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Hair cell ribbon synapses

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Abstract

Hearing and balance rely on the faithful synaptic coding of mechanical input by the auditory and vestibular hair cells of the inner ear. Mechanical deflection of their stereocilia causes the opening of mechanosensitive channels, resulting in hair cell depolarization, which controls the release of glutamate at ribbon-type synapses. Hair cells have a compact shape with strong polarity. Mechano-electrical transduction and active membrane turnover associated with stereociliar renewal dominate the apical compartment. Transmitter release occurs at several active zones along the basolateral membrane. The astonishing capability of the hair cell ribbon synapse for temporally precise and reliable sensory coding has been the subject of intense investigation over the past few years. This research has been facilitated by the excellent experimental accessibility of the hair cell. For the same reason, the hair cell serves as an important model for studying presynaptic Ca^{2+} signaling and stimulus-secretion coupling. In addition to common principles, hair cell synapses differ in their anatomical and functional properties among species, among the auditory and vestibular organs, and among hair cell positions within the organ. Here, we briefly review synaptic morphology and connectivity and then focus on stimulus-secretion coupling at hair cell synapses.

Keywords

Hair cell; Ribbon synapse; Cochlear; Vestibular; Inner ear

Introduction

The coding of information with respect to sounds and head movements by the hair cell afferent synapse requires synaptic transmission to be both reliable and temporally precise. Even in the absence of stimulation, the hair cell ribbon-type active zone drives “spontaneous” spiking in the afferent neuron at rates of up to or even beyond 100 Hz (Kiang

et al. 1965; Liberman 1982; Brichta and Goldberg 2000). To ensure such remarkable temporal fidelity and high throughput, the hair cell afferent synapse relies on unique molecular and structural specializations, some of which have been elucidated only recently.

Mature afferent hair cell synapses usually display one single synaptic ribbon or synaptic body facing one postsynaptic density (Fig. 1). Ribbons tether a monolayer of synaptic vesicles, with a high packing density (Lenzi et al. 1999). A fraction of these ribbon-associated vesicles “docks” onto the presynaptic membrane. In addition, some docked vesicles are not associated with the ribbon, and some cytosolic vesicles neither touch the presynaptic membrane nor bind to the ribbon. The synaptic vesicles that populate the hair cell active zones comprise only a tiny fraction of the huge number of synaptic vesicles contained in a hair cell (Spicer et al. 1999).

To study hair cell transmitter release in vivo and in vitro, microelectrode (e.g., Kiang et al. 1965; Rose et al. 1967; Liberman 1982; Furukawa 1986; Goldberg et al. 1990; Holt et al. 2006), patch-clamp recordings from afferent neurons (Glowatzki and Fuchs 2002; Keen and Hudspeth 2006), patch-clamp measurements of presynaptic exocytic membrane capacitance changes (e.g., Parsons et al. 1994; Moser and Beutner 2000), and fluorescence microscopy of presynaptic membrane turnover (e.g., Griesinger et al. 2005), have been used. These experiments have provided insights into the mechanisms of synaptic coding of auditory and vestibular stimuli, such as stimulus secretion coupling, synaptic vesicle pool dynamics, and the kinetics of transmitter release.

Once the stereocilia are mechanically stimulated, mechanosensitive channels open and mediate the receptor current. The resulting depolarization activates voltage-gated Ca^{2+} channels, mostly $\text{Ca}_v1.3$ L-type Ca^{2+} channels. Ca^{2+} channels cluster at the active zone (Lewis and Hudspeth 1983; Art and Fettiplace 1987; Roberts et al. 1990; Rodriguez-Contreras and Yamoah 2001; Zenisek et al. 2003; Sidi et al. 2004; Brandt et al. 2005) and are the source of the Ca^{2+} signal that drives transmitter release (Platzner et al. 2000; Brandt et al. 2003). Two competing hypotheses of stimulus-secretion coupling, viz., the control of synaptic vesicle exocytosis by Ca^{2+} microdomains (Roberts et al. 1990; Roberts 1994; Tucker and Fettiplace 1995) versus Ca^{2+} nanodomains (Brandt et al. 2005), have been put forward and will be discussed in this review. Evidence has been provided for the existence of a readily releasable vesicle pool (RRP) that mediates synchronous synaptic transmission. The ribbon stabilizes a large RRP that supports the release of several vesicles within a short time at each active zone (Moser and Beutner 2000; Khimich et al. 2005). Close inspection of the transmitter release permitted by sensitive postsynaptic recordings has indicated that the exocytosis of multiple vesicles can be highly synchronized (Glowatzki and Fuchs 2002; but for an alternative explanation of quantal size variability in vestibular hair cells, see Holt et al. 2006). Loss of the ribbon reduces the RRP and impairs synchronous postsynaptic activation, leading to the conclusion that the ribbon synapse uses the parallel release of several synaptic vesicles to improve the temporal precision of postsynaptic spiking (Khimich et al. 2005). Whereas some hair cells form multiple synaptic contacts with one postsynaptic neuron, each active zone of mammalian auditory inner hair cells (IHCs) drives just one postsynaptic neuron. Thus, in contrast to large central auditory synapses such as the endbulb and the calyx of Held, which achieve highly reliable and temporally precise

transmission with many small active zones (for a review, see Schneggenburger and Forsythe 2006, this issue), the ribbon synapse of mammalian auditory hair cells uses a single active zone holding a large RRP, as another structure for the same task.

Short-term synaptic depression, involving partial RRP depletion, most likely contributes to peripheral auditory adaptation (Furukawa and Matsuura 1978; Moser and Beutner 2000; Spassova et al. 2004), which has been implicated in the peripheral processing of complex sound signals (Delgutte 1980). Interestingly, whereas the onset of the postsynaptic response varies greatly across different stimulus intensities applied to goldfish saccular hair cells, the time course of adaptation is invariant (Furukawa and Matsuura 1978). In agreement with the hypothesis that RRP depletion underlies rapid peripheral auditory adaptation, the amplitude of the receptor potential has been found to determine the RRP size, but not the time course of pool depletion to the same extent (Brandt et al. 2005). The putative mechanism underlying this behavior will be discussed later in this review. A detailed discussion of vesicle pools, their dynamics, and potential morphological correlates has been provided recently (Nouvian et al. 2006).

Once released, glutamate binds and activates α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Glowatzki and Fuchs 2002) that are clustered in the postsynaptic density (Matsubara et al. 1996). Finally, glutamate is taken up by glutamate transporters (GLAST) of glia-like supporting cells (Furness and Lehre 1997; Furness and Lawton 2003) avoiding glutamate accumulation and hence limiting excitotoxic damage in the case of strong stimulation (Hakuba et al. 2000). The type I vestibular hair cell-calyx synapse isolates the hair cell from the supporting cells.

Progress in understanding the molecular anatomy of hair cell synapses (Safieddine and Wenthold 1999; Eybalin et al. 2002; Khimich et al. 2005) has been slow, mostly because of the limited amount of material (a few thousand hair cells per ear). Although we are far from a detailed description (for a recent review, see Nouvian et al. 2006), we can state that the molecular composition is similar to that of retinal synapses (for a review, see tom Dieck and Brandstätter 2006, this issue). Important differences include the dominance of $\text{Ca}_v1.3$ channels and the presence of the putative Ca^{2+} sensor of exocytosis otoferlin (Yasunaga et al. 1999).

Here, we will first review the common features and different aspects of active zone morphology and synaptic connectivity in auditory and vestibular hair cells. We will then discuss the general properties of presynaptic function in hair cells. We will also focus on ion channels and Ca^{2+} signaling related to transmitter release.

Structure of hair cell ribbon synapses and synaptic connectivity

Specialization of auditory and vestibular hair cell synapses results in substantial anatomical diversification. Hence, few “typical hair cell afferent synapses” exist. Hair cell ribbons vary greatly in size (from ~ 0.1 to $\sim 0.4 \mu\text{m}$) and shape (from plate-like ribbons to spheroid bodies) among the various preparations (Fig. 1, Table 1). Thus, the number of vesicles tethered by a ribbon ranges from 20 to 30 in peripheral vestibular type II hair cells (Lysakowski and Goldberg 1997), from 100 to 200 in mouse cochlear IHCs (Khimich et al. 2005), and from

150 to 300 in central type II vestibular hair cells (Lysakowski and Goldberg 1997) and is ~400 in frog saccular hair cells (Lenzi et al. 1999). The relationship between structural and functional vesicle populations at ribbon-type active zones is still a matter of debate (for a review, see Nouvian et al. 2006). There seems to be an agreement at least for mouse IHCs (Moser and Beutner 2000), turtle hair cells (Schnee et al. 2005), and frog saccular hair cells (Rutherford and Roberts 2006) that the first exhaustible kinetic component (the RRP) corresponds to the docked vesicles (but, for an opposing view, see Edmonds et al. 2004; Spassova et al. 2004).

The way that a hair cell makes synaptic contact with postsynaptic neuron or neurons (synaptic connectivity) varies among auditory and vestibular hair cells and between hair cells of different species. Synaptic connectivity is probably still most uniform in the auditory end organ (Fig. 2, Table 1). Here, we will illustrate this for the mammalian cochlea and the avian basilar papilla. In the mammalian cochlea, a clear functional segregation of the hair cells exists. The IHCs are the genuine sensory cells coding sound information, whereas the outer hair cells (OHCs) have been implicated in the mechanical amplification of sound-induced cochlear vibration. Depending on the species and the position along the tonotopic axis, IHCs contain 5–30 active zones driving a corresponding number of unbranched processes of myelinated spiral ganglion neurons. These type I spiral ganglion neurons (95% of the spiral ganglion neurons) vary in their spontaneous rate and threshold (e.g., Liberman 1982). Synapses of low spontaneous rate fibers, mostly located at the neural (modiolar) side of the IHC, tend to have larger ribbons than those of the high spontaneous rate fibers on the abneural (pillar) side (Merchan-Perez and Liberman 1996; Tsuji and Liberman 1997; Slepecky et al. 2000). OHCs in the apical cochlear turns form ribbon synapses with unmyelinated type II spiral ganglion neurons (Berglund and Ryugo 1987; Liberman et al. 1990). The function of OHC afferent synaptic transmission remains to be clarified.

The avian auditory end organ, the basilar papilla, does not show a strict dichotomy of hair cell types. Rather, a continuum ranges from tall hair cells with closest proximity to the neural edge, to the short hair cells near the abneural side of the organ. Likewise, the innervation pattern is not as segregated as in the mammalian cochlea. The number of afferent fibers per tall hair cell is lower than that of IHCs in the cochlea. Most fibers exclusively contact one tall hair cell (Fischer 1992) and collect input from several synapses.

The vestibular system is particularly rich in synaptic specializations (Fig. 2), featuring bouton-, dimorphic-, and calyx-type afferent endings (Fernandez et al. 1988, 1990, 1995; Goldberg et al. 1990). Each vestibular neuron receives input from multiple ribbon synapses of one (or more than one) hair cell in the case of a calyx or dimorphic afferent, or of many hair cells in the case of a bouton or dimorphic afferent (Lysakowski and Goldberg 1997). Calyx-type terminals not only receive ribbon synapse input from the enclosed type I hair cells on their inner face, but can also receive this input from neighboring type II hair cells on their outer face, although whether these outer face synapses have equal weight is unknown. Figure 2 illustrates the spectrum of afferent hair cell connectivity observed in the various preparations.

Convergent input from several synapses of one or multiple hair cells onto an afferent neuron (e.g., vestibular system, avian basilar papilla) is likely to enhance the evoked activity over background. Detection of coincident synaptic inputs by the action-potential-generating element would help to suppress uncorrelated spontaneous synaptic input. On the other hand, the number of statistically independent channels carrying sensory information to second order neurons will be lower than, for example, in the mammalian cochlea with potential consequences for processing in the brainstem. In the vestibular system, with its large variation in synaptic ribbons, afferent fibers, and terminal endings, it would be surprising if these did not permit a wide latitude in coding mechanisms relevant to vestibular function (Goldberg 2000). Future work will be needed to clarify the exact way that the different connectivities relate to coding strategies of auditory and vestibular stimuli.

Ion channels affecting hair cell presynaptic function

Hair cells display a resting membrane conductance based on basolateral potassium channels (KCNQ and erg families) and stereociliar mechanoelectrical transduction channels. Mutation of the gene coding for KCNQ4 causes autosomal dominant hearing impairment DFNA2 in humans (Kubisch et al. 1999). The hearing loss of mouse models of DFNA2 can mostly be explained by the dysfunction/degeneration of mechanically active OHCs (Kharkovets et al. 2006), as had been suggested before in a pharmacological model of DFNA2 (Nouvian et al. 2003). The DFNA2 mouse models show no major change of IHCs synaptic function and no obvious vestibular dysfunction. In addition to KCNQ channels, vestibular type I hair cells express erg channels (Eatock et al. 2002; Wong et al. 2004), which may contribute to a distinctive current found in type I hair cells, $I_{K,L}$ (K.M. Hurley, S. Gaboyard, J.R. Woollorton, J.L. Garcia, S.D. Price, A. Lysakowski, R.A. Eatock, submitted for publication).

$Ca_v1.3$ Ca^{2+} channels of hair cells (see below) become activated at the resting potential of the hair cell even in the absence of sensory stimuli (Koschak et al. 2001; Xu and Lipscombe 2001; Brandt et al. 2005; Johnson et al. 2005). Afferent neuron “spontaneous” activity seems to rely entirely on transmitter release from hair cells (Liberman and Kiang 1978; Sewell 1984). Once mechanoelectrical transduction occurs, the apical potassium influx drives receptor potential generation, which further activates voltage-gated K^+ and Ca^{2+} channels. BK (large conductance) currents are activated within hundreds of microseconds and have been implicated in shortening the hair cell membrane time constant required for high temporal precision sound coding, in limiting of the receptor potential, and in electrical frequency tuning (in non-mammalian hair cells). Electrical hair cell tuning to a certain stimulus frequency builds on an intimate interplay of BK and Ca^{2+} channels, which require close spatial colocalization, most likely at the active zones (Lewis and Hudspeth 1983; Art et al. 1986; Fuchs et al. 1988; Roberts et al. 1990; Tucker and Fettiplace 1995). This has fostered the use of BK channels as a readout of active zone Ca^{2+} signaling of hair cells (see next section). Surprisingly, BK channels of mammalian cochlear IHCs cluster remotely from the active zones at the apical part of the cell (neck; Pyott et al. 2004; Hafidi et al. 2005) and are largely unaffected by manipulations of Ca^{2+} channels (Marcotti et al. 2004; Thurm et al. 2005). This finding might be related to the evolution of other efficient tuning mechanisms in mammalian cochlea. Deletion of the *Kcnma1* gene in mice has surprisingly little primary

impact on hearing as revealed by auditory brainstem responses (Rüttiger et al. 2004), which reflect the sound-evoked synchronized activation of neuronal populations along the auditory pathway. However, single hair cell and single spiral ganglion neuron analysis has revealed defects in the timing of receptor potentials and subsequently of postsynaptic action potential generation (Oliver et al. 2006). Activation of K_v -type channels causes a millisecond time course decline of the hair cell receptor potential and may thereby contribute to rapid auditory adaptation (Kros 1996; Moser and Beutner 2000).

Evidence for an essential role of L-type Ca^{2+} channels for hair cell transmitter release is well documented (Lewis and Hudspeth 1983; Art and Fettiplace 1987; Fuchs et al. 1990; Roberts et al. 1990; Zhang et al. 1999; Moser and Beutner 2000; Platzer et al. 2000; Spassova et al. 2001; Robertson and Paki 2002; Brandt et al. 2003; Dou et al. 2004; Schnee et al. 2005). Two independently generated $Ca_v1.3$ knockout mice show complete deafness (lack of auditory brainstem responses) and more than a 90% reduction of hair cell Ca^{2+} currents (Platzer et al. 2000; Brandt et al. 2003; Michna et al. 2003; Dou et al. 2004), but no obvious balance problems. Interestingly, Ca^{2+} uncaging, bypassing the defect in Ca^{2+} influx, elicits robust exocytic capacitance changes (Brandt et al. 2003). This result together with the finding of a largely unchanged molecular synaptic anatomy in young mice (Nemzou et al. 2006) argues that the presynaptic machinery is assembled, despite the lack of Ca^{2+} influx. The remaining Ca^{2+} current of cochlear hair cells is at least partially mediated by L-type Ca^{2+} channels and is insensitive to blockers of N, P/Q, and R-type Ca^{2+} channels. The Yamoah group has presented single channel evidence for the presence of non-L, possibly N-type, channels in frog saccular hair cells (Rodriguez-Contreras and Yamoah 2001; Rodriguez-Contreras et al. 2002). In frog crista hair cells, R-type Ca^{2+} channels have been suggested by pharmacological analysis of whole-cell Ca^{2+} currents (Martini et al. 2000). This, together with normal performance of $Ca_v1.3$ -deficient mice in screening tests of vestibular function (Dou et al. 2004), has led the authors to conclude that non-L-type channel can mediate transmitter release in vestibular hair cells.

Stimulus-secretion coupling at the hair cell ribbon synapse

Many aspects of presynaptic Ca^{2+} signaling are inaccessible in small presynaptic boutons of the CNS but can be readily studied and manipulated in hair cells. The classical work of Roberts and Fettiplace and their colleagues on Ca^{2+} channel number and distribution and on cytosolic Ca^{2+} buffers has largely contributed to making the hair cell a valuable model system for presynaptic Ca^{2+} signaling (for previous reviews, see Lenzi and Roberts 1994; Tucker and Fettiplace 1996; Augustine 2001; Augustine et al. 2003). Moreover, the characterization of stimulus-secretion coupling in hair cells is the key to understanding the coding of information regarding sound or vestibular stimuli.

The actual Ca^{2+} channel release-site topography at a given active zone remains unknown for hair cell ribbon synapses. Do Ca^{2+} channels cooperate to impose an elevation of the Ca^{2+} concentration (Ca^{2+} microdomain), or do individual channels drive “their” vesicles, which are positioned in nanometer proximity (Ca^{2+} nanodomain)? Figure 3 illustrates both concepts. Roberts and colleagues (1990) have inferred a specific topography of Ca^{2+} and K^+ channels at the synapses of frog saccular hair cells based on the finding of clusters of

intramembrane particles in freeze-fracture electron micrographs at presumptive synapses and the numerical correspondence of particle count and physiologically determined numbers of Ca^{2+} and BK channels. However, numerous particles also exist outside the arrays, and the number and distribution of Ca^{2+} channels relative to the docked vesicles still awaits quantitative analysis, e.g., by immunoelectron microscopy.

Even if the Ca^{2+} channel/release site topography at the active zone were known, it remains difficult to predict whether release is controlled by the Ca^{2+} nanodomain shaped by stochastic gating of one or few Ca^{2+} channels (as suggested by Brandt et al. 2005) or by a Ca^{2+} microdomain produced by many channels (Roberts 1994; Tucker and Fettiplace 1995) where the single channel properties are averaged. This difficulty arises because the functional stimulus-secretion coupling further depends on the channel number, distribution, and open probability (set by the stimulus and the maximal open probability), the width of the single channel Ca^{2+} domain (set by the single channel current and the cytosolic Ca^{2+} buffers), and the intrinsic Ca^{2+} sensitivity of exocytosis. Substantial work has been performed in the past to quantify these parameters and will be reviewed here.

Number and distribution of Ca^{2+} channels in hair cells

Evidence arguing for a micrometer-scale clustering of Ca^{2+} channels at the hair cell ribbon-type active zone comes from loose patch recordings (Roberts et al. 1990), cell-attached recordings (Rodriguez-Contreras and Yamoah 2001), Ca^{2+} imaging (Issa and Hudspeth 1994; Tucker and Fettiplace 1995; Zenisek et al. 2003), and immunohistochemistry (Sidi et al. 2004; Brandt et al. 2005). Table 2 summarizes the available information on Ca^{2+} channel number in hair cells and the approximate total and maximal open Ca^{2+} channel numbers per active zone for the various hair cell types. As pointed out by Martinez-Dunst et al. (1997), hair cell Ca^{2+} current seems to scale not only with the number of synapses, but also with the size of the active zone. Hair cell Ca^{2+} channel numbers have been obtained by (1) non-stationary fluctuation analysis, (2) dividing the whole cell Ca^{2+} current (tail or pulse current) by the single channel current. Approximations of the number of Ca^{2+} channels per active zone either assume a purely synaptic distribution (Roberts et al. 1990; Tucker and Fettiplace 1995; Martinez-Dunst et al. 1997) or consider a certain extrasynaptic density of Ca^{2+} channels in hair cells (Brandt et al. 2005).

The assumption of a purely synaptic localization has been motivated by the co-variation of Ca^{2+} current and active zone number (e.g., Martinez-Dunst et al. 1997; Wu et al. 1995; Schnee et al. 2005). However, a substantial, albeit much lower, abundance of L-type Ca^{2+} channels has been found outside the observed dense clusters (Rodriguez-Contreras and Yamoah 2001) in cell-attached measurements of Ca^{2+} channel distribution in frog saccular hair cell. Imaging of Ca^{2+} entry (low affinity dyes and excess non-fluorescent high-affinity exogenous Ca^{2+} buffers) and of immunostained Ca^{2+} channels is not capable of detecting extrasynaptic channels, which probably occur at low density. Hence, further experiments, such as immunoelectron microscopy and cell-attached recordings of Ca^{2+} channel distribution in hair cells with a known relationship to the synapse position, are needed.

Ca²⁺ buffering in hair cells

Work by the groups of Hudspeth and Roberts and Fettiplace and colleagues has indicated a millimolar concentration of fast mobile Ca²⁺-binding proteins in frog (Roberts et al. 1990; Edmonds et al. 2000; Heller et al. 2002) and turtle (Tucker and Fettiplace 1995) hair cells (see Table 3). The presence of the mobile proteinaceous Ca²⁺ buffers calretinin, calbindin, and parvalbumin has now been documented in a variety of cochlear and vestibular hair cells (Edmonds et al. 2000; Heller et al. 2002; Hackney et al. 2003, 2005; Desai et al. 2005a,b). Their relevance for local hair cell Ca²⁺ signaling has been inferred from recordings of Ca²⁺-activated large conductance K⁺ channels (Fettiplace 1992; Roberts 1993; Tucker and Fettiplace 1996; Edmonds et al. 2000), from investigations of the adaptation of mechanoelectrical transduction current (Ricci et al. 1998), from simulations (Roberts 1994), and from capacitance measurements (Moser and Beutner 2000; Spassova et al. 2004). Most likely, they spatiotemporally restrict the presynaptic Ca²⁺ domains and hence improve the timing of synaptic transmission. Table 3 summarizes currently available information on Ca²⁺ buffers in hair cells of various species. Not only are the concentrations of the buffers different between the various hair cells, but these proteins also differ in their kinetics of Ca²⁺ binding. Calbindin and calretinin bind Ca²⁺ rapidly and are therefore often compared with the fast Ca²⁺ chelator BAPTA, whereas the two slow Ca²⁺-binding sites of parvalbumin are mostly considered to be equivalent to two molecules of EGTA. These comparisons are, however, oversimplifications, not taking into consideration distinct properties of each of the four or five binding sites in calbindin D-28k and calretinin, respectively nor cooperativity between binding sites (Schwaller et al. 2002).

The major differences in the amount of buffers in the various hair cells suggest that our understanding of the role of calcium buffers for stimulus-secretion coupling at the hair cell active zone synapse is far from complete. Within the mammalian cochlea, immunohistochemistry and physiology suggest that IHCs contain submillimolar concentrations of Ca²⁺-binding sites. Surprisingly, OHCs of the cochlea, whose primary function is mechanical amplification, but who have little if any role in sound coding, contain much higher concentrations of Ca²⁺ buffers (Hackney et al. 2005). A high buffer concentration might be important for stereociliar Ca²⁺ signaling and hair-bundle-based amplification in these cells (Kennedy et al. 2005). Examination of hair cells from mutant mice lacking the major proteinaceous Ca²⁺ buffers promises further insights into the role and impact of fast Ca²⁺ buffering in amplification and synaptic sound coding in the mammalian cochlea.

Ca²⁺ sensitivity of release

Transmitter release depends on cooperative binding of more than one incoming Ca²⁺ ion to the fusion apparatus in most presynaptic cells (Augustine 2001). The cooperativity of incoming Ca²⁺ ions in regulating release (“apparent” Ca²⁺ cooperativity) depends on several factors: (1) the binding properties of the Ca²⁺ sensor of exocytosis (“intrinsic” Ca²⁺ cooperativity), (2) the topography of the vesicle release site and the Ca²⁺ channels triggering release at the active zone, and (3) the way that Ca²⁺ influx is experimentally manipulated. When transmitter release is evoked by depolarization to varying levels, a low Ca²⁺ cooperativity (close to unity) is observed in presynaptic capacitance measurements (Moser

and Beutner 2000; Brandt et al. 2005; Johnson et al. 2005; Schnee et al. 2005) and in paired pre- and postsynaptic recordings (Keen and Hudspeth 2006). This is in sharp contrast to the high intrinsic Ca^{2+} cooperativity of transmitter release elicited by Ca^{2+} uncaging (Beutner et al. 2001) in the low micromolar $[\text{Ca}^{2+}]$ range (cooperative binding of five ions). In these experiments, the Ca^{2+} -dependent kinetics of exocytosis display saturation at $[\text{Ca}^{2+}]$ exceeding 50 μM .

Brandt and colleagues (2005) have compared the effects of changes in open Ca^{2+} channel number and in single Ca^{2+} channel current on exocytosis of the RRP in mouse IHCs. A high apparent Ca^{2+} cooperativity of RRP exocytosis, approaching the intrinsic Ca^{2+} cooperativity, has been observed during changes of single channel current (changes of extracellular Ca^{2+}) and rapid flicker block (Zn^{2+}) in the range of small Ca^{2+} currents. On the other hand, near linear changes of RRP exocytosis with the Ca^{2+} current have been found during pharmacological (dihydropyridine) modulation of Ca^{2+} channel open probability, mimicking the Ca^{2+} cooperativity of RRP exocytosis observed upon depolarization to different levels. In contrast to their findings, a Ca^{2+} microdomain control of exocytosis should have revealed a high Ca^{2+} cooperativity, irrespective of the way that the Ca^{2+} influx was manipulated (Augustine et al. 1991; Mintz et al. 1995). Hence, stimulus-secretion coupling in mouse IHCs has been interpreted within the framework of a Ca^{2+} nanodomain control of synaptic vesicle exocytosis. This hypothesis has further been supported by the small number of open Ca^{2+} channels per active zone (maximal 30 at saturating stimuli), the low sensitivity to the exogenously added, slow-binding, Ca^{2+} chelator EGTA (Moser and Beutner 2000), the evidence for nanodomain overlap for strong depolarization (Augustine et al. 1991), and the low Ca^{2+} affinity of the hair cell Ca^{2+} sensor (Beutner et al. 2001). The depolarization level has been concluded to determine the number of active channel-release site units, i.e. the RRP, whereas the kinetics of pool depletion are much less dependent on the stimulus (Brandt et al. 2005). This Ca^{2+} nanodomain control of exocytosis has been postulated to enable the hair cell to be involved in sound coding with high temporal precision even at low sound intensities. The temporal precision of synaptic transmission would be further improved with stronger stimuli, because then more vesicles could be released in parallel, thereby reducing the jitter of postsynaptic spiking.

Concluding remarks

The various hair cell types share common presynaptic properties. These include a high abundance of potassium channels shortening the membrane time constant and the presence of ribbons providing a large RRP for the exocytosis of glutamate driven by Ca^{2+} influx through $\text{Ca}_v1.3$ L-type Ca^{2+} channels. However, they differ extensively in synaptic morphology and connectivity. More research is required to relate the specific molecular anatomy and physiology of a particular hair cell synapse to their specific coding properties.

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Fig. 1.

Electron micrographs of ribbon synapses from hair cells. **a–i** Ribbons from type I and type II vestibular hair cells in the adult chinchilla crista ampullaris (from Lysakowski and Goldberg 1997, *J. Comp. Neurol.* vol. 491, pp. 438–439, modified with permission from John Wiley and Sons, Inc.). Type I hair cells generally have small spherical ribbons, regardless of their location within the sensory epithelium (central, **a, b**; peripheral, **c, d**). The elongated ribbon from a central type I hair cell in **b** is an exception in the chinchilla, although much more common in the monkey (Engstrom et al. 1972). Type II hair cells, particularly those in the central part of the sensory epithelium, where sensitivity is highest, show a large variation in shapes and sizes. They can be large and hollow (**e, g**), spheroid (**f**), and elongated and plate-like (**i**), extending through 10–12 ultrathin sections. They can occur singly (**e, f, i**) or in clusters (**g, h**). Ribbons in type II hair cells in the peripheral part of the epithelium tend to be small and spheroid (similar to the peripheral type I hair cell ribbons in **c, d**). **j–o** Ribbons from cochlear hair cells in diverse species. Inner hair cells (**j–m, IHC**) have variable ribbon shapes, whereas outer hair cell (**OHC**) ribbons tend to be elongated rods (**n, o**), although the ribbon in **o** is a less common spheroid ribbon. **j, k** Middle turn of guinea pig cochlea, inner

hair cells (*DP* dense plaques found presynaptically); modified from Saito (1980) J. Ultrastructural Research, vol. 71, p. 225, with permission from Elsevier. **l** Cat cochlea, tonotopic location approximately 2.4 kHz, modified from Liberman (1980) Hear. Res., vol. 3, p. 56, with permission from Elsevier. **m, n** Cat cochlea; inner hair cell (*IHC*) at 0.5 kHz, and outer hair cell (*OHC*), respectively, probably from an apical turn, given the multiple ribbons (*RF* radial fiber afferent, *arrows* indicate extent of synaptic plaque) *arrowheads* docked vehicles; modified with permission from Liberman et al. (1990) J. Comp. Neurol. vol. 301, p. 447, with permission from John Wiley and Sons, Inc. **(o)** Taken from macaque cochlea, outer hair cell, location unknown; from Kimura (1984), modified with permission from Friedmann and Ballantyne (1984) Ultrastructural Atlas of the Inner Ear, Taylor and Francis Publishers

Fig. 2.

Afferent synaptic connectivity of hair cells. Four different types of afferent connections are found in the auditory and/or vestibular system. For simplification, all other cellular elements (e.g., efferent contacts and supporting cells) have been ignored. **a** Several unbranched processes, each forming one synapse (blue fiber and postsynaptic density, green synaptic ribbon) with one hair cell, as is typical for mammalian cochlear inner hair cells. **b** Branched process innervating two hair cells. Each branch collects input from one or more ribbon synapses. **c** Calyx-type postsynaptic ending enclosing a type I vestibular hair cell and forming several ribbon synapses with the enclosed hair cell in addition to some synapses on its outer surface with a neighboring type II hair cell. Such “outer face” synapses are more

common in the central part of the sensory epithelium. **d** A dimorphic fiber that forms a calyx synapse with a type I hair cell and bouton synapses with type II hair cells

Fig. 3.

Contrasting views of Ca^{2+} signaling at hair cell active zones. **a** Modeling of active zone Ca^{2+} concentration for frog saccular hair cells; from Roberts (1994), reprinted with permission from the Journal of Neuroscience. The model was based on measurements of voltage-gated Ca^{2+} entry, the accompanying activation of BK current in frog saccular hair cells, and an estimation of cytosolic Ca^{2+} buffering capacity. Ca^{2+} microdomains soak the entire active zone with some locally active maxima. **b** Representation of the inner hair cell active zone seen from the hair cell cytosolic side with the ribbon (*medium gray ellipse* projection) removed (from Brandt et al. 2005; reprinted with permission from the Journal of Neuroscience). Synaptic vesicles (*gray spheres*) preferentially dock to the plasma membrane (*transparent*) opposite the postsynaptic density (*light gray*). Approximately 80 Ca^{2+} channels (*black dots*) were pseudo-randomly scattered at the active zone with a higher density underneath the ribbon (*dark gray ellipsoid*) than for the rest of the active zone (*light gray ellipsoid* postsynaptic density). Synaptic vesicles were placed in a more regular array according to electron microscopic findings (c.f. Fig. 1). Domains of elevated $[\text{Ca}^{2+}]$ are

indicated in *black*. In the case of weak stimulation, only a few channels open and drive exocytosis of “their” vesicles, whereas an overlap of Ca^{2+} domains is expected for saturating stimulation

Table 1

Synaptic morphology and synaptic connectivity (*HC* hair cell, *Fib* fiber, *LF* low frequency, *HF* high frequency, *Syn* synapse, *ND* not determined). The table summarizes part of the available information obtained in the various preparations. The references are examples from which information was obtained, but which do not represent a complete list. Data were obtained by electron microscopy except for those obtained by immunofluorescence (Khimich et al. 2005)

Species/Organ	Hair cell	Ribbon: shape, size (nm), and number	Afferent connectivity	References
Mammalian cochlea	Inner hair cells	Ellipsoid; 3 axes: 55, 100–200, 200–600; 5–30	1 HC: 5–30 Fib; 1 Fib: 1 HC; 1 Fib: 1 Syn	Liberman 1980; Francis et al. 2004; Khimich et al. 2005
Avian papilla	Outer hair cell (apex)	Elongate; 100–200; 8–10		Liberman et al. 1990
	Tall hair cells:	Round; 120 (apex), 210 (base); 15	1 HC: 1–3 Fib; 1 Fib: 1(–3) HC*; 1 Fib: several Syn	Fischer 1992; Martinez-Dunst et al. 1997
	Short hair cells	0 (HF) –10 (LF)		
Mammalian crista ampullaris	Type I hair cell	Spheroid; 90, Rod: 200–300. ~17	1 HC: 1 Fib; 1 Fib: 1–5 HC; 1 Fib: 1–5 Calyces with 15 Syn each	Goldberg et al. 1990
	Type II hair cell	Barrel: 200 Elongate 150–300. ~18	1 HC: 3–6 Fib; 1 Fib (bouton): ~30 HC; 1 Fib (bouton): 50–80 Syn	Lysakowski and Goldberg 1997 Fernandez et al. 1988
Avian crista ampullaris	Type I hair cell type II hair cell	Spheroid; rod ND	1 Fib: up to 12 HCs	Lysakowski 1996
Turtle crista ampullaris	Type I hair cell type II hair cell	Spheroid; ND	1 Fib: up to 8 HCs	Lysakowski 1996; Brichta and Peterson 1994
Frog crista ampullaris	Type II hair cell	Spheroid; ND		Lysakowski 1996
Fish crista ampullaris	Type II hair cell	Spheroid 15–25 central; 4–5 peripheral		Wegner 1982; Chang et al. 1992; Lysakowski 1996

Table 2

Number of Ca^{2+} channel at hair cell synapses. This table summarizes part of the available information obtained in diverse preparations. The analysis is complicated by the low open probability of hair cell L-type Ca^{2+} channels in the absence of the dihydropyridine agonist of L-type Ca^{2+} channels, BayK8644. The numbers provided are rough approximations relying on several assumptions (e.g., extrasynaptic Ca^{2+} channel density) and should be taken with caution (*LF* low frequency, *HF* high frequency)

Species/organ	Hair cell	Total number of channels	Channels per active zone (total/maximal open)	References
Frog sacculus		~1,800	~90/~20	Roberts et al. 1990 ^a , assuming an exclusively synaptic Ca^{2+} channel localization
Turtle basilar papilla		~2,240 (HF) ~400 (LF)	~40 ~20	Wu et al. 1995 ^b ; Sneyers 1988, assuming an exclusively synaptic Ca^{2+} channel localization
Mammalian cochlea	Inner hair cells	~1,700	~80/~30	Brandt et al. 2005, ^a assuming an extrasynaptic channel density of 1 channel/ μm^2
Avian basilar papilla	Tall hair cells	~350 (HF) ~200 (LF)	~15 ~23	Martinez-Dunst et al. 1997 ^b , assuming an exclusively synaptic Ca^{2+} channel localization
	Short hair cells	~100		

^a Estimates obtained by non-stationary fluctuation analysis on Ca^{2+} tail currents estimates

^b Approximations provided by the cited reference based on whole-cell Ca^{2+} or Ba^{2+} current measurements and single channel currents

Presence and concentrations of calcium-binding proteins in hair cells. The table summarizes part of the available information on calcium binding obtained from various preparations. Hair cells vary in the type and amount of calcium-binding proteins, as determined by the various approaches. The number of binding sites per protein also varies (for additional discussion, see Hackney et al. 2005; Desai et al. 2005b), e.g., mammalian calbindin D-28k has four and calretinin five functional calcium-binding sites, while parvalbumin binds only two calcium ions. The Ca²⁺ affinity under physiological conditions is rather similar for all three proteins (in the range of 0.3–1.5 μM), while kinetic properties differ considerably: calbindin D-28k and calretinin are considered “fast” buffers, while parvalbumin has properties similar to EGTA (Schwaller et al. 2002) (*LF* low frequency, *HF* high frequency, *IHC* inner hair cell, *OHC* outer hair cell, ⁺ positive, – negative)

Table 3

Species/organ	Calbindin-D28k (μM)	Calretinin (μM)	Parvalbumin-α (μM)	Parvalbumin-β (μM)	Parvalbumin-3 (μM)	Ca-binding sites (mM)	Approach	References
Tall frog saccular	–	1,200±400				6	Western blot, patch-clamp	Edmonds et al. 2000
Turtle auditory	627±151 (HF)	9±3 (HF)		700–3000			Western blot	Heller et al. 2002
	129±95 (LF)	11±2 (LF)		256±72 (HF)	3.0 (HF)		Calibrated immunogold	Hackney et al. 2003
Mature rat cochlear IHC	–			223±51 (LF)	1.0 (LF)			
					1		Patch-clamp	Tucker and Fettiplace 1996
					0.1–0.4		Patch-clamp	Ricci et al. 1998
			~160 (HF)	–	0.49 (HF)		Calibrated immunogold	Hackney et al. 2005
Mature rat cochlear OHC		~35 (HF)	~170 (LF)			0.53 (LF)		
	15±11 (HF)	~70 (HF)	~100 (HF)	2892±120 (HF)	6.0 (HF)		Calibrated immunogold	Hackney et al. 2005
	230±38 (LF)	~40 (LF)	~290 (LF)	1954±151 (LF)	5.4 (LF)			
Mouse cochlear IHC	+	++	++				Immunofluorescence	Sendin et al., unpublished
Mature rat and mouse type II vestibular hair cells						Submillimolar	Patch-clamp	Moser and Beutner 2000
		++ (70&–80% of type II hair cells)					Immunoperoxidase	Desai et al. 2005a,b

⁺ medium staining
⁺⁺ strong staining