Heterogeneous Voltage Dependence of Inward Rectifier Currents in Spiral Ganglion Neurons

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Mo, Zun-Li and Robin L. Davis. Heterogeneous voltage dependence of inward rectifier currents in spiral ganglion neurons. J. Neurophysiol. 78: 3019–3027, 1997. Inward rectification was characterized in neonatal spiral ganglion neurons maintained in tissue culture. Whole cell current and voltage-clamp techniques were used to show that the hyperpolarization-activated cationic ($I_h$) current underlies most or all of the inward rectification demonstrated in these neurons. The average reversal potential ($-41.3 \text{ mV}$) and cesium sensitivity were typical of that found in other neurons and cell types. What was unique about the hyperpolarization-activated currents, however, was that the half-maximal voltages ($V_{1/2}$) and slope factors ($k$) that characterized $I_h$ current activation were graded from neuron to neuron. Voltage-clamp recordings made with standard bath and pipette solutions revealed $V_{1/2}$ values that ranged from $-78.1$ to $-122.1 \text{ mV}$, with slope factors from 7.6 to 13.1. These gradations in the voltage-dependent features of the $I_h$ current did not result from variability in the recording conditions because independently measured Na$^+$ current-to-voltage relationships were found to be uniform (peak current at $-20 \text{ mV}$). Moreover, the range and average $V_{1/2}$ and slope values could be altered with activators [8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate in combination with okadaic acid] or inhibitors [N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinoline sulfonamide] of protein indicating that $I_h$ current heterogeneity most likely resulted from differential phosphorylation.

INTRODUCTION
Precision in the auditory system is critical to coding stimulus parameters such as frequency, intensity, and direction, where the number and timing of incoming action potentials must be preserved at higher neuronal levels. It is likely, therefore, that auditory cell types possess specializations that ensure accurate transmission. For example, neurons in the cochlear nucleus (CN), medial nucleus of the trapezoid body (MNTB), and medial geniculate body (MGB) possess distinctive voltage-dependent ion channels that produce neuronal firing properties that complement synaptic transmission (Banks et al. 1993; Hu 1995; Manis and Marx 1991; Manis et al. 1993, 1994; Oertel et al. 1988). One channel type that is prevalent in specific populations of cells that compose part of the sound localization pathway (bushy cells of the anteroventral CN, and principal cells in the MNTB), is the hyperpolarization-activated ($I_h$) cationic channel (Banks et al. 1993; Oertel 1983). Because the $I_h$ current is found in neurons that must follow input at high rates, its elementary features may, in combination with other current types, contribute to their temporal fidelity.

Originally identified in heart cells (Brown and DiFrancesco 1980; DiFrancesco 1984; Yanagihara and Irisawa 1980), and subsequently in central and peripheral neurons (Crepel and Penit-Soria 1986; Fletcher and Chiappinelli 1992; Halliwell and Adams 1982; Mayer and Westbrook 1983; Spain et al. 1987), the $I_h$ inward rectifier is characterized by its unique combination of voltage dependence, positive reversal potential, slow time course, second-messenger modulation, and pharmacological profile (Banks et al. 1993; Hagiwara and Irisawa 1989; Li et al. 1993; McCormick and Pape 1990a; Pape and McCormick 1989; Schwindt et al. 1992; Wollmuth and Hille 1992). Because the reversal potential of the $I_h$ current is more positive than the typical resting potential, these ion currents are important regulators of firing patterns (Destexhe et al. 1993a,b; Erickson et al. 1993; McCormick and Huguenard 1992; McCormick and Pape 1990a; Pape and McCormick 1989; Soltész et al. 1991) by shifting the resting voltage to more depolarized levels. Second-messenger modulation via adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) activated by neurotransmitter receptors has been shown to alter the voltage dependence of $I_h$ current activation (Banks et al. 1993; Li et al. 1993; McCormick and Pape 1990b; Pape and McCormick 1989). In thalamocortical neurons, a shift of steady-state $I_h$ current activation by $\sim 5 \text{ mV}$ drastically alters firing from single action potentials to bursts of activity (McCormick and Pape 1990a). Furthermore, at the termination of transient hyperpolarizing voltages, $I_h$ current activation can produce a rebound depolarization that reaches firing threshold (Mayer and Westbrook 1983; Solomon and Nerbonne 1993). Thus the resulting rebound action potentials could potentially enhance firing rate following inhibitory input.

In our previous analysis of spiral ganglion endogenous firing patterns (Mo and Davis 1997), we observed inward rectification that was suggestive of the $I_h$ current, yet showed substantial cell-to-cell gradation. In the spiral ganglion, this variation in current magnitude appeared to result from voltage dependence of current activation rather than simple differences in magnitude, indicating that spiral ganglion neurons possessed inwardly rectifying current types with heterogeneous characteristics.

Differential channel distribution is a feature that has previously been identified in the inner ear sensory epithelia (Fuchs 1996; Navaratnam et al. 1995) and primary-auditory neurons (Davis 1996). The density of the inward rectifier current carried by K$^+$ ions, the $I_K$ current, was localized solely to the low-frequency apical region of the chick cochlea (Navaratnam et al. 1995), whereas the Ca$^{2+}$-activated K$^+$ current [$I_{K(Ca)}$] is found predominantly in receptor hair cells of the basal cochlea (Fuchs 1992) and in the caudal cells of goldfish auditory nerve (Davis 1996). Furthermore,
temperature-dependent changes in the voltage dependence of a delayed rectifier current type was also found in vestibular hair cells (Rüsch and Eaton 1996). Electrophysiological studies have also shown that, in addition to characteristic frequency, type I spiral ganglion neurons do not all display identical firing properties in vivo (Kiang et al. 1965; Liberman 1978; Schmiedt 1989; Winter et al. 1990). Some of these characteristics could result from nonneuronal sources, such as mechanical nonlinearities, or from synaptic regulation of neuronal firing (Evans 1992; Javel 1986; Kiang 1990; Kiang et al. 1986; Ruggiero 1992), yet others could result from endogenous electrical properties of the neurons themselves (Davis 1996; Mo and Davis 1997).

Our observations of graded electrophysiological characteristics in spiral ganglion neurons may reveal an important aspect of spiral ganglion cellular physiology that could contribute to specific aspects of signal coding. A definitive test of this hypothesis, however, requires a better understanding of the underlying regulatory mechanisms. The results reported herein, based on whole cell patch-clamp recordings from cultured neurons, confirm that the hyperpolarization-activated current, the I_h current, is the predominant current type that underlies the inward rectification in spiral ganglion neurons. Furthermore, we demonstrate directly that voltage dependence of I_h activation accounts for variations in the inward rectification among the spiral ganglion neurons. By selecting cells from limited regions of the cochlea, we determined that this feature was not related to specific characteristic frequencies. Moreover, the heterogeneity of steady-state current activation was modified by substances that alter PKA-mediated second-messenger pathways, suggesting that it is a dynamic property that could be altered by extrinsic influences such as efferent neurotransmitters (Altschuler and Fex 1986; Eybalin 1993).

**METHODS**

**Tissue culture**

Experiments were performed on murine spiral ganglion neurons. The procedures involving animals in this study have been approved by The Rutgers University Institutional Review Board for the Use and Care of Animals (IRB-UCA), protocol 90-073 and 92-062. Neonatal animals were decapitated, and both inner ears were removed from the base of the cranium for further dissociation. Cochlea were extracted from the outer bony labyrinth, the outer ligament/stria vascularis, and organ of Corti were dissected away from the central core of the cochlea that contained the spiral ganglion. Sections of the nerve were plated as small explants in culture dishes coated with poly-L-lysine without the use of enzymes. Cells were maintained in growth medium: Dulbecco’s modified Eagle’s medium (DMEM; Sigma, St. Louis, MO; D–6717), supplemented with 10% fetal bovine serum (Sigma, F-2442), 4 mM L-glutamine (Sigma, G-6392), and 0.1% penicillin-streptomycin (Sigma, P–0781). Neurotrophins (brain-derived neurotrophic factor or neurotrophin-4, each at 5 ng/ml) were added to most cultures for enhanced neuronal survival; however, they did not alter the magnitude or voltage dependence of inward rectification. Under these conditions neurons were maintained for up to 25 days at 37°C in a humidified incubator with CO_2 levels adjusted to 5%.

**Electrophysiology**

The whole cell configuration of the patch-clamp technique was used to obtain current- and voltage-clamp recordings from spiral ganglion neurons in vitro (Hamill et al. 1981). Whole cell, rather than perforated patch-clamp recording, was utilized to obtain optimal electrical access to the cell soma. Electrodes were pulled on a two-stage vertical puller (Narishige, PP-83), and the shafts were coated with silicone elastomer (Sylgard; Dow Corning) to reduce the pipette capacitance. Just before use, electrode tips were fire-polished (Narishige MF-83 microforge); electrode resistances typically range from 2 to 5 MΩ in standard pipette and bathing solutions (see below). Pipette offset current was zeroed immediately before contacting the cell membrane; current-clamp measurements were made exclusively with low-resistance electrodes to avoid the need for bridge-balance compensation.

A standard set of solutions was used to approximate physiological conditions. The basic internal solution was (in mM) 112 KCl, 2 MgCl_2, 0.1 CaCl_2, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.45. On the day of the recording, 2 mM NaATP was added to the stock solution, and the pH was readjusted to 7.45. Neurons were exposed to the following bath solution (in mM): 137 NaCl, 5 KCl, 1.7 CaCl_2, 1 MgCl_2, 17 glucose, 50 sucrose, 10 HEPES, pH 7.45 neuron recording solution (NRS) (Davis 1996). The osmolarity of the solution was adjusted with sucrose to 350 mosM to match the osmolarity of the growth medium. For selected experiments, rapid solution changes were achieved with a micropipet perfusion system (Ogata and Tatebayashi 1991).

To determine the role of cAMP-dependent phosphorylation on voltage-dependent parameters of the I_h current, we examined the effects of the membrane-permeable cAMP analogue 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (8-CPT-cAMP; Sigma, St. Louis, MI) and the protein kinase A inhibitor N-[2-(p-bromocinnamylamino)ethyl]-5-isouquinolinesulfonamide (H-89; Calbiochem, La Jolla, CA). Unless otherwise indicated, 8-CPT-cAMP and H-89 were added to both the bath and pipette solutions. Okadaic acid (OA; Calbiochem) was used in conjunction with 8-CPT-cAMP and was added to the pipette solution only. H-89 was dissolved in dimethyl sulfoxide and diluted 1:10,000 in recording solution for both preincubation and recording.

Because our cultures contained neurons as well as satellite cells, we only evaluated cells that possessed tetrodotoxin (TTX) sensitive, rapidly activating Na_+ currents in voltage clamp and action potentials in current clamp. Voltage-clamp recordings of slow non-regenerative currents could be made from neurons that possessed neurites (McCormick and Pape 1990a,b) despite the unknown nature of the extent of neuronal membrane under precise voltage control. Empirical evaluations of the magnitude and voltage dependence of I_h current activation showed unequivocally that measurements were smooth functions of membrane potential. Compensated series resistance was typically below 7 MΩ. Uncompensated series resistance did not vary systematically with I_h current parameters (see below). Recordings were made at room temperature (19–22°C) using an Axopatch 200 (Axon Instruments) patch-clamp amplifier. Data were digitized with an Indec IDA 15125 interface in an IBM-compatible personal computer; the programs for data acquisition and analysis were written in Borland C++ and Microsoft Visual Basic (generously contributed by Dr. Mark R. Plummer). Unless otherwise indicated, each segment of data was digitized at 5 kHz and filtered at 1 kHz.

**Data analysis**

Current-clamp recordings were considered acceptable when they met the following criteria: stable holding potentials, low noise levels, discernible membrane time constant on step current injection, and overshooting action potentials (magnitudes of at least 70
nV). If any of these parameters changed during an experiment, the remaining data were not analyzed.

Because our experiments were designed to quantify the heterogeneity of inward rectifier currents in spiral ganglion neurons, we took a number of precautions to eliminate variability arising from recording and measurement errors. In addition to using solutions prepared in the same manner for the entire series of experiments, we also sought to establish uniformity by examining different parameters of our measurements. To establish the graded nature of the currents and to control for variability in recording conditions, we routinely measured the current-to-voltage relationship for the TTX-sensitive Na⁺ current (as in Fig. 3D), and only used recordings that showed a peak at −20 mV. This ruled out variations in Iₙ activation arising from subtle differences in recording solutions, leak currents, or series resistance.

We also evaluated the correlation between the current-to-voltage relationship and the tail currents. Iₙ activation data (from tail currents) were fitted with the Boltzmann relationship, \( P = P_{\text{max}}/[1 + \exp((V - V_{1/2})/k)] \), estimated with a least-squares fit. The \( V_{1/2} \) (half-maximal voltage) and \( k \) (slope factor) values calculated for a particular recording were used in the following equation to fit the current-to-voltage relationship: \( I_{\text{trans}} = G(V - E_{\text{rev}})/[1 + \exp((V - V_{1/2})/k)] \), where \( G \) is conductance and \( E_{\text{rev}} \) is reversal potential.

Therefore the equation utilized to fit the current-to-voltage relationship of the \( I_{\text{h}} \) current incorporated the slope factor and half-activation voltage calculated from fitting the activation data obtained from tail currents. All recordings that showed stable Na⁺ current-to-voltage relationships were satisfactory according to this criterion.

We also only accepted experiments in which the activation parameters of the slow \( I_{\text{h}} \) current remained unchanged during a recording. Although changes in current magnitude sometimes occurred, the \( V_{1/2} \) remained constant throughout most experiments. If the voltage dependence of steady-state activation changed, however, it was eliminated from the data set. Furthermore, there was no correlation between the \( V_{1/2} \) or \( k \) levels to the recording sequence.

**RESULTS**

Recordings were made from a total of 157 spiral ganglion neurons that demonstrated rapidly activating Na⁺ current, of which 81 were analyzed in detail. Evidence that inward rectification differs from cell to cell was noted in current-clamp recordings from neurons maintained at different holding potentials. By examining the inward rectification at more than a single holding potential, these differences appeared to be attributable to variation in the voltage dependence of the underlying current or currents rather than due solely to differences in current magnitude. For example, a comparison of two neurons at a holding potential of −80 mV shows a moderate amount of inward rectification in one cell (Fig. 1B, bottom traces) and little or no inward rectification in the other (Fig. 1A, bottom traces). This was quantified using hyperpolarizing constant-current injections and measuring the difference between the onset (filled triangle) and offset (filled diamond) of the voltage response (Fig. 1, C and D, right panels). The cell shown in Fig. 1B displayed significant divergence of the peak and steady-state levels, whereas the cell shown in A did not.

If these cells were examined solely at a −80 mV holding potential, one might erroneously conclude that only one possessed inward rectification. However, current injections delivered from a holding potential of −60 mV revealed that inward rectification was actually present in both cells (Fig. 1, A and B, top panels). Furthermore, the difference between the peak and steady-state voltage levels was approximately the same for the two cells (Fig. 1, C and D, left panels). Because inward rectifier conductance increases with hyperpolarization, the apparent lack of inward rectification at −80 mV for the cell in Fig. 1A most likely represents a voltage at which the channels were fully activated, thus resulting in an absence of the time-dependent changes in voltage. In contrast, the voltage dependence of the cell illustrated in Fig. 1B must be shifted negative relative to that shown in Fig. 1A.

Hyperpolarization-activated current-clamp and voltage-clamp recordings obtained from a single spiral ganglion neuron are shown in Fig. 2A and C, respectively. These recordings were unaffected by 1 mM barium, which ruled out a substantial contribution from the rapidly activating K⁺ inward rectifier current (\( I_{\text{wo}} \)). Application of 5 mM cesium, however, completely blocked the slowly activating current (Fig. 2, B and D), thus demonstrating a pharmacological profile characteristic of the \( I_{\text{h}} \) current (Banks et al. 1993; Bayliss et al. 1994; Crepel and Penit-Soria 1986; Mayer and Westbrook 1983; McCormick and Pape 1990a; Spain et al. 1987; Wollmuth and Hille 1992). Consistent with this, the subtracted current in Fig. 3A shows the expected slow time course of activation and deactivation typical of the \( I_{\text{h}} \) current. From all of the recordings of this type, application of barium (1–5 mM) either had no action or caused effects so small that they could not easily be discriminated from changes that may occur over time in prolonged recordings. Therefore we concluded that there was little or no contribution of \( I_{\text{wo}} \) to the hyperpolarization-activated currents of spiral ganglion neurons, providing evidence against the hypothesis that heterogeneous inward rectification results from graded combinations of multiple ionic channel types.

Steady-state activation of the \( I_{\text{h}} \) current was determined from analysis of the slow tail currents at −60 mV that resulted from graded hyperpolarizing voltage steps to −160 mV (Fig. 3B). Measurements were made after the capacity transient settled and were normalized to maximum and minimum current levels and fitted with the Boltzmann equation to determine the half-maximal voltage (\( V_{1/2} \)) and slope factor (\( k \)). Values obtained from recordings made in NRS (control trace; filled diamonds) and those obtained from cesium-subtracted traces (shaded circles) showed similar half-maximal voltages (−96.1 and −97.1 mV, respectively) and slope factors (10.4 and 10.3, respectively).

Amplitudes of the \( I_{\text{h}} \) current from recordings in NRS were obtained by measuring the difference between the initial level after the settling of the uncompensated capacity current and the steady-state level at the termination of the voltage excursion (see Fig. 3A, 1st series of superimposed traces). The cesium-subtracted traces, measured from baseline to maximum amplitude, were almost identical to the control traces (Fig. 3C). The Na⁺ current-to-voltage relationship measured from this and subsequent recordings used to establish the heterogeneity under standard recording conditions displayed a peak current at −20 mV (Fig. 3D).

As expected from our current-clamp recordings (Fig. 1), the \( I_{\text{h}} \) currents measured with voltage-clamp recordings showed heterogeneity in the voltage dependence of activation. Half-maximal voltages and slope factors calculated

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**REFERENCES**

Bayliss et al. 1994; Crepel and Penit-Soria 1986; Mayer and Westbrook 1983; McCormick and Pape 1990a; Spain et al. 1987; Wollmuth and Hille 1992.
from Boltzmann functions fitted to tail current data ranged from $-83.3$ to $-109.3$ mV, and 7.6 to 13.1, respectively (Fig. 4C). As can be seen from two examples shown in Fig. 4A, the data from different experiments were well fitted despite having divergent $V_{1/2}$ values (filled triangles: $-92.4$ mV; filled diamonds: $-109.3$ mV). Furthermore, the dissimilar voltage dependence was also reflected in the current-to-voltage relationships for the same two recordings (Fig. 4B). Normalized current-to-voltage relationships for the full data set also displayed the expected shift along the voltage axis for modest hyperpolarizations, although they all converged to a relatively uniform linear component at voltages corresponding to the maximum activation levels (Fig. 4D). Least-squares fits to these linear portions of the current-to-voltage relationships yielded a mean extrapolated reversal potential of $-41.3 \pm 4$ mV (mean $\pm$ SE; $n = 12$). This value corresponds well with the reversal potential reported for the $I_h$ current from thalamocortical projection neurons, principle cells of the MNTB, and hypoglossal motoneurons (Banks et al. 1993; Bayliss et al. 1994; McCormick and Pape 1990a), consistent with mixed K$^+$ and Na$^+$ current through the $I_h$ channels in spiral ganglion neurons, as demonstrated elsewhere (DiFrancesco 1984; Mayer and Westbrook 1983; McCormick and Pape 1990a; Solomon and Nérbonne 1993; Spain et al. 1987; Travagli and Gillis 1994; Wollmuth and Hille 1992).

To determine whether variations in animal age, days in culture, or cochlear location could account for the heterogeneity in steady-state $I_h$ current activation, multiple recordings were made on the same culture day from neurons isolated from a single animal at a restricted cochlear location. $I_h$ current heterogeneity was still observed in neurons from the apical third of a cochlea removed from an animal one postnatal day ($P1$) of age and maintained in culture for 13 days (Fig. 5). Tail current data were well fitted with the Boltzmann equation (Fig. 5A) revealing steady-state current activation $V_{1/2}$ and $k$ values that ranged from $-78.1$ to $-122.1$ mV and 8.6 to 12.1, respectively (Fig. 5C). Calculated $I_h$ current-to-voltage relationships were similarly shifted along the voltage axis (Fig. 5, B and D). Thus the variations in $I_h$ current cannot be accounted for by differences in the age of the animal, time in culture, or cochlear location.

These observations were consistent with the hypothesis that spiral ganglion neurons possess endogenous hyperpolarization-activated currents that show nonuniform voltage dependence. Yet, if this heterogeneity is a consequence of $I_h$ phosphorylation (Banks et al. 1993; Li et al. 1993; McCormick and Pape 1990b), then one might expect that manipulation of cAMP-dependent phosphorylation could reduce the variation in $I_h$ activation. To test this, we included membrane-permeable 8-CPT-cAMP in both the bath and pipette solutions and the phosphatase inhibitor OA in the pipette solution while evaluating $I_h$ voltage dependence. To ensure adequate delivery of the cAMP analogue, cells were incubated with 2 mM 8-CPT-cAMP for at least 1 h before recording. Recordings made under these conditions were compared with those from control neurons (as in Figs. 4 and 5).

The major effect of 8-CPT-cAMP and OA on the $I_h$ current was to reduce the range of the half-maximal voltages; this can be observed by comparing Fig. 6C with Figs. 4C and 5C. Tail current data were again well fitted by Boltzmann functions (Fig. 6A), and current-to-voltage relationships shifted along the voltage axis in correspondence with the $I_h$ activation curves (Fig. 6, B and D). The range of $V_{1/2}$ values obtained from the 11 neurons exposed to 8-CPT-cAMP and OA was only 7.5 mV. This indicated that the activation curves were clustered around their mean ($-95.6 \pm 0.9$; $n = 11$) compared with the large range of $V_{1/2}$ values observed from neurons exposed to standard recording solutions (Fig. 7E). Although the average $V_{1/2}$ levels were more positive when neurons were exposed to 8-CPT-cAMP and OA, the differences were not significant ($P > 0.6$; Fig. 7D), most likely owing, in part, to the wide ranges found in control experiments. The slope factors of the activation curves from
Furthermore, the slope factor of the activation curve declined significantly \( (11.0 \pm 0.4; n = 10) \) when compared with that obtained in 8-CPT-cAMP and OA (Fig. 7C; \( P < 0.05 \)).

**Discussion**

Inward rectification is prominent in auditory neurons at several processing levels. It has been observed in spiral ganglion neurons (Mo and Davis 1997) and in cells that compose the anteroventral CN (Oertel 1983), dorsal CN (Manis et al. 1994), MNTB (Banks et al. 1993), and the MGB (Hu 1995). Proposed functions for inward rectification in auditory neurons are augmented temporal acuity during heightened states of arousal (Banks et al. 1993) and enhancement of spontaneous activity and excitability (Hu 1995). Although neurons within the spiral ganglion possess endogenous voltage-dependent channel types that are similar to those described for brain stem auditory neurons (unpublished observations), they also possess inward rectifier currents that display heterogeneous voltage dependence of activation under baseline conditions.

Our pharmacological characterization revealed that inward rectification in spiral ganglion cells was primarily due to current through the \( I_h \) channel. This current has been found in restricted nuclei throughout the central and peripheral nervous systems (Hu 1995; Scroggs et al. 1994; Solomon et al. 1993; Travagli and Gillis 1994), which has heretofore been described as displaying a uniform \( V_{1/2} \) and slope factor for each class of neuron. Typical values were \(-75 \text{ mV} (k = 5.5)\) for thalamocortical relay neurons (McCormick and Pape 1990a), \(-75.7 \text{ mV} (k = 5.7)\) for MNTB cells (Banks et al. 1993), \(-79.8 \text{ mV} (k = 5.3)\) for hypoglossal motoneurons (Bayliss et al. 1994), \(-81.0 \text{ mV} (k = 7.2)\) for superior colliculus-projecting neurons (Solomon and Nernboine 1993), \(-81.1 \text{ mV} (k = 3.64 \text{ to } 4.33)\) for spinal sensory ganglion neurons (Mayer and Westbrook 1983), \(-82 \text{ nV} (k = 7)\) for sensorimotor cortical neurons (Spain et al. 1987), and \(-85.5 \text{ mV}\) for photoreceptors (Wollmuth and Hille 1992). The most negative reported \( V_{1/2} \) for the \( I_h \) current was that from hippocampal neurons \( (V_{1/2} \text{ approximately } -95 \text{ mV}) \) (Halliwell and Adams 1982). This level was close to the average \( V_{1/2} \) level in spiral ganglion neurons \( (-97 \text{ mV}) \). Using a single \( V_{1/2} \) value for the \( I_h \) current in spiral ganglion neurons is misleading, however, because our recordings showed that it could range from \(-78.1 \text{ to } -122.1 \text{ mV} \). Although the full range of \( V_{1/2} \) values was 44 mV, it is important to note that >95% of the neurons \( (41 \text{ of } 43) \) fell within a more restricted range of \(-78.1 \text{ to } -109.3 \text{ mV} \) \( \text{(range, } 31.2 \text{ mV}) \). This suggests that the two most negative \( V_{1/2} \) values may not be representative of the entire population of spiral ganglion neurons. In addition, the slope factors obtained from \( I_h \) activation curves from spiral ganglion neurons \( (7.6 \text{ to } 13.1) \) were greater than those observed in other neurons \( \text{(ranging from } 3.64 \text{ to } 7.2) \).

The relatively negative \( V_{1/2} \) values found in the present study could be due, in part, to the buffering of intracellular \( \text{Ca}^{2+} \) with EGTA in our pipette solution. Although changes in internal calcium concentration do not alter the slope of the \( I_h \) activation curve, low levels of internal calcium have been shown to shift the activation threshold of the \( I_h \) current to more negative voltages in sinoatrial node cells and neocor-
tical neurons (Hagiwara and Irisawa 1989; Schwindt et al. 1992). The uniformity of the solutions that we used, however, precludes their contribution to the heterogeneity of $I_h$ currents. Shifts in the $I_h$ activation curve can also be produced by monoamines and acetylcholine, acting through cAMP. These shifts are generally within the range of 5–10 mV for a variety of different cell types (McCormick and Pape 1990b; Tokimasa and Akasu 1990; Kiehn and Harris-Warrick 1992; Li et al. 1993). Much larger changes in $I_h$ activation have been found in a particular class of auditory neuron. Modulation of $I_h$ current via 8-Br-cAMP induced voltage shifts as great as 23 mV in MNTB neurons (Banks et al. 1993).

**FIG. 3.** The predominant inwardly rectifying current in spiral ganglion neurons is the $I_h$ current. A: voltage-clamp traces obtained under control conditions (left) and in the presence of 5 mM cesium (middle). Subtraction of the 2 sets of sweeps shows the characteristics of the cesium-sensitive current (right). B: tail current analysis was utilized to determine the voltage dependence of activation of the $I_h$ current. Measurements from control and subtracted traces were depicted with filled diamonds and shaded circles, respectively. Data were fitted with a Boltzmann relationship using a least-squares fit. C: current-to-voltage relationship obtained from the control (filled diamonds) and subtracted (shaded circles) currents. Measurements were taken as the difference between the instantaneous and peak current. D: current-to-voltage relationship of the tetrodotoxin-sensitive Na$^+$ current. Peak current amplitude occurred at ~20 mV.

**FIG. 4.** Analysis of whole cell voltage-clamp recordings from multiple neurons showed heterogeneous steady-state activation of the $I_h$ current. A: Boltzmann relationships fitted to tail current data obtained from 2 different neurons. The half-maximal voltages of the 2 cells differed by 17 mV (filled diamonds $V_{1/2} = -92.4$ mV, $k = 11.1$; filled triangles $V_{1/2} = -109.3$ mV, $k = 10.7$). B: normalized current-to-voltage relationships obtained from the same neurons shown in A. The smooth curve was fitted using $V_{1/2}$ and $k$ values calculated from the Boltzmann fits. The shapes and relative shifts in voltage are consistent with tail current analysis. C: Boltzmann relationships fitted to a total of 12 neurons to show the range of $V_{1/2}$ (from ~83.3 to ~109.3) and $k$ values (7.6 to 13.1). For clarity, individual data points are not shown. Cultured spiral ganglion neurons from P1 to P8 animals maintained in vitro from 4 to 21 days. For the data set shown, voltage dependence ($V_{1/2}$) and slope factor ($k$) of the steady-state activation of the $I_h$ current did not vary systematically with age, time in culture, or the combination of these 2 parameters. All 12 neurons showed peak Na$^+$ current at ~20 mV. D: normalized current-to-voltage relationships fitted as in C for all 12 neurons. For clarity, individual data points are not shown.

**FIG. 5.** Graded $I_h$ activation curves of neurons obtained from a single animal (P1) and limited to the apical cochlea. A: Boltzmann relationships fitted to tail current data obtained from 4 different neurons. The half-maximal voltages for the tail current data depicted with filled diamonds, squares, triangles, and circles were ~122.1, ~102.5, ~93.5, and ~79.9 mV, respectively. B: normalized current-to-voltage relationships obtained from the same neurons shown in A. The smooth curve was fitted using $V_{1/2}$ and $k$ values calculated from the Boltzmann fits. The shapes and relative shifts in voltage are consistent with tail current analysis. C: Boltzmann relationships fitted to a total of 11 recordings, including the 4 in A, showed a range of $V_{1/2}$ (~78.1 to ~122.1 mV) and $k$ values (7.9 to 12.1). For clarity, individual data points are not shown. D: normalized current-to-voltage relationships fitted as in C for all 11 neurons. For clarity, individual data points are not shown.
Heterogeneity in the voltage dependence of $I_h$ activation was dramatically reduced ($V_{1/2}$ range, 7.5 mV) after exposure to 8-CPT-cAMP and OA. Correspondingly, exposure to H-89 also diminished the $V_{1/2}$ variability. Moreover, treatment with 8-CPT-cAMP shifted the mean $V_{1/2}$ to a value significantly more positive than that seen with H-89 ($-95.6$ vs. $-103.6$ mV). These changes in $V_{1/2}$ were important for two reasons. First, they showed that second-messenger-mediated regulation of phosphorylation may underlie the graded voltage dependence of $I_h$ current activation in spiral ganglion cells. Second, it provided strong support for the validity of $I_h$ heterogeneity under control conditions. There is little reason to believe that artifactual voltage shifts would be affected by either 8-CPT-cAMP or H-89. Because recordings under those conditions produced more uniform $V_{1/2}$ values, the graded $V_{1/2}$ values seen under baseline conditions are likely to be genuine.

Experiments performed to eliminate variability in recording and growing conditions were achieved by examining multiple neurons isolated from a single animal and maintained in the same culture dish. We found that gradients in both voltage dependence and slope persisted. Additionally, in all of our recordings we found no correlation between age of the animal, time in culture, or a combination of these two parameters, with either $V_{1/2}$ or slope factor.

**FIG. 6.** Heterogeneity of the $I_h$ current was reduced and shifted to depolarized voltages when neurons were exposed to the membrane permeable adenosine 3',5'-cyclic monophosphate (cAMP) analogue 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (8-CPT-cAMP). Neurons were pre-treated with 2 mM 8-CPT-cAMP for at least 1 h before recording. A: Boltzmann relationships fitted to tail current data obtained from 2 different neurons. The half-maximal voltages for the tail current data depicted with filled diamonds and triangles were $-99.5$ and $-92.2$ mV, respectively. B: normalized current-to-voltage relationships obtained from the same neurons shown in A the smooth curve was fitted using $V_{1/2}$ and $k$ values calculated from the Boltzmann fits. The shapes and relative shifts in voltage are consistent with tail current analysis. C: Boltzmann relationships fitted to a total of 11 neurons to show the range of $V_{1/2}$ ($-92.1$ to $-99.5$ mV) and $k$ values (8.2 to 11.6). For clarity, individual data points are not shown. D: normalized current-to-voltage relationships fitted as in B for all 11 neurons. For clarity, individual data points are not shown. 8-CPT-cAMP was present in the both the bath and pipette solutions; 0.5 mM okadaic acid was also in the pipette solution.

The neurons we studied were isolated from their afferent and efferent connections, so there should be little or no transmitter present to activate second-messenger systems. We observed no evidence for synaptic transmission in any of the recordings presented herein; like their counterparts in vivo, spiral ganglion neurons do not appear to form synapses on one another in vitro. Furthermore, any neurotransmitter released extrasynaptically would most likely be washed away by our perfusion system. Therefore, in the absence of exogenous elements that could account for $I_h$ current heterogeneity, we instead suspect that spiral ganglion neurons possess endogenous regulatory mechanisms that establish distinct $I_h$ current activation set points on a neuron-by-neuron basis. Additional experiments are necessary to support this hypothesis by determining whether relative amounts of channel phosphorylation vary within the population of spiral ganglion neurons in vivo.

Spiral ganglion neurons are distributed systematically throughout the periphery according to the frequency that they code, with apical neurons coding the lowest and basal...
neurons coding the highest frequencies (Kiang 1984; Yost 1994). We were curious whether \( I_h \) activation parameters were similarly distributed. By examining neurons removed from discrete regions of the cochlea, we found that the full ranges of \( V_{1/2} \) and slope values were apparently independent of cochlear location. One caveat to this conclusion, however, is that cells maintained in culture may establish new sets of contacts once removed from cell-cell interactions in situ.

The question of how the heterogeneity in \( I_h \) activation contributes to signal coding in the auditory periphery will be important to answer. Graded inward rectification was noted in both of the two previously identified electrophysiological classes that compose the spiral ganglion (Mo and Davis 1997) and thus does not discriminate between rapidly and slowly adapting neurons. Variations in \( I_h \) were noted in nodose ganglion cells (Gallego and Eyzaguirre 1978; Görke and Pierau 1980), and, like the spiral ganglion neurons, the magnitude of the inward rectification was not associated with rapidly or slowly adapting categories of firing. Graded \( I_h \) activation parameters may instead be related to features such as efferent innervation (Altschuler and Fex 1986; Eybalin 1993) rate-intensity functions (Kiang et al. 1965; Winter et al. 1990) and/or spontaneous rate (Kiang et al. 1965; Liberman 1982; Kawase and Liberman 1992; Schmiedt 1989). Future experiments will be aimed at examining the functional significance of the gradations in steady-state half-activation voltage and slope of the \( I_h \) current in the spiral ganglion.

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