isolated from 21 cochleae. Animals were anesthetized by intraperitoneal injection of a mixture of 100 mg/kg ketamine and 4 mg/kg xylazine and were killed by cervical dislocation. The dissected temporal bones were placed in an ice-chilled Hanks’ balanced salt solution (HBSS; Biochrom KG, Berlin, Germany), containing: 137 mM NaCl, 5.4 mM KCl, 1.25 mM CaCl₂, 4.2 mM NaHCO₃, 0.81 mM MgSO₄·7H₂O, 0.44 mM KH₂PO₄, 0.34 mM
were 74±7μm.

As the extracellular fluid throughout the cochlea; the pH was 7.3. HBSS was used.

310 mOsm/l adjusted with D-(+)-glucose. The fluorescent fluid-phase marker Lucifer yellow (Lucifer yellow carbocyanide, potassium salt) was used to examine pinoctyosis. Lucifer yellow is a well-known dye for studying fluid-phase endocytosis [15]. The dye is hydrophilic, but being anionic cannot permeate the cell membrane by passive diffusion. The potassium-salt of Lucifer yellow has excitation/emission spectral maxima at 428/536 nm, respectively. A 1-mg/ml stock solution of DiOC₆ was prepared in DMSO. Just before the experiments began, it was diluted with HBSS to a final concentration of 0.87 μM [23].

The fluorescent fluid-phase marker Lucifer yellow (Lucifer yellow carbocyanide, potassium salt) was used to examine pinoctyosis. Lucifer yellow is a well-known dye for studying fluid-phase endocytosis [15]. The dye is hydrophilic, but being anionic cannot permeate the cell membrane by passive diffusion. The potassium-salt of Lucifer yellow has excitation/emission spectral maxima at 428/536 nm, respectively. A 1-mg/ml stock solution of DiOC₆ was prepared in DMSO. Just before the experiments began, it was diluted with HBSS to a final concentration of 0.87 μM [23].

Using a single-barrel perfusor enabled homogeneous labeling of the entire PM; the flow rate was 14μl/min. By contrast, using a double-barrel perfusor enabled labeling of a single pole of the OHC (Fig. 1). The double-barrel perfusor was fabricated from a borosilicate-glass theta capillary with an external diameter of 2 mm (Harvard Apparatus, MA, USA) using a DMZ-Universal Puller (Zeitz Instruments, Augsburg, Germany). To achieve single-pole staining, the perfusor tip was positioned as close as possible to the cell. The coverslip was coated with a cell-and-tissue adhesive (Cell-Tak™) to facilitate cell adhesion.

The output flow rate was 3μl/min per barrel. As recently demonstrated [18], the double-barrel perfusion method is an effective tool for exclusively labeling one pole of bipolar cells such as OHCs.

Fluorescence microscopy

Imaging was performed using a Zeiss LSM 510 confocal laser-scanning system based on a Zeiss Axioskop2 FS mot microscope (Zeiss, Heidelberg, Germany) equipped with a two-photon laser system (Mira™ 900 Ti:Sapphire Laser pumped by a Verdi V5 Diode-Pumped Laser from Coherent, Santa Clara, USA) and a pinhole diameter of 1 AU. A Zeiss 40×IR-Acroplan water-immersion objective with NA 0.8 and ZEN2009 software was used.

The fluorescent membrane markers FM1-43 [N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino)styryl) pyridinium dibromide] and FM4-64 [N-(3-triethylammoniumpropyl)-4-(6-(4-diethylamino)phenyl)hexatrienyl) pyridinium dibromide] were used to visualize endocytosis. FM1-43 is a green-fluorescent, lipophilic styrylpyridinium dye that is commonly used to visualize endocytic and exocytic processes [3], also in cochlear hair cells [15, 16, 18, 23]. Virtually non-fluorescent in aqueous media, the dye rapidly inserts into the PM where it becomes intensely fluorescent; excitation/emission spectral maxima are 473/579 nm, respectively. FM4-64 is a derivative of FM1-43 with similar membrane labeling properties, but exhibits longer wavelength (red) fluorescence making it suitable for double-labeling experiments with lower wavelength dyes [18]; the excitation/emission spectral maxima are 505/725 nm, respectively. Stock solutions of these dyes in a concentration of 10 mM were prepared in DMSO. On the day of the experiment, the dyes were diluted in HBSS to a final concentration of 10 μM.

The fluorescent endoplasmic marker DiOC₆(3,3′-dihexyloxacarbocyanine iodide) was used to examine possible targets of endocytosed vesicles in the endoplasmic reticulum (ER). DiOC₆ is a cell-permeant, green-fluorescent, lipophilic dye, which at low concentrations is selective for mitochondria of live cells and at high concentrations labels other intracellular structures, such as ER [24, 34]. Excitation/emission spectral maxima are 489/506 nm, respectively. For double-labeling experiments with FM4-64 and DiOC₆, OHCs were first incubated in DiOC₆ for 1 min, the DiOC₆ was washed out with extracellular solution, and then the FM4-64 was applied. This FM dye was used instead of FM1-43 because the fluorescence spectra of FM4-64 and DiOC₆ can be readily separated using bandpass filters. A 1-mg/ml stock solution of DiOC₆ was prepared in DMSO. Just before the experiments began, it was diluted with HBSS to a final concentration of 0.87 μM [23].

The fluorescent fluid-phase marker Lucifer yellow (Lucifer yellow carbocyanide, potassium salt) was used to examine pinoctyosis. Lucifer yellow is a well-known dye for studying fluid-phase endocytosis [15]. The dye is hydrophilic, but being anionic cannot permeate the cell membrane by passive diffusion. The potassium-salt of Lucifer yellow has excitation/emission spectral maxima at 428/536 nm, respectively. A 50-mM stock solution of Lucifer yellow was prepared in DMSO. On the day of the experiments, it was diluted with HBSS to a final concentration of 50 μM. This concentration was chosen to be of the same order of magnitude as that of the FM dyes (10 μM).

FM and DiOC₆ were excited with an argon laser with a wavelength of 488 nm and the emitted light was collected with bandpass filters: 505–750 nm for
Different rates of endocytic activity and vesicle transport from the apical and synaptic poles of the outer hair cell

Abstract

Background. Intense endocytic activity at the apex of outer hair cells (OHCs)—the electromechanical cells of the cochlea—has been demonstrated using the vital plasma-membrane marker FM1-43 and confocal laser-scanning microscopy. Vesicular traffic toward the cell nucleus to distinct locations of the endoplasmic reticulum has also been shown.

Objective. The current study characterizes the dynamics of endocytic activity, as well as apicobasal and basoapical trafficking, using a local perfusion technique that we recently developed and published to visualize bidirectional trafficking in isolated bipolar cells.

Materials and methods. The fluorescent plasma-membrane markers FM1-43 (10 μM) and FM4-64 (10 μM), together with a fluid-phase marker, Lucifer yellow (50 μM), were used to label endocytosed vesicles in isolated OHCs of the guinea pig cochlea. Targets of endocytosed vesicles were examined with a fluorescent marker of subsurface cisternae, DiOC6 (0.87 μM). Single- and two-photon confocal laser-scanning microscopy was used to visualize labeled vesicles.

Results. The plasma-membrane markers presented more intense vesicle internalization at the synaptic pole than at the apical pole of the OHC. Intracellular basoapical vesicle trafficking was faster than apicobasal trafficking. Vesicles endocytosed at the synaptic pole were transcytosed to the endoplasmic reticulum system. An intracellular Lucifer yellow signal was not detected.

Conclusion. The larger endocytic fluorescent signals in the synaptic pole and the faster basoapical trafficking imply that membrane internalization and vesicle trafficking are more efficient at the synaptic pole than at the apical pole of the OHC.

Keywords

Cochlea · Endocytosis · Pinocytosis · Transcytosis · FM dyes

Unterschiedliche Raten von endozytischer Aktivität und Vesikeltransport am apikalen und synthetischen Pol der äußeren Haarsinnzelle

Zusammenfassung

Hintergrund. Intensive endozytische Aktivität am apikalen Pol der äußeren Haarsinnzelle (ÄHZ) – der elektromechanischen Zelle der Cochlea – wurde mit dem vitalen Plasmamembranmarker FM1-43 unter Einsatz konfokaler Laserscanningmikroskopie nachgewiesen. Es wird gezeigt, dass Vesikel in Richtung Zellkern zu unterschiedlichen Bereichen des endoplasmatischen Retikulums (ER) transportiert werden.

Fragenstellung. Ziel der aktuellen Studie war es, die Dynamik der endozytischen Aktivität sowie des apikobasalen und basoapikalen Vesikeltransportes mittels einer vor Kurzem von den Autoren entwickelten lokalen Perfusionstechnik zu charakterisieren, welche die Visualisierung von bidirektionalem Vesikeltransport in isolierten bipolaren Zellen ermöglicht.

Material und Methode. Die fluoreszierenden Plasmamembranmarker FM1-43 (10 μM) und FM4-64 (10 μM) sowie der fluoreszierende Fluidphasenmarker Lucifer Yellow (50 μM) wurden verwendet, um endozytierte Vesikel in isolierten ÄHZ der Meerschweinchencochlea zu markieren. Ziele der endozytierten Vesikel wurden mit einem ER-Fluoreszenzmarker, DiOC6 (0.87 μM), untersucht. Fluoreszierende Vesikel wurden mittels Einzel- und Zwei-Photonen-Laserscanningmikroskopie sichtbar gemacht.

Ergebnisse. Die Plasmamembranmarker zeigten eine intensivere Vesikelaufnahme am synthetischen als am apikalen Pol der ÄHZ. Der intrazelluläre Vesikeltransport in basoapikaler Richtung war schneller als in apikobasaler Richtung. Am synthetischen Pol endozytierte Vesikel wurden zum ER transportiert. Ein intrazelluläres fluoreszierendes „Lucifer-Yellow-Signal“ wurde nicht detektiert.

Schlussfolgerungen. Die intensiveren endozytischen Fluoreszenzsignale im synthetischen Pol und der schnellere Transport in die basoapikale Richtung deuten darauf hin, dass Membranaufnahme und Vesikeltransport effizienter am synthetischen als am apikalen Pol der ÄHZ sind.

Schlüsselwörter

Cochlea · Endozytose · Pinozytose · Transzytose · FM-Farbstoffe
**Results**

**Apicobasal and basoapical trafficking**

Endocytic activity was investigated separately at the apical and basal poles of the isolated OHC (**Fig. 2**). In the first example shown (**Fig. 2a–c**), the PM marker FM1-43 was applied to the apical pole of the OHC using the double-barrel perfusor (**Fig. 2a**). Channel A (ChA) contained 10 μM FM1-43 in HBSS while channel B (ChB) contained dye-free HBSS to prevent apical pole labeling. The spatial distribution and the temporal course of the intracellular dye (**Fig. 2b**) imply that FM-labeled vesicles traffic toward the base of the cell. **Fig. 2c** shows the time course of the fluorescence signal intensities in the ROIs indicated in **Fig. 2a**. The dashed line denotes 20% of the saturation intensity from the apically located PM ROI. This 20% value, arbitrarily set as a threshold signal value, was used to calculate the signal delays to the ROIs relative to the PM ROI. Dashed black line: FM1-43 application period. **Fig. 2g** shows the average distance of ROIs from a cell pole as a function of time delays of the ROIs relative to the PM ROI for apicobasal (8 cells) and basoapical (5 cells) vesicle traffic. Error bars: standard deviations. Dashed lines: linear regression lines used to estimate trafficking speed.
distance-axis intercept locates the PM ROI, on average, at 1.52 ± 3.09 μm from the apical pole, which is not significantly different from 0 μm (t = 1.39, p = 0.21).

The same protocol was used to investigate basoapical trafficking, the FM1-43 dye being applied to the basal half of the cell (ChB) and the dye-free HBSS to the apical half (ChA) to prevent access of dye to that region (Fig. 2d–f). Signal delay as a function of distance from the basal pole is shown in Fig. 2g (circles) for five cells. The average apicobasal speed is 0.18 ± 0.01 μm/s. The distance-axis intercept locates the PM ROI, on average, at 6.25 ± 2.73 μm from the basal pole. In other words, the basoapical speed is significantly greater than the apicobasal speed (t0 = 12.17, p = 9.37·10^-6), indicating more intense trafficking toward the apex of the cell.

For the apical application, the fluorescent signal in the infracuticular ROI required, on average, 45.1 ± 22.8 s (N = 8) to reach the 20% threshold, whereas for the basal application, on average, only 13.6 ± 6.4 s (N = 5) was required for the infranuclear ROI, relative to the respective PM ROIs at or near the poles. The significantly smaller delay for the infranuclear ROI (t1 = 3.44, p = 0.003) indicates that vesicle formation in the synaptic pole is more intense than that at the apex of the OHC.

**Targets of basal endocytosed vesicles**

Until now, targets of vesicles formed at the synaptic pole of OHCs had not been demonstrated. Therefore, here, the endoplasmic reticulum of OHCs was labeled with DiOC₆ (Fig. 3a, green) and compared with vesicle targets of the fluorescent PM marker FM4-64 applied to the basal pole (Fig. 3b, red). The yellow signal in Fig. 3c indicates colocalization of DiOC₆ and FM4-64. Similar observations were made in a total of 14 cells. These observations imply that basally endocytosed vesicles are transcytosed to the endoplasmic reticulum.

**Molecular weight limit of endocytosed molecules**

To investigate the maximum possible molecular weight (MW) for a substance to be internalized by fluid-phase endocytosis (pinocytosis), Lucifer yellow (MW = 522 Da) was applied to the OHC and the development of intracellular fluorescence recorded (Fig. 4). In these experiments (N = 10), a single-barrel perfusor was used to apply the stain homogeneously around the entire cell. To avoid recording of scattered emitted light from extracellular dye, fluorescence was induced and recorded with two-photon confocal microscopy. Despite the presence of a constant and high-intensity extracellular signal and an extensive application time of the dye (>30 min), an intracellular fluorescence signal was not detected above the background noise level (Fig. 4). Importantly, the displayed intracellular signals represent noise derived from the extracellular signal because the intracellular signal dropped to zero when the Lucifer yellow application was terminated and extracellular solution applied (not illustrated). In the absence of a detectability problem, this result implies that anionic molecules with an MW greater than 500 Da cannot be internalized by pinocytosis.

**Discussion**

Different types and sizes of vesicles have been demonstrated at the infracuticular [21] and synaptic [33] pole of hair cells using electron microscopy and horseradish peroxidase labeling, respectively. Investigations using the PM marker FM1-43 show that vesicles formed from the reticular lamina transcytose to distinct intracellular compartments that are part of...
Fig. 4 a Molecular weight limit for uptake by pinocytosis. a Phase-contrast image showing intracellular (red and blue circles) and extracellular (green circle) positioned ROIs. Scale bar: 10 μm. b Fluorescence images showing Lucifer yellow labeling of extracellular fluid only. Numbers in the bottom right corners indicate experimental time in seconds. c Fluorescence signal intensities for the ROIs in panel a. Horizontal black line: Lucifer yellow application period

Here, using the double-barrel perfusor, it was found that endocytosis at the synaptic pole and basoapical trafficking are more intense than endocytosis at the apical pole and apico basal trafficking. Double-labeling experiments with DiOC₆ and FM4-64 imply that vesicles internalized at the synaptic pole traffic to the endoplasmic reticulum. The findings are summarized graphically in □ Fig. 5.

Experiments with the low-molecular-weight, fluid-phase endocytic, fluorescent marker Lucifer yellow indicated that pinocytic internalization of anionic molecules is limited to MWs of no more than 500 Da. However, the Lucifer yellow experiments are considered preliminary because if, indeed, there were pinocytic uptake, the number of vesicles might have been insufficient to produce a detectable signal. In these experiments, we limited the concentration of Lucifer yellow (50 μM) to be similar to that used for the PM endocytosis markers (10 μM). Griesinger et al. (2002) demonstrated pinocytic uptake of Lucifer yellow by inner hair cells for high concentrations (20 mM) and long incubation times (60 min). Future MW experiments should examine the pinocytic uptake of fluorescent markers of much greater extinction coefficient and a higher quantum yield than for Lucifer yellow.

Possible routes of FM1-43 entry into hair cells

It has been shown that FM1-43 can penetrate mechanoelectrical transducer (MET) channels in hair cells of immature cochlea cultures [12], hair cells of embryonic mice [13], developing hair and sensory cells [26], cultured chick
Fig. 5  Graphical summary. Endocytic activity of the outer hair cell is more pronounced at the synaptic pole and vesicle trafficking is faster toward the apex.

auditory papilla hair cells [32], cultured zebrafish larvae lateral line organs [31], and Xenopus larvae lateral line organs [28]. In addition, Crumling et al. [5] used hair cells of chicks 5–10 days post-hatch and showed PPADS- and suramin-dependent AM1-43 entry, implying that FM dyes can also penetrate P2X receptors. To avoid this type of nonspecific labeling, recently two techniques were developed: (1) photo-oxidation electron microscopy [22], whereby the FM dye taken up by the organelles is converted to a dark precipitate visible electron microscopically, and (2) instead of using an FM dye, a novel endocytotic probe impermeable to MET-channels, called membrane-binding fluorophore-cysteine-lysine-palmitoyl group, was used [30]. For both techniques, experiments with IHCs from mice at postnatal day 14–18 yield vastly different patterns of endocytotic signals compared with those found for when uptake is also through the MET channels. Therefore, there is still ongoing controversy about whether FM1-43 does indeed reliably report endocytosis in (P14–18) mice.

However, for the mature cochlea of the guinea pig, there is no evidence of FM-1-43 uptake through the MET channels or P2X receptors. For the inner hair cell (IHC), it has been demonstrated, using an in-situ cochlea preparation from young adult guinea pigs, that neither the MET-channel blockers dihydrostreptomycin or D-tubocurarine nor the lesioning of tip links with BAPTA treatment influences FM1-43 labeling [15]. Similar results were reported for OHCs in situ using the same animal and preparation as in the IHC study; namely, that FM1-43 labeling was not influenced by these MET-channel blockers or BAPTA [16]. By applying FM1-43 to isolated OHCs from the mature, pigmented guinea pig cochlea, we also demonstrated that block of MET channels by dihydrostreptomycin or of P2X receptors by PPADS had no influence on the FM1-43 labeling [23]. Therefore, for the experimental model on which the present results are based, there is no evidence of FM1-43 entering through MET channels or P2X receptors of hair cells of the functionally mature cochlea. In other words, the available evidence convincingly suggests that in the present experiments FM1-43 uptake was via endocytic activity.

**Physiological relevance of endocytosis in OHCs**

Plasma-membrane Ca\(^{2+}\) ATPase (PMCA) activity is crucial for removing Ca\(^{2+}\) from the cytosol of the stereocilia and, therefore, for protecting hair cells from Ca\(^{2+}\) overload [4]. It has been proposed that apical endocytic activity of hair cells is involved in recycling of PMCA molecules of the hair bundle [14].

Receptors and ion channels of the efferents and afferents located at the synaptic pole of the OHC may require continuous replacement. However, the colocalization data with DiO\(_c\) and FM4-64 show that vesicles formed at the synaptic pole of OHCs traffic basoapically, targeting the endoplasmic reticulum. Therefore, in addition to FM recycling, proteins such as acetylcholine receptors might also be involved.

It has been proposed that aminoglycoside uptake might be regulated by endocytosis [7]. Although it has been suggested that aminoglycosides enter hair cells through transducer channels in cultured organs from newborn rats [1], the significance of endocytosis-dependent aminoglycoside uptake at the synaptic pole cannot be excluded because aminoglycosides such as gentamicin have a relatively low MW (450–478 Da), which is below the MW limit suggested in this study for pinocytic internalization. With the aid of the double-barrel perfusor, experiments with fluorescent-labeled gentamicin could address the possibility of its endocytic internalization.

**Practical conclusion**

- Fluorescent membrane markers enable the investigation of the dynamics of endocytic activity and vesicle trafficking in outer hair cells.
- Such investigations are essential for characterizing cellular mechanisms required not only for membrane and for protein recycling but also for intra- and intercellular communication.
- Mechanisms of vesicle formation and the cargo-specific targets are still debated for outer hair cells.
- Using a recently designed local-perfusion system, the current study demonstrates that basal endocytosis and basoapical trafficking are more intense than apical endocytosis and apicobasal trafficking.
- When coupled with molecular and pharmacological investigations of uptake and trafficking mechanisms, this finding should offer insight into
underlying cellular mechanisms, which is a prerequisite for designing cell-specific medication for hair cells in the future.

References

1. Alharazneh A, Luk L, Huth M et al (2011) Functional hair cell mechanotransducer channels are required for aminoglycoside ototoxicity. PLoS ONE 6:e22347
2. Ashmore J (2008) Cochlear outer hair cell motility. Physiol Rev 88:173–210
3. Betz WJ, Bewick GS (1992) Optical analysis of synaptic vesicle recycling at the frog neuromuscular junction. Science 255:200–203
4. Bourg M, Nam JH, Chen Q et al (2010) Calcium balance and mechanotransduction in rat cochlear hair cells. J Neurophysiol 104:18–34
5. Cramling MA, Tong M, Aschenbach KL et al (2009) P2X antagonists inhibit styril dye entry into hair cells. Neuroscience 161:1144–1153
6. Dallos P (2008) Cochlear amplification, outer hair cells and prestin. Curr Opin Neurobiol 18:370–376
7. De Groot JC, Meeuwsen F, Ruizendaal WE et al (1990) Ultrastructural localization of gentamicin in the cochlea. Hear Res 50:35–42
8. Dong W, Olson ES (2013) Detection of cochlear amplification and its activation. Biophys J 105:1067–1078
9. Fettplace R (2017) Hair cell transduction, tuning, and synaptic transmission in the mammalian cochlea. Compr Physiol 7:1197–1227
10. Frank G, Hemmert W, Gummer AW (1999) Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. Proc Natl Acad Sci USA 96:4420–4425
11. Froud KE, Wong ACY, Cederholm JME et al (2015) Type II spiral ganglion afferent neurons drive medial olivocochlear reflex suppression of the cochlear amplifier. Nat Commun 6:7115
12. Gale JE, Marcotti W, Kennedy HJ et al (2001) FM1-43 dye behaves as a permeant blocker of the hair-cell mechanotransducer channel. J Neurosci 21:7013–7025
13. Géloëc GSG, Holt JR (2003) Developmental acquisition of sensory transduction in hair cells of the mouse inner ear. Nat Neurosci 6:1019–1020
14. Grati M, Schneider ME, Lipkow K et al (2006) Rapid turnover of stereocilia membrane proteins: evidence from the trafficking and mobility of plasma membrane Ca2+-ATPase 2. J Neurosci 26:6386–6395
15. Griesinger CB, Richards CD, Ashmore JF (2002) FM1-43 reveals membrane recycling in adult inner hair cells of the mammalian cochlea. J Neurosci 22:3939–3952
16. Griesinger CB, Richards CD, Ashmore JF (2004) Apical endocytosis in outer hair cells of the mammalian cochlea. Eur J Neurosci 20:41–50
17. Guinan JJ Jr. (2018) Olivocochlear efferents: Their action, effects, measurement and uses, and the impact of the new conception of cochlear mechanical responses. Hear Res 362:38–47
18. Harasztosi C, Klensek E, Badum S et al (2018) Double fluorescent labelling of a bipolar epithelial cell in vitro: the outer hair cell. J Neurosci Methods 293:310–320
19. Huang G, Santos-Sacchi J (1993) Mapping the distribution of the outer hair cell motility voltage sensor by electrical amputation. Biophys J 65:2228–2236
20. Hudspeth AJ (2008) Making an effort to listen: mechanical amplification in the ear. Neuron 59:530–545
21. Kachar B, Battaglia A, Fox J (1997) Compartmentalized vesicular traffic around the hair cell cuticular plate. Hear Res 107:102–112
22. Kamin D, Revelo NH, Rizzoli SO (2014) FM dye photo-oxidation as a tool for monitoring membrane recycle in inner hair cells. PLoS ONE 9:e88353
23. Kaneko T, Harasztosi C, Mack AF et al (2006) Membrane traffic in outer hair cells of the adult mammalian cochlea. Eur J Neurosci 23:2712–2722
24. Konig AJ, Lum PY, Williams JM, Wright R (1993) DiOC5 staining reveals organelle structure and dynamics in living yeast cells. Cell Motil Cytoskeleton 25:111–128
25. Meyer J, Mack AF, Gummer AW (2001) Pronounced infracuticular endocytosis in mammalian outer hair cells. Hear Res 161:10–22
26. Meyers JR, MacDonald RB, Duggan A et al (2003) Lighting up the senses: FM1-43 loading of sensory cells through nonselective ion channels. J Neurosci 23:4054–4065
27. Nadd JB Jr. (1983) Serial section reconstruction of the neural poles of hair cells in the human organ of Corti. II. Outer hair cells. Laryngoscope 93:780–791
28. Nishikawa S, Sasaki F (1996) Internalization of amiloride-sensitive Na+ channels in organ of Corti. II. Outer hair cells. Laryngoscope 106:799–810
29. Osen KE, Widdicombe JH (1970) Acoustic reflex and the physiology of normal middle ear function. J Acoust Soc Am 47:333–471
30. Plontke S, Salt AN (2018) Local drug delivery to the inner ear: principles, practice, and future challenges. Hear Res 368:1–2
31. Revelo NH, Kamin D, Truckenbrodt S et al (2014) A new probe for super-resolution imaging of membranes elucidates trafficking pathways. J Cell Biol 205:591–606

Funding. This work was supported by the Deutsche Forschungsgemeinschaft (DFG), grant GU 194/9–1, 2.

Compliance with ethical guidelines

Conflict of interest. C. Harasztosi and A. W. Gummer declare that they have no competing interests.
Fachnachrichten

31. Seiler C, Nicolson T (1999) Defective calmodulin-dependent rapid apical endocytosis in zebrafish sensory hair cell mutants. J Neurobiol 41:424–434
32. SiF, Brodie H, Gillespie P et al (2003) Developmental assembly of transduction apparatus in chick basilar papilla. J Neurosci 23:10815–10826
33. Siegel JH, Brownell WE (1986) Synaptic and Golgi membrane recycling in cochlear hair cells. J Neurocytol 15:311–328
34. Terasaki M (1988) Fluorescent labeling of endoplasmic reticulum. Methods Cell Biol 29:125–135
35. Zheng J, Shen W, He DZ et al (2000) Prestin is the motor protein of cochlear outer hair cells. Nature 405:149–155

Folgenreiche Fake News: Beispiel HPV-Impfung in Japan

Die Behandlung von Patienten auf Basis einer evidenzbasierten Medizin sollte die Richtschnur ärztlichen Handelns sein. Doch was passiert, wenn seriöse Medizininfos übertönt werden von zweifelhaften Erkenntnissen? Ein Fall aus Japan zeigt die Folgen.

In Deutschland rühren verschiedene Ärzteverbände die Werbetrommel, um die Impfantei- ten gegen humane Papillomviren (HPV) zu steigern – nur knapp die Hälfte der Zielgruppe genießt diesen Impfschutz bereits, obwohl die Evidenzlage klar ist. Japan hingegen verzeichnete 2013 bereits eine Quote von 70 %. Die Impfrate ist aber schlagartig auf das niedrige Niveau von rund 1 % gefallen. Für die japanische Ärztin Riko Muranaka ist die plötzliche Ablehnung der HPV-Impfung vor allem auf eines zurückzuführen: Fake Science und Fake News.

Kehrtwende nach Erfolgsstory

Nachdem die HPV-Impfung unter jungen Mädchen rasant zur Erfolgsstory geworden war, seien plötzlich Schlagzeilen in den Medien aufgetaucht, die den Impfgegner das Wort redeten – untermauert mit Videos von Mädchen, die behaupteten, geschädigt worden zu sein. Ursache für das plötzliche Medieninteresse seien wissenschaftlich nicht haltbare Studien gewesen – allerdings interessierte sich offenbar niemand für deren Evidenz, führte die Ärztin aus.

Eine Maus sorgt für viel Aufregung

Im Jahr 2016 fand Muranaka eigenen Angaben zufolge dann heraus, dass Dr. Shuichi Ikeda, der auf allen Fernsehkanälen mit seiner Maus-Studie präsent war, die vor der HPV-Impfung warnen sollte, massive wissenschaftliche Defizite aufgewiesen habe. Unter anderem habe Ikeda für seine Studie nur eine einzige Maus benutzt, deren Gehirn durch den Impfstoff geschädigt worden sei. Die Studie sei zudem nie am Menschen repliziert worden.

Sammelklage von über 200 Frauen

Aktuell sehe sich die japanische Regierung einer Sammelklage im Namen von über 200 Frauen ausgesetzt, deren Anwälte sie als Opfer der HPV-Impfung darstellen. Muranaka erwartet eine Prozessdauer von rund 10 Jahren und rechnet in dieser Zeit mit mehreren tausend Toten wegen unterlassener HPV-Impfung.

Staatliche Empfehlung abgeschwächt

Die Regierung stehe unter Feuer, weil es ursprünglich eine staatliche Empfehlung für die Impfung gegeben habe, die inzwischen abgeschwächt worden sei. Es wird lange dauern, bis sich die HPV-Impfrate wieder erholen kann, so Muranaka. Aus ihrer Sicht liegt ein erheblicher Teil der Verantwortung bei den Medien: „Die Medien berichten immer noch eher auf Basis von Emotionen als von wissenschaftlichen Fakten.”

Nährboden für Impfgegner

Sie habe den Eindruck, dass die japanische Medienlandschaft nicht so vielfältig sei wie in anderen Ländern, erläuterte sie. Der Erfolg der Impfung war nach ihrer Einschätzung mit verantwortlich, dass die Botschaften der Impfkritiker auf fruchtbaren Boden fallen konnten. In der Folge seien die entsprechenden Infectionen kaum noch sichtbar gewesen, das Problembewusstsein sei entsprechend gesunken.

Quelle: Ärzte Zeitung
www.aerztezeitung.de