Physiological role of extracellular ATP in the inner ear

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Painfully sustained loud sound (90-120 dB, >6h) causes protective or withdrawal responses, rather than continued listening. Purinergic P2X receptors are ligand-gated cation permeable channels which mediate adaptation to loud sounds. In the cochlea, extracellular ATP-activated P2X2 receptor channels plays an important role in adaptation to elevated sound by producing a cation shunt that reduces the endolymphatic potential and the driving force for sound transduction. These receptors are expressed in the cochlear hair cells and the supporting cells and Reissner's membrane. There has been no clear evidence that how extracellular ATP modulates the function of cochlea in regulating cochlear sensitivity in order to provide protection during loud sound exposure.

In this study, we have investigated the physiological role of extracellular ATP upon activation of ATP gated cation P2X receptor channels in the inner ear. To answer that, we performed physiological and electrophysiological recordings in combination with confocal imaging, and measurements of loud trauma.

Temporal bones were excised from young normal hearing guinea pigs, and a small opening was made at the cochlea's base and at the apex. Opening at the cochlea base allowed to perfuse ATP (1.0 mM). and Yo Pro dye (5 µM). Micro electrodes were used for cochlear microphonic (CM), endocochlear potential (EP), summating potential (SP) recordings, electrical stimulation, staining of the bundle membrane with the membrane dye Di-3-ANEPPDHQ, and delivery Yo Pro dye and ATP. In order to visualize the sound-evoked motion of stereocilia, sequences of confocal images were acquired during sound stimulation. The extracellular potentials are tuned to a particular sound stimulus of 84 dB SPL frequency near 200 Hz to get a maximum response. The acquired image sequences were low-pass filtered and motion quantified through optical flow analysis using Matlab. High-resolution quantitative confocal imaging was performed on a Zeiss LSM 780. Sound-evoked motion of the hearing organ at Hensen cells level was performed at laser interferometer combined with confocal imaging.

Through confocal imaging, we confirmed the localization of P2X receptor channels in the mature guinea pig hearing organ displaying a strong distribution in the Reissner's membrane and faint expression in Hensen's and hair cells. After application of ATP extracellularly in the organ of Corti via perfusion, a dramatic effect on sound-evoked electrical potentials and stereocilia defelction was observed. After removal of ATP, the effects disappeared. No effect was observed on the hearing organ motion at Hensen cells level. Structural changes in the hearing organ were observed at supporting cells. Loud sounds had effects on the release of ATP inside the organ of Corti. Our data shows the influence of extracellular ATP on sound-evoked responses in the perilymphatic compartment of the hearing organ.

Collectively, the results reveal distinct mechanism in cochlea on how extracellular ATP plays role in protecting the hearing organ from loud sound.