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Purinergic signaling in the organ of Corti: potential therapeutic targets of sensorineural hearing losses

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Highlights

- Supporting cells are important in the organ of Corti
- Both P1 and P2 purinergic receptors are widely distributed in the cochlea
- Purines are major regulators of supporting and epithelial cell functions
- Purinergic signalling modulates hearing sensitivity through different pathways
- Lowering hearing sensitivity protects in sensorineural hearing losses

Abstract

Purinergic signaling is deeply involved in the development, functions and protective mechanisms of the cochlea. Release of ATP and activation of purinergic receptors on sensory and supporting/epithelial cells play a substantial role in cochlear (patho)physiology. Both the ionotropic P2X and the metabotropic P2Y receptors are widely distributed on the inner and outer hair cells as well as on the different supporting cells in the organ of Corti and on other epithelial cells in the scala media. Among others, they are implicated in the sensitivity adjustment of the receptor cells by a K^+ shunt and can attenuate the cochlear amplification by modifying cochlear micromechanics acting on outer hair cells and supporting cells. Cochlear blood flow is also regulated by purines. Sensorineural hearing losses currently lack any specific or efficient pharmacotherapy. Decreasing hearing sensitivity and increasing cochlear blood supply by pharmacological targeting of purinergic signaling in the cochlea are potential new therapeutic approaches in these hearing disabilities, especially in the noise-induced ones.

Abbreviations:

ATP, adenosine triphosphate; RGS4, regulator of G-protein signaling 4; NIHL, noise induced hearing loss; SNHL, sensorineural hearing loss

Keywords:

purinergic signaling; organ of Corti; supporting cells; hearing sensitivity; sensorineural hearing losses; noise induced hearing loss

1. Introduction

The mammalian organ of Corti, the sensory epithelium of the hearing organ, is a spiralled structure located in the inner ear bony capsule called cochlea. The number of turns of the organ depends on the species, in mice it has two and a half turns (Keiler and Richter, 2001). Inside the ossified labyrinth, membranes divide the spiral canal into three functional compartments (membranous labyrinth, Fig. 1A, B). The upper (scala vestibuli) and the lower part (scala tympani) is filled with perilymph – an extracellular fluid with electrolyte composition resembling to that of the cerebrospinal fluid (Patuzzi, 2011a; Wan et al., 2013). The middle part (scala media) contains endolymph, with high K^+ and low Na^+ concentration, similar to the intracellular solution (Patuzzi, 2011a; Wan et al., 2013). The endolymph is about 80-100 mV more positive than the perilymph (endocochlear potential). The electrochemical difference is even larger between the endolymph and the intracellular space of the hair cells at their resting membrane potential: the inside of the hair cells is about 125-145 mV more negative than the endolymph (Patuzzi, 2011a). This electrochemical gradient generates the force for the ion-flow into the hair cells thereby leading to their depolarization during activation (Patuzzi, 2011b, 2011a; Wangemann, 2006).

The organ of Corti (Fig. 1.) lies on the basilar membrane separating the endo- and the perilymphatic compartment. The cells are surrounded by the perilymph, but the stereocilia of the hair cells are bathed in the endolymph. The two different compartments are separated by the reticular lamina which is formed by the apical parts of the cells connected by tight junctions establishing a barrier between the endo- and perilymphatic fluid compartments (Fig. 1B). The three rows of outer hair cells (OHCs) and the one row of inner hair cells (IHCs) are surrounded by different types of supporting cells. These non-sensory cells support the hair cells mechanically and separate them from each other and from the basilar membrane. Supporting cells in the organ of Corti and other epithelial cells in the scala media had been considered solely as structural and metabolic elements, but growing evidence indicates their important role in the development of the organ, the regulation of ion homeostasis of the endo- and perilymph, the modulation of hearing sensitivity and in the control of responses to insults damaging the cochlea. Cochlear damage leads to different sensorineural hearing losses (SNHLs), like noise (NIHL) or ototoxic drug induced hearing losses and age-dependent hearing loss. Similarly to supporting cells of the nervous system, i.e., glial cells, the role of purinergic system in the functions of cochlear supporting and other epithelial cells has been clearly emerged. Here we overview the purinergic signaling in the organ of Corti, with special

emphasis on the supporting cells, and the possible therapeutic targets this purinergic regulation provides for curing or preventing SNHLs.

2. Supporting cells of the organ of Corti

The central axis in the cochlea is the modiolus (Fig. 1A). The modiolar side of the organ of Corti is built up by cuboidal epithelial cells forming the inner sulcus. Next to the inner sulcus starts the organ of Corti (Fig. 1B and C) where the single line of inner hair cells is covered by the inner border and the inner phalangeal supporting cells. In the middle of the organ the tunnel of Corti is bordered by the two (inner and outer) pillar cells. Lateral to them the three rows of outer hair cells are supported by three rows of Deiters' cells. Deiters' cells have a special polarized shape: their soma contacts and supports the basis of an outer hair cell while their phalangeal processes pass by the side of the OHCs and connect the apical part of them, participating in the formation of the reticular lamina. The processes are not connected to the same OHC as the soma, usually they touch receptor cells located two lines towards the apical turn in mice (Richard P Bobbin, 2001; Chen et al., 2018; Parsa et al., 2012; Zetes et al., 2012). The phalangeal processes are especially rich in microtubule bundles. Based on their accurate localization and angle, they are supposed to be important mechanical elements of the organ of Corti (Chen et al., 2018; Jensen-Smith et al., 2003; Zetes et al., 2012). Deiters' cells also receive efferent innervation (Burgess et al., 1997; Nadol and Burgess, 1994; Raphael and Altschuler, 2003). The inner border, inner phalangeal and Deiters' cells are thought to mediate the growth and formation of the tectorial membrane (Wan et al., 2013; Xu et al., 2016)**,** which leans over the sensory epithelium and causes the deflection of stereocili of the hair cells, thereby activating them.

Lateral to the outer hair cells Hensen's cells close the classical organ of Corti structure. The Claudius and Boettcher cells, located further lateral in the outer sulcus (Fig. 1B and C) are considered by many researchers as part of the organ of Corti as they are also involved in one of the most important supporting cell function, the K^+ spatial buffering pathway (see below in 5.1). The lateral wall of the turns is covered by the marginal cells of the stria vasularis. This structure is responsible for the production of the endolymph (Ciuman, 2017; Patuzzi, 2011a; Rybak et al., 1992).

Supporting cells have been considered promising targets in gene therapy and regenerative medicine (Bermingham-McDonogh and Reh, 2011; Devare et al., 2018; Liu et al., 2012; Mellado Lagarde et al., 2014; Monzack and Cunningham, 2013; Wan et al., 2013).

3. Purinergic receptors in the cochlea

Purinergic signaling is involved in the proper functioning of virtually all organs of the body. ATP is released from healthy cells and considered as an important messenger molecule and modulator in cell-cell communication in the central and peripheral nervous system, in neurons, neuron-glia or glia-glia communications (Burnstock, 1972, Fields and Burnstock, 2006; Köles et al., 2011). Besides well-documented effects in the cardiovascular, gastrointestinal, genitourinary, respiratory and nervous systems ATP has been also proposed to play an important role in the afferent auditory neurotransmission by acting as either a neurotransmitter or a neuromodulator (Gary D Housley et al., 1999; Housley et al., 2009). However, pathologically high concentrations of ATP (e.g. from injured or dying cells) can also initiate and aggregate harmful mechanisms leading to further destruction and damage, thereby the purinergic mechanisms can also provide a possibility for therapeutic interventions (Burnstock et al., 2011; Köles et al., 2005).

Several types of purinergic receptors have been detected in the cells of the organ of Corti which could mediate both physiological and pathophysiological actions. Some of them show age and species dependent expression patterns (Table 1.) which hinders the elucidation of their role in the hearing system. One of the most widely expressed purinergic receptor in the hearing organ is the P2X2 subtype. Its presence has been demonstrated in different species, in various ages and with different methods both on sensory cells (Glowatzki et al., 1997; G D Housley et al., 1999; Housley et al., 2013, 1998, Järlebark et al., 2002, 2000; Parker et al., 1998; Salih et al., 1999; Sueta et al., 2003; Szücs et al., 2004; Telang et al., 2010; Wang et al., 2003; Yan et al., 2013; Zhao et al., 2005b) and on supporting cells (Chen and Bobbin, 1998; G D Housley et al., 1999; Housley et al., 1998; Järlebark et al., 2002, 2000; Parker et al., 1998; Salih et al., 1999; Telang et al., 2010; Wang et al., 2003; Zhu and Zhao, 2010). This receptor subtype seems to be located on the endolymphatic surface of the cells and on the stereocilia of the hair cells. Other ionotropic purinergic receptors showed more restricted, often strong age-dependent expression profile. P2X3 subtype is typically expressed on hair cells around the period of synaptic maturation in rodents (Huang et al., 2005; Xiang et al.,

1999). P2X1 and P2X4 have been reported to be present only on isolated guinea pig outer hair cells (Szücs et al., 2004). Other studies demonstrated that P2X1 had transient expression profile during the development, but it was limited to structures out of the organ of Corti (i.e. Reissner's membrane, spiral limbus) and the spiral ganglion neurones between P0-P6 (Nikolic et al., 2001). The P2X7 subtype, which is typically activated by high concentration of ATP and hypothesised to be involved mostly in pathological processes, is also present in the hearing organ. It has been found to be expressed in guinea pig OHC (Szücs et al., 2004), in mature rat IHC (Nikolic et al., 2003) and on supporting cells of both species (Nikolic et al., 2003; Zhu and Zhao, 2010).

The metabotropic P2Y receptor subtypes are also expressed in the hearing organ. P2Y4 is widespread in all developmental stages. It has been detected both on hair cells (Huang et al., 2010; Parker et al., 2003; Szűcs et al., 2006) and on supporting cells (Huang et al., 2010; Parker et al., 2003; Piazza et al., 2007). P2Y1, P2Y2 and P2Y6 have been found on rat and guinea pig OHC (Huang et al., 2010; Szücs et al., 2004) while IHCs were positive for P2Y12 but not for P2Y1 in rat (Huang et al., 2010). Supporting cells around IHC were not specifically tested for P2Y receptor subtypes, but several functional data support the view that they express them (Tritsch et al., 2010, 2007; Tritsch and Bergles, 2010). P2Y2 and P2Y12 have been detected on unmatured pillar cells, next to the P2Y4 (Huang et al., 2010; Piazza et al., 2007). Matured PCs were additionally positive for P2Y6 but not for P2Y12 anymore (Huang et al., 2010). Both DCs and HCs expressed P2Y2 receptor subtypes before hearing onset, but not after that (Huang et al., 2010). In contrast, outer sulcus cells remained P2Y2 positive after maturation and started to express P2Y1 after the hearing onset in rat (Huang et al., 2010).

4. Purinergic regulation during the development of the organ of Corti

4.1. Synaptic maturation

Purinergic receptors in the immature cochlea start to be expressed around the second embryonic week and their expression profile and level change until the end of the maturation on the second postnatal week (Table 1) (Brändle et al., 1999; Chen et al., 2000; G D Housley et al., 1999; Huang et al., 2010, 2006, Nikolic et al., 2003, 2001; Parker et al., 2003; Wang et al., 2003; Xiang et al., 1999).

From birth (P0) to the onset of hearing (~P15 in mice) the auditory neurons fire spontaneous action potentials (Dayaratne et al., 2014; Kreinest et al., 2009; Searchfield et al., 2004; Tritsch et al., 2007; Tritsch and Bergles, 2010; Wang et al., 2015). The immature hair cell-neuron synapses are covered by the neighbouring supporting cells. In this period of the development the rodent organ of Corti is not functional and transient anatomical structures are present: medial to the pillar cells the Kölliker's organ or greater epithelial ridge (GER), lateral to the pillar cells the lesser epithelial ridge. The supporting cells of the GER have high columnar shape and hypothesised to cause the spontaneous activity observed in the whole auditory pathway during the development (Dayaratne et al., 2014; Dietz et al., 2012; Tritsch et al., 2007). The spontaneous activity has the highest frequency around P10-12 during synapse maturation (Dayaratne et al., 2015, 2014; Tritsch and Bergles, 2010). The expression of the purinergic receptors also reaches the highest level to this time, and then it drops to the adult level (Housley et al., 1998).

Supporting cells in GER release ATP spontaneously, which by acting on purinergic receptors of the hair cells, depolarizes them (Tritsch et al., 2007; Zhao et al., 2005a). The depolarized hair cells release glutamate and activate the spiral ganglion neurons (SGNs) promoting their survival and the maturation of the hair cell-primary auditory neuron synapses (Delacroix and Malgrange, 2015; Housley et al., 2002; Huang et al., 2006; Jovanovic et al., 2017; Robertson and Paki, 2002) (Fig. 2.). Several studies indicated that the release of glutamate from the hair cells is necessary for the activation of the SGNs (Tritsch et al., 2010, 2007). Furthermore, these neurons also express purinergic receptors, especially of the P2X2, P2X3 subtypes and the P2X2/3 heteromers (Brändle et al., 1999; Greenwood et al., 2007; Huang et al., 2005; Salih et al., 1999, 1998; Telang et al., 2010; Wang et al., 2003; Xiang et al., 1999) (Fig. 2.). The cultured embryonic SGNs also exhibit spontaneous activity and they can be activated by ATP or ADP (Greenwood et al., 2007). The ADP activation indicates functional expression of P2Y receptors on the SGNs, which is also supported by immune labelling (Huang et al., 2010).

During maturation some synapses are strengthened while others are eliminated. These processes are driven by growth factors released by the active synapses (Dulon et al., 2006; Greenwood et al., 2007; Johnson Chacko et al., 2017; Pirvola and Ylikoski, 2003). P2X2 receptor activation inhibited the brain derived neurotrophic factor (BDNF) expression as well as BDNF-induced neurite outgrowth and branching in the SGNs in cultured neurons (Greenwood et al., 2007). Hence, ATP secreted in different compartments of the cochlea can

regulate the synapse maturation in opposing way: ATP released from supporting cells into the endolymphatic space activates hair cells and promotes the strengthening of the synapses, whilst ATP in the perilymphatic compartment acts directly on the SGNs and could lead to the elimination of the weaker synapses.

4.2. Tonotopy

ATP released from the supporting cells, possibly through connexin or pannexin hemichannels (Forge et al., 2013, 2002; Gossman and Zhao, 2008; Lazarowski, 2012; Majumder et al., 2010; Piazza et al., 2007; Wang et al., 2009), acts as a paracrine mediator molecule on neighbouring supporting and hair cells. It results in the synchronized depolarization of neighbouring hair cells and their glutamate release with the consequent activity of SGNs located nearby. The ATP-mediated coordination of primary auditory neuron firing contributes to the proper organization of the auditory system (Dale, 2008; Dayaratne et al., 2014).

5. Purinergic regulation of hearing sensitivity

5.1. K^+ shunt

As mentioned above, the peri- and endolymphatic compartments differ in electrolyte composition (Patuzzi, 2011a; Wan et al., 2013). The resultant endocochlear potential is the external driving force for cations causing hair cell activation (D. J. B. Muñoz et al., 1995a; Rybak et al., 1992). The deflection of the stereocilia opens the mechanoelectrical transduction channels and cations (especially K^+) enters the cell (Housley et al., 2006; Wangemann, 2006; Zhu and Zhao, 2010). This K^+ will be removed to the perilymph by voltage gated K^+ channels located in the lateral membranes of the cell. The locally elevated K^+ concentration is reduced by the supporting cells and passed to the neighbouring cells via gap junctions (Szűcs et al., 2006; Wangemann, 2006; Zhu and Zhao, 2010). K^+ flows to the lateral wall of the cochlea and the cells of the stria vascularis can resecrete it to the endolymph (Okamura et al., 2001; Rybak et al., 1992; Zhu and Zhao, 2010) (Fig. 3.).

In physiological conditions the ATP concentration in the endolymph and perilymph is about 10 nM which can be elevated to the micromolar range in case of harmful stimuli (e.g. noise, hypoxia, ischaemia) (D. J. B. Muñoz et al., 1995a). K^+ can enter the cells lining the endolymphatic compartment through P2X channels activated by the elevated ATP

concentrations. As a result, K^+ concentration can be reduced in the endolymph resulting in a decrease in the endocochlear potential and hearing sensitivity (Housley et al., 2013, 2002; D. J. B. Muñoz et al., 1995b; Telang et al., 2010; Thorne et al., 2002; Wangemann, 2006) (Fig. 3.). ATP can also disconnect the gap junction coupling between the supporting cells and inhibit voltage gated channels thereby disrupting the K^+ recycling through the supporting cells to the stria vascularis thus helping K^+-sinking (Eckhard et al., 2012; Ye et al., 2016; Yu and Zhao, 2009; Zhu and Zhao, 2012).

In case of harmful stimuli purinergic receptors can be both up- and down-regulated. Indeed, chronic noise exposure elevated the P2X2 subunit expression in hair and supporting cells, as well as in SGNs in mice, guinea pigs and rats (Chen et al., 1995; Wang et al., 2003). Furthermore, ischemia and noise induced trauma usually shifts the pH to acidic levels and most of the purinergic receptors are potentiated by acidic pH. ATP induced ion currents in isolated Deiters' cells were almost duplicated at pH 6.5 (Kanjhan et al., 2003). This phenomenon may facilitate the protective effect of ATP (decrease in the hearing sensitivity).

5.2. Membrane rigidity

Cochlear (mechanical) amplification of acoustic signals is a common feature of mammalian hearing. Prestin, a membrane protein of OHCs, and prestin-based electromotility are key factors in cochlear amplification (Mahendrasingam et al., 2010; Xia et al., 2013; Yu and Zhao, 2009). Prestin is located in the lateral wall of OHCs and converts the electricity into motion as a piezo crystal. OHC motility enforces the activation of the IHCs whose threshold is higher than that of the OHCs. This amplification process decreases the hearing threshold by about 40 dB and improves frequency selectivity (Fukazawa, 2002; Nam and Fettiplace, 2012; Zhu et al., 2013).

ATP induced cytoskeleton reorganization in PC12 cells (Homma et al., 2008). Formation of cofilin rods – actin filament bindig protein – was driven by the elevation of intracellular Ca^{2+} concentration evoked by the administration of ATP or UTP. Hence, both P2X and P2Y receptor subtypes can be involved in actin filament rebuilding.

Considering that the activation of purinergic receptors can directly influence cell morphology and rigidity, they may affect the cochlear amplification by decreasing the OHC motility and thereby increasing the inner hair cell threshold level. Indeed, extracellular ATP induced

movement of phalangeal processes of isolated Dieters' cells (Richard P Bobbin, 2001). Contraction of the phalangeal process can directly modulate OHC electromotility through Dieters' cells-OHC mechanical coupling. ATP also evoked an inward current in supporting cells, including the Deiters' cells (see the ATP-induced K^+-sink above), that may regulate the OHC electromotility via the Deiters' cells (Yu and Zhao, 2009). In acute preparations, as the hemicochlea, this motion was not observed (T. Horváth et al., 2016), probably because of the strong coupling between the cells which limits the detection of visible motion of the cellular compartments.

5.3. Intercellular Ca^{2+} waves

In case of hair cell damage elevated intracellular Ca^{2+} levels was detected in the neighbouring supporting cells which spread as Ca^{2+} waves to several 10-100 μm from the damaged area (Gale et al., 2004; Lahne and Gale, 2010; Piazza et al., 2007; Wong and Ryan, 2015). These intracellular Ca^{2+} level elevations are supposed to be caused by ATP (Berekméri et al., 2019; T Horváth et al., 2016) liberated from the injured cells. The waves could propagate in two ways: a) a faster wave implicates ATP release via hemichannels and activation of both P2X (i.e. P2X2 and/or P2X4) and P2Y (i.e. P2Y2) receptors of neighbouring cells, and b) a slower wave caused by the IP_3 flow through gap junctions activating internal Ca^{2+} stores of the neighbouring cells (Anselmi et al., 2008; Gossman and Zhao, 2008; Lahne and Gale, 2010, 2008; Majumder et al., 2010; Mistrík and Ashmore, 2010; Ogawa and Schacht, 1993; Piazza et al., 2007; Zhao et al., 2005a). Recently, this intercellular Ca^{2+} signaling was also recognized in adult mouse organ of Corti (Sirko et al., 2019). These intercellular Ca^{2+} waves are probably the early detectors of cochlear injury and also thought to be essential for the K^+ recycling through the supporting cells. Furthermore, their involvement in cell regeneration, apoptosis or synaptic maintenance was also suggested (Chan and Rouse, 2016; De Bock et al., 2014).

6. Possible medical targeting of the purinergic signaling in sensorineural hearing losses

Hearing impairment is the most common sensory deficit, affecting more than 360 million people worldwide (www.who.int/mediacentre/factsheets/fs300, WHO 2017). Deafness may occur at any age with any degree of severity. Hearing loss threatens personal autonomy

resulting in major difficulties in daily life and, ultimately, could lead to social isolation and mental depression. Contrary to the conductive hearing losses, specific pharmacotherapy is not available for the sensorineural forms (e.g., ototoxic drug- and noise-induced hearing loss or presbycusis). Noise-induced hearing loss (NIHL) is one of the most common form of SNHLs and its prevalence is increasing by changes in our recent music listening habits. Acute or chronic noise exposure can cause temporary shift of the auditory threshold but hearing loss frequently becomes permanent.

Human therapeutic targeting of purinergic signalling in the treatment of SNHLs needs preclinical data in animal models. The role of purinergic control is best characterized in sound sensitivity of the cochlea and in NIHLs (Morton-Jones et al., 2015; Srdjan M Vlajkovic et al., 2017).

Up- and down regulation of ATP receptors and elevation of the level of ectonucleotidases were all detected after noise exposure (Chen et al., 1995; Szűcs et al., 2006; Vlajkovic et al., 2004; Wang et al., 2003), suggesting that the purinergic system is involved in the machinery activated in hearing impairments caused by noise trauma. Functional in vivo experiments supported this idea, but also revealed some contradictions.

Purinergic ligands have been reported to be involved in protective mechanisms in response to noise stimuli. ATP perfusion into the endolymph in vivo decreased the endocochlear potential in both guinea pigs and mice. This decrease, supposed to be protective through the reduction of the driving force on K^+ influx activating the hair cells, was blocked by PPADS or suramin confirming the role of ATP and the involvement of P2 receptors in the observed protective effect (D. J. Muñoz et al., 1995; Telang et al., 2010; Thorne et al., 2004). Diminished cochlear sensitivity after application of ATP and related agonists into the perilymph was also demonstrated on one hand as reduced cochlear potentials in electrocochleographic measurements (cochlear microphonic, summating potential and compound action potential) and on the other hand as suppressed values in distortion product otoacoustic emission (DPOAE), indicative of the OHC-driven cochlear amplification (S G Kujawa et al., 1994). Furthermore, infusion of ATP into the perilymph also facilitated the recovery from the temporary shift of hearing threshold evoked by acoustic trauma (Sugahara et al., 2004). Endolymphatic ATP can reduce hearing sensitivity by the K^+ shunt conductance through P2X2 receptors on a range of epithelial cells facing the scala media (Housley et al., 2013), while perilymphatic ATP can reduce that by modifying the cochlear micromechanics acting on supporting cells and OHCs (Chen et al., 1998; Housley et al., 2002; Yu and Zhao, 2009).

Interestingly, in other reports suppression of the cochlear potentials and DPOAE were evoked not by ATP, but by the perilymphatic administration of its antagonists PPADS and suramin (Chen et al., 1998; S. G. Kujawa et al., 1994). LeBlanc and Bobbin (LeBlanc and Bobbin, 1999) also showed that the cochlear potential suppression effect of perilymphatic PPADS was similar to that effect of moderately intense sound exposure and the combination of PPADS and noise produced additivity and potentiation. While Bobbin (R P Bobbin, 2001) demonstrated the protective effect of perilymphatic administration of PPADS against moderately intense sound, using DPOPAE measurements.

Drawing a clear conclusion is difficult because the effect of ATP probably depends on i) its concentration and way of administration (peri- or endolymphatic), ii) on the intensity of the noise exposure iii) on the experimental method used to assess the function of hearing and the ATP effect is mediated by iv) the diversity of the purinergic receptor subtypes located at different sites in the cochlea. Predominantly otoprotection seems to be the primary effect of ATP and the P2 receptor agonists.

Unfortunately, the enthusiasm in research focusing on P2 receptor mediated mechanisms and exploration of P2 receptor based pharmacotherapeutic targets were reduced in the last years - no new in vivo result was published recently. Besides the necessary advances in purinergic pharmacology, new impulses are needed to revitalize this research field.

The P1 adenosine receptors – mostly A1 and A2A on the Deiters' cells and inner hair cells as well as A3 on all types of the supporting and hair cells – are also widely distributed in the cochlea (Ford et al., 1997b, 1997a; Nehlig et al., 1994; Ramkumar et al., 2004, 1994, Tabuchi et al., 2012, 1999, Vlajkovic et al., 2010, 2007; Whitworth et al., 2004). In various hearing impairments activation of adenosine receptors were indicated to be preventive. Up-regulation of adenosine receptors was detected after noise exposure and in response to perfusion of cisplatin (an ototoxic chemotherapeutic drug) in the perilymph of guinea pigs (Ford et al., 1997b; Ramkumar et al., 2004). Cisplatin-induced ototoxicity was prevented by A1 agonists CCPA and R-PIA, which in turn was reversed by the A1 antagonist DPCPX. However, the A2A agonist CGS-21680 significantly increased the cisplatin-induced threshold changes (Whitworth et al., 2004). Excitotoxicity caused by applying kainic acid into the perilymph was also attenuated by A1 agonists, but not effected by A2A agonists (Tabuchi et al., 2012). The idea that adenosine receptor activation recruits protective mechanism against the increased level of reactive oxygen species was supported by the elevation of superoxide

dismutase, catalase or glutathione peroxidase activity detected in different models (Ford et al., 1997b, 1997a; Whitworth et al., 2004).

The increase of cochlear blood flow by adenosine could contribute to its protective effect. It was shown that adenosine and ATP increased significantly the cochlear blood flow in guinea pigs measured by laser Doppler flowmetry on the bone overlying the stria vascularis (Muñoz et al., 1999). It is well substantiated that loud sound exposure decreases cochlear blood flow and the oxygen tension (pO_2) of the endolymph contributing probably to the cochlear damage in NIHLs (Okamoto et al., 1990; Thorne and Nuttall, 1989, 1987). Atherosclerosis or other disease of the inner ear supplying labyrinthine artery or of the stria vascularis may have similar effect (Ciccone et al., 2012; Tanigawa et al., 2014). Administration of an A1 adenosine receptor agonist i.p. exerted a protective effect on the cochlear injury induced by transient occlusion of the labyrinthine artery (Tabuchi et al., 1999).

Susceptibility to NIHL is also influenced by adenosine. In A1A receptor knock-out mice a 2 h intense noise exposure caused higher threshold shifts and decrease in amplitudes of the primary auditory neurons in ABR measurements as well as enhanced loss of OHCs and synapses compared with wide-type and A2A receptor knock-out mice. A2A receptor null mice, however, showed increased cochlear resistance to the acoustic trauma (Srdjan M. Vlajkovic et al., 2017). In a “similar to life” pilot study adenosine proved to be a promising future target. Guinea pigs were exposed to noise and then caffeine – a non-selective adenosine receptor antagonist – was administered for 2 weeks. Caffeine inhibited the spontaneous regeneration of the hearing thresholds indicating that adenosine has important effects in the regeneration from harmful-stimuli (Mujica-Mota et al., 2014). A selective A1 receptor agonist, an adenosine amine congener has been proposed as a cochlear rescue agent. It can reduce noise- and cisplatin-induced cochlear injury in rodents and inhibit the hair cell loss in a time window after the exposure of the harmful effects (Vlajkovic et al., 2014, 2010). In a rat model after intravenous administration it was able to enter the perilymph and reach the therapeutic concentration there (Chang et al., 2017). Despite these promising results, the therapeutic use of direct adenosine receptor ligands is limited by their cardiovascular adverse effects such as vasodilation and heart blocks. This handicap could be bypassed by a tissue-specific intervention at the intracellular level of adenosine receptor signaling (Chen et al., 2012). Bogosanovich from the Thorne lab at the University of Auckland (Masters thesis, http://hdl.handle.net/2292/27523) reported the protective role of CCG-4986, a regulator of G-protein signaling 4 (RGS4) protein inhibitor against acoustic trauma. RGS proteins limit and

shorten the response of heterotrimeric G-protein coupled receptors, like A1 adenosine receptors. Tissue-specific expression of RGS4 subtype, including in spiral ganglion neurons, supporting and sensory cells in the cochlea, provides the possibility of enhancing the action of endogenous adenosine on A1 receptor by disinhibition, i.e., inhibiting the limiting factor of its intracellular signaling pathway. The approach skips the administration of adenosine receptor ligands and their possible adverse effects on the cardiovascular system.

Conclusion

Supporting cells – besides their structural and homeostatic functions – are also important in the development and regulation of the sensitivity of the hearing organ. Both P1 and P2 receptors are expressed in the sensory, supporting and other epithelial cells in the cochlea and probably involved in their reactions to different – both physiological and pathophysiological – stimuli. Numerous subtypes of P2X and P2Y receptors, the lack of selective ligands, their species and age dependent expression may explain the puzzling experimental results and hinders the elucidation of their precise role in hearing (patho)physiology. It also hampers the utilization of drugs targeting them in hearing disorders. Adenosine A1 receptor seems to be the most promising purinergic target to treat hearing impairments. However, some problems must be still solved. Especially pharmacokinetic properties of drugs are a serious issue in the treatment of hearing losses because of the special anatomy of the inner ear and the different barriers (Sun and Wang, 2015) limiting drugs in reaching their targets.

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Figure legend

Figure 1. Anatomy of the cochlea and the organ of Corti. Schematic illustration of the cochlea (A), organ of Corti (B) and a representative image of an adult mouse hemicochlea preparation visualized by oblique illumination (C; (Berekméri et al., 2019; T Horváth et al., 2016)). The cochlea is divided into three chambers (scalae) by two membranes. The organ of Corti is located in the scala media. The three rows of outer hair cells (OHCs) and the one row of inner hair cells (IHCs) are surrounded by different types of supporting cells. The cells are surrounded by the perilymph, but the stereocilia of the hair cells are bathed in the endolymph. The reticular lamina is formed by the apical parts of the cells establishing a barrier between the endo- and perilymphatic fluid compartments. The basilar membrane separates the scala media and tympani. Supporting cells (IBCs, IPhCs, IPC, OPC, DCs, HCs, CCs, BCs) span through the cellular layer of the organ while hair cells (IHC and OHCs) are not in direct contact with the basilar membrane (BM), but their stereocilia reaches the tectorial membrane (TM). ISCs: inner sulcus cells; IBCs: inner boarder cells; IPhCs: inner phalangeal cells; IPC: inner pillar cell; OPC: outer pillar cell; DCs: Deiters' cell; HCs: Hensen's cell; CCs: Claudius' cells; BCs: Boettcher cells. IHC and OHCs: inner and outer hair cells; SGNs: spiral ganglion neurons.

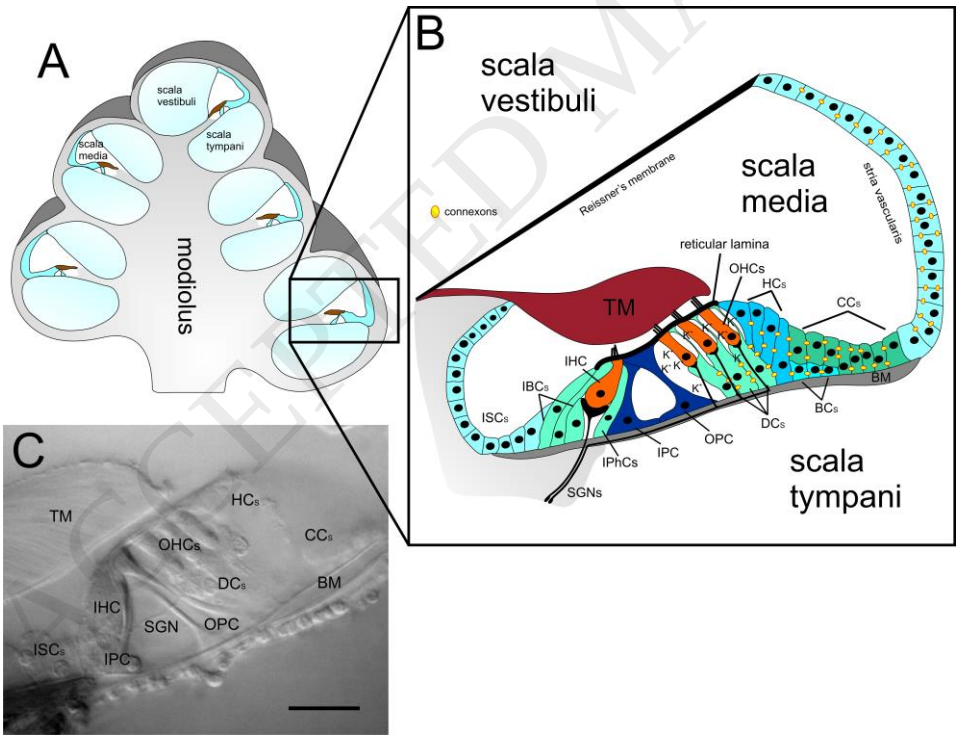

Figure 2. Purinergic receptors are involved in spontaneous activity of hair cells and spiral ganglion neurons in the immature cochlea. Supporting cells (the columnar shaped cells) of the Kölliker's organ initiate spontaneous activity observed in hair cells (bottle shape cell) and

ganglion neurons during development. ATP, released through hemichannels from the adjacent supporting cells activates ionotropic purinergic receptors expressed on the IHCs. The evoked depolarization releases glutamate from hair cells causing action potential firing in the contacting ganglion cells. The firing activity in the neurons promotes synapse formation and maintenance and contributes to the correct organization of the auditory system. Supporting cells also express purinergic receptors through which they modulate each other's activity.

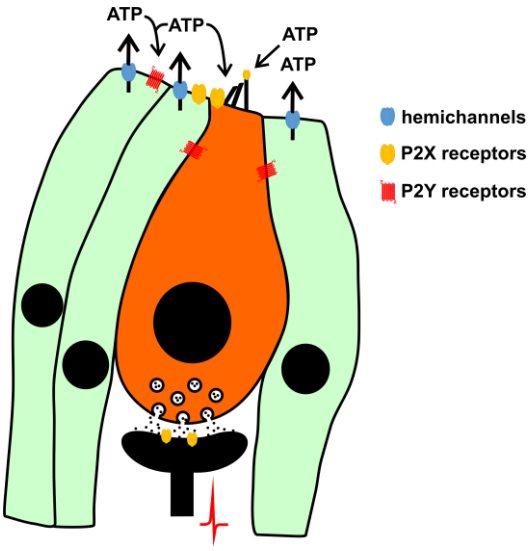

Figure 3. K^+ shunt in the cochlea and its regulation by the purinergic system. The high K^+ concentration and positive potential of the endolymph drives K^+ into the hair cells upon deflection of their stereocilia and opening of their mechanoelectrical transduction channels. K^+ is removed from hair cells to the perilymph by voltage gated K^+ channels. The locally elevated K^+ concentration is reduced by the adjacent supporting cells which pass the K^+ to the neighbouring cells via gap junctions. K^+ flows to the lateral wall of the cochlea and the cells of the stria vascularis can resecrete it to the endolymph. In response to noise trauma ATP concentrations in the endolymph is elevated from the nanomolar to the micromolar range. Activation of P2X channels on cells lining the endolymphatic compartment is resulted in K^+ entry and reduced K^+ concentration in the endolymph. The consequent decrease in the endocochlear potential reduces the sensitivity of hearing. ISCs: inner sulcus cells; IBCs: inner boarder cells; IPhCs: inner phalangeal cells; IPC: inner pillar cell; OPC: outer pillar cell; DCs: Deiters' cell; HCs: Hensen's cell; CCs: Claudius' cells; BCs: Boettcher cells. IHC and OHCs: inner and outer hair cells; SGNs: spiral ganglion neurons.

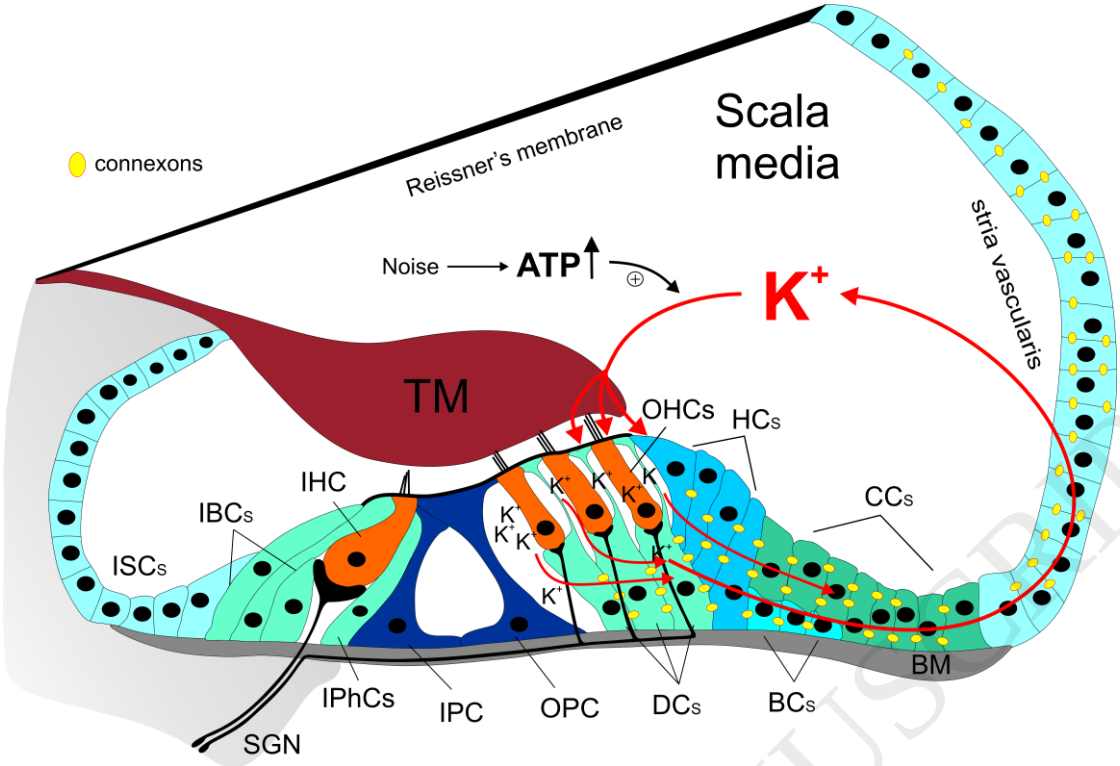

Table legend:

Table 1.: Expression of purinergic receptors in the cells of the organ of Corti before and after hearing onset (P15) in rodents and guinea pigs. It includes only subtype and cell specific results. mRNA detection means PCR analysis and in situ hybridization, protein detection means Western-blot analysis and immunohistochemistry, while functional detection indicates pharmacological analysis in electrophysiological or Ca^{2+} imaging experiments. Results from guinea pigs are not separated by age as these animals have *in utero* hearing.

cell type	species	detected	P0-15	>P15
outer hair cell	guinea pig	mRNA	P2X2, P2X7 (Parker et al., 1998)	
		protein	P2X1, P2X2, P2X4, P2X7; P2Y1, P2Y2, P2Y4 (G D Housley et al., 1999; Parker et al., 2003; Shen et al., 2005; Szücs et al., 2004; Szűcs et al., 2006; Zhao et al., 2005b)	
		function	P2X2 (Sueta et al., 2003; Zhao et al., 2005b)	
	rat	mRNA	P2X2 (Housley et al., 1998)	P2X2 (Housley et al., 1998; Wang et al., 2003)
		protein	P2X2, P2X3; P2Y1, P2Y2, P2Y4, P2Y6, P2Y12 (Huang et al., 2010; Järlebark et al., 2000; Salih et al., 1999)	P2X2; P2Y1, P2Y4, P2Y6 (Huang et al., 2010; Järlebark et al., 2000)
	mouse	protein	P2X2, P2X3 (Huang et al., 2006; Järlebark et al., 2002; Yan et al., 2013)	P2X2 (Telang et al., 2010; Yan et al., 2013)
		function	P2X2 (Glowatzki et al., 1997; Järlebark et al., 2002; Yan et al., 2013)	P2X2 (Housley et al., 2013; Yan et al., 2013)
inner hair cell	guinea pig	mRNA	P2X2 (Parker et al., 1998)	
		protein	P2X2; P2Y4 (G D Housley et al., 1999; Parker et al., 2003)	
		function	P2X2 (Shen et al., 2005; Sueta et al., 2003)	
	rat	mRNA	P2X2 (Housley et al., 1998)	P2X2 (Housley et al., 1998; Wang et al., 2003)
		protein	P2X2, P2X3, P2X7; P2Y2, P2Y4, P2Y6, P2Y12 (Huang et al., 2010; Järlebark et al., 2000; Salih et al., 1999)	P2X2, P2X7; P2Y4 (Huang et al., 2010; Nikolic et al., 2003; Wang et al., 2003)
	mouse	protein	P2X3 (Huang et al., 2006; Järlebark et al., 2002)	-
		function	P2X2 (Järlebark et al., 2002)	P2X2 (Housley et al., 2013)
supporting cells of IHC	rat	mRNA	P2X2 (Housley et al., 1998)	P2X2 (Housley et al., 1998)
		protein	P2X2 (Järlebark et al., 2000)	P2X2 (Järlebark et al., 2000)
pillar cells	guinea pig	mRNS	P2X2 (Parker et al., 1998)	
		protein	P2X2; P2Y4 (G D Housley et al., 1999; Parker et al., 2003)	
		function	P2X7 (Zhu and Zhao, 2010)	
	rat	mRNA	P2X2 P2Y2, P2Y12 (Housley et al., 1998)(Huang et al., 2010)	P2X2, P2Y2, P2Y4, P2Y6 (Housley et al., 1998; Huang et al., 2010; Wang et al., 2003)
		protein	P2Y2, P2Y12 (Huang et al., 2010)	P2X2, P2X7; P2Y2, P2Y4, P2Y6 (Huang et al., 2010; Nikolic et al., 2003)
		function	P2Y2, P2Y4 (Piazza et al., 2007)	-
	mouse	protein	-	P2X2 (Telang et al., 2010)s
Deiters'cell	guinea pig	mRNA	P2X2 (Parker et al., 1998)	
		protein	P2X2; P2Y4 (G D Housley et al., 1999; Parker et al., 2003)	
		function	P2X2, P2X7 (Chen and Bobbin, 1998; Zhu and Zhao, 2010)	
	rat	mRNA	P2X2; P2Y2 (Housley et al., 1998; Huang et al., 2010)	P2X2 (Housley et al., 1998; Wang et al., 2003)
		protein	P2X2, P2X7; P2Y2 (Huang et al., 2010; Järlebark et al., 2000; Nikolic et al., 2003; Salih et al., 1999)	P2X2, P2X7 (Järlebark et al., 2000; Nikolic et al., 2003; Wang et al., 2003)
	mouse	protein	P2X2 (Järlebark et al., 2002)	P2X2 (Telang et al., 2010)
Hensen's cell	guinea pig	mRNA	P2X2 (Parker et al., 1998)	
		protein	P2X2; P2Y4 (G D Housley et al., 1999; Parker et al., 2003)	
		function	P2X7 (Zhu and Zhao, 2010)	
	rat	mRNA	P2X2; P2Y2 (Housley et al., 1998; Huang et al., 2010)	P2X2 (Housley et al., 1998)
		protein	P2X2; P2Y2 (Huang et al., 2010; Järlebark et al., 2000)	P2X2 (Järlebark et al., 2000)
		function	P2Y2, P2Y4 (Piazza et al., 2007)	-

	mouse	protein	P2X2 (Järlebark et al., 2002)	-
outer sulcus cells	guinea pig	function	P2X7 (Zhu and Zhao, 2010)	
	rat	mRNA	P2X2; P2Y2 (Housley et al., 1998; Huang et al., 2010)	P2X2; P2Y1, P2Y2 (Housley et al., 1998; Huang et al., 2010)
		protein	P2X2; P2Y2 (Huang et al., 2010; Järlebark et al., 2000)	P2X2; P2Y1, P2Y2 (Huang et al., 2010; Järlebark et al., 2000)
		function	P2X4; P2Y2, P2Y4 (Lahne and Gale, 2010; Piazza et al., 2007)	-