Examination of the supporting cells in the organ of Corti in hemicochlea preparation of mice with and without developed hearing

Ph.D. thesis

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1. Introduction

1.1. Supporting cells in the organ of Corti

The mammalian auditory organ, the organ of Corti, is the sensory epithelium in the bony capsule of the inner ear. Next to the widely researched hair cells, it contains supporting cells that are actively involved in the mechanisms of hearing. The inner hair cells are surrounded by the inner boarder and the inner phalangeal cell. Lateral to them two rows of pillar cells hold the tunnel of Corti. Next to it the three rows of Deiters' cells are located. The outer hair cells are placed on the apical pole of these cells. Rows of Deiters' cells are followed by Hensen's cells, which are bound to cubic Claudius' cells. Claudius' cells continue in the outer sulcus, which reaches the stria vascularis, forming the side wall of the cochlea.

The pillar and the Deiters' cell have an advanced microtubular system. Without them, cochlear (macro-) mechanics cannot be realized. The Deiters' cell is also thought to play a role in micromechanics: its long process touches the apical part of the outer hair cells and affects its movement. The movement of outer hair cells reduces the hearing threshold by a phenomenon called cochlear amplification.

1.2. The role of the purinergic signaling in the hearing

ATP, as a paracrine signaling molecule, can modify the hearing. Some of its receptors are more prominent at certain stages of development, coordinating the maturation process of the hair cell-auditory neuron synapses. By regulating the intracellular Ca^{2+} level ($[Ca^{2+}]_i$) of the supporting cells, they can modulate the ionic concentration of the cochlear fluid spaces and decrease the excitability

of the hair cells. During damage ATP-induced ATP release triggers intercellular Ca^{2+} waves among the supporting cells, followed by changes in expression pattern to initiate defense mechanisms.

Ionotropic P2X and metabotropic P2Y receptors have been described on the supporting and hair cells of young animals.

1.3. Other signaling systems in the supporting cells

Anatomical works have shown the expression of the transient receptor potential (TRP) channels in the inner ear. Prominent expression of the TRP ankirin 1 (TRPA1) channel has been found on Hensen's cells, but has also been reported on other supporting and hair cell types. Because of its many properties, it was thought to be part of the mechanoelectric transduction complex of hair cells. However, this hypothesis later failed.

The TRP vanilloid 1 (TRPV1) receptor has also been found on supporting cells and is still considered a promising target molecule for the treatment of hearing impairment. Both of the above-mentioned TRP channels are non-selective cation channels with high Ca^{2+} permeability.

The efferent nerve fibers running to outer hair cells containing acetylcholine (ACh) add collaterals to Deiters' and Hensen's cells. Isolated Deiters' cells have been shown to react with Ca^{2+} influx to ACh, likely via the α9 subunit containing nicotinic ACh receptor with high Ca^{2+} permeability. To our knowledge, there is no data on Hensen's cells that functionally support efferent innervation.

2. Aims

Efficient drug therapy for sensorineural hearing losses are missing. The reason for this is the lack of knowledge due to the complex anatomical structure and difficult location of the auditory organ. In order to facilitate investigations, our aim was the:

Improvement of the used bulk-loading method for Ca^{2+} imaging to reach better signal-to-noise ratio during the examination of the cells of the organ in mice with matured hearing:

- Setting up a targeted, high selectivity indicator loading method in hemicochlea preparation.
- During Ca^{2+} imaging measurements the source of the transient should be clearly identifiable.
- Achieve a better signal-to-noise ratio during experiments.
- The set method should be applicable to several cell types.

The unique Deiters' cell can be studied with its thin process only in selectively loaded cells. Our goals were:

To monitor purinergic signaling changes in Deiters' cells during the postnatal period which is critical for hearing development:

- Purinergic receptor-mediated Ca^{2+} signaling of Deiters' cells in two subcellular compartments (soma and process) during auditory development (P5-25).
- Subcellular monitoring of morphological changes of these compartments allowed by unique cell labeling.

3. Methods

3.1. Animals

All animal care and experimental procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The Animal Use Committee of Semmelweis University, Budapest, approved procedures.

The preparation was made from BALB/c mice from P5 to P25.

3.2. Hemicochlea preparation

Hemicochlea preparation was made from acutely dissected cochleae of BALB/c mice from P5 to P25. Briefly, after decapitation in isoflurane anesthesia, the cochleae were removed and placed in ice-cold solution similar to perilymph (composition in mM: NaCl 22.5; KCl 3.5; CaCl2 1; MgCl2 1; Hepes 10; Na-gluconate 120; glucose 5.55; pH 7.4; 320 mOsm/L). It was continuously oxygenated. Medial surface of each cochlea was glued onto a plastic plate and placed in a cutting chamber of a vibratome while being continuously bathed in the ice-cold solution. Cochlea was cut half in the modiolar plane under visual control through a stereomicroscope and the glued half was used for the imaging experiments.

3.3. Targeted single-cell electroporation dye-loading and Ca^{2+} imaging

The hemicochleae were placed into an imaging chamber filled with the oxygenated perilymph-like solution on the microscope stage. The perfusion speed was 3.5 ml/min in the chamber. The cells were chosen in oblique illumination under a LUMPlanFl 40x/0.80w water immersion objective. Borosilicate pipette (5-7MΩ) was filled with the Ca^{2+} indicators OGB-1 or fura-2/K^+ (1 mM) dissolved in distilled water. The pipette was mounted onto an electrode holder attached to a micromanipulator. Each chosen cell was approached and gently touched by the pipette under visual control; a single square wave current impulse (10 ms duration and 10 μA amplitude) was sufficient to load the cell with the Ca^{2+} dye.

The OGB-1 dye-filled cells were illuminated by 494 ± 5nm excitation light and the emitted light was monitored after passage through a band-pass filter (535 ± 25 nm). Fura-2/K^+ loaded cells were alternately illuminated by 340 ± 5 nm and 380 ± 5 nm excitation light and the emitted light was detected behind a 510 ± 20 nm band-pass

filter. Fluorescent images were obtained with an fluorescence microscope equipped with a CCD camera. The image frame rate was 1 or 0.5 Hz during the ATP-evoked responses and 0.1 or 0.05 Hz otherwise (OGB-1 or fura-2/K^+, respectively). Fura-2/AM was used to contrast the difference between single-cell and bulk-loading. Briefly, the hemicochlea was incubated with 10 mM fura-2/AM in the presence of pluronic F-127 for 30 min, then deesterified in standard experimental solution for 15 min before recording. The whole experiment was performed within 1.5-2 h after decapitation.

3.4. Drug delivery

ATP, UTP, allyl isothiocyanate (AITC), capsaicin and carbachol were added to the perfusion for 30 s. The buffer volume in the perfusion chamber was about 1.9 ml. ATP, as a standard stimulus on supporting cells, was always administered at the beginning and at the end of validation experiments to confirm the cellular responsiveness and viability. Before the first drug application, at least a 3 min long baseline period was registered in each experiment. Minimum 10 min had to elapse between two drug stimuli, except in the case of the Ca^{2+} free solution (composition in mM: NaCl 22.5; KCl 3.5; MgCl2 2; Hepes 10; Na-gluconate 120; glucose 5.55; EGTA 1; pH 7.4; 320 mOsm/l) when we waited at least 15 mins between two ATP applications.

3.5. Data analysis

Data analysis was performed off-line. Pixel intensity within a polygonal region of interest (ROI) was averaged for each frame. Fluorescence intensities were background-corrected using a nearby area devoid of loaded cells. Using OGB-1, the relative fluorescence changes were calculated as follows:

$$\frac{dF}{F_0} = \frac{F_t - F_0}{F_0}$$

where F_0 is the fluorescence intensity of the baseline, and F_t is the fluorescence intensity at time t. In case of fura-2/K^+, the ratio of emitted fluorescence intensities (F340/F380) were calculated. The response amplitudes were defined as the maximal change in intensity.

Area under curves (AUC) and average curves of the responses were calculated in Igor Pro 6.37. Signal-to-noise ratio (S/N) in fura-2/AM and fura-2/K^+ loaded cells were calculated from ATP response curves of 12-12 randomly selected cells as follows:

$$S/N = \Delta R/\delta R$$

where ΔR is the amplitude of the ATP-evoked transients and δR is the standard deviation of the baseline ratio prior to the ATP administration.

Data are presented as mean ± standard error of the mean (s.e.m.). The number of experiments (n) indicates the number of cells. Testing of significance ($p < 0.05$) was performed based on the distribution of the data (tested by Shapiro-Wilk test). In case of normal distribution ANOVA, otherwise Kruskal-Wallis test were used (followed by Bonferroni post-hoc test). In developmental experiments testing is based on the multiple linear regression models in R version 3.2.3.. Explanatory variables were the location of the cell (apical or middle turn; tonotopy) and the postnatal days (age). The period of P5–7 was not included in the models (except in case of the morphological development), as we hypostatized that the P10–11 period is a starting point in case of hearing functions.

3.6. Measurement of the morphological parameters

Subcellular compartments of Deiters' cells were measured using overview Z-stack images of cells filled with electroporation (7-9 images/cell). Measurements were made with FIJI/ImageJ software. After overlaying the images, the parameters were measured. Width measurements were made in the half of the soma and in the process.

Processes were seen only on the fluorescent images, but to be able to reliably apply the fluorescent image to the morphological measurements, the parameters of the soma were measured both on fluorescent and oblique images. If the data of the parameters measured under oblique illumination and the parameters measured under fluorescent illumination were within 5% error, then the cell could be included in the analysis.

4. Results

4.1. Targeted single-cell electroporation dye-loading method set up and validation

Targeted single-cell electroporation is suitable to load Ca^{2+} indicators into cells in the hemicochlea prepared from hearing mice

After the onset of the first hearing optimum (> P14 ~ adult like hearing), Deiters', Hensen's, and Claudius' cells were loaded with Ca^{2+} indicator in hemicochlea preparation using targeted single-cell electroporation technique (10 μA and 10 ms electrical stimuli, at 1 mM indicator concentration). In addition to the 3 supporting cells, both hair cell types were successfully loaded.

Single-cell electroporation was suitable for loading single cell in all three turns of the hemicochlea preparation and provided a better signal-to-noise ratio compared to the bulk-loading procedure. Subcellular measurements were also performed on the process of the Deiters' cells.

ATP evoked reversible and repeatable Ca^{2+} transients in Deiters' cell soma and process, Hensen's and Claudius' cells

Administration of ATP (100 μM, 30 s) to perfusion induced repetitive and repeatable Ca^{2+} transients on Deiters', Hensen's and Claudius' cells. In addition to the soma, responses of process of the

Deiters' cells were detected. The responses differed in their characteristics: the process of the Deiters' cells had the highest amplitude and AUC value, whereas in Hensen's cells two-peak Ca^{2+} transients and in Claudius' cells short-term transients were measured.

To identify the purinergic receptors of supporting cells, we also induced ATP-induced Ca^{2+} transients in Ca^{2+}-free medium, which typically exhibited lower amplitudes than in control solution. This was significant in both compartments of Deiters' cells.

Stimulation of TRPA1 and TRPV1 channels did not induce Ca^{2+} signaling, but TRPA1 activation resulted in the slight movement of the tissue

Administration of TRPA1 agonist, AITC (200, 400 and 2000 μM, 30 s) did not induce Ca^{2+} transients in the measured supporting cells. However, a concentration-dependent shift from the focal plane was observed in cells filled with single-wavelength OGB-1 indicator. For better monitoring of intensity changes, cells were loaded with the fura-2/K^+, ratiometric indicator. No increase in $[Ca^{2+}]_i$ was observed.

After AITC treatment, the ATP-induced Ca^{2+} transients of Deiters' and Hensen's cells approximate the ATP-induced signal before AITC, but transients in Claudius' cells were lower.

Perfusion of the TRPV1 agonist, capsaicin (330 and 990 nM, 30 s), did not induce Ca^{2+} transients in either of the supporting cells or compartments. In this case, no shift from the focal plane was observed either.

Activation of ACh receptors by carbachol induced Ca^{2+} response in Deiters' and Hensen's cells

The effect of carbachol (100 μM, 30 s) was investigated on innervated supporting cells (Deiters' and Hensen's cells). Ca^{2+} transients were measured in both compartments of Deiters' cells in ~ 33% of cases, while in Hensen's cells one in five responded to the AChR agonist.

4.2 Morphological and purinergic Ca^{2+} signaling change during the development of Deiters' cell

The morphological changes in Deiters' cell somata and processes during postnatal development have no tonotopic preference

The entire shape of Deiters' cells filled with single-cell electroporation can be detected. This allows not only functional but also morphological observations. We examined the development of Deiters' cells at two tonotopic sites - apical and middle turns - at critical periods of postnatal development (P5-25). In the hemicochlea preparation the development of the organ of Corti can also be observed (e.g. opening of the tunnel of Corti or the Nuel's space). Cell growth in Deiters' cells reaches adult height by about P14-15 and does not change significantly in width. The length of the process is slowly extended. Its width is reduced until P17-18. Beyond age, tonotopic location also determines the size: apical turn cells are larger in both width and length.

The maturation-dependent spontaneous Ca^{2+} activity is tonotopically heterogeneous and implies subcellular difference

Spontaneous Ca^{2+} transients produced without stimulation are typical of the developmental period. The frequency of occurrence is inversely proportional to age in both turns, and can be considered to disappeared by >P20.

Spontaneous signals are present in both compartments. The activity of the process tends to exceed that of the cell body. A major point in the apical turn is the developmental stage of P10-11, when cells were much more active than other periods.

Exogenous ATP-evoked Ca^{2+} responses are maturation-dependent

The ATP-induced Ca^{2+} transients of Deiters' cells undergo developmental changes. During the maturation, the amplitudes of the process will become larger, while their duration will gradually decrease from the P10-11 period. The AUC values can be considered constant over the development. The responses in the middle turn tend to assume higher values.

As the soma develops, the amplitude of the Ca^{2+} transients decreases from P10-11. Response duration and AUC show the same pattern. No tonotopic differences were observed.

Selective P2Y receptor activation evokes maturation and tonotopy-dependent Ca^{2+} transients with a P2Y receptor dominance in the apical turn

In addition to ATP, Ca^{2+} transients were measured after stimulation with P2Y receptor-specific UTP (100 μM, 30 s), in both compartments and turns, for 3 crucial developmental periods (P10-11, P14-15, P17-18). The UTP-induced Ca^{2+} transients remained below the ATP-evoked values in all parameters, but their direction of change was similar to ATP-induced responses.

The amplitude of the process increased, but the duration of the responses decreased with development. There was no change in AUC. When comparing turns, we found only differences in amplitudes and apically located cells had higher values.

All measured values (amplitude, duration, AUC) of soma decreased during development. Here, too, cells in the apical turn showed higher values.

5. Discussion

5.1. Targeted single-cell electroporation dye-loading method in the supporting cells of the organ of Corti

Advantages of the mature hemicochlea preparation and drawbacks of bulk-loadings in Ca^{2+} imaging

Hemicochlea preparation has several advantages that allows us for examine the auditory organ: retained anatomical structure, access to multiple turns (tonotopic examinations), cross-sectional view of the organ of Corti and adult-like auditory organ.

In our previous work, we used a bulk indicator loading process with the AM form of the indicator. In these experiments the molecules are trapped in the extracellular spaces, so the background staining were significant, causing a reduction in the signal-to-noise ratio. AM-bound indicator molecules can be picked up by any cell, and we cannot selectively focus on one cell. More over the scattering of fluorescence degrades the ability to identify the source of the sign. In addition, the dyeing process and de-esterification take longer time, reducing the preparation's survival.

We set up and validated single-cell electroporation indicator loading method that provides the opportunity to test individual supporting cells in the hearing mouse hemicochlea. The electroporation technique provides a faster loading and better signal-to-noise ratio, thus increasing the spatial resolution of our functional imaging assays, and the source of fluorescence is clearly identifiable due to minimal background staining.

Single-cell electroporation is a rapid and specific Ca^{2+} indicator loading of supporting cells with low S/N and retained viability

Single-cell electroporation allows dye loading of selected cells. The quick approach of the selected cell and the lack of pressure on the pipette minimized the spillover of the indicator. The method enabled

subcellular functional imaging of the soma and the process of Deiters' cells as it was not obscured by the fluorescence of outer hair cells.

The electroporation worked well for the Deiters', Hensen's and Claudius' cells. However, the pillar cells could not be loaded homogenously, because the dye did not diffuse through the stalk part of the cell. Inner and outer hair cells could also be loaded successfully.

ATP evoked Ca^{2+} transients in the soma of Deiters', Hensen's and Claudius' cells and the phalangeal process of the Deiters' cells - validation of (sub)cellular imaging

The suitability and reliability of single-cell electroporation was demonstrated by the P2 receptor agonist ATP.

In electroporated cells, an ATP induced, recurrent and repeatable increase in $[Ca^{2+}]_i$ was observed. The signal-to-noise ratio of the responses was better than with bulk-loading. In Deiters' cells, selective loading allowed for subcellular studies.

The ATP-evoked responses differed in their characteristics: Hensen's cells often exhibited two peaks in the Ca^{2+} response; process of the Deiters' cells produced the largest amplitudes. This may be because of the highest density of ATP-activated receptors in the cells/compartments. However, the lower baseline intensity and the smaller compartment can also play a role in generating high amplitude.

To separate the P2X ionotropic and P2Y metabotropic receptor groups, ATP-induced Ca^{2+} transients were induced in Ca^{2+} -free solution. In these cases the release of internal stores were observed, and the presence of P2Y receptors can be confirmed. The self-controlled design of the experiment allows the comparison of the total ATP-evoked Ca^{2+} response and the smaller Ca^{2+} response produced with P2Y alone on the same cell. The use of a third stimulus, in the presence of Ca^{2+}, proves that the cell's viability and response were maintained until the end of the experiment.

In the Ca^{2+}-free medium, ATP-induced Ca^{2+} transients were observed in each cell, suggesting functional P2Y receptors. The decrease in the amplitude of the transients indicates that external Ca^{2+} influx also contributes to the ATP response, thus demonstrating the presence of P2X receptors.

TRPA1 stimulation did not induce Ca^{2+} response in Deiters' and Claudius' cells but raised the possibility of TRPA1 role in Hensen's cell Ca^{2+} homeostasis

The presence of TRP channels in the cochlea was examined by anatomical methods. In our validation experiments, we used a functional approach: TRPA1 agonist AITC and TRPV1 agonist capsaicin were added to the perfusion.

Expression of TRPA1 channels has previously been demonstrated on supporting cells in neonatal mice. In older animals, only SGN fibers are expressed this protein. Our experiments confirm the latter opinion. The agonist AITC could not induce Ca^{2+} transients in the cells at any concentration. A single low-amplitude Ca^{2+} response in Hensen's cells was observed (400 μM AITC), but response of the same cell was not detected at higher concentrations.

TRPA1 stimulation displaced the organ of Corti

A dose-dependent shift from the focal plane of the organ of Corti was observed during administration of AITC. This was caused by AITC-induced cell contraction, which was also implicated in the activation of TRPA1 in hair cells, Deiters' cells and pillar cells in P0-7. This contraction was not observed in mice with Trpa1 -/- genotype. We did not experience direct contraction of Deiters' cells during our work. The presence of TRPA1 channels in the adult organ of Corti cannot be excluded.

The presence of these channels may also be indicated by a decrease in the amplitude of the second ATP response of Claudius' cells after

AITC stimulation, which can be explained by cross-inhibition of the co-expressing TRPA1-P2X receptors.

TRPV1 stimulation did not evoke any Ca^{2+} response in the supporting cells

The presence of TRPV1 channels in the inner ear was also deduced from anatomical work. TRPV1 expression has previously been shown to exhibit species and age-dependent patterns in the cochlear epithelium. In the auditory organ, RNA levels rise from embryonic 18 to P8 days. Other work did not find TRPV1 RNA during this period. However, TRPV1 protein was detected by immuno-labeling.

No Ca^{2+} response to capsaicin was detected in our experiments, suggesting a lack of TRPV1 channel in Deiters', Hensen's and Claudius' cells. We did not experience any deviation from the focal plane during excitation.

Based on these results, our experiments cannot confirm the functional expression of TRPV1 channels on mast cells.

ACh receptor activation evoked Ca^{2+} transients in some Deiters' and Hensen's cells

ACh-containing efferents innervate outer hair cells, negatively regulating cochlear amplification. Efferent collaterals run to Deiters' and Hensen's cells, in anatomical path marks. To confirm this, functional assays have already been performed: in isolated Deiters' cells ACh induced currents, through nicotinic ACh channel containing the α9 subunit. There are only anatomical observations on the innervation of Hensen's cells.

A broad spectrum ACh receptor agonist, carbachol, was used in our experiments. The proportion of reactive Deiters' cells (~ 33%) was similar to that of isolated Deiters' cells. 20% of Hensen's cells were able to induce Ca^{2+} transients with carbachol, which had a lower amplitude than ATP-induced ones.

5.2. Morphological changes and maturation of purinergic signaling in Deiters' cells during the development

Postnatal morphological development of Deiters' cells in the mouse cochlea

In mouse hearing maturation is approximately complete by the end of the third week after birth.

The hemicochlea preparation has already been used to measure morphological parameters as it gives a good view of the organ of Corti in cross section and gives access to 3 turns. However, in the wide field view, the process of the Deiters' cells is not visible due to the covering of the outer hair cells. The development of this subcellular region has not yet been explored. Research has shown that this compartment is important in cochlear micromechanics. With the single-cell electroporation indicator loading, the processn of the developing Deiters' cells could be studied.

Morphological development was monitored from the end of the first week. Deiters' cells reached adult size by P14-15 and the initial tonotopic differences were diminished. This is similar to that found in Mongolian mice. The adult cell height we measured is higher than that measured in other mouse strains. However, several studies showed morphological differences between mouse strains. There has been no such data about BALB/c mouse strain so far.

The process showed no change in length, but cells from the apical turn had longer processes, as expected: since the height of the hair cells also changes along the tonotopical axis. The processes and the hair cells form the reticular lamina, so their length correlates. The hair cells extend ~ 1.2-fold between the middle and apical curves. This increase in mid-apical turn was ~1.5-fold in the process of Deiters' cell. This may be a BALB / c strain feature.

The Nuel space between the hair cells and the processes of the Deiters' cells opens in P6-10. This intercellular space appears as the thickness of the processes decrease. The slimming of the process continued until P17-18.

Spontaneous Ca^{2+} activity in phalangeal process and soma of the Deiters' cells decreases by development with different pattern in the middle and the apical cochlear turns

Spontaneous Ca^{2+} activity and intercellular Ca^{2+} waves are well-described processes in the young organ of Corti. Ca^{2+} signals are triggered by ATP released from the supporting cells via hemichannels, in a paracrine fashion. They play a role in maturation, enhancement and pruning of hair cell-SGN synapses.

The process of Deiters' cells have a higher spontaneous Ca^{2+} activity than the soma. This may be due to multiple hemichannels on the endolymphatic surface: released ATP reaches higher concentrations and induce receptor activation. In addition, purinergic receptors are denser on the endolymphatic surface.

The frequency of spontaneous signals in apical turn reached its maximum around P10. The decrease in activity may be due to the transient presence of some purinergic receptors. Several types have been shown to be present during the maturation period of P10 (e.g. P2X1, P2X2/3) and then gradually disappear from the organ of Corti. We also know receptors that decrease in density with age but do not lose expression. In addition, it is possible a change in the expression profile of the receptor pattern, the expression of the hemichannels may also change.

P10-11 seems to be an important period in apical turn. This is when the prestin motor protein begins to be expressed in outer hair cells. This prominent period was not present in the middle turn. This is because in the cochlea, the maturation of the organ of Corti shows a

baso-apical gradient, so it is possible that we could have found increased spontaneous signal activity earlier.

Amplitude and shape of exogenous ATP-induced Ca^{2+} transients depend on the developmental stage

A purinergic receptor expression in the cochlea is an age-dependent process. Receptor density increases in Deiters' cells in the first week of postnatal development and begins to decrease around P11-12. This receptor pattern explains the changes we observed in amplitude, response time, and AUCs observed at different ages.

Parameters increased from P5-7 to P10-11, where values peaked, followed by a slower, decreasing period.

The amplitude of the process increased and showed greater values in the middle turn. This indicates an uneven purinergic receptor distribution within the cochlea.

Response duration and AUC showed a decreasing trend in both compartments. This could be due to the more uniform Ca^{2+} buffering and removal mechanisms that have been studied so far only in hair cells.

Both P2X and P2Y receptors are involved in the maturation-dependent purinergic signaling, with tonotopically different cellular distribution

ATP is an endogenous agonist at P2X and P2Y receptors. In order to study the role of P2Y receptors, UTP, a P2Y agonist, was added to the perfusion during a critical period of development.

UTP-induced Ca^{2+} transients were also age-dependent. At the same time, values were less than the ATP evoked signals. Thus, both receptor subtypes are thought to play a role in ATP-mediated signaling in developing Deiters' cells. This is independent of the cell's position.

The two turns have different P2X and P2Y profiles. ATP-evoked responses are greater in the middle turn, while UTP-evoked responses are larger in the apical turn.

6. Publications

The thesis is based on these publications:

Berekméri, E., Deák, O., Téglás, T., Sághy, É., Horváth, T., Aller, M., Fekete, Á., Köles, L., Zelles, T., 2019. Targeted single-cell electroporation loading of Ca2+ indicators in the mature hemicochlea preparation. Hear. Res. 371, 75–86. https://doi.org/10.1016/j.heares.2018.11.004

Berekméri, E., Fekete, Á., Köles, L., Zelles, T., 2019. Postnatal Development of the Subcellular Structures and Purinergic Signaling of Deiters' Cells along the Tonotopic Axis of the Cochlea. Cells 8. https://doi.org/10.3390/cells8101266

Berekméri, E., Szepesy, J., Köles, L., Zelles, T., 2019. Purinergic signaling in the organ of Corti: Potential therapeutic targets of sensorineural hearing losses. Brain Res. Bull. 1–10. https://doi.org/10.1016/j.brainresbull.2019.01.029

Köles, L., Szepesy, J., Berekméri, E., Zelles, T., 2019. Purinergic Signaling and Cochlear Injury-Targeting the Immune System? Int. J. Mol. Sci. 20, 2979. https://doi.org/10.3390/ijms20122979

Other(s):

Kalász, H., Tekes, K., Faigl, E.B., Pöstényi, Z., Berekméri, E., Karvaly, G., Adeghate, E., 2017. Monitoring the Level of 14C-Labelled Selegiline Following Oral Administration. Open Med. Chem. J. 11, 1–8. https://doi.org/10.2174/1874104501711010001