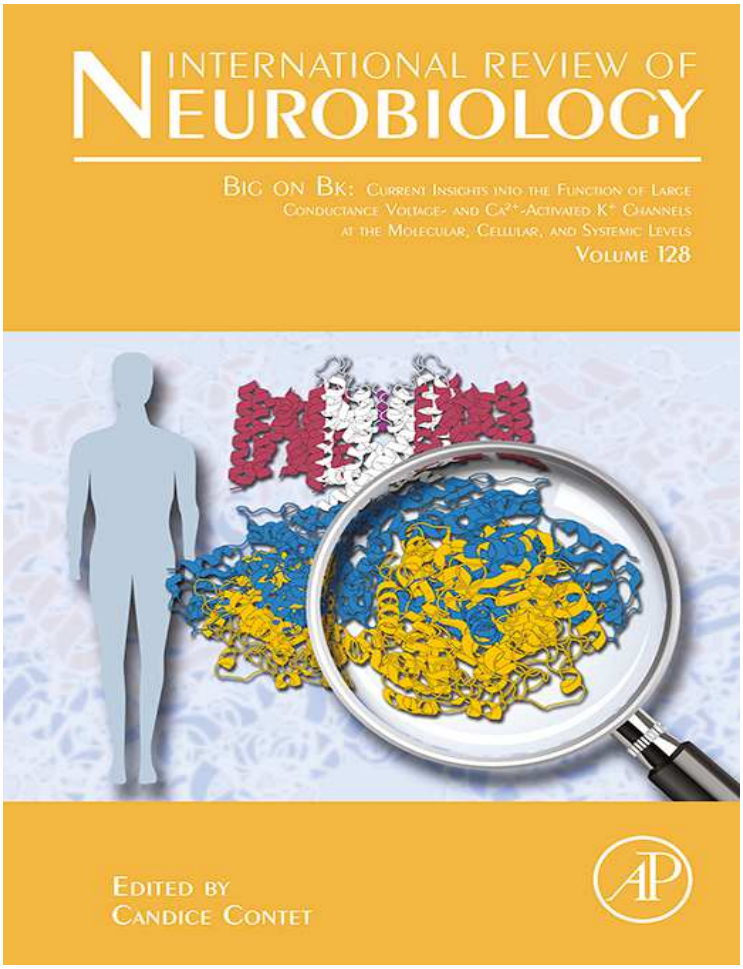

From S.J. Pyott and R.K. Duncan, BK Channels in the Vertebrate Inner Ear. In: Candice Contet, editor, *International Review of Neurobiology, Vol. 128*, Burlington: Academic Press, 2016, pp. 369-399.
ISBN: 978-0-12-803619-8

CHAPTER TEN

BK Channels in the Vertebrate Inner Ear

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Abstract

The perception of complex acoustic stimuli begins with the deconstruction of sound into its frequency components. This spectral processing occurs first and foremost in the inner ear. In vertebrates, two very different strategies of frequency analysis have evolved. In nonmammalian vertebrates, the sensory hair cells of the inner ear are intrinsically electrically tuned to a narrow band of acoustic frequencies. This electrical tuning relies on the interplay between BK channels and voltage-gated calcium channels. Systematic variations in BK channel density and kinetics establish a gradient in electrical resonance that enables the coding of a broad range of acoustic frequencies. In contrast, mammalian hair cells are extrinsically tuned by mechanical properties of the cochlear duct. Even so, mammalian hair cells also express BK channels. These BK channels play critical roles in various aspects of mammalian auditory signaling, from developmental maturation to protection against acoustic trauma. This review summarizes the anatomical localization, biophysical properties, and functional contributions of BK channels in vertebrate inner ears. Areas of future research, based on an updated understanding of

International Review of Neurobiology, Volume 128
ISSN 0074-7742
http://dx.doi.org/10.1016/bs.irn.2016.03.016

the biology of both BK channels and the inner ear, are also highlighted. Investigation of BK channels in the inner ear continues to provide fertile research grounds for examining both BK channel biophysics and the molecular mechanisms underlying signal processing in the auditory periphery.

1. INTRODUCTION TO THE AUDITORY PERIPHERY

All around us at any given moment is an ever-changing, complex auditory scene. In order to follow a friend's voice through a crowded room or segregate a bubbling brook from buzzing cicadas, the auditory system must separate rich acoustic signals into individual frequency components and transmit this information to the brain. This spectral analysis begins in the auditory periphery, primarily within the auditory sensory epithelium of the inner ear. The auditory epithelium is comprised of sensory hair cells, support cells, and the neurons that connect the hair cells to the central nervous system. A place code exists along this epithelium. Specifically, any one hair cell is tuned to a narrow band of acoustic frequencies in the audible spectrum. The location of the hair cells then creates a "tonotopic" or frequency–place map along the length of the epithelium. In all vertebrate species, the sensory hair cells and their neural connections are exquisitely organized in this tonotopic pattern, but the mechanisms involved in achieving frequency tuning of the individual hair cells are distinct between mammals and nonmammals.

In nonmammalian vertebrates, an intrinsic, electrical tuning mechanism is primarily responsible for resolving frequency information. Electrical tuning arises from electrical resonance in the hair cell's membrane potential. Current stimulation results in a damped oscillation of the membrane voltage response, and the frequency of this oscillation is systematically graded along the auditory epithelium. In this way, hair cells at the apex of the cochlea resonate at low frequencies and those at the base are tuned to high frequencies. The interplay between BK channels and voltage-gated calcium channels (VGCCs) are the primary determinants of this phenomenon, underscoring the importance of BK channels in auditory processing in these species. The structure and function of BK channels in electrical tuning is considered further in Section 2.

In contrast, mammalian hair cells are extrinsically or mechanically tuned by accessory structures of the inner ear. Although mammalian hair cells are not electrically tuned as they are in other vertebrates, BK channels

nevertheless carry a significant component of the total outward potassium conductances in these hair cells. Moreover, recent research has shown that the expression of BK channels is not restricted to the hair cells. In sum, a variety of evidence indicates that BK channels play critical roles in mammalian auditory signaling. BK channels in the mammalian inner ear are considered further in Section 3.

As in other systems, BK channels in the auditory system have been (sometimes confusingly) referred to by many names, including MaxiK, BK, BK_{Ca}, Slo1, $K_{Ca}1.1$ channels, and (in inner hair cells) $I_{K,f}$. Despite differences in nomenclature, these channels are encoded by the KCNMA1 gene and generally characterized by a large conductance to potassium and dual activation by depolarization and intracellular calcium. More general reviews of BK channels can be found elsewhere in this book (as well as Toro et al., 2014). As this chapter illustrates, the investigation of BK channels in the vertebrate inner ear has provided reciprocal insight into the fundamental molecular mechanisms underlying BK channel structure and function and systems-level auditory processing.

2. BK CHANNEL STRUCTURE AND FUNCTION IN NONMAMMALIAN AUDITORY ORGANS

2.1 Electrical Tuning of Nonmammalian Hair Cells

Auditory physiology in the 1970s was marked by a raging debate about the sources of tuning in the cochlea. Recordings from single auditory neurons revealed sharp, narrow-band tuning to sound stimulation (Kiang, 1965). Likewise, intracellular recordings from presynaptic hair cells were sharply tuned (Russell & Sellick, 1978). Yet, mechanical vibrations in the cochlear partition, that is, displacements of the basilar membrane, appeared poorly sensitive and broadly tuned (Evans & Wilson, 1975). The discrepancy suggested the actions of another amplifier somewhere within the sensory epithelium, and so the hunt for a "second filter" ensued. In many ways, this search provided the context for major discoveries about electrical and mechanical tuning mechanisms, including insight into the diversity and function of BK channels in the inner ear.

Over the next two decades, the search for a second filter revealed a startling evolutionary divergence in coding strategies between mammals and nonmammals. Advances in recording techniques and preparation health revealed sharply tuned mechanics in mammalian cochleae (Khanna & Leonard, 1982), discarding the need for a second filter in those species. However,

comparable measurements in nonmammalian auditory organs continued to show broadly tuned basilar membranes (turtle, O'Neill & Bearden, 1995). Moreover, in some lower vertebrates, a basilar membrane is entirely absent even though a place code exists along the epithelium (frog; Pitchford & Ashmore, 1987).

In a series of now landmark papers, Andrew Crawford and Robert Fettiplace described an intrinsic electrical tuning in turtle hair cells that served as the primary means of frequency tuning in the turtle cochlea (Crawford & Fettiplace, 1980, 1981; Fettiplace & Crawford, 1980). Current injection into these hair cells caused a damped oscillation in the membrane voltage response that matched the tonotopic position of the cells and the narrow-band tuning of auditory nerve fibers at those characteristic frequencies (Fettiplace & Crawford, 1980). Feedback from voltage-gated potassium conductances was suggested as a possible mechanism, since application of the potassium channel blocker tetraethylammonium (TEA) reduced or eliminated membrane potential oscillations upon current injection (Art, Crawford, Fettiplace, & Fuchs, 1985). Electrical tuning was ultimately described in other auditory organs of nonmammalian vertebrates, including frog (Pitchford & Ashmore, 1987), alligator (Fuchs & Evans, 1988), and chicken (Fuchs, Nagai, & Evans, 1988), but was absent in hair cells from the mammalian cochlea (Kros & Crawford, 1990).

The advent of whole-cell voltage-clamp recording techniques solidified the role of voltage-gated ion channels in hair cell electrical tuning. The major outward potassium conductance in these cells was consistent with the features of BK channels, namely sensitivity to extracellular TEA and intracellular cesium (Art & Fettiplace, 1987), a large single-channel conductance (Fuchs & Evans, 1988; Hudspeth & Lewis, 1988b), and activation by calcium influx through VGCCs (Hudspeth & Lewis, 1988a). The interplay between VGCCs and BK channels, along with a leak conductance and membrane capacitance, is necessary and sufficient to achieve membrane resonance and electrical tuning (Hudspeth & Lewis, 1988a). In this model, damped oscillations are initiated by depolarizing current steps and rapid activation of VGCCs. The resulting calcium influx further depolarizes the hair cell membrane and, with some delay, activates BK channels. The delayed but fast activation of large-conductance BK channels opposes a regenerative calcium action potential and drives the voltage response downward. As the membrane potential becomes more negative, VGCCs close and BK channels begin to deactivate. The persisting current injection initiates the next phase of depolarization. The combined delays in activation and deactivation

of these channels cause neither to reach their resting values during each oscillation, resulting in a damped resonance that eventually reaches a steady-state level. The two inescapable conclusions are that (1) the channels must be in close proximity to one another to provide rapid feedback and (2) some parameter must systematically change along the auditory epithelium to enable a smooth tonotopic gradient in electrical frequency tuning.

2.2 Interplay of BK Channels and VGCCs

Several functional studies support the close proximity of VGCCs and BK channels in nonmammalian hair cells. In these hair cells, and in contrast to that in mammalian analogs (see Section 3.2.1), BK channel activation requires calcium influx through VGCCs (Art & Fettiplace, 1987). Moreover, the high degree of calcium buffering in hair cells limits the degree of calcium diffusion and thereby restricts the size of BK channel VGCC domains to within 250 nm (Roberts, 1993). Additionally, BK channel activation is not eliminated by high intracellular concentrations of the fast calcium chelator BAPTA (Roberts, Jacobs, & Hudspeth, 1990), suggesting proximity at the single-channel level, possibly within tens of nanometers (Fakler & Adelman, 2008). Consequently, clusters of VGCC and BK channels are found in loose patch recordings from the basolateral membrane of frog saccular hair cells (Roberts et al., 1990). The average number of clusters per hair cell matched the average number of synaptic active zones, suggesting a tight correlation between these two critical calcium signaling domains. Similarly, cell-attached patch recordings at fluorescent calcium hotspots typically contained a small number of BK channels (ie, about 60% of the patches contained both channel types; Issa & Hudspeth, 1994). Importantly, membrane patches from regions outside calcium hotspots contained no BK currents. However, the number of recordings was small, so the basolateral membrane surface was not thoroughly scanned.

Immunocytochemistry studies have provided further evidence of channel clustering and colocalization (Li et al., 2009; Rodriguez-Contreras & Yamoah, 2001; Samaranayake, Saunders, Greene, & Navaratnam, 2004), but differences in epitope targeting, particularly in the C-terminus of the highly alternatively spliced BK channels, dramatically affect clustering and subcellular localization (Kim et al., 2010). Interestingly, although over 100 interaction partners have been identified for cochlear BK channels (Kathiresan, Harvey, Orchard, Sakai, & Sokolowski, 2009; Sokolowski, Orchard, Harvey, Sridhar, & Sakai, 2011) and physical coassembly of BK

channels and VGCCs has been described in the rat brain (Berkefeld et al., 2006; Grunnet & Kaufmann, 2004; Loane, Lima, & Marrion, 2007), biochemical evidence supporting such direct interaction of BK channels and VGCCs in hair cells remains elusive (Lesage, Hibino, & Hudspeth, 2004; Sokolowski et al., 2011). Consequently, the fidelity of colocalization and the mechanisms behind targeting to the ribbon synapse remains an active area of research.

2.3 Tonotopic Distribution of BK Channel Properties

BK currents are the primary determinants of tuning frequency, since they gate more slowly than VGCCs. Therefore, BK currents are the rate-limiting step in electrical resonance (Art & Fettiplace, 1987). Accordingly, it is the kinetics of this current that is smoothly graded along the auditory epithelium, with higher resonant frequencies arising from faster BK currents (Art & Fettiplace, 1987; Fuchs & Evans, 1988; Fuchs et al., 1988; Smotherman & Narins, 1999). The startling conclusion from these data is that some molecular feature contributing to BK channel conductance is systematically graded along the cochlea. Modeling schemes suggest that gradients in a small number of variables can achieve resonant frequencies spanning 40–4000 Hz, a range sufficient to encode relevant acoustic signals in chicks and lower vertebrates (Wu & Fettiplace, 1996). The speed and size of the BK channel potassium conductance could be influenced by the calcium signal (eg, influx, diffusion, and extrusion) or intrinsic differences in BK channel gating. Although the magnitude of the calcium current increases with increasing resonant frequency, reduction of extracellular calcium by two orders of magnitude had little impact on BK channel relaxation time constants in turtle hair cells (Art, Fettiplace, & Wu, 1993). Likewise, tuning frequency is largely insensitive to intracellular BAPTA (Ricci, Gray-Keller, & Fettiplace, 2000), indicating that resonance is set by activity within BAPTA-resistant microdomains independent of calcium diffusion and extrusion.

Single-channel recordings of BK channel activity were first obtained from inside-out patches from frog saccular hair cells (Hudspeth & Lewis, 1988b), confirming a high unitary conductance ($\approx$200 pS) in symmetrical potassium and prototypical sensitivity to both membrane voltage and intracellular calcium concentration. Subsequently, extensive recordings in auditory epithelia demonstrated that variations in intrinsic BK channel kinetics correlated with estimates of resonant frequency in isolated turtle hair cells (Art, Wu, & Fettiplace, 1995) and direct measures of tonotopic position

in chick epithelia (Duncan & Fuchs, 2003). Deactivation rates were strongly associated with resonant frequency in both species, but other gating parameters were independent of tuning or only weakly correlated (Duncan & Fuchs, 2003). Fig. 1 illustrates the variations in BK channel density and kinetics with hair cell tonotopic location that serves electrical tuning in non-mammalian hair cells.

Early predictions from findings in turtle cochlea suggested that BK channels from high-frequency hair cells would exhibit lower calcium sensitivity

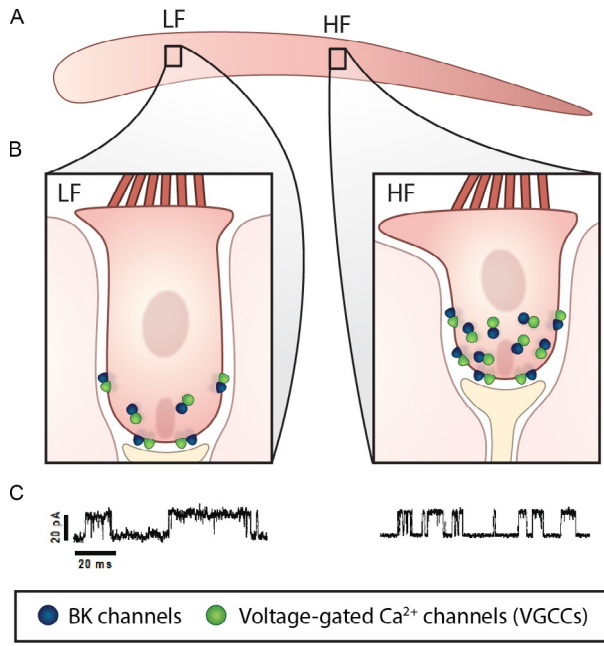

Fig. 1 Variations in BK channel density and kinetics with tonotopic location. (A) A schematic of the chick auditory epithelium with *boxes* indicating the positions of hair cells at two tonotopic locations, a low-frequency (LF) hair cell tuned to 250 Hz and a high-frequency (HF) hair cell tuned to 825 Hz. (B) Hair cells from these two locations are illustrated to show relative differences in cell morphology and ion channel density. BK and VGCC channels are clustered so that calcium influx from VGCCs activates neighboring BK channels (*arrows*). The density of these clusters increases with increasing tuning frequency. (C) Single-channel recordings from hair cells at these locations (R. K. Duncan, unpublished data) demonstrate kinetic differences that underlie electrical tuning. The mean open duration from these recordings was 9.8 and 1.9 ms for the LF and HF hair cells, respectively. Correspondingly, deactivation rates from whole-cell BK recordings increase systematically with tuning frequency. Recordings were made at −60 mV in the presence of 5 μM $[Ca^{2+}]_i$ using inside-out patches in symmetric potassium solutions.

(Art et al., 1993). However, single-channel data from these hair cells showed no tonotopic gradient in calcium affinity (Art et al., 1995). In chick, a weak correlation between calcium sensitivity and tuning was found, possibly due to the expanded range in resonant frequencies encoded in that animal. Interestingly, the average $K_D(0)$—the $[Ca^{2+}]_i$ required for half-activation at 0 mV—was greater in patches from the high-frequency end of the cochlea, but the variance (diversity) in calcium sensitivity increased with frequency as well. High-affinity channels were distributed throughout the cochlea, but the distribution of $K_D(0)$ broadened to include BK channels with reduced calcium affinity as location moved toward high frequencies. These data underscore the diversity in channel properties at any one location and stand in contrast to early models of tuning that mixed a small number of BK channel variants expressed at discrete locations to achieve smooth gradients in electrical tuning (Wu & Fettiplace, 1996). Indeed, in the chick, excised patches with greater numbers of channels showed better correlation between the predicted resonant frequency for that cell and its tonotopic location; patches with single or few channels showed a greater discrepancy between predicted and actual tuning frequency. The data suggest significant BK channel diversity even within single cells.

2.4 Splice Variation

What then is the source of BK channel diversity in nonmammalian auditory epithelia? BK channels are comprised of four pore-forming α subunits encoded by a single gene, KCNMA1, as well as several potential auxiliary subunits. Initial cloning experiments in the turtle identified two primary splice sites in the BK α subunit mRNA (Jones, Gray-Keller, Art, & Fettiplace, 1999). Alternative exons at these two sites reside in the cytoplasmic tail between the two calcium regulatory domains RCK1 and RCK2 (see Chapter "Posttranscriptional and Posttranslational Regulation of BK Channels" by Shipston and Tian). Alternative splicing in the turtle cochlea results in six naturally occurring variants with a range of calcium affinity and gating kinetics when heterologously expressed in oocytes (Jones et al., 1999). Interestingly, kinetics varied systematically with insertion/deletion (indel) length. Hypothetical regional distribution of these variants, some combined with accessory β1 subunits, could account for tuning in the turtle. However, it remains unclear whether the variants are expressed in these distributions in the epithelium. Moreover, evidence of β1 expression in the turtle cochlea is lacking.

Electrical tuning in chick spans a larger range of frequencies than in turtle, so a larger number of unique isoforms may be required. Indeed, there appears to be a larger number of splice sites utilized in the chick (ie, seven alternative splice sites within the coding region plus alternative start sites; see for review, Fettiplace & Fuchs, 1999). Sixteen alternative exons have been identified (Miranda-Rottmann, Kozlov, & Hudspeth, 2010). If each were incorporated independently and combined with others in a nonobligatory manner, then a staggering array of unique variants could be formed, particularly if unique isoforms formed heteromultimers with intermediate properties.

The potential diversity is large, but the question remains whether alternative splicing is distributed in such a way to govern or contribute to tonotopic gradients in tuning. Central to the hypothesis is the expectation that variants are expressed in restricted regions in the auditory epithelium. Navaratnam and colleagues divided the chick epithelium into quadrants and examined exon expression by PCR (Navaratnam, Bell, Tu, Cohen, & Oberholtzer, 1997). Several alternative exons were differentially distributed, and individual hair cells expressed multiple variants at the same splice site. Unfortunately, these data gave no indication about naturally occurring combinations of alternative exons or the relative abundance of these variants among the total pool of BK channel transcripts.

In a recent study, more than 3000 full-length variants of the BK channel α subunit were cloned and sequenced from the chick cochlea (Miranda-Rottmann et al., 2010). Transcripts were examined based on C-terminal splicing at site 7, because variation at this site has a profound influence over surface expression and clustering (Kim et al., 2010). Notably, transcripts ending in the C-terminal isoform QEDRL, which may be the predominant cell surface isoform, showed little alternative splicing. Over 90% of the QEDRL transcripts included no alternative exons at upstream splice sites. The authors found no selective inclusion of alternative exons at specific regions along the auditory epithelium and the limited degree of long, complex splice forms argues against indel length as a major contributor to tuning.

2.5 Regulatory β Subunits

In every model of electrical tuning, BK channel β subunits are employed to increase the range of calcium affinities and gating kinetics to allow for tuning in low-frequency hair cells. Four BK β subunits have been described (KCNMB1–4; see, for example, Contreras, Neely, Alvarez, Gonzalez, &

Latorre, 2012). β1 and β4 have been cloned from the chick cochlea, and gene expression studies suggest higher expression of both subunits in apical than basal hair cells (Bai, Surguchev, & Navaratnam, 2011; Ramanathan, Michael, Jiang, Hiel, & Fuchs, 1999). When coexpressed with BK channel α subunit variants in HEK293 cells or oocytes, β subunits increase calcium affinity and slow deactivation kinetics while maintaining the relative differences between alternative α splice forms (Bai et al., 2011; Ramanathan, Michael, & Fuchs, 2000). These features are consistent with enriched β expression in the low-frequency end of the cochlea. However, relaxation time constants in native BK channels from chick cochlea are about one order of magnitude faster than heterologously expressed BK channels coassembled with β1 or β4 (Bai et al., 2011; Duncan & Fuchs, 2003; Ramanathan et al., 2000).

Although siRNA knockdown of β1 and β4 in cultured chick cochlea suggests a role in BK channel surface expression (Bai et al., 2011), functional evidence is lacking. Electrophysiological data from the siRNA experiments will be important for establishing the necessity for either subunit in electrical tuning. Pharmacological experiments could also shed light on the functional impact of β subunit expression. Coassembly with β subunits modulates BK channel sensitivity to steroidal hormones and toxins. For example, BK channel activation by estradiol or the xenoestrogen tamoxifen requires coexpression with either β1 (Dick, Rossow, Smirnov, Horowitz, & Sanders, 2001; Dick & Sanders, 2001) or β4 (Behrens et al., 2000). However, when tamoxifen was applied to chick hair cell BK channels, only 1 of about 20 patches showed an increase in open probability (Tong & Duncan, 2009). The data did not support a major role for β subunits, except possibly in the most apical end of the cochlea. Since β4 confers resistance to the BK channel blocker iberiotoxin (Wang, Jaffe, & Brenner, 2014), it would be interesting to examine toxin sensitivity along the auditory epithelium in chick.

2.6 Other Possibilities

Other accessory subunits or interaction partners could play major roles in tuning. While recently identified BK γ subunits have not been identified in nonmammalian genomes (Dolan et al., 2007), the possibility remains that orthologues of these subunits or other protein partners regulate BK channel function in nonmammalian hair cells. Using tandem mass spectrometry, over 100 putative BK channel interaction partners have been identified in the chick cochlea (Sokolowski et al., 2011). Informatics analysis indicated

that these interactions may be involved in cell death/survival pathways, trafficking, calcium binding, and kinase activity. In particular, protein kinases may have a strong impact on electrical tuning, since BK channels exhibit numerous phosphorylation sites that confer significant changes to channel gating (Yan et al., 2008). These possibilities are just now beginning to be explored. For example, the cyclin-dependent kinase CKD5 is differentially expressed along the chick auditory epithelium, increasing in expression with increasing tuning frequency (Bai, Surguchev, Joshi, Gross, & Navaratnam, 2012). Interaction with CDK5 results in faster deactivation kinetics, consistent with tuning in high-frequency hair cells.

Gene expression arrays from apical and basal segments of the chick auditory epithelium have been screened by two separate groups to determine candidate signaling pathways that might govern the myriad number of tonotopically distributed parameters in the inner ear, including BK channels and VGCCs (Frucht, Uduman, Kleinstein, Santos-Sacchi, & Navaratnam, 2011; Kowalik & Hudspeth, 2011). Three major pathways were indicated, including Notch, Wnt, and FGF. MicroRNAs and protein kinases were also identified as potential regulators of tonotopic distributions. Future experiments will need to combine screens for interaction partners with screens for apical-to-basal molecular gradients to identify new candidate molecules involved in BK channel diversity and electrical tuning.

3. BK CHANNELS IN THE MAMMALIAN COCHLEA

3.1 Anatomy of the Mammalian Auditory Sensory Epithelium

In mammals, the auditory sensory epithelium, called the organ of Corti, contains two distinct classes of hair cells: a single row of inner hair cells and three to four rows of outer hair cells. Both inner and outer hair cells transduce vibrations produced by sound waves into graded changes in membrane potential. The inner hair cells, however, are the primary sensory cells. When depolarized, the inner hair cells release glutamate and activate the primary auditory neurons, called the spiral ganglion cells, which relay (afferent) information to the brain. In contrast, the outer hair cells relay little afferent information. Instead, by virtue of their electromotility, depolarization generates mechanical forces necessary for the amplification of sound-induced vibrations of the basilar membrane. They are, therefore, essential for amplifying the mechanical signals transduced by the inner hair cells. The outer hair cells receive abundant inhibitory (efferent) feedback from the brainstem

that modulates cochlear amplification. The anatomy of the mammalian cochlea and organ of Corti is more fully reviewed elsewhere (for example, Raphael & Altschuler, 2003).

In contrast to hair cells from other vertebrates, mammalian inner hair cells are extrinsically or mechanically tuned by accessory structures of the inner ear, most notably the basilar membrane. As first elucidated by von Békésy (1960), changes in mechanical properties of the basilar membrane, especially in the width and thickness, are responsible for separating sound frequencies spatially along the length of the overlying auditory sensory epithelium that contains the hair cells. Active properties of the outer hair cells refine this spatial frequency tuning. In this way, specific frequencies of sound maximally excite a subset of spatially localized inner hair cells, creating a tonotopic or frequency–place map. The distinct sensory and mechanical roles of inner and outer hair cells are nicely reviewed by Fettiplace and Hackney (2006). Although mammalian hair cells are not electrically tuned, BK channels nevertheless carry a significant component of the total outward potassium conductances in both inner and outer hair cells. Moreover, BK channel expression is not restricted to the hair cells. Work, largely over the last two decades, has shown that BK channels contribute in various and often unexpected ways to mammalian hearing.

3.2 BK Channel Expression in the Mammalian Auditory Sensory Epithelium

3.2.1 Inner Hair Cells

BK currents, initially referred to as $I_{K,f}$ to distinguish their fast rate of activation from other more slowly activating potassium currents, were first observed by Kros and Crawford (1990) using whole-cell patch-clamp recordings from isolated guinea pig inner hair cells. This conductance was found to be pharmacologically characteristic of BK channels: sensitive to TEA (Kros & Crawford, 1990), resistant to 4-AP (Kros & Crawford, 1990), and blocked by the highly specific BK channel blockers, iberiotoxin (Hafidi, Beurg, & Dulon, 2005; Kros, Ruppersberg, & Rusch, 1998; Marcotti, Johnson, & Kros, 2004a; Pyott, Glowatzki, Trimmer, & Aldrich, 2004), and charybdotoxin (Dulon, Sugasawa, Blanchet, & Erostegui, 1995; Kros et al., 1998).

Additional molecular evidence that $I_{K,f}$ is indeed carried by BK channels came from rtPCR and in situ hybridization. These techniques identified BK channel (α subunit) transcripts in the rat organ of Corti (Brändle et al., 2001)

as well as various BK channel splice variants in rat inner hair cells (Langer, Grunder, & Rusch, 2003). The diversity and tonotopic patterns of BK channel splice variant expression in inner and outer mouse inner hair cells have also been carefully documented (Beisel et al., 2007). Immunohistochemistry verified expression of the BK channel protein in guinea pig (Skinner et al., 2003) and mouse inner hair cells (Hafidi et al., 2005; Pyott et al., 2004). Finally, definitive evidence that the BK channel is responsible for $I_{K,f}$ in inner hair cells came from BK α knockout mice. Importantly, $I_{K,f}$ was absent in whole-cell patch-clamp recordings of inner hair cells from these mice (Pyott et al., 2004; Rüttiger et al., 2004).

Immunofluorescent localization of BK channels revealed an unexpected subcellular localization in inner hair cells. In nonmammalian hair cells BK channels are colocalized with VGCCs at transmitter release sites (see Section 2.2). This colocalization of BK and VGCCs is essential for electrical tuning. In contrast, BK channels are predominantly and possibly exclusively localized to the necks of mammalian inner hair cells (Hafidi et al., 2005; Pyott et al., 2004) away from the afferent ribbons and VGCCs (Fig. 2). The functional importance of this subcellular localization is not clear although it undoubtedly reflects the fundamentally different role BK channels play in nonmammalian and mammalian hair cells. Importantly, expression in other subcellular locations of the inner hair cells that remains undetected by immunofluorescence cannot be excluded. Moreover, BK channels in both inner (see Wersinger et al., 2010) and outer (Engel et al., 2006; Maison et al., 2013; Wersinger et al., 2010) hair cells appear to show tonotopic gradients of increasing expression from apical (low-frequency) to basal (high-frequency) regions. The stronger expression in high-frequency regions suggests a contribution of BK channels to high-frequency hearing in mammals (see Section 3.3.4).

The biophysical properties of BK channels in inner hair cells are curiously different from those generally attributed to BK channels observed in other cells, including nonmammalian hair cells. Importantly, the dual activation of BK channels by both membrane depolarization and intracellular calcium is key to their regulation of membrane excitability in various cells (reviewed in Toro et al., 2014) including the electrical tuning of nonmammalian hair cells. Whereas BK currents in the hair cells of nonmammalian species depend on calcium entry into the cell (see Section 2.2), BK currents in mammalian (guinea pig and mouse) inner hair cells are curiously unaffected by the removal of extracellular calcium (Kros & Crawford, 1990; Marcotti et al., 2004a).

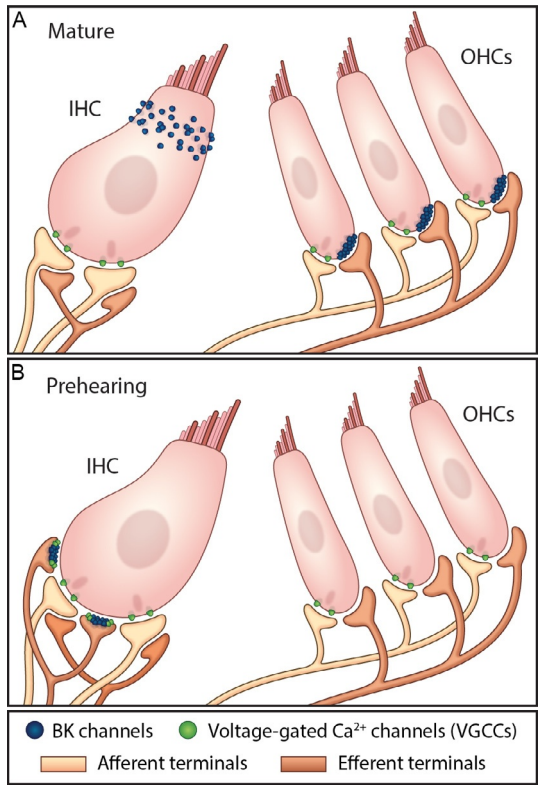

Fig. 2 Localization of BK channels in the mammalian organ of Corti. (A) In the mature organ of Corti (after the onset of hearing), BK channels are found in clusters in the necks of the inner hair cells and conspicuously distant from ribbon active zones (Pyott et al., 2004). In the higher frequency outer hair cells (>4 kHz), BK channels are found in single large clusters directly postsynaptic to (medial olivocochlear) efferent terminals (Wersinger, McLean, Fuchs, & Pyott, 2010). These clusters peak in size between 16 and 32 kHz (Maison, Pyott, Meredith, & Liberman, 2013). (B) Before the onset of hearing, BK channels are not present in either the inner or outer hair cells. However, during the period of transient efferent innervation of the inner hair cells (which peaks around postnatal days 7–9 in rat; Katz et al., 2004), BK channels are found in single smaller clusters in the presynaptic efferent terminals contacting the inner hair cells. These BK channels are activated by calcium influx from L-type VGCCs (Zorrilla de San Martin, Pyott, Ballestero, & Katz, 2010). The additional presence of BK channels in efferent terminals contacting the (type I) afferent terminals, which are themselves contacting the inner hair cells, or in nascent (prehearing) efferent terminals contacting the outer hair cells (especially higher frequency outer hair cells) has not been fully investigated (see Section 3.2.4).

Rigorous characterization of the voltage and calcium dependence of BK channel activation using whole-cell and excised patch-clamp recordings of rat inner hair cells showed that BK channel activation does not rely on VGCCs and is remarkably insensitive to local intracellular calcium concentration (Thurm, Fakler, & Oliver, 2005). The activation of BK currents at negative membrane potentials even in the absence of intracellular calcium led these investigators to speculate that BK channels in mammalian inner hair cells may function as purely voltage-gated potassium channels. In fact, the lack of activation by calcium may be a requirement of their subcellular localization away from VGCCs. This conclusion is, however, challenged by findings that intracellular photorelease of calcium activated a charybdotoxin-sensitive current in dissociated inner hair cells isolated from guinea pig (Dulon et al., 1995) and that BK currents in inner hair cells in the intact organ of Corti are modulated by voltage-dependent release from intracellular calcium stores (Marcotti et al., 2004a). Differences between the preparations may affect BK currents' properties. Nevertheless, these inconsistent findings warrant more investigation into the biophysics of BK channels in mammalian inner hair cells.

The biophysical properties of BK channels are known to be altered by alternative splicing and association with regulatory subunits (recently reviewed in Toro et al., 2014; see Chapter "Posttranscriptional and Posttranslational Regulation of BK Channels" by Shipston and Tian). As in nonmammalian hair cells (reviewed in Fettiplace & Fuchs, 1999), various alternatively spliced BK channel α (pore-forming) subunit transcripts (Langer et al., 2003; Sakai, Harvey, & Sokolowski, 2011) have been observed in the mammalian inner ear and hair cells. The contribution of alternative splicing of the BK channel in the cochlea is unclear. Based on differences in detection by monoclonal antibodies that recognize distinct epitopes of the BK channel (S. J. Pyott, unpublished findings), distinct BK channel splice variants may be present in the inner and outer hair cells (see more in Section 3.2.2). Perhaps alternatively spliced variants enable the appropriate trafficking of BK channels to the neck of the inner hair cells and the synaptic poles of the outer hair cells.

While various β (regulatory) subunit (β1, β2, and β4) transcripts have been observed in the mammalian cochlea inner ear and hair cells (Langer et al., 2003), their contribution to auditory processing is also unclear. BK channel currents in inner hair cells from BK β1/β4 double-knockout mice are comparable to those from wild-type mice (Pyott et al., 2007). Moreover, inner and outer hair cell function as assessed by auditory brainstem responses

and distortion product otoacoustic emissions are also comparable between BK β1/β4 double-knockout and wild-type mice (Pyott et al., 2007). These findings suggest that the BK β1 and β4 subunits do not regulate BK channel expression in the hair cells and are not required for normal function of the cochlea. Possible contributions of the BK β2 and β3 subunits have not been investigated yet. Importantly, the BK β2 subunit (Uebele et al., 2000; Wallner, Meera, & Toro, 1999; Xia, Ding, & Lingle, 1999) as well as particular splice variants of the BK β3 subunit (Lingle, Zeng, Ding, & Xia, 2001; Uebele et al., 2000; Xia, Ding, Zeng, Duan, & Lingle, 2000) impart inactivation to BK channels in other systems and may contribute to the inactivation of these currents in frog hair cells (Armstrong & Roberts, 2001), as well as in mouse inner hair cells, as reported by some (Pyott et al., 2004) but not others (Marcotti et al., 2004a). The presence of other recently identified regulatory subunits, BK γ1–γ4 (LRRC26, LRRC52, LRRC55, LRRC58, respectively; Zhang & Yan, 2014) has also not been investigated. Interestingly, BK γ subunits can produce BK channels with increased voltage sensitivity even in the absence of intracellular calcium (Yan & Aldrich, 2010, 2012). The expression of BK γ subunits may explain the curious biophysics of BK channels in mammalian inner hair cells.

3.2.2 Outer Hair Cells

Identification of BK channels using electrophysiology in mammalian outer hair cells has been much less straightforward compared to inner hair cells. In retrospect, these discrepancies are likely due to developmental and tonotopic gradients of BK channel expression in the mammalian organ of Corti that complicated experimental identification. The effects of enzymatic treatments used to dissociate the hair cells may also affect BK channel properties (see Armstrong & Roberts, 2001; Spreadbury, Kros, & Meech, 2004) and confound identification.

The first examination of currents in isolated guinea pig outer hair cells using patch-clamp electrophysiology was undertaken by Ashmore and Meech (1986). Based on measured single-channel conductances, they reported expression of a large-conductance calcium-activated potassium, presumably BK, current. The presence of high-conductance potassium channels was subsequently confirmed by Gitter, Fromter, and Zenner (1992) and van den Abbeele, Teulon, and Huy (1999) using cell-attached and excised inside-out patches recordings. Housley and Ashmore (1992) used patch-clamp electrophysiology to examine whole-cell currents in outer hair cells isolated from the guinea pig organ of Corti. In outer hair cells isolated from each turn, they observed a TEA- and nifedipine-sensitive

potassium current. These results indicated the presence of a potassium channel dependent on calcium influx via L-type calcium channels in outer hair cells along the length of the organ of Corti. Finally, Nenov, Norris, and Bobbin (1997) identified a charybdotoxin-sensitive current in isolated long (lower frequency) outer hair cells isolated from guinea pig. In contrast, patch-clamp recordings from undissociated outer hair cells from adult guinea pig by Mammano and Ashmore (1996) identified distinct potassium channels in outer hair cells from apical turns and basal turns and argue against the expression of BK currents in outer hair cells from either region.

Identification of BK α transcripts using in situ hybridization in the rat organ of Corti revealed labeling of outer hair cells that appeared later than the more robust expression observed in the inner hair cells just before the onset of hearing (Langer et al., 2003). BK channel transcript and also protein expression was confirmed in outer hair cells in the guinea pig organ of Corti by in situ hybridization and immunocytochemistry (Skinner et al., 2003). The first examination to utilize BK α knockout mice identified BK channel immunoreactivity in the inner hair cells and also associated with efferent terminals contacting the outer hair cells (Rüttiger et al., 2004). In this study, BK α knockout mice displayed outer hair cell degeneration in the high-frequency cochlear turns. This finding motivated Engel et al. (2006) to examine the developmental and tonotopic gradient of BK channel immunoreactivity in outer hair cells. These authors observed BK channel immunoreactivity in mouse outer hair cells that increased from low- to high-frequency turns.

The tonotopic expression and functional contribution of BK channels in mammalian outer hair cells was further elucidated by the work of Pyott and colleagues (Fig. 2). Systematic examination of BK channel expression by whole-cell patch-clamp recordings and immunofluorescence in the rat organ of Corti indicated expression of BK channels after the onset of hearing and only in higher frequency outer hair cells (Wersinger et al., 2010). Importantly, this work also showed that acetylcholine application exclusively activated BK currents in high-frequency outer hair cells as opposed to SK currents in the lower frequency outer hair cells. This finding was unexpected since all previous studies in vertebrate hair cells had shown that acetylcholine, the major efferent neurotransmitter, mediated hair cell inhibition via the combined action of nicotinic acetylcholine receptors and small-conductance calcium-activated potassium, or SK, channels. [See, for example, work in chick (Yuhas & Fuchs, 1999), turtle (Holt, Lysakowski, & Goldberg, 2006), and low-frequency outer hair cells from

the guinea pig (Nenov, Norris, & Bobbin, 1996) and rat (Oliver et al., 2000).] Subsequent work verified that BK channels indeed shape efferent inhibitory synaptic responses in the high-frequency outer hair cells (Rohmann, Wersinger, Braude, Pyott, & Fuchs, 2015). Finally, this gradient of BK channel expression was verified in mouse outer hair cells and the absence of the BK channel (in BK α knockout mice) results in an in vivo loss of outer hair cell efferent suppression and increased susceptibility to noise-induced temporary threshold shifts (Maison et al., 2013). Initial work by Pyott et al. (2004, 2007) did not observe BK channel immunoreactivity in mouse outer hair cells but later attributed this discrepancy to experimental conditions (overfixation) that abolished immunoreactivity specifically in the outer hair cells.

As in inner hair cells, the biophysical properties of BK channels in outer hair cells appear unusual. Specifically, voltage-independent activation of BK channels appears to occur. Evoked inhibitory postsynaptic currents in outer hair cells isolated from high-frequency turns of both mouse and rat organ of Corti are shaped by activation of BK channels (Rohmann et al., 2015). In these conditions, calcium influx through closely positioned acetylcholine receptors appears to raise intracellular calcium to levels sufficient to activate both SK and BK channels even in the absence of depolarization. The molecular mechanisms underlying this voltage-independent gating of BK channels in outer hair cells have not been explored but may be conferred by alternative splicing and/or the association of regulatory subunits (see Section 3.2.1).

3.2.3 Spiral Ganglion Neurons

BK channel expression has been mostly directly investigated in the sensory hair cells. However, various reports indicate the expression of BK channels in the spiral ganglion neurons. The spiral ganglion neurons are the first action potential generating neurons in the mature auditory pathway. Therefore, expression of BK channels, which regulate action potential latency and firing frequency in other neurons (N'Gouemo, 2014; see Chapter "BK Channels in Neurons" by Barth and Contet), is not necessarily surprising in the spiral ganglion neurons. Both in situ hybridization (Skinner et al., 2003) and immunocytochemistry (Hafidi et al., 2005; Skinner et al., 2003) demonstrated BK channel expression in the cell bodies of the spiral ganglion neurons (in addition to sensory hair cells) in the intact auditory sensory epithelium isolated from guinea pig (adult; Skinner et al., 2003) and mouse (postnatal day 12; Hafidi et al., 2005). BK channel immunoreactivity was detected in dissociated spiral ganglion neurons isolated from postnatal

day 3 to 8 mice (Adamson, Reid, Mo, Bowne-English, & Davis, 2002). In this work, immunoreactivity increased in intensity from apex to base. Moreover, blockade of BK channels with charybdotoxin transformed spiral ganglion neuron firing patterns from rapidly adapting (firing one or two successive action potentials) to slowly adapting (firing multiple successive action potentials). In light of this work, BK channel expression in the spiral ganglion cells may appear before the onset of hearing and, therefore, before expression in the sensory hair cells. Importantly, these findings collectively indicate that BK channels are positioned to modulate afferent transmission (discussed later in Section 3.3.2).

3.2.4 Efferent Terminals

In addition to relaying afferent sensory information to the brain, hair cells of the vertebrate cochlea also receive efferent innervation from the brain (Guinan, 1996). In the mammalian auditory system, cholinergic neurons originating in the medial superior olive in the brainstem supply inhibitory efferent innervation to the outer hair cells (Liberman, Dodds, & Pierce, 1990). As discussed in Section 3.2.2, BK channels, present in the postsynaptic high-frequency outer hair cells, carry this inhibitory efferent current (Maison et al., 2013; Rohmann et al., 2015; Wersinger et al., 2010). Just before the onset of hearing efferent terminals originating from the medial superior olive transiently contact the inner hair cells (Simmons, 2002). This efferent inhibition modulates inner hair cell spiking (Glowatzki & Fuchs, 2002; Marcotti, Johnson, & Kros, 2004b) and has, therefore, been proposed to shape establishment of the afferent auditory pathway (Clause et al., 2014). Work by Zorrilla de San Martin and colleagues (2010) used a combination of whole-cell patch-clamp recordings, quantal analysis, and immunofluorescence to show that calcium entering the presynaptic efferent terminals via L-type calcium channels activates BK channels. Activation of BK channels serves to inhibit the presynaptic terminals and negatively regulate acetylcholine release from the terminal. This work suggests that BK α knockout mice should show increased efferent inhibition of the inner hair cells before the onset of hearing. This prediction and its effect on afferent development have not been investigated yet. This work also suggests that BK channels may be present in the medial efferent terminals contacting the outer hair cells before the onset of hearing. Although electrophysiological examination of apical (low-frequency) cochlear turns from prehearing mice suggests the absence of BK channels from outer hair cell efferent terminals (Ballestero et al., 2011), these experiments do not exclude the possibility of BK channel

expression in efferent terminals contacting the higher frequency outer hair cells. Additionally, the role of BK channels in a second efferent pathway, arising from neurons originating in the lateral superior olive in the brainstem (Warr, 1992), has not been investigated yet. Fig. 2 illustrates the localization of BK channels in efferent terminals contacting the inner hair cells before the onset of hearing.

3.3 Function of BK Channels in the Mammalian Auditory System

3.3.1 Maturation of the Auditory System

BK channels appear to be involved in the maturation of mammalian hearing. Similar to early observations made in chick hair cells (Fuchs & Sokolowski, 1990; Li et al., 2009), BK channels are first observed in mammalian inner (Hafidi et al., 2005; Kros et al., 1998; Marcotti, Johnson, Holley, & Kros, 2003; Pyott et al., 2004) and outer (Rohmann et al., 2015) hair cells at the onset of hearing. The appearance of the BK channel and downregulation of VGCCs have been hypothesized to convert immature inner hair cells, which fire calcium-driven action potentials, into mature inner hair cells, which instead display graded receptor potential changes (Kros et al., 1998). Therefore, the expression of BK channels appears to be a defining biophysical indicator of the mature inner hair cell.

Consistent with this conclusion, in mice, mutations that interfere with the normal physiology of the inner hair cells and lead to deafness, including genetic deletion of the L-type calcium channel $Ca_V1.3$ (Brandt, Striessnig, & Moser, 2003; Nemzou, Bulankina, Khimich, Giese, & Moser, 2006) and hypothyroidism (Dettling et al., 2014; Dror et al., 2014; Winter et al., 2007), result in delayed or failed developmental appearance of BK currents or channels in the inner hair cells. Moreover, genetic deletion of the BK channel results in subtle morphological changes of the efferent terminals contacting the outer hair cells (Maison et al., 2013; Rohmann et al., 2015). Specifically, quantitative electron and light microscopic image analysis revealed that postsynaptic cisterns of BK α knockout outer hair cells are smaller than those of wild-type mice and that efferent presynaptic terminals of BK α knockout mice retain their smaller (prehearing) sizes compared to wild-type mice (Rohmann et al., 2015). Thus, BK channels appear to contribute to the final structural maturation of the descending efferent innervation of the outer hair cells.

Finally, although gross aspects of afferent maturation appear to be intact in BK α knockout mice, a careful characterization of the contribution of BK

channels to maturation and organization of the ascending auditory pathways has not been carried out. Specifically, prehearing expression of BK channels in efferent terminals that contact the immature inner hair cells (see Section 3.2.4; Zorrilla de San Martin et al., 2010) may regulate efferent modulation of inner hair cell activity and shape tonotopic map refinement as observed in mice that lack a particular subunit (α9) of the nicotinic acetylcholine receptors (Clause et al., 2014).

3.3.2 Afferent Synaptic Transmission

The expression of BK channels in the inner hair cells (Section 3.2.1) and spiral ganglion neurons (Section 3.2.3) indicates that BK channels are poised to regulate afferent transmission in the cochlea. Indeed, intracochlear perfusion of the BK channel toxins charybdotoxin or iberiotoxin reversibly reduces the compound action potential recorded from the auditory nerve, suggesting that BK channels regulate transmission at the synapses between the inner hair cells and spiral ganglion neurons (Skinner et al., 2003).

Careful dissection of the possible pre- and postsynaptic roles of BK channels was revealed from analysis of single-fiber recordings made from the auditory nerve of BK α knockout and wild-type mice (Oliver et al., 2006). These experiments revealed a number of properties that were unchanged in BK α knockout mice, indicating that BK channels do not contribute to spontaneous rates, dynamic range, or the shapes of tuning curves of individual auditory nerve fibers. The preserved tuning curves in BK α knockout mice is further evidence that BK channels do not contribute to the tuning of mammalian hair cells as they do in hair cells from other nonmammalian vertebrates.

In contrast, individual auditory nerve fibers from BK α knockout mice show reduced temporal precision of spike timing and reduced sound-evoked discharge rates (Oliver et al., 2006). Presynaptic BK channels (present in the inner hair cells) likely contribute to the precision of spike timing, whereas postsynaptic BK channels (present in the spiral ganglion cells) likely determine evoked discharge rates. Increased variance of the first spike latency (an indicator of reduced temporal precision of spike timing) at the tone onset is consistent with the slowed voltage responses observed in the inner hair cells of BK α knockout compared to wild-type mice. These voltage responses control transmitter release from the inner hair cells and are slower in the inner hair cells from BK α knockout mice due to the loss of voltage-dependent conductance, which in turn increases the membrane time constant. Single auditory neurons from BK α knockout mice show

reduced evoked firing rates compared to auditory neurons from wild-type mice. Because auditory neurons from knockout mice show longer absolute refractory periods, these postsynaptic BK channels presumably contribute to action potential recovery, likely by repolarizing the neurons and relieving sodium channel inactivation.

Ultimately, BK channels are important determinants of temporal precision in the mammalian auditory periphery (Oliver et al., 2006; Shaheen, Valero, & Liberman, 2015). This precision, importantly, also appears necessary to contribute to stimulus coding in the central auditory system (Kurt et al., 2012).

3.3.3 Protection from Acoustic Overexposure

BK channels are also involved in protection from acoustic overexposure. Although initial investigation showed that BK α knockout mice were more resistant to noise-induced hearing loss (Pyott et al., 2007), direct investigation reported greater noise-induced temporary threshold shifts in knockout compared to wild-type mice (Maison et al., 2013). Because activation of the outer hair cell cholinergic efferent system minimizes damage from acoustic trauma (Maison, Luebke, Liberman, & Zuo, 2002), weakened efferent inhibition would be predicted to result in increased vulnerability to acoustic injury. Thus, the observed increased sensitivity to noise exposure in the absence of BK channels is consistent with the involvement of BK channels in mediating efferent inhibition of the outer hair cells (Section 3.2.4).

Curiously, however, BK α knockout mice show comparable susceptibility to permanent thresholds shifts following noise exposure (Maison et al., 2013). Given that different patterns of structural damage distinguish noise-induced temporary vs permanent threshold shifts (Wang, Hirose, & Liberman, 2002), BK channels are likely involved in the reversible (but not irreversible) processes underlying noise-induced hearing loss. Further investigation of BK α knockout mice may, therefore, provide insight into the mechanisms contributing to the distinct pathologies of noise-induced hearing loss.

3.3.4 High-Frequency Hearing

The tonotopic increase in BK channel expression in high-frequency hair cells (Sections 3.2.1 and 3.2.2) suggests that BK channels are a necessary specialization of the expanded auditory frequency range typical of mammals. In both inner and outer hair cells, large conductance, rapidly activating BK currents result in lower input resistances and shorter membrane time constants,

providing better voltage control during rapidly changing receptor potentials. In inner hair cells, the developmental appearance of BK channels decreases the membrane time constant and likely contributes to the prevention of action potentials (Section 3.2.1). Thus, the BK channel, while not necessary for frequency tuning per se, is nevertheless required to transform hair cells into signal transducers with fast signal following responses. In outer hair cells, BK channels, compared to SK channels, additionally provide a larger and faster conductance change in response to acetylcholine (Rohmann et al., 2015), increasing the gain and speed of efferent inhibition. Thus, BK channels, which serve a different function in nonmammalian hair cells, are preadapted to serve demands required of high-frequency hearing in mammalian inner and outer hair cells.

3.4 BK Channels in Vestibular Hair Cells

The mammalian vestibular structures use sensory hair cells to detect head rotations. Hair cell BK channels were first identified in the frog sacculus (Hudspeth & Lewis, 1988a, 1988b), an auditory as well as vestibular structure. Thus, it follows that BK channels may also be expressed in mammalian vestibular hair cells. The mammalian vestibular sensory epithelia contain two types of hair cells differentiated by their shape, distribution within the epithelia, and synaptic contacts. Type I hair cells are surrounded by a calyx-shaped afferent ending, whereas type II hair cells are contacted by bouton-shaped afferent as well as efferent endings (reviewed in Lysakowski & Goldberg, 2004). Based on findings in the auditory sensory epithelium, a variety of expression patterns might be predicted for the BK channel, including expression in (1) one or both classes of vestibular hair cells, (2) afferent calyx and/or bouton endings, and (3) efferent terminals. Moreover, expression patterns may change developmentally and/or regionally.

Whole-cell patch-clamp recordings of type II vestibular hair cells from mature guinea pigs identified an acetylcholine-induced BK current (Kong et al., 2007, 2005). This current is elicited by local, extracellular application of acetylcholine and blocked by the BK channel blockers charybdotoxin and iberiotoxin but not the SK channel blocker apamin. Moreover, the BK current is blocked by the L-type calcium channel blocker nifedipine. This observation, together with subsequent work (Guo et al., 2012; Yu, Guo, Wang, Zhou, & Kong, 2014), suggests that acetylcholine acts via muscarinic acetylcholine receptors to open L-type calcium channels. The influx of

calcium via L-type calcium channels, in turn, activates BK channels. Thus, BK channels appear to mediate efferent inhibition of the type II vestibular hair cells but utilize muscarinic rather than nicotinic (α9/10-containing) acetylcholine receptors as in the auditory system (reviewed in Katz & Elgoyhen, 2014).

In contrast to this work, immunofluorescence to physically localize BK channels in the intact vestibular sensory epithelia (Schweizer, Savin, Luu, Sultemeier, & Hoffman, 2009) indicates expression only in a small subset of type I vestibular hair cells. Curiously, this expression peaks in juvenile rats and declines into adulthood. Unfortunately, BK α knockout mice have not been utilized yet to investigate the expression and/or functional contributions of the BK channel to the vestibular periphery. Global knockout of the BK channel causes gate abnormalities that may indicate dysfunction of the vestibular periphery (Meredith, Thorneloe, Werner, Nelson, & Aldrich, 2004). Inner ear-specific knockout of BK α via local Cre-mediated recombination of a floxed BK α allele in mice (Zemen et al., 2015) would be especially useful to identify peripheral contributions of the BK channel to balance.

4. CONCLUSION

Because of their dual activation by membrane voltage and intracellular calcium, BK channels are important and widely expressed molecular integrators of extracellular and intracellular signals of cell activity. Alternative splicing and association with regulatory subunits further refines the biophysical properties of BK channels and tailors their activity in cell type-specific ways. This coupled activation by membrane voltage and intracellular calcium and systematic variation in biophysical properties is exquisitely illustrated in the electrical tuning of nonmammalian hair cells. In these sensory cells, BK channels are essential for the frequency-specific responses necessary to decompose sound into its individual frequency components. Although the mammalian inner ear employs a different strategy of frequency decomposition, the sensory hair cells as well as synapses that regulate hair cell activity also express BK channels. In these structures, the subcellular localization of BK channels is regulated both tonotopically and developmentally. In the mammalian inner ear, BK channels appear to be preadapted to serve a variety of essential yet unexpected roles, including maturation of the sensory epithelium, high-frequency hearing, and protection from acoustic trauma.

Further investigation of BK channels in the inner ear will provide important insights into the molecular mechanisms underlying auditory processing in the periphery as well as BK channel biophysical properties and subcellular localization.

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