TRANSDUCTION AND ADAPTATION IN SENSORY HAIR CELLS OF THE MAMMALIAN VESTIBULAR SYSTEM

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ABSTRACT

The human vestibular apparatus detects head movements and gravitational stimuli which impinge upon the mechanosensory hair cells of the inner ear. The hair cells, in turn, transduce these stimuli into electrical signals which are transmitted to the brain. These sensory cells are exquisitely responsive, signaling deflections of their mechanosensitive organelles as small as 1–2 nanometers. Remarkably, they are able to preserve this level of sensitivity even when confronted with large tonic stimuli, such as gravity. To accomplish this feat hair cells have devised a novel adaptation process that repositions the mechano-transduction apparatus on a millisecond time scale to allow high sensitivity over a broad operating range.

Mechanotransduction in hair cells occurs via a direct gating mechanism in which hair bundle deflection focuses tension onto membrane-bound, cation-selective ion channels located near the tips of the hair bundle. Increased tension favors an open conformation of the channel and allows calcium to enter the cell. Elevated intracellular calcium promotes adaptation which has been hypothesized to result from the activity of a cluster of molecular motors that continually adjust the tension in the transduction apparatus. Although the transduction channel itself remains elusive, myosin Ic has recently been identified as a molecular component of the "adaptation" motor.

INTRODUCTION

Maintenance of balance and spatial orientation requires input from the proprioceptive, visual and vestibular systems. Proprioceptive receptors in muscles and joints provide information regarding trunk and limb position. Ocular input allows for orientation relative to surrounding objects in the environment. The vestibular organs detect both angular velocity and linear acceleration, including gravity. Here we review the anatomy and physiology of the vestibular end organs with particular attention devoted to sensory transduction and adaptation in the mechano-sensitive hair cells.

VESTIBULAR ANATOMY

The human vestibular system is composed of two clusters of motion sensing organs situated in the hard labyrinthine bone of the inner ear. The apparatus, or vestibule, is sculpted to allow detection of linear acceleration and angular velocity in orthogonal planes of orientation. Each vestibule consists of two sack-like structures, the saccule and utricle and three tubular structures known as the semicircular canals (Figure 1A). The lateral canal is

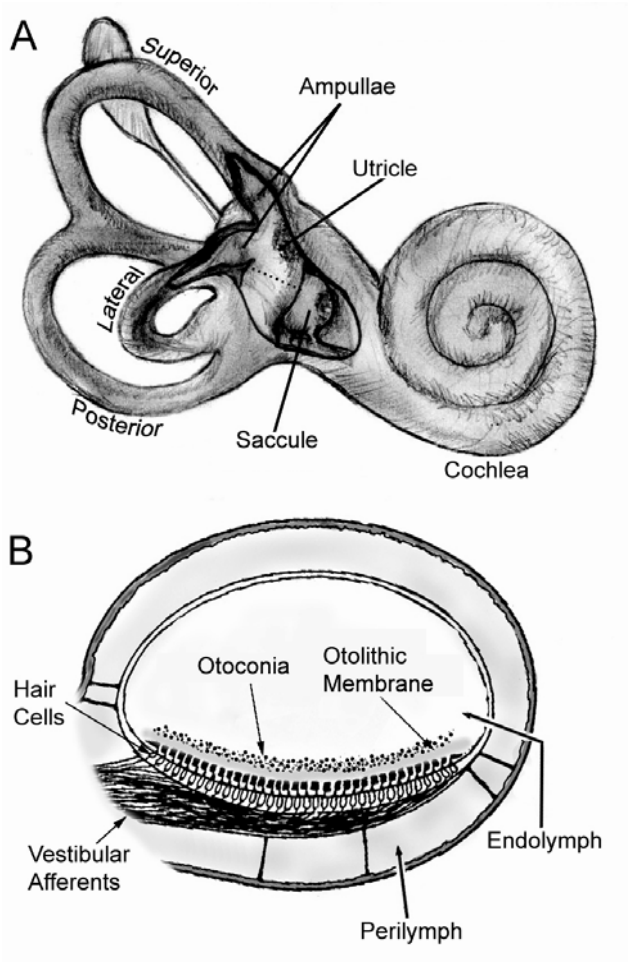

Figure 1. Schematic illustrations of the human vestibule with the utricle. ***(A) The vestibule as situated in the hard labyrinthine portion of the temporal bone.*** *The relative orientations of the three semicircular canals are represented along with their anatomic positions relative to the utricle, saccule, and the auditory organ (cochlea). A window of bone overlying the central portion of the vestibule has been removed to reveal the membranous labyrinth with its associated vestibular end organs: the ampullae, utricle, and saccule. The dotted line marks the cross-section of the utricle shown in B.* ***(B) Illustration of a cross section through the mammalian utricle.*** *The utricle is housed in the membranous labyrinth, suspended within the hard labyrinthine region of the temporal bone. Note that the membranous system is surrounded by perilymphatic fluid, while the hair bundles of the utricular epithelia and the overlying otolithic membrane face the endolymphatic compartment. The endolymph is distinctly high in potassium and is more intracellular in nature. The perilymph is low in potassium and is similar in composition to typical extracellular fluid. The otoconia are embedded in the upper portion of the otolithic membrane and are of a greater density than the surrounding endolymphatic fluid. Beneath the hair cells are sensory fibers of the vestibular branch of the eighth cranial nerve. Modified from Eatock et al., (1987).*

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oriented approximately in the horizontal plane. There are two vertically positioned canals oriented nearly 45°off the sagittal plane, the anterior (or superior) and posterior canals. Each semicircular canal is responsible for detecting angular velocity within its plane of orientation. The position of the canals within the two ears places the right anterior semicircular canal in the same orientation as the left posterior canal, i.e. these canals detect angular velocity within the same plane. Similarly, the left anterior canal and the right posterior canal are in the same orientation, as are the two horizontal canals.

In anatomic continuity with the semicircular canals of the vestibule are the otolithic organs, the saccule and utricle (Figure 1A). They are primarily accelerometers and detect changes in linear motion and gravity in both the vertical and horizontal planes (Goldberg and Fernandez, 1975).

Situated within the convoluted bony framework of the vestibule is the membranous labyrinth. This membranous system connects the semicircular canals, utricle, saccule, and the auditory organ and is filled with an endolymphatic solution (reviewed in Wangemann, 2002a). Separating the membranous labyrinth from the surrounding walls of the bony labyrinth is the perilymphatic fluid space. The compositions of the endolymph and perilymph are distinct. The endolymphatic fluid is high in potassium and low in sodium and calcium, similar to intracellular fluid. The perilymphatic fluid is similar in composition to typical extracellular fluid, like the nearby intracranial fluid which surrounds the brain and spinal cord (i.e. low potassium and high sodium and calcium). The anatomic separation of these two fluid compartments not only reflects morphologic and mechanical characteristics of the inner ear, but is also physiologically significant. The differing ion concentrations of the endolymph and perilymph lead to electrical potential differences across the membranes that separate these two systems, with observed potentials of +80 millivolts in certain portions of the vestibule (reviewed in Wangemann, 2002b). The *stria vascularis*, a strip of highly vascularized tissue, in the auditory organ and the dark cells of the vestibular organs are responsible for actively maintaining the high potassium concentration of the endolymph.

Within each membranous semicircular canal is a dilated region that contains the ampulla. The ampulla is composed of the crista (neuroepithelium), the cupula, supporting cells, connective tissue, blood vessels, and fibers of the vestibular nerve. Overlying the crista is the cupula, a gelatinous diaphragm composed of mucopolysaccharides which extend to the roof and lateral walls of the membranous labyrinth. The neuroepithelia of the cristae are mechanosensitive and detect motion of the cupula and the surrounding endolymphatic fluid. During rotational head movements the fluid within the semicircular canals lags behind and exerts pressure on the cupula causing it to bow. This afferent 'motion signal' is detected by the neuroepithelia of the canals and is transmitted to the central nervous system via a branch of the eighth cranial nerve (reviewed in Goldberg 1991).

Similar to the cristae, the maculae of the saccule and utricle are composed of neuroepithelia, supporting cells, blood vessels, and nerve fibers. Overlying each macula is an otolithic membrane (Figure 1B). The portion of the membrane adjacent to the mechanosensitive neuroepithelium is gelatinous in nature and is somewhat comparable to the cupula. The uppermost portion of the membrane is embedded with otoliths (or otoconia). These crystalline precipitants are composed of calcium carbonate and have a greater density than the surrounding tissue (reviewed in Thalmann *et al.*, 2001). During linear acceleration there is an inertial lag, due to the greater density of the otoconia, which results in displacement of the otolithic membrane. The displacement is detected by the neuroepithelia of the utricle and saccule, and the signal is transmitted to the afferent nerve fibers and ultimately to the central nervous system (Goldberg 1991).

HAIR CELL ANATOMY

The neuroepithelia of the vestibular end organs are composed of sheets of sensory epithelia and surrounding supporting cells. The primary sensory cell, termed the hair cell, is the central player. Hair cells convert vestibular stimuli into electrical impulses that are transmitted to the brain for further processing. In mammals there are two varieties of hair cells: the phylogenetically older type II cells have a cylindrical cell body, and type I cells have a flask shape with a constricted neck. Type II hair cells are contacted by bouton afferent and efferent nerve terminals, whereas, type I hair cells are only contacted by afferent terminals that engulf the entire cell body and form tight junctions that ring the apical surface of the cell (Lysakowski and Goldberg, 1997). Little is known about transduction and adaptation in type I hair cells. Here we present findings from mammalian type II hair cells and from hair cells of lower vertebrates all of which lack type I cells.

Hair cells are named for the tuft of modified microvilli which protrudes from the apical portion of the cell (Figure 2A,C). This tuft of microvilli or hair bundle projects into the fluid of the endolymphatic compartment and is coupled to the otolithic membrane in the saccule and utricle or to the cupula in the semicircular canals. Intercellular junctions between the neuroepithelial cells form a tight barrier between the endolymphatic compartment and the perilymphatic compartment and thereby maintain the electrical potential differences observed in portions of the vestibule (Wangemann, 2002b).

Hair bundles are the mechanosensitive organelles that transduce vestibular stimuli into electrical signals (reviewed by Holt and Corey, 1999). Each vestibular end organ contains thousands of hair cells. Each hair cell has one bundle that contains 30–300 microvilli, or stereocilia, depending on the organ and species. Stereocilia are composed of cores of hundreds of actin filaments (Tilney *et al.*, 1980) cross linked by fimbrin and espin and are covered by an extension of the cell membrane (Figure 2). The actin filaments taper near their base and are anchored to the cell body in a thickened region known as the cuticular plate (Jacobs and Hudspeth, 1990). This arrangement allows the relatively stiff stereocilia to pivot

around the point at which they insert into the cuticular plate. Furthermore, stereocilia are connected to each other by interdigitating links that connect their lateral membranes (Goodyear and Richardson, 1999). Thus, mechanical stimulation of the hair bundle does not splay the stereocilia apart but evokes uniform bundle deflections (Duncan *et al.*, 1999).

The stereocilia are arranged in a staircase-like array with a single true cilium, the kinocilium, located adjacent to the top of the staircase. The kinocilium does not exhibit mechanosensitivity but provides a connection of the apex of the hair bundle to overlying structures and it may also serve to organize proper formation of the bundle during development (Hudspeth and Jacobs, 1979).

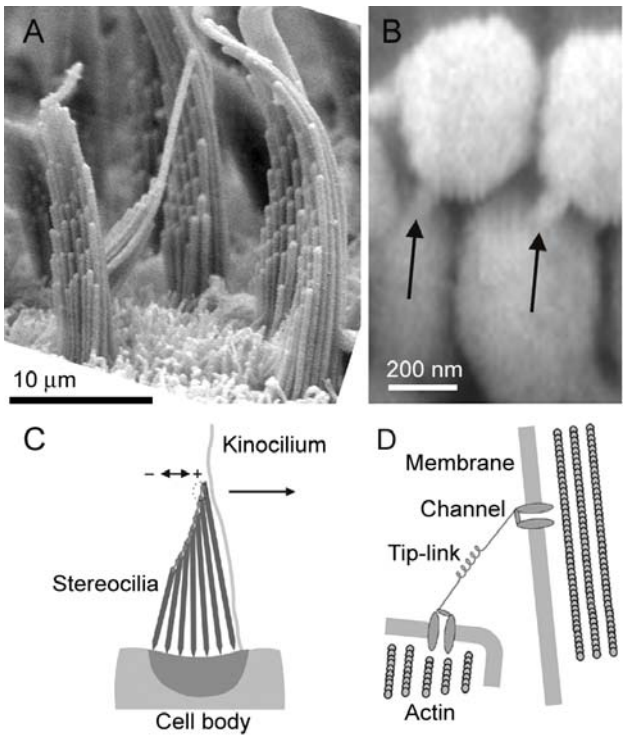

Figure 2. Morphology of hair bundles and schematic representations of the transduction apparatus. ***(A) Scanning electron micrograph of several hair bundles of the mouse utricle****. Note the staircase arrangement of the stereocilia which reflects the bundle's morphologic and functional polarity. The kinocilium is eccentrically placed and is the tallest structure of the bundle (right). These bundles are 10–-15 microns tall and contain 50–60 stereocilia.* ***(B) Scanning electron micrograph of the tips of two pair of stereocilia*** *The tip-links (arrows) are approximately 150–-200 nm in length and 5 nm in diameter; the stereocilia have cores of actin filaments and are approximately 250 nm in diameter.* ***(C) Schematic representation of the hair bundle illustrating the axis of polarity (arrow).*** *Deflections to the right are defined as positive. Note that the stereocilia taper at the base and pivot rather than bend.* ***(D) Schematic representation of a pair of stereocilia and the transduction apparatus.*** *Positive deflections (to the right) increase tip-link tension and cause the channels to open. Negative deflections reduce tension across the tip-link and allow the transduction channels to close.*

HAIR CELL TRANSDUCTION

The hair bundles of vestibular sensory cells convey a physiologic axis of polarity (reviewed in Pickles and Corey, 1992). Deflection of the bundle towards the tallest stereocilium, defined as positive, results in an influx of cations with subsequent depolarization and an associated excitatory afferent response (Figure 2C,D). At rest, approximately 10–20% of the transduction conductance is active. Deflection of the bundle away from the tallest stereocilium, defined as negative, results in a decrease in membrane conductance and is inhibitory to afferent fibers. Deflection of the bundle in a vector off the axis of physiological polarity does not result in signal transduction. Thus, each hair bundle has morphological as well as functional polarity (i.e. a single deflection vector to which it will respond). Hair bundle orientations span the range of polarities allowed by the plane of each vestibular epithelium (Corey and Hudspeth, 1979).

The mechanism of transduction of mechanical stimuli into electrical excitatory responses has been elucidated over the last several decades (reviewed in Holt and Corey, 1999; Vázquez and Yamoah, 2002). The increased membrane conductance during bundle deflection arises from the opening of mechanically gated ion channels. The transduction process is extremely fast with channels opening within about 10 microseconds of bundle deflection (Corey and Hudspeth, 1979). The speed is too fast for enzyme cascades or second messenger systems that characterize sensory transduction in the visual system and olfactory system (Corey and Hudspeth, 1983). It was proposed that mechanically gated channels were linked between stereocilia by spring-like extensions. Bundle deflection would therefore exert direct force on the channel gate placing it in the open position. This "spring" gating hypothesis was supported by the work of Howard and Hudspeth (1987) who found that hair bundle stiffness decreased as the channels opened (Howard and Hudspeth, 1987; 1988). This implicated a direct gating mechanism in series with the "springs". Structures extending between the tips of stereocilia oriented in line with the bundle's axis of sensitivity were discovered and thought to be the morphological correlates of the "springs" (Figure 2B, Pickles *et al.*, 1984). Exposure of the bundle to the calcium chelator, 1,2-bis(2-aminophenoxy)ethane-N,N,N'N'-tetraacetate (BAPTA), was found to cleave the stereocilia links, or tip-links, and concurrently abolish transduction (Assad *et al.*, 1991). Following tip-link cleavage, a recovery period of 12–24 hours allowed for regeneration of the links with concomitant return of transduction (Zhao *et al.*, 1996), thereby supporting the notion that tip-links are essential for mechanosensitivity. The tip-link is an extracellular glycoprotein composed of 2–3 coiled fibers about 150 nm long and 5 nm in diameter which extends from the tip of one stereocilium to the side of the adjacent taller stereocilium (Kachar *et al.*, 2000).

Other investigations have demonstrated that transduction channels are permeable to calcium. Calcium-sensitive dyes were used to localize transduction channels to the tips of stereocilia (Denk *et al.*, 1995; Lumpkin and

Hudspeth, 1995). Following positive bundle deflections, calcium was found to rise rapidly in the tips of stereocilia and diffuse more slowly down their lengths (Denk *et al.*, 1995; Ricci and Fettiplace, 1997; Lumpkin and Hudspeth, 1998), suggesting the channels were at the tips of the stereocilia near the insertion points of the tip-links. It is postulated that there may be up to six mechanosensitive channels per tip-link (Holt and Corey, 2000).

Figure 2D summarizes this model for hair cell mechanotransduction. Positive deflection of the hair cell bundle along its axis of polarity results in increased tension in the tip-link. This in turn evokes an immediate opening of the gated membrane-bound transduction channels, perhaps located at both ends of the tip-links. The model accounts for the bundle's directional selectivity, for its high sensitivity and for the exceptional speed of transduction.

HAIR CELL ADAPTATION

Tonic bundle deflections in the positive direction evoke rapid inward currents that decay during the subsequent 10–100 msec (Figure 3A; Holt *et al.*, 1997 and references therein). Concurrent with the decay, there is a shift of the activation curve (Figure 3B) which allows hair cells to maintain bundle sensitivity over a broad operating range (Eatock *et al.*, 1987). However, the tip-link model of transduction imposes geometric constraints that upon initial examination appear incompatible with these observations. For example, large bundle deflections, such as those imposed by gravity, would result in increased tension across the tip-links and saturate the bundle response with all the transduction channels open, thus rendering the cell insensitive to subsequent positive deflection. Large negative deflections, on the other hand, would decrease tension across the tip-link and place the bundle in a position to require a more robust deflection to achieve tension-regulated opening of the transduction channels.

In fact, hair cells do maintain sensitivity, despite pre-positioning of the bundle in either the positive or negative direction. Electrophysiological studies have shown that positive deflection of the hair bundle results in excitation, followed by a decline in the response toward the resting level (Eatock *et al.*, 1987). Subsequent positive bundle deflections reopen the transduction channels and excite the cell. The responsiveness of deflected bundles to further deflection demonstrates the hair cell's ability to adapt (reviewed by Eatock, 2000).

Two models have been proposed to explain hair cell adaptation (reviewed by Holt and Corey, 2000). One model suggests that adaptation to hair bundle deflection relaxes tension across the tip-link and thereby allows the channels to close (Howard and Hudspeth, 1987). Within the gating spring theory this implies that the hair cell has the ability to move the tip-link insertion point to adjust tip-link tension. The proposed site of adaptation is the upper end of the tip-link where it is attached to the side of the taller stereocilium. For a positive deflection calcium influx rises and allows the insertion point to descend thereby decreasing tension in the link, permitting the channels to close and restoring sensitivity. Conversely, a negative deflection results in an initial decrease in tip-link tension allowing the channels to close and thereby reducing calcium entry. Low intracellular calcium permits the upper attachment point to ascend and re-establish resting tension. A molecular motor located at the insertion site of the upper tip-link might attempt to climb up the

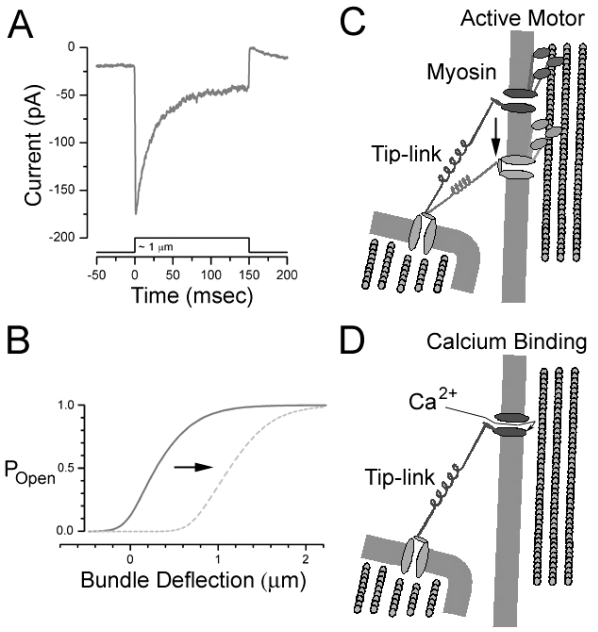

Figure 3. Two models for transducer adaptation in sensory hair cells ***(A) A representative transduction current recorded from a mouse utricle hair cell.*** *The hair bundle was deflected about 1 micron with a stiff glass probe mounted on a piezoelectric bimorph. A whole-cell pipette was used to record a rapidly activating inward current that decayed about 75% over the subsequent 150 msec.* ***(B) Adaptation shifts the stimulus-response relationship (or activation curve) in the direction of the applied stimulus (arrow)****. Transduction channel open probability is plotted as a function of bundle deflection. The solid curve shows the relationship from a resting bundle position. The dashed curve shows the shift in the stimulus-response relationship evoked by adaptation. Note that at the hair bundle's resting position, approximately 10% of the channels are open (solid line). Immediately following a one micron bundle deflection (see A) the open probability approaches 100% (solid line). After adaptation, approximately 20–30% of the channels remain in the open position (dashed line).* ***(C) Schematic diagram of a pair of stereocilia illustrating the active motor model for slow adaptation****. Following bundle deflection, tip-link tension opens transduction channels allowing calcium to enter the cell. Calcium disengages the motor and allows the upper channel to slip down the side of the taller stereocilium relieving tension and permitting both channels to close. Conversely, when the bundle is deflected in the negative direction, the reduced tension across the tip-link allows the active motor complex to engage and climb up the actin core, thus re-establishing resting tension.* ***(D) Schematic diagram illustrating the calcium-binding model for fast adaptation****. The model proposes that immediately after channel opening, calcium enters and binds to a site on or near the transduction complex causing the channels to close. Upon negative deflection, the channels close, the calcium concentration falls and calcium inhibition of the transduction channels is reduced.*

actin core of the stereocilium to increase tip-link tension and slip down the side to relieve tension and use intracellular calcium as a trigger to regulate its activity (Figure 3C).

In addition to the decay of the response, adaptation shifts the stimulus-response relationship in the direction of the applied stimulus (Figure 3B; Eatock *et al.*, 1987). The shift is approximately 60–70% of the magnitude of the bundle deflection (Shepherd and Corey, 1994; Holt *et al.*, 1997). Thus, 30–40% of the response remains following a tonic bundle deflection. This component of the sensory signal may be responsible for signaling the tonic position of the bundle, leaving the remaining 60–70% of the response available to signal subsequent bundle deflections. Several observations support this mechanical model. First, relaxation of hair bundles has been observed that match the time course of adaptation (Howard and Hudspeth, 1987). In other words, as adaptation relieves tip-link tension, a free-standing bundle moves farther in response to a constant force. Second, processes that are known to change the rate of adaptation cause hair bundles to move, indicating the adaptation motor can perform work (Assad and Corey, 1992). Lastly, electron microscopy has localized the insertion point of the tip-link closer to the top of the taller stereocilium when bundles were fixed following negative deflections and further from the top following positive deflections, suggesting the insertion point can move (Shepherd *et al.*, 1991).

An alternate model for adaptation was proposed based on work in turtle hair cells. Crawford and Fettiplace (1989) suggested that calcium that entered the cell through the transduction channels would bind to a site on or near the channel and promote channel closure (Figure 3D). In this scenario, positive deflections open the channels allowing calcium to enter and quickly bind causing a rapid decay in the response (0.5–5 msec). Subsequent deflections impose enough tip-link tension to overcome the calcium effect, and evoke further channel activation. Conversely, when the bundle is deflected in the negative direction, channels close; the local calcium concentration declines allowing a return to the resting state.

These two models of adaptation are not mutually exclusive, in fact, there is growing evidence that they may coexist to varying degrees in hair cells of different organs and species (Wu *et al.*, 1999; Holt and Corey., 2000). Several observations allow these mechanisms to be distinguished: The rate of adaptation is tens to hundreds of milliseconds for the motor model (Holt *et al.*, 1997) and as fast as 0.3 msec for the calcium-binding model (Ricci and Fettiplace, 1998). The latter form appears to be more prominent for very small bundle deflections but saturates with larger deflections of the bundle. The slower, motor-dependent, adaptation serves the bundle during larger deflections. Lastly, the calcium-binding model which involves swift alteration of the open probability of a channel at any given tension may be important for tuning of the hair bundle allowing it to amplify relatively miniscule bundle deflections (Crawford *et al.*, 1989; Ricci *et al.*, 2002).

CALCIUM AND ADAPTATION

Both adaptation mechanisms depend on calcium influx through the transduction channels. Imaging with calcium sensitive dyes has shown that the concentration of calcium rises briskly in the tips of stereocilia when the channels open, indicating significant calcium permeability (Denk *et al.*, 1995; Lumpkin *et al.*, 1997). The fate of calcium that enters the hair bundle has yet to be fully elucidated. In addition to diffusing down the stereocilium, calcium is likely taken up by fixed and free floating buffers (Lumpkin and Hudspeth, 1998) and is actively pumped out of the stereocilium (Yamoah *et al.*, 1998). The free floating buffer, calmodulin likely mediates calcium's role in adaptation. Walker and Hudspeth (1996) showed that calmodulin inhibitors block adaptation despite elevated intracellular calcium.

Work on active calcium efflux has implicated plasma membrane calcium ATPases (PMCA). Yamoah *et al.* (1998) found sufficient amounts of these molecules in the stereocilia membranes to account for the egress. Furthermore, mice with a mutation or a targeted deletion of the PMCA2 gene are deaf and have vestibular deficiencies (Street *et al.*, 1998; Kozel *et al.*, 1998).

Several observations suggest that calcium has a profound effect on the rate of adaptation. Decreases in intracellular calcium affected by lowering extracellular calcium (Corey and Hudspeth, 1983; Eatock *et al.*,1987; Assad *et al.*, 1989; Crawford *et al.*, 1989; Hacohen *et al.*, 1989; Holt *et al.*, 1997), increasing intracellular calcium buffers (Ricci and Fettiplace, 1997; Crawford *et al.*, 1989), or depolarization toward the calcium equilibrium potential (Assad *et al.*, 1989) slow the rate of adaptation and shift the activation curve to the left (negative direction). Under conditions of high extracellular calcium and low or slow intracellular calcium buffers, the intracellular calcium concentration can rise. High intracellular calcium speeds the rate of adaptation and shifts the activation curve to the right (positive direction). Under these conditions adaptation becomes very fast with a time constant as short as 0.3 msec (Ricci and Fettiplace, 1997), too fast to be explained by the motor model. Although manipulation of calcium and examination of the time course of adaptation allow the motor model (slow) and calcium-binding model (fast) to be distinguished, the precise molecular mechanisms of adaptation have remained obscure until recently.

IDENTIFICATION OF THE ADAPTATION MOTOR MOLECULE

Since the cores of the stereocilia are filled with actin, the most likely candidate for an adaptation motor that moves along the side of the stereocilia is a myosin. Which myosin isozymes mediate adaptation has been the focus of considerable attention (Gillespie and Corey, 1997). Over 40 different isozymes have been identified (Berg *et al.*, 2001) and at least five different myosins have been found in hair cells, including myosin I, VI, VII, X and XV (Solc *et al.*, 1994; Metcalf *et al.*, 1994; Liang *et al.*,

1999). Both myosin Ic (formerly known as myosin I-beta) and myosin VIIA have been localized to the hair bundle (Hasson *et al.*, 1997).

Interestingly, mutations in myosin VIIA were found to cause deafness and vestibular defects in mice and humans (Gibson *et al.*, 1995). Recent evidence suggested that hair cells from mice that expressed a mutant form of myosin VIIA had altered properties of adaptation (Kros *et al.*, 2002). However, since myosin VIIA was found only along the sides of the stereocilia, Kros *et al.* suggested that it plays a more developmental or structural role, perhaps maintaining hair bundles in the proper erect position.

Several groups have gone on to localize myosin Ic more precisely using transmission electron microscopy and immunolocalization (Metcalf, 1998; Steyger *et al.*, 1998; Garcia *et al.*, 1998). They found that myosin Ic staining was most dense at the tips of the stereocilia and along the side of the adjacent taller stereocilium, 100 nm above the tip-link attachment point. Thus, myosin Ic was ideally localized to mediate adaptation. Physiological evidence implicating specific myosin isozymes was more scarce. Pharmacological agents that inhibit myosin activity were found to block adaptation (Yamoah and Gillespie, 1996), but lacked the specificity needed to distinguish amongst the different isozymes.

To address the question, Gillespie *et al.* (1999) developed a novel chemical-genetic strategy to test the role of myosin Ic in adaptation. They discovered an amino acid residue in the ATP binding pocket of myosin Ic that when mutated rendered the molecule sensitive to inhibition by novel ADP analogs. The mutation was a tyrosine to glycine substitution at position 61 (Y61G). *In vitro* motility assays revealed that an ADP analog, N^6- 2-methyl butyl-ADP (NMB-ADP), strongly inhibited the activity of the mutant myosin, but not wild type myosin. Furthermore, the mutant myosin Ic had normal ATPase activity and normal motility in the absence of inhibitor. Lastly, they found that the mutation was dominant in the presence of the drug. The motility of clusters that consisted of as little as 10% mutant myosin Ic was inhibited by concentrations of 30 μM NMB-ADP.

Holt and colleagues (2002) went on to generate transgenic mice that expressed both wild type myosin Ic and the Y61G form. Hair cells from the utricles of wild type and transgenic mice were excised and studied electrophysiologically. Control experiments revealed normal transduction and adaptation in wild type littermates even in the presence of NMB-ADP and in mice that expressed the Y61G myosin Ic transgene in the absence of the drug. However, when the drug was included in recording pipettes sealed onto hair cells from Y61G mice, adaptation to both positive and negative bundle deflections was strongly inhibited (Figure 4). Both the current decay and the shift of the activation curve was abolished. Holt *et al.* (2002) concluded that myosin Ic mediates hair cell adaptation either on its own or together with other motor molecules. Cyr *et al.* (2002) have recently shown that myosin Ic has three IQ motifs that likely bind calmodulin to confer the calcium sensitivity.

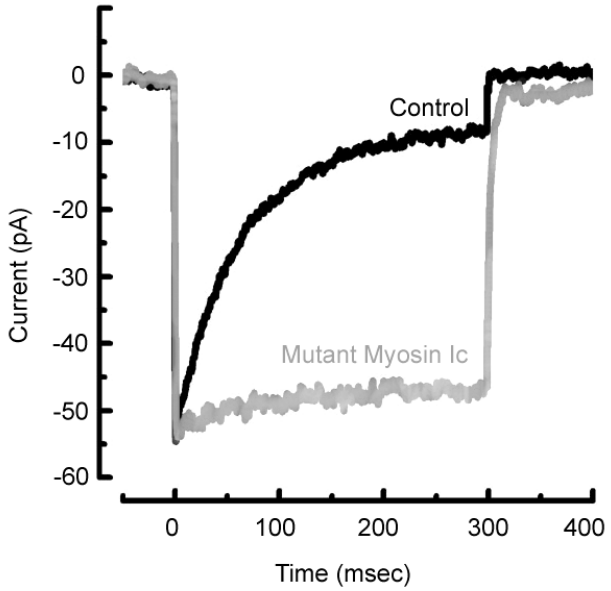

Figure 4. Transduction and adaptation recorded from hair cells of wild type and Y61G transgenic mice. *Stimulus pipettes delivered a 300-msec, 0.5 micron step deflection to the hair bundles of a wild type mouse hair cell and to the hair cell of a transgenic mouse that carried the gene for Y61G myosin Ic. The recording pipette contained 250 μM NMB-ADP in both cases. The control response (black) revealed wild type adaptation and demonstrated that the drug did not alter the properties of adaptation. The response of the mouse hair cell that expressed the mutant Y61G form of myosin Ic (gray) revealed strong inhibition of adaptation. Similar experiments with the transgenic hair cells but without the drug in the recording pipette revealed a response similar to that of the wild type response (data not shown).*

PHYSIOLOGICAL SIGNIFICANCE OF ADAPTATION

Identification and specific inhibition of the molecules that mediate slow adaptation provides a valuable tool for distinguishing between fast and slow adaptation and for addressing the function of slow adaptation in hair cells. The presence of slow adaptation has been well documented in mammalian vestibular hair cells (Holt *et al.*, 1997; 2002). Whether fast adaptation contributes to the normal behavior of mammalian vestibular hair cells remains to be seen. Adaptation acts as a high pass filter, favoring rapidly changing stimuli over slow tonic stimuli, such as gravity. Since the mammalian vestibular organs are sensitive to low frequency head movements and gravity (Goldberg *et al.*, 1990), it could be argued that fast adaptation would impede sensory signaling in these organs. Time constants of exponential fits to fast adaptation vary from 0.3 to 5 msec (Ricci and Fettiplace, 1998). At this rate, the predicted corner frequencies would range from 32 to 530 Hz, below which the hair cell response would be significantly attenuated. The time constants of slow adaptation (10–100 msec) would predict cut-off frequencies that range from 1.6 to 16 Hz (Holt *et al.*, 1998), well within the sensitive range of the human vestibular system. Thus, it could be argued from a

teleological point of view that fast adaptation might not be useful for maximal sensitivity to vestibular stimuli. Indeed, Holt *et al.* (2002) found evidence for fast adaptation in only six of 32 hair cells in which they blocked slow adaptation, suggesting that the fast component was present but not prominent in mammalian vestibular hair cells.

Myosin Ic and slow adaptation may also be important for maximizing hair bundle sensitivity. Since the adaptation motor acts through negative feedback to regulate tension in each tip-link, at any given bundle position tip-links are expected to carry an equal amount of tension. In other words, the motors of those that are too slack, climb up the stereocilia and those that are too tight, slip down the stereocilia. This preserves bundle sensitivity and maximizes the steepness of the activation curve (Figure 5A). Holt *et al.*, (2002) reported that activation curves from Y61G hair cells in which adaptation had been blocked were about twice as broad as controls, confirming a role for adaptation in maximizing sensitivity.

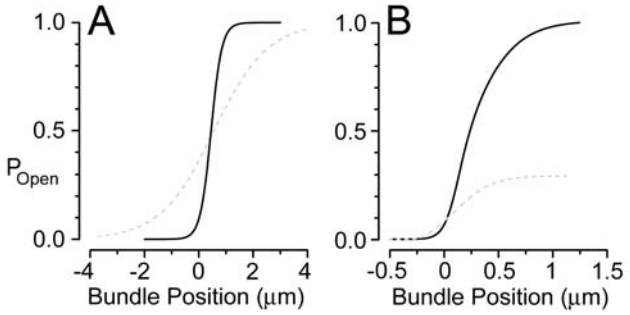

Figure 5. Open probability of transducer channels as a function of bundle position. ***(A) Adaptation allows mouse utricular hair cells to maintain maximal sensitivity as illustrated by the steepness of the activation curve (dark solid line)****. In hair cells from Y61G myosin Ic transgenic mice adaptation was abolished and the activation curve was significantly broader. Under these circumstances the tension across the tip-links was presumably unregulated because the active motor complex was disabled. Therefore, without adaptation the 40–50 tip-links in a hair bundle carry varying levels of tension, collectively reducing the sensitivity of the bundle and broadening the activation curve (gray dashed line).* ***(B) Comparison of the peak activation curve with the steady state activation curve****. The peak activation curve (dark solid line) was obtained from mouse utricle hair cells by measuring the peak transduction currents after bundle deflections and calculating transduction channel open probability (dark solid line). After a given deflection the current decayed to a steady-state level. The steady-state current was measured and open probability was plotted versus bundle position (gray dashed line). Note that for a one micron deflection the peak activation curve indicates an open probability of nearly 100%. At steady state, the open probability declines to about 30%, thereby enabling the bundle to signal tonic stimuli (such as gravity) even after transducer adaptation has occurred.*

In wild type hair cells the activation curve shifts in the direction of the applied stimulus (Figure 5B). The shift was abolished in Y61G cells exposed to NMB-ADP. Thus, block of myosin Ic, inhibited the hair cell's ability to compensate for tonic offsets in bundle position and its ability to maintain sensitivity over a broad operating range.

Lastly, adaptation is incomplete: 60–70% of the response decays following a positive deflection from the rest position (Shepherd and Corey, 1994; Holt *et al.*, 1997). In mouse utricle hair cells the remaining 30–40% of the response does not adapt. Comparison of the peak activation curve with the steady-state activation curve, measured after the current has decayed to a steady level, illustrates the proportion of the hair cell response available to signal phasic and tonic vestibular stimuli (Figure 5B). Here, the peak activation curve represents the portion of the response that adapts and is able to signal phasic stimuli over a broad range of bundle positions. The steady-state curve does not shift and therefore this portion of the response is always available to signal tonic stimuli, such as gravity. Thus, the vestibular system can detect phasic head motion centered around any given tonic head position.

CONCLUSIONS

The accessory structures of the mammalian vestibular system serve to focus motion induced by rotational and linear head movements onto the mechanosensitive organelles of sensory hair cells. Hair cells are exquisitely engineered to detect motion with remarkable temporal fidelity and unparalleled mechanosensitivity. Yet, they are robust enough to be able to signal stimuli over several orders of magnitude. An adaptation mechanism reconfigures the mechanotransduction apparatus on a millisecond time scale. Adaptation thereby allows the cell to maintain maximal sensitivity over a broad operating range by adjusting to tonic offsets in bundle position. Myosin Ic has recently been identified as a component of the adaptation complex and together with calmodulin they represent the first molecules determined to be part of the transduction apparatus. Although the molecular identity of the tip-link and the transduction channel remain to be elucidated, identification may not be far off. With the human and mouse genomes complete and the adaptation motor "in hand" we now have a handle on the transduction complex and thus techniques such as yeast-two-hybrid may be used to identify the other key players.

The properties of adaptation such as rate, extent and calcium sensitivity vary among hair cells, organs and species. Nonetheless, hair cells from all organs and species examined adapt, suggesting adaptation is a requisite component of mechanotransduction in vestibular organs. Yet, several intriguing questions remain. 1) Do the properties of adaptation summarized here accurately reflect the hair cell response when the hair bundle is bathed in the endogenous high potassium, low calcium endolymph? 2) What role does fast adaptation play in low-frequency sensitive vestibular organs? 3) Are there other components of the adaptation motor? Myosin VIIA perhaps? 4) Does adaptation contribute to sensory signaling *in vivo?* 5) Does the non-adapting component of mechanotransduction signal tonic stimuli *in vivo*?

Experiments that take advantage of the chemical-genetic strategy devised by Gillespie *et al.* (1999) may be able to address some of these issues. For example, inhibition of slow adaptation in hair cells where fast adaptation is more prominent might allow these mechanisms to be distinguished and attribute specific functions to each form of adaptation. Chemical-genetic inhibition of myosin VIIA might illuminate its contribution to hair bundle development, structure and to slow adaptation. Development of a membrane permeable form of NMB-ADP might allow inhibition of adaptation *in vivo* and reveal its contribution to signal detection and processing of vestibular stimuli.

Lastly, if the non-adapting component of hair cell mechanotransduction signals tonic stimuli, it is possible it could be selectively abolished to minimize the false sensation of gravity in microgravity environments.

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