Development of $K^+$ and $Na^+$ conductances in rodent postnatal semicircular canal type I hair cells

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Li GQ, Meredith FL, Rennie KJ. Development of $K^+$ and $Na^+$ conductances in rodent postnatal semicircular canal type I hair cells. Am J Physiol Regul Integr Comp Physiol 298: R351–R358, 2010. First published November 25, 2009; doi:10.1152/ajpregu.00460.2009.—The rodent vestibular system is immature at birth. During the first postnatal week, vestibular type I and type II hair cells start to acquire their characteristic morphology and afferent innervation. We have studied postnatal changes in the membrane properties of type I hair cells acutely isolated from the semicircular canals (SCC) of gerbils and rats using whole cell patch clamp and report for the first time developmental changes in ionic conductances in these cells. At postnatal day (P) 5 immature hair cells expressed a delayed rectifier $K^+$ conductance ($G_{K,I}$) which activated at potentials above approximately $-50$ mV in both species. Hair cells also expressed a transient $Na^+$ conductance ($G_{Na}$) with a mean half-inactivation of approximately $-90$ mV. At P6 in rat and P7 in gerbil, a low-voltage activated $K^+$ conductance ($G_{K,L}$) was first observed and conferred a low-input resistance, typical of adult type I hair cells, on SCC type I hair cells. $G_{K,L}$ expression in hair cells increased markedly during the second postnatal week and was present in all rat type I hair cells by P14. In gerbil hair cells, $G_{K,L}$ appeared later and was present in all type I hair cells by P19. During the third postnatal week, $G_{Na}$ expression declined and was absent by the fourth postnatal week in rat and the sixth postnatal week in gerbils. Understanding the ionic changes associated with hair cell maturation could help elucidate development and regeneration mechanisms in the inner ear.

vestibular system; rat; gerbil; tetrodotoxin

THE VESTIBULAR SYSTEM OF THE mammalian inner ear contains the semicircular canals (SCC), which detect angular acceleration and the otolith organs (utricle and saccule), which sense linear accelerations of the head. Mature vestibular epithelia contain mechanoelectroreceptive hair cells, supporting cells and nerve terminals. In mouse vestibular system, hair cells are first apparent at embryonic day (E) 12, and terminal mitosis is virtually complete at birth (31). Hair bundles at the apex of the hair cell become sensitive to mechanical stimuli between E16 and E17 (13). However, the rodent vestibular system is not functionally mature in neonates (4). Hair cells have not acquired their adult morphology at birth (P0), and in the cochlea and utricle, it has been shown that hair cell electrophysiological properties continue to mature for several days postnatally (11). Afferent calyx terminals, which innervate and define type I hair cells, are rarely seen at P0 in mouse and rat utricles, but the numbers of calyx terminals in both species increase dramatically during the first postnatal week as calyces form cup-shaped terminations around type I hair cells (10, 12, 32).

Mature type I and type II vestibular hair cells differ markedly in their electrophysiological properties. Adult type I hair cells consistently express a low-voltage activated $K^+$ conductance ($G_{K,L}$) or current ($I_{K,L}$), which is substantially activated at the resting potential, whereas type II hair cells do not have a significant resting conductance (25, 32). In the early postnatal period, the properties of immature hair cells are more homogeneous. At P0, mouse utricle hair cells express a delayed rectifier current ($I_{DR}$) and fast inward rectifier ($I_{K1}$) (14, 32). In rat utricle, $G_{K,L}$ was not found in hair cells younger than P7, but type I hair cell properties reached maturation by P16 (17). In mouse utricle $G_{K,L}$ appeared earlier in development, and the proportion of hair cells expressing $G_{K,L}$ was the same at P8 as in mature animals (32). Two types of $Na^+$ currents in developing rat utricle hair cells have also been described (35).

Little is known about the maturation of ionic conductances in SCC hair cells and their afferent fibers. Spontaneous activity in horizontal SCC afferents was reported to increase during the first postnatal month in rats and mice (8, 9), suggesting underlying maturation of hair cell and/or afferent electrophysiological properties. Here, we report the time course of acquisition of ionic conductances in type I hair cells nonenzymatically isolated from early postnatal and mature rodent cristae. The functional maturation of auditory hair cells has been studied in mice, gerbils, and rats, in which hearing onset occurs at around P12 (18, 20, 24). Eye opening occurs around P12 in mice, but later in rats (P13–P14) and gerbils (P17–21). Neuroanatomical data and behavioral testing also indicate a more protracted development of the peripheral vestibular system in rats and gerbils. Our goal is to correlate development of rodent hair cell properties with afferent maturation and other developmental milestones in the postnatal vestibular system.

MATERIALS AND METHODS

Cell dissociation. Vestibular hair cells and afferent terminals were dissociated using methods outlined previously (28). Animal procedures were performed under protocols approved by the University of Colorado’s Institutional Animal Care and Use Committee and were within guiding principles established by the American Physiological Society. Mongolian gerbils (Meriones unguiculatus) were obtained from Charles River Laboratories (P4 onward, where P0 is the day of birth) and were injected with pentobarbital sodium (Nembutal, 50 mg/kg ip) and ketamine (40 mg/kg im). SCC were removed from both ears following decapitation (<3 wk old) or surgically removed under deep anesthesia with decapitation immediately following (>3 wk old). Sprague-Dawley rat pups were obtained from timed pregnant females (Charles River Laboratories) and the SCC removed following decapitation. SCC ampullas were trimmed and placed in “high Mg2+/low Ca2+” saline containing (in mM): 135 NaCl, 5 KCl, 10 MgCl2, 0.02 CaCl2, 10 HEPES, and 3 D-glucose, pH 7.4 with NaOH and osmolality 300–305 mmol/kg. Gerbil ampullas were incubated at...
37°C for 30–32 min and then placed in Leibovitz’s L-15 medium with bovine albumin (0.5–1 mg/ml) for at least 50 min at room temperature (18–24°C) before dissociation. Rat ampullas were maintained in chilled high Mg²⁺/low Ca²⁺ (8–9°C) ≥ 15 min and L-15 with bovine albumin (0.5–1 mg/ml) ≥ 15 min before transferring to chilled L-15 medium. Hair cell dissociation was performed in the recording chamber in L-15 medium. Each entire ampulla was stroked with a probe to dislodge cells under a stereomicroscope. Cells were observed using an Olympus upright microscope (BX50WI or BX51WI) equipped with water immersion objectives and differential interference contrast optics.

**Cell identification.** Mature type I cells are amphora shaped with a constricted neck region, whereas mature type II hair cells are more cylindrical in shape. The two cell types can be distinguished by their neck to plate and neck to body ratios (30). However, during the first few postnatal days in both species, many immature SCC hair cells (identified by the presence of a hair bundle) were ovoid in shape, as described for immature hair cells in mouse utricle (32). Therefore, it was not always possible to morphologically differentiate between type I and type II hair cells. In some instances, a calyx terminal remained attached to a hair cell, which could, therefore, be defined as a type I hair cell. The presence of $G_{K,L}$ also defined a type I hair cell. Other early postnatal hair cells were classified as “unknown” hair cell type.

**Electrophysiological recording and solutions.** Patch pipettes were pulled from capillary glass tubing on a Sutter Instruments horizontal micropipette puller (Flaming/Brown P-87 or P-90), heat-polished on a micromasive (Sylgard, Dow Corning). The normal patch pipette solution used in the majority of experiments comprised (in mM): 110 KF, 15 KCl, 1 or 2 NaCl, 10 HEPES, 3 d-glucose, 1.8 MgCl₂ and 10 EGTA, pH 7.4 with KOH (≈ 27 mM). In some experiments, KCl was entirely replaced with KF, and the solution included 2 mM ATP magnesium and 0.2 mM GTP lithium salt. To record Na⁺ currents, patch electrode K⁺ was replaced with Cs⁺. Conventional whole cell tight-seal patch-clamp experiments were carried out at room temperature (18–24°C). Patch pipettes with an open tip resistance of 1–5 MΩ were used to obtain high-resistance seals onto cells for whole cell recordings. Currents were amplified with an Axopatch-1D or Axopatch 200B patch amplifier (Axon Instruments) connected to a PC through an AD converter (Axon Instruments). Clampex software (ver. 8 or ver. 10) was used for data acquisition and analysis. Signals were low-pass filtered on-line at 2 or 5 kHz, and the sampling rate was set between 7.2 to 9.7 mV and were corrected off-line. Series resistance was not typically compensated. The standard extracellular solution was Leibovitz’s L-15 media. 4-Aminopyridine (4-AP, 1.3–5 mM) was dissolved in the external solution, and the pH was readjusted with 0.1 M KOH (≈ 0.5–1 ml/min. Solutions containing drugs were bath applied or perfused locally.

**Data analysis.** Input resistance values were calculated in voltage clamp using a linear regression of a voltage vs. steady state current plot at potentials between −90 and −60 mV.

Steady-state inactivation for Na⁺ currents was obtained using voltage protocols with 120- to 150-ms prepulses prior to a test pulse from a holding potential of −78 mV. Data were normalized to the peak inward Na⁺ current and fitted with a Boltzmann function of the form (Eq. 1): $I/I_{max} = 1/1 + exp [(V - V_{1/2})/S]$, where $V$ is the conditioning potential, $V_{1/2}$ is the half-maximum inactivation potential, and $S$ is the slope factor for inactivation.

Values are presented as means ± SE. Data were analyzed by one-way ANOVA and a post hoc test (Bonferroni) was used to determine individual differences between means. A P value of <0.05 was considered significant.

**RESULTS**

$K^+$ conductances differ in mature type I and type II hair cells. Fig. 1 compares the voltage-dependent $K^+$ conductances in mature (older than 10 wk) gerbil type I and type II hair cells in response to a standard voltage protocol. Cells were held at −78 mV and stepped to −128 mV for 40 ms before stepping to a series of depolarized potentials in 10-mV increments. The type I hair cell in Fig. 1A shows a low-voltage activated $K^+$ conductance ($G_{K,L}$) that has been shown to be a characteristic feature of mature type I hair cells across different vestibular end organs and species (25, 29, 32). $G_{K,L}$ is active at the resting potential of the cell and deactivates on stepping to hyperpolarized potentials as shown by the arrow. Following depolarizations to potentials above −70 mV, large sigmoidally activating outward currents are seen. The effect of the $K^+$ channel blocker 4-aminopyridine (4-AP) on the type I hair cell $K^+$ conductance is also shown in Fig. 1A. 4-AP reduced the current active at the holding potential and also reduced outward currents revealing a slowly developing outward current, which first activated at potentials above −50 mV. Fig. 1B shows currents recorded from a type II hair cell isolated from adult gerbil SCC in response to the same voltage protocol. No large conductance is active at the holding potential in this cell and following depolarizations to potentials above −40 mV outward currents activate rapidly and partially inactivate over the duration of the pulse. Application of 2.5 mM 4-AP blocks a large component of the type II outward conductance.
ductance is different from $G_{K,L}$. A major difference between mature type I and type II hair cells is the presence of $G_{K,L}$ in type I hair cells.

Developmental growth of $K^+$ conductances in gerbil and rat SCC hair cells. Fig. 2 shows ionic currents recorded from hair cells isolated from gerbil SCC at four different postnatal stages. We refer to the type I conductance active at rest as $G_{K,L}$. By P10, type I hair cell morphology was evident, and the majority of these hair cells (8/11) expressed $G_{K,L}$ as shown by deactivating currents on stepping from hyperpolarized potentials (arrow, Fig. 2). At P21, the $K^+$ currents in morphologically identified type I hair cells were much larger (Fig. 2). At P25, the peak outward current amplitude at 0 mV was $4.2 \pm 1.1$ nA (mean ± SD, $n = 10$) compared with $3.3 \pm 1.9$ nA ($n = 11$) at P10 and $2.1 \pm 1.2$ nA at P6 and P7 combined ($n = 9$). The type I-specific $K^+$ current $I_{K,L}$, therefore, first makes its appearance in gerbil SCC type I hair cells at P7 and is present in an increasing number of cells during the second postnatal week.

Figure 3A shows the development of ionic conductances in postnatal hair cells isolated from rat SCC. At P5, a slowly activating outward current is present at potentials above $-50$ mV. The kinetics and voltage dependence of this conductance...
are consistent with \(G_{\text{DR}}\). The signature type I hair cell conductance \(G_{K,L}\) is seen in rat crista hair cells as early as P6 and by P20, all type I hair cells expressed \(G_{K,L}\). A small transient inward \(\text{Na}^+\) current (described in the next section) is also apparent at P5 and P6. Peak currents over a range of voltages at three different stages of development in representative cells (P5, P10, and P18) are shown in the current voltage (I-V) plot of Fig. 3B. Currents were smallest at P5 and both inward and outward currents were much larger at P18. The mean zero-current potential for rat hair cells is plotted vs. postnatal age in days in Fig. 3C. The mean zero-current potential at P6 was significantly different from the mean zero-current potential measured in cells from P15 onward. Cell capacitance did not change significantly when hair cells from P6 and the third postnatal week were compared, suggesting that hair cell size did not change (Fig. 3C).

Appearance of \(G_{K,L}\) is associated with a decrease in input resistance. During the first few postnatal days, immature hair cells from rat and gerbil SCC express \(G_{\text{DR}}\), and we first observed the type I-specific conductance \(G_{K,L}\) at P6 in rat and P7 in gerbil SCC hair cells. We defined cells as having \(G_{K,L}\) if a current was active at the holding potential of \(-78\) mV and deactivated with hyperpolarization. A hallmark of mature type I hair cells is their low input resistance, which occurs as a result of \(G_{K,L}\). Previous reports have reported mean input resistances <100 M\(\Omega\) in adult type I hair cells from rat and gerbil SCC (3, 26) and in embryonic mouse utricle an input resistance of <200 M\(\Omega\) was correlated with the presence of \(G_{K,L}\) in a subpopulation of hair cells (14). We, therefore, estimated the input resistance in rat (Fig. 4A) and gerbil SCC hair cells (Fig. 4B) at different developmental stages. As \(G_{K,L}\) develops, a corresponding decrease in input resistance is seen during the second postnatal week. Solid symbols indicate positive identification of type I hair cells whereas, hair cells of unknown type are represented by open symbols (Fig. 4). At P14, all rat type I hair cells (14/14) expressed \(G_{K,L}\) and beyond P15, all rat cells \((n = 60)\) had \(G_{K,L}\) and input resistances of less than 100 M\(\Omega\) (Fig. 4A).

A similar trend was seen with gerbil hair cells, although in this species type I hair cells lacking \(G_{K,L}\) were still present at P18. After P20, all type I hair cells expressed \(G_{K,L}\) and had input resistances of less than 200 M\(\Omega\) (Fig. 4B).

As shown in Fig. 1A, 4-AP blocks a major component of the outward current in mature type I hair cells, revealing a current with slower activation kinetics. Although 4-AP is a relatively nonselective blocker of \(\text{K}^+\) channels, it has proved useful in identifying components of the \(\text{K}^+\) current in developing outer and inner hair cells (15, 20). 4-AP at low millimolar concentrations is also an effective blocker of \(I_{K,L}\) in type I hair cells in several species (1, 25, 32). We also investigated the effect of 4-AP on postnatal hair cells lacking \(G_{K,L}\) and compared the effect to those cells expressing \(I_{K,L}\). Newly formed inner hair cells express a small outward \(\text{K}^+\) current, which is relatively insensitive to 4-AP (20). However, we found that 4-AP reversibly blocked most of the outward currents in vestibular hair cells as young as P7. In the presence of 4-AP, a small current remained at depolarized potentials (Fig. 5A), but ~80% of outward current was blocked in cells regardless of whether they expressed \(I_{K,L}\) or \(I_{\text{DR}}\) alone (not shown). Therefore, even before the appearance of \(I_{K,L}\), the \(\text{K}^+\) current in immature type I hair cells is highly sensitive to 4-AP.

In cells that lacked \(I_{K,L}\), outward currents were reduced following 4-AP application, but the activation kinetics were relatively unchanged (Fig. 5A). This was in contrast to cells with \(I_{K,L}\), where outward current activation was much slower in the presence of 4-AP (Fig. 5B). We investigated the kinetics of the 4-AP-sensitive current in type I hair cells that did or did not express \(I_{K,L}\). The activation of 4-AP-sensitive outward current was best fit with a sigmoidal function. At steps to potentials above ~30 mV the 4-AP-sensitive current in cells with \(I_{K,L}\) activated with a smaller time constant than the 4-AP-sensitive current in cells without \(I_{K,L}\). Therefore, cells expressing \(I_{K,L}\) have outward currents that activate faster than cells without \(I_{K,L}\), and 4-AP application blocks \(I_{K,L}\), revealing a more slowly activating conductance. It remains to be established whether the postnatal appearance of \(I_{K,L}\) during type I hair cell development is due to the addition of new \(\text{K}^+\) channels, or a modification of existing channels that produces a leftward shift of the activation curve and a resulting decrease in input resistance.

Postnatal expression of \(\text{Na}^+\) currents in SCC hair cells. A rapid and transient inward current was often observed preceding \(\text{K}^+\) currents in rat and gerbil SCC hair cells and is shown in detail in Fig. 6A. The inward current was more obvious at younger ages before \(G_{K,L}\) became prominent and tended to mask it. To isolate the inward currents, outward \(\text{K}^+\) currents were reduced by replacing patch electrode \(\text{K}^+\) with Cs\(^+\) and in

**Fig. 4.** Input resistance in rat and gerbil SCC type I hair cells declines during early postnatal development as \(G_{K,L}\) is increasingly expressed in type I hair cells. A: morphologically identified rat type I hair cells (black symbols) express \(G_{K,L}\) as early as P6. Unidentified hair cells at early postnatal days are represented by open circles. By P15, all hair cells expressed \(G_{K,L}\), and input resistance values for all cells but 1 were less than 100 M\(\Omega\) (indicated by dashed line). B: input resistance in gerbil type I hair cells decreases with age as \(G_{K,L}\) is expressed. Identified type I hair cells (solid symbols) and unidentified hair cells (open squares) are shown. Input resistance was measured between ~90 and ~60 mV.
some cases 4-AP (2.5–5 mM) was added to the external solution. $G_{K,L}$ is unusual in that it shows permeability to Cs\(^+\) (25, 32), but a combination of Cs\(^+\) and 4-AP was effective in blocking most of the outward current in cells expressing $G_{K,L}$. Depolarizing steps to potentials above $-60$ mV following a hyperpolarizing step resulted in rapidly activating and rapidly inactivating inward currents (Fig. 6A), which showed steady-state inactivation (Fig. 6E). Inward currents were not present when choline was substituted for external Na\(^+\) (data not shown), confirming their identity as Na\(^+\) currents ($I_{Na}$). Transient Na\(^+\) currents have also been described previously in rat and mouse immature utricular hair cells (14, 19, 35). The $I-V$ plot in Fig. 6B shows peak inward currents measured in a gerbil hair cell (P8, open symbols) and a rat hair cell (P14, solid symbols). The expression of $I_{Na}$ as a function of postnatal day is shown for SCC hair cells in Fig. 6C. Between P5 and P7, $I_{Na}$ was seen in $70$–$100\%$ of cells in both species. $I_{Na}$ was still discernible in many type I cells between P15 and P20, but the percentage of cells expressing $I_{Na}$ declined during this period. We continued to observe a small but detectable transient inward current in a minority of rat SCC cells at P20 and gerbil SCC cells at P35. In rat type I cells, $I_{Na}$ was absent by P25 and in gerbil, it was gone by P42 (Fig. 6C).

Na\(^+\) currents can be sensitive or insensitive to low nanomolar concentrations of the blocker TTX depending on the underlying subunits. In chick SCC hair cells, 300 nM TTX blocked $>$90\% of $I_{Na}$ (23), but in rat utricular hair cells, two types of $I_{Na}$ were described with different sensitivities to TTX (35). Therefore, we tested the effects of TTX at three different concentrations. Following application of 50 nM TTX, $I_{Na}$ was reduced by a mean value of $40.2 \pm 1.4\%$ in 3 gerbil type I hair cells (2 cells at P8 and 1 at P9). At 500 nM, $I_{Na}$ was reduced by $68.7 \pm 4.4\%$ ($n = 5$, P8–P9), and the effect was reversible (Fig. 6D). Even at higher concentrations ($700$–$750$ nM), TTX did not completely block the inward current in two P8 gerbil cells tested (data not shown). On the basis of the high concentration needed to reduce the current, $I_{Na}$ appears relatively insensitive to TTX. $I_{Na}$ was also studied using an inactivation protocol where the membrane was stepped to a series of hyperpolarized potentials before the test potential to generate an inactivation curve as shown for a P6 rat type I cell and P8 gerbil cell in Fig. 6E. The mean half-inactivation ($V_{1/2}$) for rat hair cells between the ages of P5 and P14 was $-93.7 \pm 3.7$ mV, and the mean slope factor was $4.7 \pm 0.6$ mV ($n = 10$). For gerbil hair cells, inactivation $V_{1/2}$ was $-90.0 \pm 1.7$ mV with a mean slope factor of $6.0 \pm 0.5$ mV ($n = 10$, P8–P9). These average values are quite negative in both species but are similar to those reported in other types of hair cell (23, 24, 35). Apparently, only a small amount of $I_{Na}$ would be available at the zero-current potential, and hair cells would be unlikely to fire Na\(^+\)-driven action potentials. Activation and inactivation kinetics of $I_{Na}$ were also studied. The time to peak for $I_{Na}$ decreased with progressive depolarizations and for a step to $-8$ mV was typically between 0.4–0.5 ms. The time constant for inactivation [0.36 ± 0.02 ms ($n = 7$) following a step to $-8$ mV] was also voltage dependent and decreased with depolarization as shown in Fig. 6F.

**DISCUSSION**

This is the first report to study developmental changes in ionic conductances in hair cells isolated from rodent semicircular canals. We compared rats and gerbils during the early postnatal period when many sensory systems, including the inner ear are still undergoing maturation. Eyelid opening in Sprague-Dawley rats occurs toward the end of the second postnatal week (P13–P14), whereas eyelid opening in Mongolian gerbils occurs a few days later, between P17 and P21 (5). Rats are able to right themselves after being put on their backs by P12. The vestibular-ocular reflex is reported to be mature by week 3 (7), and the rate of spontaneous firing in rat SCC afferents increases slowly after P0 and reaches mature levels within the first postnatal month (8). Concomitant developmental changes in SCC hair cell ionic conductances occur during this period.

Developmental expression of potassium conductances $G_{DR}$ and $G_{K,L}$. At early postnatal days, all rat and gerbil SCC hair cells expressed a conventional delayed rectifier K\(^+\) conductance, $G_{DR}$, which activated at potentials depolarized to $-50$ mV. At early postnatal days, many SCC hair cells had not acquired their typical morphology and innervation, and therefore, could not be classified as type I or type II. At P6 some rat hair cells expressed the low-voltage-activated conductance $G_{K,L}$. The incidence of this current increased during the second postnatal week, and it was present in all rat SCC type I hair cells by P15, 1–2 days later than eye opening in this species. As
a direct consequence of $G_{K,L}$ expression, the input resistance of type I hair cells declined dramatically during this period. The zero-current potential also became more negative during the second postnatal week. In contrast, $G_{K,L}$ was expressed at later times in gerbil type I hair cells. It was first observed at P7, and $G_{K,L}$ was present in all type I hair cells by the start of the fourth postnatal week.

The differentiation and expression of ion channels in developing vestibular hair cells has been described previously in rodent utricle. Gélécó et al. (2004) reported the presence of $G_{K,L}$ as early as E18 in utricles from Swiss Webster mice. In another study $G_{K,L}$ was first observed at P3 in mouse utricle and by P6 50–75% of all hair cells expressed $G_{K,L}$, consistent with mature expression levels (32). The incidence of $G_{K,L}$, therefore, increases rapidly in the first postnatal week and is mature at P7–P8 in mouse utricle (14, 32). $G_{K,L}$ appears later in hair cells from Long-Evans rat utricle since prior to P7, all outward currents were reported to activate above rest. In the utricle $G_{K,L}$ appeared after the first postnatal week and reached mature levels by the third postnatal week (17). In contrast, the magnitude and activation of outward $K^+$ currents in maturing type II cells did not change during the same postnatal period (17).

We studied SCC hair cells from P4 onward to investigate the electrophysiological changes associated with synapse formation and functional maturation of the type I hair cell/calyx synapse. During the first postnatal week, calyces form terminations, and type I cells start to acquire their typical adult morphology (12, 32). Our data show that most cells with type I morphology expressed $G_{K,L}$, but not all cells morphologically characterized as type I had $G_{K,L}$. The acquisition of SCC hair cell $K^+$ conductances is similar to that described in mouse utricle in that hair cells first express $G_{DR}$, and type I cells subsequently express $G_{K,L}$. However, electrophysiological properties of type I cells in rat and gerbil SCC mature later than...
in mouse utricle. This allows a developmental window encompassing several postnatal days for studying cellular processes that may be important for hair cell development.

The molecular composition of K⁺ channels underlying whole cell K⁺ currents in hair cells remains elusive. A 4-AP-sensitive current was described in outer hair cells from the basal cochlea at E18, whereas K⁺ currents in outer hair cells from the E18 apical cochlea and in neonatal inner hair cells were not blocked by 4-AP (15, 20). In inner hair cells, K⁺ currents increase in size prior to the onset of hearing at ~P12, and a progressively larger component of the outward K⁺ current is sensitive to 4-AP during postnatal development (20). In contrast, we observed that most of the macroscopic K⁺ current \( I_{\text{Na}} \) in immature type I hair cells, before the onset of \( I_{\text{K,L}} \), was sensitive to 4-AP and that \( I_{\text{K,L}} \) was also blocked by 4-AP. 4-AP-sensitivity did not change significantly during development. Regenerated type I hair cells in adult pigeon vestibular epithelia also expressed a 4-AP-sensitive \( I_{\text{K,L}} \) (6). The molecular identity of the 4-AP-sensitive K⁺ channels in type I and type II hair cells remains unclear. It has been suggested that KCNQ and erg-like K⁺ channels contribute to the macroscopic current in type I hair cells (16, 17, 27). However, KCNQ and erg-mediated currents are not blocked by 4-AP, indicating that other K⁺ channels may also contribute substantially to the type I hair cell conductance.

Postnatal expression of sodium conductance \( G_{\text{Na}} \). We found a rapidly activating, rapidly inactivating inward Na⁺ current in postnatal SCC rat and gerbil hair cells. A transient sodium current, \( I_{\text{Na}} \), has been described previously in hair cells from mouse and rat utricle (2, 14, 19, 35), mouse, rat and guinea pig cochlea (20, 24, 34) and chicken vestibular system (23, 33). In mouse utricle \( I_{\text{Na}} \) peaked at E16–E18 and subsequently declined to almost zero at birth (14). In rat utricle, a TTX-insensitive Na⁺ current named \( I_{\text{Na1}} \) and a TTX-sensitive current \( I_{\text{Na2}} \) were studied in hair cells during P0–P22 (35). \( I_{\text{Na1}} \) was present in type I hair cells and predominated in the striolar regions of the utricle, although its expression decreased dramatically after the first postnatal week (35). We found a current with similar kinetic properties in type I hair cells from rat and gerbil SCC. We found a mean inactivation \( V_{1/2} \) of approximately −90 mV in gerbil SCC hair cells, similar to a mean value of −94 mV for \( I_{\text{Na1}} \) in rat utricle hair cells (35) and −93 mV in neonatal rat outer hair cells (24). Masetto et al. (23) also described \( I_{\text{Na}} \) in SCC hair cells from chick embryo and adults with a half-inactivation of −96 mV. \( I_{\text{Na}} \) was reduced by ~70% but not completely blocked by 500 nM TTX (Fig. 6D). TTX-insensitive subunits include \( \text{NaV}1.5, \text{NaV}1.8, \) and \( \text{NaV}1.9 \). \( \text{NaV}1.5 \) subunits are likely candidates for \( I_{\text{Na}} \) based on the current’s negative inactivation and relative insensitivity to TTX. In addition \( \text{NaV}1.5 \)-like immunoreactivity was found in hair cells and calyces in rat utricular macula, and mRNA for \( \text{NaV}1.5 \) was detected in both utricle and SCC epithelia at P1 and P21 (35). In another study of rat utricle, \( G_{\text{Na}} \) was absent in type I hair cells, but expression peaked in immature hair cells at P1 and was found in only 5% of hair cells by P21 (2). In contrast, we found that the transient inward current was present in most SCC hair cells at early postnatal days and could still be detected in about one-third of rat and gerbil type I hair cells at P20 and P19, respectively. \( I_{\text{Na}} \) was absent in rat in the fourth postnatal week but was detected in a small number of gerbil type I cells as late as P35.

Role of innervation in development and regeneration. Calyces form around type I hair cells during the first postnatal week. In regenerating bird vestibular epithelia, type II hair cells repopulate SCC epithelia before type I hair cells, and ionic currents in hair cells are acquired in a similar pattern to that seen during development (22, 33). It has been suggested that contact with a calyx may drive expression of the type I ion channel phenotype (6, 22). In support of this, we recorded from early postnatal hair cells that had attached calyces but lacked \( G_{\text{K,L}} \). However, hair cells from denervated cultures of mouse utricle showed similar developmental patterns to acutely isolated hair cells, suggesting that calyces were not required for the postnatal differentiation of type I hair cells (32). Further studies of developing and regenerating hair cells and associated afferents are needed to elucidate synaptogenesis and underlying mechanisms important in establishing vestibular signals.

Perspectives and Significance

Postnatal maturation of hair cells and their associated afferents enables inner ear epithelia to become fully functional. Data presented here show that type I hair cells, which are only present in amniotes, show a mature K⁺ channel phenotype by the third postnatal week, coincident with the time of eye opening in two rodent species. \( I_{\text{Na}} \) is expressed transiently during postnatal development, but its physiological role is unclear since hair cells would need to hyperpolarize several millivolts from rest to remove inactivation of \( G_{\text{Na}} \) and enable action potentials to fire. However, the prominence of \( G_{\text{Na}} \) in several different types of hair cells during embryonic and early postnatal development strongly suggests an underlying role in maturation. In inner hair cells, Na⁺ and Ca²⁺ currents contribute to spontaneous spiking activity prior to hearing onset (11). Indeed, it has been suggested that Na⁺-driven action potentials may drive BDNF release from utricular hair cells, which may be important for vestibular neuron guidance and synapse formation (2, 21).

Vestibular receptors clearly contribute to the development and maturation of vestibular reflexes, but the specific roles of type I and type II hair cells in these processes remain to be determined. Further studies targeting ionic changes that occur in the vestibular periphery during development should elucidate hair cell/afferent signaling cues and may offer insight into inner ear epithelia regrowth mechanisms.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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