Regional Analysis of Whole Cell Currents From Hair Cells of the Turtle Posterior Crista

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Brichta, Alan M., Anne Aubert, Ruth Anne Eatock, and Jay M. Goldberg. Regional analysis of whole cell currents from hair cells of the turtle posterior crist. J Neurophysiol 88: 3259–3278, 2002; 10.1152/jn.00770.2001. The posterior crist is made up of two hemicristae, each consisting of a central zone containing type I and type II hair cells and a surrounding peripheral zone containing only type II hair cells and extending from the planum semilunatum to the sensory torus. Afferents from various regions of a hemicrist differ in their discharge properties. To see if afferent diversity is related to the basolateral currents of the hair cells innervated, we selectively harvested type I and II hair cells from the central zone and type II hair cells from two parts of the peripheral zone, one near the planum and the other near the torus. Voltage-dependent currents were studied with the whole cell, ruptured-patch method and characterized in voltage-clamp mode. We found regional differences in both outwardly and inwardly rectifying voltage-sensitive currents. As in birds and mammals, type I hair cells have a distinctive outwardly rectifying current (I\textsubscript{K,L}), which begins activating at more hyperpolarized voltages than do the outward currents of type II hair cells. Activation of I\textsubscript{K,L} is slow and sigmoidal. Maximal outward conductances are large. Outward current in type II cells vary in their activation kinetics. Cells with fast kinetics are associated with small conductances and with partial inactivation during 200-ms depolarizing voltage steps. Almost all type II cells in the peripheral zone and many in the central zone have fast kinetics. Some type II cells in the central zone have large outward currents with slow kinetics and little inactivation. Although these currents resemble I\textsubscript{K,L}, they can be distinguished from the latter both electrophysiologically and pharmacologically. There are two varieties of inwardly rectifying currents in type II hair cells: activation of I\textsubscript{K,L} is rapid and monooxygened, whereas that of I\textsubscript{K}; very few cells only have I\textsubscript{K}; inward currents are less conspicuous in type I cells. Type II cells near the torus have smaller outwardly rectifying currents and larger inwardly rectifying currents than those near the planum, but the differences are too small to account for variations in discharge properties of bouton afferents innervating the two regions of the peripheral zone. The large outward conductances seen in central cells, by lowering impedances, may contribute to the low rotational gains of some central-zone afferents.

INTRODUCTION

Voltage-sensitive ionic currents flowing across the basolateral surfaces of hair cells can shape receptor potentials and thereby modify neurotransmitter release and the response of afferent neurons. The importance of basolateral currents is clear in auditory and vibratory organs of lower vertebrates, where such currents participate in the electrical tuning of hair cells (Fettiplace and Fuchs 1999). Basolateral currents have also been described in vestibular organs, which monitor head movements (Correia et al. 1989; Masetto and Correia 1997; Masetto et al. 1994, 2000; Ohmori 1984; Rennie and Correia 1994; Rüschi and Eatock 1996). Despite the large number of such studies, the roles of basolateral currents in vestibular processing remain a matter of speculation. In particular, it is unclear how the currents influence afferent discharge properties, which vary between different regions of the neuroepithelium (Baird and Lewis 1986; Baird et al. 1988; Boyle et al. 1991; Goldberg et al. 1990; Honrubia et al. 1989; Myers and Lewis 1990).

There may be several reasons for this situation. First, only a few studies have compared the electrophysiological properties of vestibular hair cells with their neuroepithelial locations. Such studies have been done in frog (Baird 1994a,b; Marcotti et al. 1999a,b; Masetto et al. 1994; Prigioni et al. 1996) and in bird vestibular organs (Masetto and Correia 1997; Masetto et al. 2000; Weng and Correia 1999). Regional studies of afferent discharge have been done in the frog (Baird and Lewis 1986; Honrubia et al. 1989; Myers and Lewis 1990) but not in birds. Second, the protocols used on vestibular hair cells have been of relatively short duration and have not included a background current. In these ways, the protocols may not simulate normal conditions of vestibular transduction (Goldberg and Brichta 2002). Third, to the extent that they display resonant behavior, vestibular hair cells show low-quality tuning with best frequencies of 30–100 Hz (Correia et al. 1989; Holt et al. 1999; Housley et al. 1989; Rennie and Ashmore 1991; Ricci and Correia 1999), well above the bandwidth of naturally occurring head movements (Grossman et al. 1988; Pozzo et al. 1990). A theoretical framework is needed to explain the poor and seemingly inappropriate tuning. A framework is provided in the companion paper (Goldberg and Brichta 2002), which considers how basolateral currents shape receptor potentials. A fourth reason relates to the presence of type I and II hair cells in vestibular organs.
As was first described by Wersäll (1956), type II hair cells, which are found in the vestibular organs of all vertebrates, resemble hair cells in nonvestibular organs in being innervated by bouton endings derived from several afferent and efferent fibers. Type I hair cells, which are only found in the vestibular organs of reptiles, birds, and mammals (Lewis et al. 1985; Lysakowski 1996; Wersäll and Bagger-Sjöbäck 1974), have a distinctive shape, and each of them is innervated by a calyx ending derived from a single afferent fiber. Another distinguishing feature of type I hair cells is an outwardly rectifying potassium current called $I_{K,L}$ (Rennie and Correia 1994) to reflect its presence in type I hair cells or $I_{K,L}$ (Rüschi and Eatock 1996) because it activates at more hyperpolarized (lower, L) potentials than the outward currents of type II cells. $I_{K,L}$ differs from type II currents not only in its activation range but in having slower kinetics and larger whole cell currents. Possibly distinctive roles of type I and II hair cells in vestibular transduction have been considered (Eatock et al. 1998; Goldberg 1996; Rennie et al. 1996), but none of the suggestions have been conclusively established.

The turtle posterior crista provides an opportunity to compare the respective roles of type I and II hair cells in vestibular transduction and to explore the relation between hair-cell and afferent physiology. As illustrated in Fig. 1A, the turtle posterior crista consists of two triangularly shaped hemicristae, each of which extends from the planum semilunatum to a non-sensory torus. Within a hemicrista, there is a central zone and a surrounding peripheral zone. Type I hair cells are confined to the central zone, which also contains a smaller number of type II hair cells (Brichta and Peterson 1994; Jørgensen 1974; Lysakowski 1996). Only type II hair cells are found in the peripheral zone.

To consider possible relations between hair-cell and afferent physiology, we briefly consider regional variations in afferent discharge properties. Vestibular-nerve afferents are referred to by the endings they possess (Fernández et al. 1988; Schessel 1982). Calyx fibers contact type I hair cells, bouton fibers terminate on type II hair cells, and dimorphic fibers contain both calyx and bouton endings and synapse on both kinds of hair cells. The central zone in the turtle posterior crista is supplied by calyx, dimorphic, and bouton fibers, while the peripheral zone is innervated only by bouton fibers (Brichta and Peterson 1994). Morphophysiological studies have related the discharge properties of afferents with the kinds and locations of the hair cells they innervate (Brichta and Goldberg 2000a,b). In their responses to head rotations, bouton afferents show a single longitudinal gradient with those ending near the planum having a more regular discharge, lower gains, and more tonic response dynamics than those ending near the torus. Calyx-bearing units, including calyx and dimorphic fibers, have an irregular discharge and can be distinguished from irregularly discharging bouton fibers by their lower gains and less phasic response dynamics.

In the present study, we used voltage-clamp protocols to characterize the voltage-sensitive ionic conductances of solitary hair cells selectively harvested from the central zone and from the peripheral zone near the planum or near the torus. Morphological criteria were used to distinguish between type I and II hair cells. We had two aims. First, we were interested in comparing the electrophysiology of the two kinds of hair cells as well as determining whether there were differences in the potassium current called $I_{K,L}$ (Rennie and Correia 1994) to reflect its presence in type I hair cells or $I_{K,L}$ (Rüschi and Eatock 1996) because it activates at more hyperpolarized (lower, L) potentials than the outward currents of type II cells. $I_{K,L}$ differs from type II currents not only in its activation range but in having slower kinetics and larger whole cell currents. Possibly distinctive roles of type I and II hair cells in vestibular transduction have been considered (Eatock et al. 1998; Goldberg 1996; Rennie et al. 1996), but none of the suggestions have been conclusively established.

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dressed two questions: 1) Did the conductance seen in the turtle resemble that described in other species? 2) Were conductances other than \( I_{\text{K,L}} \) present in type I hair cells? In a second paper (Goldberg and Brichta 2002), current steps and sinusoids were used to examine how the various conductances might help to determine the gain and response dynamics of vestibular transduction.

**METHODS**

**Dissociation of hair cells**

Red-eared turtles (Trachemys scripta elegans, 150–250 g, 10- to 13-cm carapace length) were decapitated, their heads bisected, and the half-heads placed in a standard external solution (see Solutions). Animals were handled according to procedures approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Chicago. The posterior ampulla was opened to reveal its crista. To loosen hair cells for mechanical dissociation, we treated the crista with the following agents dissolved in dissociation solution (see Solutions): protease XXVII (50 \( \mu \)g/ml, Sigma, St. Louis, MO) for 20 min; papain (500 \( \mu \)g/ml, Sigma) and \( \lambda \)-cysteine (300 \( \mu \)g/ml, Sigma) for 45 min; and bovine serum albumin (500 \( \mu \)g/ml, Sigma) for 20 min. The crista was then transferred to a low-Ca\(^{2+}\) medium in a recording chamber and viewed at \( \times 60 \) (Zeiss Stemi 2000 stereomicroscope). The neuroepithelium was stroked with an eyelash, releasing hair cells from one of three selected regions (peripheral zone near the planum, peripheral zone near the torus, or central zone; Fig. 1A). Given the topography of the hemicrista, it was relatively easy to get uncontaminated samples from the torus or the central zone. Our planum samples were restricted to the corners of the neuroepithelium. Even so, they could easily have been contaminated from the central zone.

Isolated cells, which were allowed to settle on the clean glass floor of the recording chamber, were viewed at \( \times 600 \) with Nomarski optics on an inverted microscope (Zeiss Axiovert 100), and were continuously perfused at a rate of 500 \( \mu \)l/min with the standard external solution. All procedures, including recording, were done at 22°C.

**Solutions**

The standard external solution used for dissection and recording and was a modified Leibowitz-15 medium (L-15; Gibco BRL, Buffalo, NY). Ion concentrations (in mM) were: 118 Na\(^{+}\), 4 K\(^{+}\), 4 Ca\(^{2+}\), 1 Mg\(^{2+}\), 131.5 Cl\(^{-}\), 0.5 H\(_2\)PO\(_4\)\(^{-}\), 5 glucose, and 5 N-2-hydroxymethylpyperazine-N\(^{-}\)-2-ethanesulfonic acid (HEPES). The final osmolality was 270 mmol/kg, and the pH of all solutions was 7.4.

Patch-clamp recording pipettes were filled with a standard internal solution containing (in mM) 140 K\(^{+}\), 0.1 Ca\(^{2+}\), 140 Cl\(^{-}\), 2 MgATP, 10 HEPES, and 11 ethylene glycol bis-(\( \beta \)-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) with an osmolality of 260 mmol/kg and a pH of 7.4.

The dissociation solution was identical to the standard external solution except that Ca\(^{2+}\) was lowered to 100 \( \mu \)M by adding 1.2 mM EGTA.

When external blockers were used, we used a phosphate-free external solution to avoid precipitates with divalent cations. The phosphate-free external solution contained (in mM) 127 Na\(^{+}\), 4 K\(^{+}\), 4 Ca\(^{2+}\), 1 Mg\(^{2+}\), 141 Cl\(^{-}\), 5 HEPES, and 5 d-glucose, supplemented with 7.5 m\(l/l\) MEM vitamin mixture (Gibco BRL) and 45 m\(l/l\) MEM amino acids solution (Gibco BRL). Low concentrations of Cd\(^{2+}\) (<200 \( \mu \)M) and of 4-aminopyridine (4-AP, Sigma; 0.01–1 m\(M\)) were obtained by adding the blockers to the phosphate-free external solution. For experiments with external Ba\(^{2+}\) and higher concentrations of external Ca\(^{2+}\), Na\(^{+}\) concentration was reduced to preserve osmolarity. For experiments with external Cd\(^{2+}\), at concentrations exceeding 1 m\(M\), Cd\(^{2+}\) was substituted for Ca\(^{2+}\). When external K\(^{+}\) was elevated, an equivalent amount of Na\(^{+}\) was removed.

**Recording**

Borosilicate pipettes were drawn and heat-polished. When filled with the standard internal solution, the pipettes had impedances of 2.5–4 M\(\Omega\). Recordings were made in whole cell mode with a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Foster City, CA). Recordings were measured in whole cell mode with a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Foster City, CA). Experiments were controlled by a Digidata 1200 interface connected to a 486-DX2 computer running pClamp 6.1 software (Axon Instruments).

A cell was first photographed with an MC 80 camera (Zeiss). Once a gigohm seal was established, the recording pipette was raised to lift the cell off the chamber floor. During these procedures, the amplifier was in “tracking mode,” which allowed us to determine the resting or zero-current (\( V_z \)) potential on breakthrough. We then went into voltage-clamp mode at a standard holding potential, \( V_H = -67 \) m\(V\), chosen because it was near the mean \( V_z \) of our cells. A standard voltage-clamp protocol was run. It consisted of eighteen 200-ms steps from \( V_H \) to voltages ranging from \(-137\) to \(+33\) m\(V\) in 10-m\(V\) increments. Each step was followed by a 20-ms step to \(-52\) m\(V\) and then by a return to \( V_H \) for a duration \(\approx 780\) ms. All voltages have been corrected for a junction potential (see following text).

To allow for off-line calculation of series resistance (\( R_S \)) and membrane capacitance (\( C_M \)), we next recorded the currents evoked by 3-ms voltage-clamp steps of \( \pm 10 \) m\(V\) from a holding potential of \(-77\) m\(V\) through a 4-pole Bessel filter with a corner frequency, \( f_c = 10 \) kHz and a sampling frequency of 100 kHz. In the analysis, the effects of the filter were removed by deconvolution. For many of our cells, a steady-state current was not abolished even when the membrane was hyperpolarized. In calculating \( R_S \) and \( C_M \), we used equations that allowed for this possibility (Gillis 1995).

Capacitive transients were canceled, and series resistance, typically 5–15 \( M \Omega \), was compensated by 70–90%. Compensation was calculated off-line by comparing the dial settings on the amplifier with the actual value of \( R_S \) determined from the 3-ms voltage clamps. In every cell, once compensation was achieved, the standard protocol was run once again followed by other protocols as required. Amplifier outputs were passed through 4-pole Bessel filters with a corner frequency, unless otherwise stated, of \( f_c = 3 \) kHz. Most data were sampled at 2 kHz, below the Nyquist sampling frequency of 6 kHz. Not conforming to the Nyquist theorem led to no serious problems because all of the analyses were done in the time domain and activation time constants were relatively long compared with the sampling interval.

Local exchange was used to study the effects of changing external solutions. Four glass delivery pipettes (each 200-\(\mu\)m diameter) were placed in parallel on a micromanipulator and were connected to separate perfusion lines. The pipettes were lowered into the bath. To deliver a particular solution, the cell was positioned within 10–20 \( \mu \)m of the orifice of the appropriate pipette. A peristaltic pump (Rainin) regulated fluid flow at 5 \( \mu \)l/min. Closure of a solenoid valve (General Valves) diverted the solution from a recirculating line to the delivery pipette. A separate valve controlled flow to each pipette. To aid in visualizing flow, we added an aqueous solution of polystyrene beads (LB-8, Sigma) to each solution at a dilution of 1:10\(^6\).

**Morphological classification**

Photomicrographs of recorded cells were projected at \(\times 5,000\) total magnification and independently ranked by two investigators without reference to the physiological results. A scale of 1.1–1.9 was used with 1.1 representing a clear type I hair cell with a constricted neck and 1.9 representing a definite type II cell with a cylindrical cell body and parallel sides. The two scores, which seldom differed by \(>0.1\), were averaged. In the text, cells with a score of 1.1–1.3 are designated type I and those with a 1.7–1.9 score are considered type II. When the score was 1.4–1.6, the cell was placed in an “unassigned” category.
OUTWARDLY RECTIFYING CURRENTS. Most type I hair cells have an outwardly rectifying current called $I_{K,L}$ (Rennie and Correia 1994) or $I_{K,L}$ (Rüschi and Eatoek 1996). In our type I hair cells, $I_{K,L}$ usually begins activating 5–15 mV more negative than our standard holding potential, $V_H = -67$ mV. Such type I hair cells show large instantaneous currents on being stepped from $V_H$ (Fig. 2, A and D). The instantaneous current, which we refer to as $i_{-67}$, is deactivated when the cell is hyperpolarized beyond $-80$ mV. In some cases, almost all of $I_{K,L}$ is activated at $V_H$ so the response to depolarizing steps is dominated by the instantaneous current (Fig. 2, B and E). More typically, depolarizing steps evoke both the instantaneous component and a slow, sigmoidally activating component (Fig. 2, A and D). In type I hair cells from other organs, outwardly rectifying currents besides $I_{K,L}$ may contribute to the slow, sigmoidally activating currents (Masetto et al. 2000; Rennie and Correia 1994; Rüschi and Eatoek 1996; Rüschi et al. 1998).

In the turtle, however, most conductance-voltage curves from type I cells would appear to be dominated by a single, $I_{K,L}$ current. Given this interpretation, we attribute the instantaneous current to $I_{K,L}$ channels active at $V_H$ and the sigmoidal component to additional $I_{K,L}$ channels becoming active with depolarizations above $V_H$.

A small percentage of type I cells (<10%) do not have an obvious instantaneous current on being stepped from $V_H$ (Fig. 2, C and F). One possibility is that cells without the instantaneous current possess an $I_{K,L}$ current whose activation range does not extend below $V_H$. Two observations are consistent with the suggestion. First, type I cells, whether or not they have $i_{-67}$, have outward currents characterized by a large size and slow, sigmoidal activation kinetics. Second, a few type I cells had an instantaneous current on initial breakthrough, but lost this component over the next several minutes as the activation range shifted in a depolarizing direction. We never encountered the reverse situation, in which an instantaneous component developed only after prolonged recording. Such observations, which have also been made in mammals (Chen and Eatoek 2000; Hurley and Eatoek 1999), would seem inconsistent with the suggestion that the hyperpolarized activation range of type I cells reflects the washout of normal intracellular constituents during whole cell recording (Lennan et al. 1999).

Other evidence (see following text) supports the contention that virtually all type I hair cells have $I_{K,L}$ whether or not they have the instantaneous current.

Table 1 shows that 15–20% of the cells from the peripheral zone (PZ) showed an instantaneous ($i_{-67}$) current large enough to qualify as $I_{K,L}$, as did 75–80% of central-zone (CZ) cells. To decide whether a cell had $i_{-67}$, we required that the instantaneous conductance measured on stepping from $V_H$ be $\geq 1 \text{nS}$ larger than that obtained after $I_{K,L}$ had been deactivated by holding the cell at $-127$ mV for 200 ms.) About 5% of the peripheral cells had the morphology of type I hair cells and may have been strays from the CZ. The same may be true for some unassigned PZ cells. Results for hair cells morphologically classified as type II are more difficult to interpret. As summarized in Table 1, $\approx 30\%$ of CZ type II cells and $\approx 5\%$ of PZ type II cells had an instantaneous current in our standard protocol, implying that they had $I_{K,L}$. There are two possible interpretations for these results: a substantial fraction of type II cells possess $I_{K,L}$ or presumed type II cells with $I_{K,L}$ are, in fact, type I hair cells. The second alternative is consistent with
our finding that many cells in the intact crista are enclosed by calyces but otherwise resemble type II cells in their morphology. Given the uncertainties associated with cells having type II morphology and an $I_{-67}$ current, we have excluded them from further consideration.

Outwardly rectifying currents in the remaining type II cells differed from $I_{K,L}$ in their activation range, activation kinetics, conductance magnitudes, and inactivation. In addition, there were differences in the outward currents of type II hair cells obtained from different zones of the neuroepithelium. Heterogeneity in the outward currents of type II cells is illustrated in Fig. 3 and summarized in scatterplots relating half-activation and inactivation (Fig. 4).

**FIG. 2.** Voltage-clamp records (top) and current-voltage ($I$-$V$) curves (bottom) for 3 type I hair cells having $I_{K,L}$ currents. A and D: the current is only partly activated at the holding potential, $V_H = -67$ mV. As a result, there is an instantaneous current at the start of all voltage steps. Depolarizing steps result in a slow, sigmoidal activation of additional current, whereas hyperpolarizing steps deactivate the instantaneous current. Both activation and deactivation can be seen in the $I$-$V$ curves as departures of the late current from the early current. B and E: most of $I_{K,L}$ is activated at $V_H$. A large instantaneous current is seen. Hyperpolarizing steps deactivate the current; depolarization steps result in only a slight activation. The late curve shows a large departure from the early curve only for hyperpolarizing steps. There is no deactivation for hyperpolarizing steps nor is there any evidence of inwardly rectifying currents. In all panels, voltage steps are 200-ms duration from a holding potential, $V_H = -67$ mV, to voltages in 10-mV steps from $-137$ to $-7$ mV. In Figs. 4–7, currents were measured 0.5 or 1 ms (early) and 195–200 ms (late) after the start of the step; voltages are corrected for series resistance. The horizontal and vertical lines for each $I$-$V$ curve indicate zero current and the resting potential, respectively.

**TABLE 1.** Correlation between hair-cell morphology and the presence of an instantaneous ($I_{-67}$) conductance at $-67$ mV

<table>
<thead>
<tr>
<th>Class</th>
<th>Central Zone</th>
<th></th>
<th>Planum</th>
<th></th>
<th>Torus</th>
<th></th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I_{-67}$/Total</td>
<td>Percent</td>
<td>$I_{-67}$/Total</td>
<td>Percent</td>
<td>$I_{-67}$/Total</td>
<td>Percent</td>
<td>$I_{-67}$/Total</td>
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<tr>
<td>Type I</td>
<td>129/140</td>
<td>92.1</td>
<td>4/5</td>
<td>80.0</td>
<td>1/1</td>
<td>100.0</td>
<td>134/146</td>
</tr>
<tr>
<td>Type II</td>
<td>11/35</td>
<td>31.4</td>
<td>1/27</td>
<td>3.7</td>
<td>2/30</td>
<td>6.7</td>
<td>14/92</td>
</tr>
<tr>
<td>Unassigned</td>
<td>107/143</td>
<td>74.8</td>
<td>9/29</td>
<td>31.0</td>
<td>1/19</td>
<td>11.1</td>
<td>117/181</td>
</tr>
<tr>
<td>All</td>
<td>247/318</td>
<td>77.7</td>
<td>14/61</td>
<td>23.0</td>
<td>4/40</td>
<td>10.0</td>
<td>265/419</td>
</tr>
</tbody>
</table>

The number of hair cells showing $I_{-67}$, a nonleak instantaneous current ($\geq 1$ nS), when the voltage was stepped from a holding potential, $V_H = -67$ mV, to other voltages. For each zone, the 1st column gives the ratio of the number of hair cells with the instantaneous current over the total number of hair cells; the 2nd column is the percentage of cells with the instantaneous current. Data are shown for solitary hair cells assigned to 3 classes based on their morphology. The cells were harvested from 3 regions of the crista, the central zone, the peripheral zone near the planum, and the peripheral zone near the torus.

**ACTIVATION RANGE.** Outwardly rectifying currents from type II hair cells usually require depolarizations to between $-50$ and $-60$ mV to begin activating (Fig. 3). In contrast, most type I hair cells are already partly activated at our holding potential ($V_H = -67$ mV) or just above it (Figs. 2 and 7F).

**ACTIVATION KINETICS.** We measured half-activation times during 200-ms depolarizations to $-37$ mV. For type I hair cells, half activation typically took 20–100 ms (Figs. 2 and 4A; Table 2).

Most type II hair cells from the planum and torus have relatively fast $t_{1/2}$ values (Fig. 3, A and B), typically 4–7 ms (Fig. 4A; Table 2). Activation kinetics are especially heterogeneous among central type II hair cells with $t_{1/2}$ values ranging from further consideration.
FIG. 3. Voltage-clamp records (top) and current-voltage (I-V) curves (bottom) for 3 type II hair cells harvested from the peripheral zone near the planum (A and A1), near the torus (B and B1), or from the central zone (C and C1). All 3 cells have outwardly rectifying currents that activate between −57 and −47 mV. The 2 cells from the peripheral zone have fast activation kinetics, while the central cell has slow activation kinetics. Inactivation is most conspicuous in the planum cell, while inwardly rectifying currents are largest in the torus cell.

FIG. 4. Scatterplots relating the half-activation times of outwardly rectifying currents with the magnitude of the associated conductance (A) and the amount of inactivation (B). Half-activation times and inactivation index $= (1-I_{end}/I_{peak})$ were measured during 200-ms, 30-mV depolarizing steps from a holding potential, $V_H = −67$ mV. $I_{peak}$ was the maximal current during the step and $I_{end}$ was the average current during the last 5 ms of the step. Conductance was measured from the instantaneous current obtained at the end of a step to $−52$ mV after the voltage was held at $−7$ mV for 200 ms; residual capacitative current, as estimated from extrapolation of tail current, was subtracted. There is a trend for currents with slower activation kinetics to be larger and to show less inactivation. Data from type I and II hair cells from different regions of the neuroepithelium (see key) are consistent with these trends. Data in each panel were fit to power laws, $y = ax^b$, where $x$ is half-activation time in milliseconds. In A, $y$ is conductance in nS, $a = 2.20 ± 0.47$, and $b = 0.97 ± 0.08$; in B, $y$ is inactivation index, $a = 1.20 ± 0.26$ and $b = −1.26 ± 0.08$. 
Different zones (P case. reviewed in the following sections, indicate that this is not the conceivable that our slow CZ type II cells were type I cells quarter of our PZ type II cells (Table 2).

Inactivation was studied in detail in 10 type II cells, 7 of which had a prominent inactivating or transient component. Results are illustrated for a planum cell with a conspicuous transient component (Fig. 5, A and A1). A test depolarization to 3 mV was delivered after a 200-ms conditioning prepulse to voltages ranging from −127 to −7 mV. The hyperpolarizing prepulse to −127 mV almost doubled the peak current relative to its value with no prepulse, whereas a depolarizing prepulse to −27 mV eliminated the peak (Fig. 5, A and A1), leaving a sustained component. The latter also showed a large increase following the −127-mV prepulse and a smaller decrease following the −27-mV prepulse.

In a torus cell (Fig. 5, B and B1), the peak or transient component was much smaller than the sustained component even after a prepulse to −127 mV. On the other hand, the same hyperpolarizing prepulse led to a large increase in the sustained component, while a depolarizing prepulse to −27 mV produced a smaller decrease. One interpretation for these effects is that a slow inactivation occurs at the holding potential and may be relieved by a preceding hyperpolarizing step and exacerbated to a lesser extent by a preceding depolarizing step. As exemplified by this cell, a slow inactivation can occur even in the almost complete absence of a fast inactivation. Slow inactivation is not associated with a decline in outward current during conditioning or test pulses. This would suggest that slow inactivation has kinetics much longer than the 400-ms combined duration of the two pulses.

In 5/10 peripheral type II cells studied, hyperpolarizing prepulses increased the sustained response by <10%. More
substantial increases, ranging from 40 to 160%, were seen in the remaining five cells. Even in the former cells, it is possible that they would have showed a slow inactivation had they been held at potentials more positive than $-67$ mV. This is suggested by the observation that depolarizing prepulses decreased sustained responses by 15–25% in all 10 cells. The suggestion is confirmed in the next paper (Goldberg and Brichta 2002) where it is shown that prolonged depolarizations to $-47$ mV invariably result in a slow inactivation of fast type II cells.

**INWARDLY RECTIFYING CURRENTS.** Most (>90%) type II cells had inward currents that activated negative to $-87$ mV. In hair cells from the frog sacculus (Holt and Eatock 1995), two inward currents ($I_{K1}$ and $I_h$) were distinguished by their activation kinetics, ion selectivity, and sensitivity to divalent cations. In the present study, we relied on activation kinetics to recognize the two currents. As was the case in other hair cells (Eatock et al. 1998; Holt and Eatock 1995; Weng and Correia 1999), $I_{K1}$ has rapid monoexponential kinetics and is fully activated by 10–25 ms (Fig. 6A). At very large negative voltages near $-137$ mV, $I_{K1}$ declines with time, suggestive of a multi-ion block (Hille 1992). Many cells had a mixture of $I_{K1}$ and $I_h$, this resulted in a rapid activation followed by a slow sigmoidal activation of inward current (Fig. 6B). Table 3 summarizes the incidence of the two currents in type II hair cells.
cells. Cells with a mixture of $I_{K1}$ and $I_h$ and those only having $I_{K1}$ each occurred in slightly less than half the cases. Only a few cells had $I_h$ but not $I_{K1}$.

To estimate the sizes of $I_{K1}$ and $I_h$ in individual type II cells, we used records obtained on stepping from $V_H = -67$ to $-107$ mV for 200 ms. Presumed leak currents were eliminated by subtracting a scaled version of the response during a step to $-77$ mV. For each cell, a time ranging from 10 to 25 ms was selected as a compromise between the complete activation of $I_{K1}$ and negligible activation of $I_h$. The current activated at this time was taken to be $I_{K1}$ and the additional current activated by 200 ms was considered to be $I_h$. Conductances were calculated by assuming that $I_{K1}$ and $I_h$ had reversal potentials of $-87$ and $-47$ mV, respectively (Holt and Eatock 1995). Results are summarized in the last two columns of Table 3.

Differences in $I_{K1}$ currents were noted among the type II hair cells categorized by the activation kinetics of their outward currents and by the regions from which they were harvested. Fast hair cells had larger values of $I_{K1}$ than did those with slower kinetics. Cells from the planum had smaller $I_{K1}$ currents than did torus cells or fast cells from the central zone. $I_h$ was present in $<30\%$ of torus cells, as compared with $>60\%$ of planum cells.

Inwardly rectifying currents were less easily recognized in type I cells than in type II cells. One reason is that the deactivation of $I_{K1}$ with hyperpolarizing voltage steps could obscure the presence of other inward currents (see, for example, Fig. 2, A and B). To remove the confounding effects of $I_{K1}$, deactivation, pharmacological agents were used. The current was blocked by 4-AP in doses of 10–300 μM (n = 3) and by 2 mM Cd$^{2+}$ (n = 2). Of the five cases, one had $I_h$, one had $I_{K1}$, and three had both currents. The currents were small. Mean conductances were $0.63 \pm 0.30$ nS for $I_{K1}$ and $0.23 \pm 0.08$ nS for $I_h$, smaller than the values of slow type II cells in the central zone (Table 3).

In summary, type I cells are distinguished from type II cells by the presence of $I_{K1}$ and by the small magnitude of inwardly rectifying currents. Most type II cells harvested from the PZ have outwardly rectifying currents with fast activation kinetics as well as inwardly rectifying currents. Outwardly rectifying

### Table 3. Inwardly rectifying in type II hair cells, turtle posterior crista

<table>
<thead>
<tr>
<th>Class</th>
<th>$n$</th>
<th>$I_{K1}$</th>
<th>$I_h$</th>
<th>Both</th>
<th>Neither</th>
<th>Conductance, nS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$g_{K1}$</td>
</tr>
<tr>
<td>Central</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>$10.3 \pm 3.9$</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>$5.3 \pm 0.7$</td>
</tr>
<tr>
<td>Slow</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>$2.5 \pm 1.2$</td>
</tr>
<tr>
<td>Torus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>11</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>$7.0 \pm 1.1$</td>
</tr>
<tr>
<td>Intermediate + Slow</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>$4.0 \pm 2.2$</td>
</tr>
<tr>
<td>Planum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>$3.4 \pm 1.3$</td>
</tr>
<tr>
<td>Intermediate + Slow</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>$1.2 \pm 0.5$</td>
</tr>
</tbody>
</table>

Presence of various inwardly rectifying currents in morphologically classified type II hair cells based on the region of the crista from which they were harvested and the activation kinetics of their outwardly rectifying conductances (see Table 2). Conductances were measured from leak-subtracted voltage-clamp records obtained on stepping from $V_H = -67$ to $-107$ mV for 200 ms; other details in text. To be considered present in a cell, the conductance associated with $I_{K1}$ or with $I_h$ had to be $>0.2$ nS. $n$, total number of hair cells in each class; other entries, number of cells with stated inward rectifiers. Values are means $\pm SE$, each of 2 conductances. Among type II (fast), there were proportionately more cells only having $I_{K1}$ in the torus than in the other 2 zones ($X^2, P < 0.02$). $g_{K1}$ is inversely related to half-activation time ($P < 0.05$) and, independent of the half-activation time, is smaller near the planum than near the torus ($P < 0.05$) or in the central zone ($P < 0.01$). $g_h$ is not significantly related to half-activation time nor is it significantly different in the various regions ($P > 0.5$).
FIG. 7. Activation of $I_{K,L}$ current in type I hair cells. A: voltage-clamp records for a hair cell. $I_{K,L}$ is deactivated by a 200-ms step to $-127$ mV from a holding potential of $-67$ mV. The current is then activated by a 500-ms step to voltages between $-97$ and $-17$ mV. This is followed by a step to $-52$ mV to measure tail currents. B: activation is first seen at $-77$ mV and becomes faster with larger depolarizations. Kinetics well fit by text Eq. 1 until $-37$ mV, where there are small discrepancies. C: time constants, obtained from the nonlinear fits of text Eq. 1 to the points in B, are plotted as a function of voltage. Both time constants decline as voltage increases. D: mean values of the time constants are plotted as a function of voltage for 10 type I cells; bars indicate SE. E: tail currents extrapolated to the beginning of the step to $-52$ mV and an empirically determined reversal potential of $-84.3$ mV were used to estimate conductances at the end of the preceding step for the cell illustrated in A–C. Curve is fit by a Boltzmann equation (Eq. 3). No attempt was made to fit the decline in conductance for depolarizations beyond $-34$ mV. F: normalized conductances are shown for 35 type I hair cells, including 28 cells (+ – –) with and 7 cells (——) without an instantaneous $(I_{-67})$ current. Thicker dashed curve (– – –) is the activation curve of a central type II cell that had a typical activation curve. Five of the 7 type I cells without $I_{-67}$ have activation curves that fall between the curves for type I cells with $I_{-67}$ and the type II cell.
was used. The leak current (\(I_{\text{LEAK}} = -0.31\) nA) was obtained by taking the average tail current between \(-87\) and \(-97\) mV, and the reversal potential (\(V_L = -84.3\)) was estimated as described in REVERSAL POTENTIAL AND ION SELECTIVITY. \(g_{K,L}\) began increasing near \(-80\) mV, reached a maximum of \(119\) nS at \(-37\) mV, and then declined by \(9.5\%\) over the next \(14\) mV. The decline seen with large depolarizations may reflect an inactivation of \(g_{K,L}\) or the extracellular accumulation of \(K^+\) ions during large, prolonged outward currents (Rennie and Correa 2000). Such an accumulation would decrease the driving force (\(V_{\text{TAIL}} - V_L\)), which would explain why there is a larger decline in the tail currents than in the currents during the preceding voltage step. Data points between \(-97\) and \(-34\) mV were fit by a Boltzmann equation

\[
g_{K,L} = \frac{g_{\text{MAX}}}{1 + \exp[-(V - V_{1/2})]/V_S]
\]

with \(g_{\text{MAX}} = 118.0 \pm 2.0\) nS, \(V_{1/2} = -65.9 \pm 0.3\) mV, and \(V_S = 5.7 \pm 0.3\) mV.

Individual normalized activation curves are shown in Fig. 7F for 35 type I cells with \(g_{K,L}\), including 28 with \(I_{-67}\) and 7 without \(I_{-67}\) (see key). A conductance decline of \(>10\%\) at large depolarizations was present in 20/35 cells. Equation 3 was fit to all 35 cells, but in each case, only one point beyond the maximum was included. In none of the cells was there a suggestion of a second conductance activating at voltages above that at which \(I_{K,L}\) reached a maximum. Mean values of the activation parameters are presented in Table 4.

The activation curves bear on the interpretation of type I hair cells lacking an instantaneous \((I_{-67})\) current. In a previous section, we suggested that these cells have \(I_{K,L}\), but that the current activates only at voltages more positive than \(V_H\). Consistent with this suggestion, many type I cells without \(I_{-67}\) activate at voltages intermediate between those of type I cells with \(I_{-67}\) and type II cells. This can be seen in Fig. 7F, which includes activation curves not only for type I cells with and without \(I_{-67}\) but also for a type II cell. The latter was chosen because it had a typical activation curve in that its \(V_{1/2}\) was almost identical to the mean value for all type II cells in Table 4. Curves for five of seven type I cells without \(I_{-67}\) clearly fall to the left of the type II curve and only one type I curve clearly falls to the right of it. In addition, type I cells with and without \(I_{-67}\) resemble each other in their activation parameters more than they do type II hair cells (Table 4). The two groups of type I cells showed statistically significant differences in \(V_{1/2}\) but not in \(g_{\text{MAX}}\) or \(V_S\). There were significant differences between type I cells not activated at \(V_H\) and slow type II cells in \(V_{1/2}\) and in the other two parameters of Eq. 3. Even larger differences were seen when comparisons were made between all of the type I and type II cells in the table. In short, we were able to distinguish type I and central type II cells electrophysiologically.

DEACTIVATION of \(I_{K,L}\). To study the kinetics of \(I_{K,L}\) at hyperpolarized potentials, we used a deactivation protocol (Fig. 8A). The cell was first stepped to \(-57\) mV to increase activation of \(I_{K,L}\) and then to potentials ranging from \(-67\) to \(-127\) mV to deactivate the current. Deactivation becomes faster with increasing hyperpolarization. As a result, the traces for more negative voltages cross those for less negative voltages (Fig. 8, A and B). Deactivation cannot be fit with a single exponential. Rather, a sum of two exponentials

\[
I(t) = I_L + I_{SLOW} \exp(-t/\tau_{SLOW}) + I_{FAST} \exp(-t/\tau_{FAST})
\]

is needed (Fig. 8B). Although this might suggest the presence of two distinct currents, it is also to be expected from a three-state \(C_1 \leftrightarrow C_2 \leftrightarrow C_0\) model provided that the hyperpolarizing step does not result in a complete steady-state deactivation. Figure 8C plots the fast and slow time constants as functions of membrane potential. Included are the results of an activation analysis as well as the deactivation analysis. Over the voltage range from \(-127\) to \(-87\) mV, \(\tau_{SLOW}\) increases from \(20\) to nearly \(200\) ms, while \(\tau_{FAST}\) only increases from \(7\) to \(11\) ms. Over the same range, the relative magnitude of the fast component decreases so that the ratio of \(\tau_{FAST}\) to \(\tau_{SLOW}\) in Eq. 4 falls from \(1.1\) at \(-127\) mV to \(0.2\) at \(-87\) mV.

Similar data were collected in a total of 11 type I cells (Fig. 8D). All cells in the sample showed a shortening of the slow time constant as the deactivating voltage was changed from \(-87\) to \(-127\) mV. Concurrent trends, including a shortening of the fast time constant and an increase in the relative magnitude of the fast component, were less consistent.

REVERSAL POTENTIAL AND ION SELECTIVITY. The reversal potential (\(V_{\text{REV}}\)) for \(I_{K,L}\) was determined as the intersection

**TABLE 4. Steady-state activation parameters in type I and II hair cells, central zone of turtle posterior crista**

<table>
<thead>
<tr>
<th>Class</th>
<th>(n)</th>
<th>(t_{1/2}), ms</th>
<th>(g_{\text{MAX}}), nS</th>
<th>(V_{1/2}), mV</th>
<th>(V_S), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>35</td>
<td>27.6 ± 1.9</td>
<td>152 ± 19</td>
<td>-57 ± 1.6</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>(I_{-67})</td>
<td>28</td>
<td>25.4 ± 2.1</td>
<td>157 ± 23</td>
<td>-60 ± 1.3</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>No (I_{-67})</td>
<td>7</td>
<td>36.2 ± 2.7</td>
<td>131 ± 8</td>
<td>-44 ± 2.7</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Type II</td>
<td>19</td>
<td>22.0 ± 2.9</td>
<td>37.9 ± 8.4</td>
<td>-37 ± 1.4</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>Intermediate*</td>
<td>8</td>
<td>9.2 ± 0.7</td>
<td>22.4 ± 3.6</td>
<td>-36 ± 2.7</td>
<td>8.9 ± 1.1</td>
</tr>
<tr>
<td>Slow</td>
<td>11</td>
<td>31.2 ± 2.5</td>
<td>49.3 ± 13.4</td>
<td>-37 ± 1.5</td>
<td>7.8 ± 0.6</td>
</tr>
</tbody>
</table>

\(n\), number of hair cells; values are means ± SE. For type I cells, voltage was first stepped to \(-127\) mV for \(200\) ms to deactivate \(I_{K,L}\). Type II cells were stepped from \(V_H = -67\) mV to more depolarized potentials, \(t_{1/2}\), half-activation time on stepping to \(-37\) mV. Other parameters from Boltzmann functions providing best fits to conductance vs. voltage functions obtained from a tail-current analysis (see text for details). Conductances calculated by assuming a reversal potential of \(-87\) mV, \(g_{\text{MAX}}\), maximal conductance; \(V_{1/2}\), voltage leading to half-maximal conductance; \(V_S\), voltage increment resulting in an \(e\)-fold conductance change. All cells from the central zone. Cells classified as type I were divided into those with and without an instantaneous \((I_{-67})\) current on being stepped from holding potential, \(V_H = -67\) mV. Type II cells sorted by their half-activation times (see text). * Includes 1 cell with fast activation kinetics. \(g_{\text{MAX}}\) values for type I with or without \(I_{-67}\) similar (\(P > 0.5\)), but either class larger than for type II, slow (\(P < 0.05\)) or type II, intermediate (\(P < 0.001\)); \(V_{1/2}\), values more negative for type I with \(I_{-67}\) than for other 3 groups (\(P < 0.001\)); type I without \(I_{-67}\) more negative than type II categories (\(P < 0.05\)); the 2 type II categories are not significantly different. \(V_S\), values for either type I category than for type II, intermediate (\(P < 0.001\)) or type II, slow (\(P < 0.05\)); the 2 type II classes do not differ significantly (\(P > 0.1\)), nor do the 2 type I categories (\(P > 0.5\)).
were reduced for outward currents in response to depolarizations beyond the resting potential. A simple explanation for these observations is that outward currents are carried by Cs\(^+\), which does not permeate the channel as easily as does K\(^+\) (Rüssch and Eatock 1996).

**Pharmacology of I\(_{K,L}\)**

Previous studies had characterized the effects of several external blockers on the I\(_{K,L}\) current in type I hair cells harvested from the gerbil and pigeon cristae (Rennie and Correia 1994) or recorded from explants of the neonatal mouse utricle (Rüssch and Eatock 1996). We wished to determine if I\(_{K,L}\) had a similar pharmacology in type I hair cells from the turtle posterior crista. Although our results are consistent with those previously reported, some of our interpretations are different. In particular, we found that the actions of some external blockers can only be appreciated by a complete activation analysis.

4-AP. I\(_{K,L}\) was blocked by low concentrations of 4-AP in a state-dependent manner. This is illustrated by a type I hair cell studied with our usual activation protocol (Fig. 9, A and B). A 30-μM dose almost completely blocked the current at −67 mV but had proportionately smaller effects as depolarization was increased to −7 mV. The result is a 10- to 15-mV shift in the activation curve (Fig. 9C) with only a small (25–30%) reduction in \(s_{MAX}\). Other effects include an approximately twofold slowing of activation kinetics (Fig. 9D) and a reduction in the inactivation produced by large depolarizations (Fig. 9B). With
the exception of the reduction in $g_{\text{MAX}}$, effects are consistent
with a so-called reverse-use dependent block in which the
blocker only attaches to the channel in the closed state and
prevents transitions to the open and inactivated states (Remil-
lard and Leblanc 1996).

The block can be measured as the proportion, $1 - p$, of
closed channels in the blocked state. An estimate of the pro-
portion of unblocked channels is provided by the equation,
$p = \frac{1}{\exp(-\Delta V_{1/2}/V_S)}$, where $\Delta V_{1/2}$ is the depolarizing shift in the
activation curve produced by the blocker. For the hair cell in
Fig. 9C, $\Delta V_{1/2} = 13.6$ mV, $V_S = 4.5$ mV, and $p = 0.044$. Values of $p$
are plotted in Fig. 9E against 4-AP concentration for nine type I cells, including four that were studied at mul-
tiple concentrations between 3 and 300 $\mu$M. A 50% block was
typically achieved at 3 $\mu$M.

Quite different results were obtained in central type II hair cells.
A slow type II hair cell is illustrative (Fig. 10). Application of 100
$\mu$M 4-AP caused a reduction in the outward currents evoked by
depolarizing voltage steps from $-67$ mV. The reduction
amounted to 40–60%, virtually independent of the size of the
voltage step (Fig. 10, A and B). Reflecting the lack of state
dependence, there was a negligible shift in the activation curve
(Fig. 10C) and no effect on activation kinetics (Fig. 10D).

A state-independent block was obtained in all eight central
type II cells studied with 100–300 $\mu$M 4-AP, including cells
whose activation kinetics were fast ($n = 1$), intermediate ($n = 1$),
or slow ($n = 6$). The mean block was 62.8 $\pm$ 6.5%, quite
similar to the calculated reduction of 62.1 $\pm$ 3.6% in $g_{\text{MAX}}$. In
none of the cells was there a shift in the activation curve. Only
small (<20%) blocks were produced by 3–30 $\mu$M 4-AP ($n = 3$; Fig. 9E). One cell was studied with low (30 $\mu$M) and high
(300 $\mu$M) doses: the lower dose produced a 7% block; the
higher dose a 46% block. These results indicate that 50%
blocking requires $\approx 10$-fold higher doses in type II cells than in
type I cells.

The differential effects of 4-AP on type I and central type II
cells reinforce the conclusion that the two kinds of hair cells
have different outward currents.
Divalent cations. Cd$^{2+}$ in a concentration of 200 μM blocked $I_{K,L}$ in six type I cells by an average of 60 ± 8%. In two other type I cells, 2 mM Cd$^{2+}$ completely blocked $I_{K,L}$, revealing in both cells small inward and outward currents that were presumably present under control conditions but masked by $I_{K,L}$ (Fig. 11). Concerning inward currents seen after $I_{K,L}$ was blocked, $I_{K1}$ was present in both cells and $I_{h}$ in one of them. The outward currents resembled those seen in many type II cells in being small, fast, and showing a fast inactivation. The fast outward currents, which were 10–20 times smaller than the $I_{K,L}$ current recorded in the absence of Cd$^{2+}$, only began activating at voltages more positive than −20 mV. This is 30–40 mV more depolarized than needed to activate similar currents in type II cells. The shift in activation range, rather than being a peculiarity of fast currents in type I cells, may reflect the presence of Cd$^{2+}$. Consistent with this interpretation, a similar shift in activation range was observed in one type II cell whose outward currents were reduced, but not abolished by 2 mM Cd$^{2+}$. Further evidence comes from two cells morphologically classified as type I that had fast outward currents in the absence of blockers. Similar to the situation in type II cells, these currents were partially activated by small depolarizing steps to −47 mV.

The fact that Cd$^{2+}$ blocks $I_{K,L}$ suggested that the latter might be a Ca$^{2+}$-activated $K^+$ current. We tested this possibility in four type I cells. Lowering Ca$^{2+}$ from 4 mM to 20 μM, while keeping Mg$^{2+}$ at its standard value (1 mM), had no consistent effect on $g_{MAX}$ but, as has been reported for other voltage-dependent channels (Hille 1992), shifted the activation range by 5–20 mV in a hyperpolarizing direction. These results show that $I_{K,L}$ is not Ca$^{2+}$-activated, in agreement with results in the mouse utricle (Rüscher and Eatock 1996).

Barium. High concentrations of external Ba$^{2+}$ block the instantaneous $I_{r,67}$ current obtained on stepping from −67 mV (Fig. 12, A and B). At the same time, the slow currents evoked by depolarization steps to −50 mV and beyond are not abolished. Similar observations were made in the neonatal mouse utricle by Rüscher and Eatock (1996), who interpreted their results as indicating the presence of $I_{K,L}$ and a second delayed rectifier, which was called $I_{DR,I}$. The two currents were thought to differ not only in their activation ranges but also in their sensitivity to external Ba$^{2+}$.

In particular, high concentrations of Ba$^{2+}$ were hypothesized to selectively abolish $I_{K,L}$, whereas the slow currents seen above −50 mV in high Ba$^{2+}$ were attributed to $I_{DR,I}$. Our data cannot be explained in this way. According to the two-current interpretation, the current evident in 30 mM Ba$^{2+}$ should also be present in the control activation curve. This should have resulted in the control curve continu-
ing to increase for depolarizations above which it actually reached a plateau. The discrepancy was present in the particular cell (Fig. 12C) or in six other cells studied with 30 mM Ba²⁺. Our results are consistent with Ba²⁺ shifting the activation curve of \( I_{K,L} \) as well as reducing \( g_{\text{MAX}} \) (Fig. 12, A and B). An implication of this interpretation is that \( I_{K,L} \) is the dominant outwardly rectifying current in our type I hair cells.

Both the voltage shift and the \( g_{\text{MAX}} \) reduction with Ba²⁺ were dose dependent. Activation curves were obtained in separate cells at 3, 10, and 30 mM. Mean values of \( \Delta V_{1/2} \) were \(-2.3 \pm 1.1 \) (SE) mV (3 mM, \( n = 7 \)), \( 5.8 \pm 1.4 \) mV (10 mM, \( n = 8 \)), and \( 20.3 \pm 2.4 \) mV (30 mM, \( n = 7 \)). Corresponding values for \( g_{\text{MAX}} \), stated as a percentage of the control, were \( 84 \pm 7\% \) (3 mM), \( 61 \pm 8\% \) (10 mM), and \( 43 \pm 7\% \) (30 mM). Application of 30 mM external Ca²⁺ was studied in two hair cells and gave similar results, shifting \( V_{1/2} \) by 18 and 32 mV. The observations with Ca²⁺ suggest that the shift in activation reflects the concentration of divalent ions and is not a specific action of Ba²⁺. A mechanism based on a screening of surface charges (Frankenhaeuser and Hodgkin 1957; Hille 1992) might be suggested. One prediction from this model is that there should be an identical shift in the voltage dependency of steady-state activation (Fig. 12C) and activation kinetics (Fig. 12D). The shift in activation kinetics falls short of expectations. Similarly, the reduction in \( g_{\text{MAX}} \) cannot be explained by charge screening and may be a specific action of Ba²⁺.

**DISCUSSION**

We have surveyed the basolateral currents found in the turtle posterior crista. This was considered an important preliminary step in the use of this organ to study regional variations in transduction mechanisms. There are four obvious advantages to this vestibular organ. First, it contains both type I and type II cells (Brichta and Peterson 1994; Jørgensen 1974; Lysakovski 1996). Second, type I hair cells are restricted to a central zone, whereas in mammals they are distributed throughout the neuroepithelium, which suggests that type I cells may have a more distinctive and possibly a more discernible function in turtles. Third, transduction appears virtually unchanged between the intact animal and the isolated ear (Brichta and Goldberg 2000a). Fourth we have reasonably complete regional maps of the afferent innervation patterns of the organ (Brichta and Peterson 1994), of its afferent discharge properties (Brichta and Goldberg 2000a), and of the effects on afferents resulting from electrical stimulation of efferents (Brichta and Goldberg 2000b). The question arises whether the electrophysiology of type I and II hair cells resembles that seen in other species. We begin with a consideration of type I cells and then consider type II cells.

**Is \( I_{K,L} \) only found in type I hair cells?**

As was first described by Correia and Lang (1990) in pigeon cristae and subsequently confirmed in vestibular organs from mammals (Behrend et al. 1997; Chen and Eatock 2000; Rennie and Correia 1994; Rüschi and Eatock 1996) and birds (Masetto et al. 2000; Rennie and Correia 1994), type I hair cells have a distinctive outwardly rectifying \( I_{K,L} \) current. Among its other distinguishing features, \( I_{K,L} \) is a large current with slow sigmoidal activation and deactivation kinetics and a relatively hyperpolarized activation range. Unlike many other outward \( K^+ \) currents, it is not blocked by internal \( Cs^+ \). A current with these properties was seen in >98% of our hair cells morphologically classified as type I. The classification was based on the presence of a constricted neck below the cuticular plate.
Others have used the ratio of the width of the hair-cell neck to that of the cuticular plate as an objective measure of this feature (Kevetter et al. 1994). Although we used a more subjective scoring method, we ascertained that the two measures were highly correlated and led to similar conclusions.

In >90% of the type I cells, \( I_{K,\text{L}} \) was already activated at our holding potential, \( V_H = -67 \text{ mV} \). The remaining type I cells had to be depolarized beyond \( V_H \) to activate \( I_{K,\text{L}} \), but even in these cases, the current was activated at more hyperpolarized potentials than required to activate outwardly rectifying currents in type II cells and could be identified by its distinctive electrophysiology and pharmacology. \( I_{K,\text{L}} \) was also present in about one-third of central hair cells that were classified as type II by the absence of a constricted neck. A possible explanation for the presence of \( I_{K,\text{L}} \) in presumed type II hair cells is that they were, in fact, type I hair cells. To investigate this possibility, we compared the shapes of the two kinds of hair cells in an intact crista, where type I hair cells could be unambiguously identified by their being enclosed in calyx endings. We were impressed that turtle type I cells had less distinctive shapes than did comparable hair cells in mammals (Kevetter et al. 1994; Lysakowski and Goldberg 1997) or in birds (Lysakowski 1996). In fact, some turtle type I cells in the intact crista lacked a constricted neck and, based solely on their shape, would have been left unclassified or would have been classified as type II.

Another explanation was also considered. \( I_{K,\text{L}} \) observed in cells without constricted necks might be an extreme form of the large, slow current observed in a substantial fraction of central type II cells. This explanation is considered unlikely as the slow type II current can be readily distinguished from \( I_{K,\text{L}} \) by its activation parameters (Table 4) and by the mechanism of its block by 4-AP. \( I_{K,\text{L}} \) was blocked in a reverse-use dependent manner at 10-μm concentrations, whereas the block of the slow type II current required higher concentrations and appeared state-independent.

**Are there ionic currents other than \( I_{K,\text{L}} \) in type I hair cells?**

It has been suggested that in mammals there were two slowly activating currents in type I hair cells (Eatock et al. 1998; Rüssch and Eaton 1996; Rüssch et al. 1998). \( I_{K,\text{L}} \) was activated at very hyperpolarized potentials and was blocked by high concentrations of external Ba2+. The other delayed rectifier (\( I_{\text{DR-L}} \)) activated at more typical membrane potentials and was unaffected by external Ba2+. Our results suggest a different mechanism for the apparent block of \( I_{K,\text{L}} \), viz., that high concentrations of Ba2+ result in a depolarizing shift in the activation range of \( I_{K,\text{L}} \) by neutralizing fixed charges on the external face of the membrane (Frankenhaeuser and Hodgkin 1957; Hille 1992).

In turtle type I hair cells, there is little evidence for a second delayed rectifier with slow activation kinetics. A small fraction of our type I cells were unusual: rather than having \( I_{K,\text{L}} \), they had fast outward currents resembling those seen in type II hair cells. Fast currents might be present in other type I hair cells but masked by the presence of a large \( I_{K,\text{L}} \). This possibility is suggested not only by the unusual cells but also by the observation of small, fast, partially inactivating currents after \( I_{K,\text{L}} \) was blocked by Cd2+. Similar currents were seen after \( I_{K,\text{L}} \) was blocked by internal tetraethylammonium (TEA) ions (Rennie and Correia 1994).

The presence of inward rectifiers may also be masked by \( I_{K,\text{L}} \). Once again, the use of blockers clarified the situation. At least two inward rectifiers can be distinguished in hair cells by their activation kinetics, a rapidly monoexponentially activating \( I_{K,\text{L}} \) and a slower sigmoidally activating \( I_K \) (Eatock et al. 1998; Holt and Eaton 1995; Weng and Correia 1999). After \( I_{K,\text{L}} \) was blocked, both kinds of inward rectifiers were seen but were small compared with the comparable currents found in many type II hair cells.

**Comparison of \( I_{K,\text{L}} \) in turtles and other species**

\( I_{K,\text{L}} \) has been studied in mammals (Chen and Eaton 2000; Rennie and Correa 1994; Rüssch and Eaton 1996), in pigeons (Masetto and Correia 1997; Rennie and Correa 1994, 2000), and in embryonic chicks (Masetto et al. 2000). In its slow activation kinetics, large size, lack of fast inactivation, and hyperpolarized activation range, the current in type I hair cells from the turtle posterior crista resembles that seen in other species. Results are similar in enzymatically dissociated cells (Chen and Eaton 2000; Rennie and Correa 1994; the present study), in slice preparations (Masetto and Correa 1997; Masetto et al. 2000), in intact organs (Rüssch and Eaton 1996), and when recordings are made with ruptured (Chen and Eaton 2000; Masetto and Correa 1997; Masetto et al. 2000; Rüssch and Eaton 1996) or perforated patches (Hurley and Eaton 1999; Rennie and Correa 1994). One difference concerns activation range. In turtle type I hair cells, on average, the current is half-activated between −55 to −60 mV, whereas this occurs more negative than −70 mV in other preparations. Concerning the difference in activation ranges between the turtle and other species, two comments are appropriate. First, the activation range of \( I_{K,\text{L}} \) can be labile. In particular, the range is observed to shift in a depolarizing direction when type I hair cells are held for several minutes during ruptured-patch, whole cell recordings (Chen and Eaton 2000; Hurley and Eaton 1999; the present study). Second, Rüssch and Eaton (1996) report that the activation range shifts in a depolarizing direction when temperature is elevated in the mammalian utricle so that the difference in \( V_{1/2} \) almost disappears when type I hair cells in turtles and mouse pups are compared at their respective normal body temperatures.

The pharmacology of \( I_{K,\text{L}} \) is also similar across species. External 4-AP blocks the current (Chen and Eaton 2000; Rennie and Correa 1994; Rüssch and Eaton 1996). As confirmed in the present study, the effective concentration is in the micromolar range. Externally applied Ba2+ affects the current in the millimolar range although the present study suggests that some of the block may be nonspecific (Rennie and Correa 1994; Rüssch and Eaton 1996). Other divalent cations, Ni2+ (Rüssch and Eaton 1996) or Cd2+ (Griguer et al. 1993b; Rennie and Correia 1994, the present study), may be more effective blockers. We found that \( I_{K,\text{L}} \) can be completely blocked by external Cd2+ in millimolar concentrations. An unusual property of the current, which we verified, is that it is not blocked by internal Cs+ (Chen and Eaton 2000; Griguer et al. 1993b; Rennie and Correa 1994, 2000; Rüssch and Eaton 1996).

We can summarize the situation for type I hair cells from the turtle posterior crista as follows. There is no convincing evidence for a second slow outward rectifier. Outward and inward currents, revealed after \( I_{K,\text{L}} \) is blocked, are of small size. These
observations emphasize that the electrophysiology of type I cells is dominated by $I_{K_{CL}}$. In both its electrophysiology and pharmacology, with the possible exception of its $V_{1/2}$, the current in turtle type I cells is similar to that seen in mammals and birds.

**Type II hair cells in the PZ**

As was mentioned in the introduction, bouton afferents located near the planum have a more regular discharge and considerably lower rotational gains and phases than those located near the torus (Brichta and Goldberg 2000a). A focus of our studies concerned the possibility that the large differences in afferent discharge properties could be related to the properties of basolateral currents in the corresponding hair cells. The suggestion receives little support from the present study. We concentrate on cells with fast activation kinetics because there were relatively few cells with intermediate or slow kinetics harvested from the PZ, suggesting that the latter cells might have been strays from the CZ.

Fast cells near the torus and near the planum have similar activation kinetics (Table 2). Torus cells have, on average, a slightly smaller fast inactivation coupled with maximal outward conductances that are two- to threefold smaller than those measured in planum cells. Conductance may be viewed as the reciprocal of hair-cell gain (Goldberg and Brichta 2002). So the difference in conductances is in the correct direction to account for the larger gains of torus afferents. But there are three caveats concerning the importance of the observed conductance differences. First, the difference in afferent gains is 200 times, not the 2–3 times predicted from hair-cell conductances.

There is one other difference between torus and planum hair cells in the turtle crista. $I_{K1}$, an inwardly rectifying current present in most type II hair cells, is approximately twice as large in torus as in planum cells. Furthermore, a much larger fraction of planum cells also have an $I_{K'}$. Based on studies in the frog sacculus (Holt and Eatock 1995), one might expect that these differences would result in planum cells having more depolarized resting potentials than torus cells. This expectation, although reasonable, was not confirmed by the statistics of Table 2 or when cells were considered on an individual basis (data not shown).

**Type II hair cells in the CZ**

Type II hair cells in the CZ show much more variability than those harvested from the PZ in the turtle crista. Not only do the CZ cells vary in their activation kinetics but also in their fast inactivation and in the maximal size of their outward conductances. Moreover, the three variables are correlated. Cells with rapidly activating $K^{+}$ currents show fast inactivation and small outward conductances. Slowly activating currents are of large size and do not inactivate during 200-ms voltage clamps.

Comparison of our type II cells with other vestibular organs

Basolateral currents have been characterized in type II hair cells from a variety of vestibular organs, including the horizontal crista of the toadfish (Steinacker et al. 1997), various cristae of frogs (Marcotti et al. 1999a; Masetto et al. 1994; Norris et al. 1992; Prigioni et al. 1996; Russo et al. 1995, 1996), and vestibular organs of mammals (Eatock et al. 1998; Griguer et al. 1993a; Holt et al. 1999; Lennan et al. 1999; Rennie and Ashmore 1991; Rennie et al. 2001) and birds (Lang and Correia 1989; Masetto and Correia 1997; Masetto et al. 2000; Weng and Correa 1999). Many of these studies are similar to ours in using solitary cells isolated by enzymatic dissociation (Griguer et al. 1993a; Holt and Correia 1989; Lennan et al. 1999; Rennie and Ashmore 1991; Rennie et al. 2001). This raises a question because it has been shown that enzyme treatment can alter basolateral currents (Armstrong and Roberts 1998). Studies of solitary cells can be compared with those in which enzymes have been avoided by the use of slice (Marcotti et al. 1999a; Masetto and Correia 1997; Masetto et al. 1994, 2000; Russo et al. 1995, 1996; Weng and Correa 1999) or epithelial preparations (Eatock et al. 1998; Holt et al. 1999; Rüschn et al. 1998). Such comparisons indicate that the use of enzymes does not have a marked influence on the properties of outwardly or inwardly rectifying currents of type II hair cells, including maximal conductances, activation ranges and kinetics. Moreover, as reviewed in a previous section, enzymatic dissociation did not greatly alter the electrophysiology of type I hair cells.
Most outward currents described in type II hair cells of other preparations resemble those of our so-called fast cells in having relatively fast activation kinetics and small maximal conductances. In most type II cells from these other preparations, as in our fast cells, outward currents can be divided into transient and sustained components. Because the transient component is blocked by 4-AP in the millimolar range (Lang and Correia 1989; Masetto et al. 1994; Russo et al. 1995), it has been identified as an A $I_{K_{A}}$ and $I_{K_{C}}$ components of $I_{K_{V}}$. In frogs, a component of $I_{K_{V}}$ has slow activation kinetics but is insensitive to 4-AP (Masetto et al. 1994; Prigioni et al. 1996). In contrast, our slow type II current was blocked in a state-independent manner by external 4-AP at concentrations near 100 mM. But even the currents in pigeon slow cells are too fast to resemble our slow type II currents. There are quite slow outward currents in chick embryos (Masetto et al. 2000), but their absence in adult pigeons (Weng and Correia 1999) suggests that the corresponding channels disappear during development or that activation kinetics become faster as the channels mature. None of the currents described in mammalian type II cells, including the sustained delayed rectifier in type II hair cells, have very slow kinetics (Eatock et al. 1998; Griguier et al. 1993a; Holt et al. 1999; Lennan et al. 1999; Rüsch et al. 1998). These remarks suggest, but hardly prove, that our slow type II current has not been previously described.

Relation of voltage-dependent currents to afferent discharge

The present study shows that basolateral currents differ depending on the type and neuroepithelial location of the hair cells in the turtle posterior crista. To what extent are the currents related to the discharge properties of afferents? We suggest that the relatively low rotational gains of calyx-bearing afferents might reflect the large whole cell conductances of type I and slow type II hair cells from the CZ. At the same time, the differences in basolateral currents of type II cells harvested near the torus and near the planum would seem too small to account for the very large differences in discharge of fibers innervating the two ends of the peripheral zone.

A limitation of the present study is that the voltage-clamp protocols we used are not well matched with the rotational stimuli used to characterize afferent discharge (Brichta and Goldberg 2000a). In the next paper (Goldberg and Brichta 2002), we study the voltage responses of hair cells to sinusoidal currents similar in frequency to the sinusoidal currents used in our afferent studies. In addition, the sinusoidal currents are superimposed on a background current intended to mimic a resting transducer current. Although we still conclude that basolateral currents are not responsible for regional differences in the discharge properties of bouton afferents, the new conditions reveal that the electrophysiological properties of some type II hair cells can be dramatically altered under these presumably more physiological stimulating conditions.

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