Major Potassium Conductance in Type I Hair Cells From Rat Semicircular Canals: Characterization and Modulation by Nitric Oxide

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Chen, James W. Y. and Ruth Anne Eatock. Major potassium conductance in type I hair cells from rat semicircular canals: characterization and modulation by nitric oxide. J Neurophysiol 84: 139–151, 2000. Mammalian vestibular organs have two types of hair cell, type I and type II, which differ morphologically and electrophysiologically. Type I hair cells alone express an outwardly rectifying current, IK,L, which activates at relatively negative voltages. We used whole cell and patch configurations to study IK,L in hair cells isolated from the sensory epithelia of rat semicircular canals. IK,L was potassium selective, blocked by 4-aminopyridine, and permeable to internal cesium. It activated with sigmoidal kinetics and was half-maximally activated at −74.5 ± 1.6 mV (n = 35; range −91 to −50 mV). It was a very large conductance (91 ± 8 nS at −37 mV; 35 nS/pF for a cell of average size). Patch recordings from type I cells revealed a candidate ion channel with a conductance of 20–30 pS. Because IK,L was activated at the resting potential, the cells had low input resistances (Rm): median 25 MΩ at −67 mV versus 1.3 GΩ for type II cells. Consequently, injected currents comparable to large transduction currents (300 pA) evoked small (±10 mV) voltage responses. The cells’ small voltage responses and negative resting potentials (Vr = −81.3 ± 0.2 mV, n = 144) pose a problem for afferent neurotransmission: how does the receptor potential depolarize the cell into the activation range of Ca2+ channels (positive to −60 mV) that mediate transmitter release? One possibility, suggested by spontaneous positive shifts in the activation range of IK,L during whole cell recording, is that the activation range might be modulated in vivo. Any factor that reduces the number of IK,L channels open at Vr will increase Rm and depolarize Vr. Nitric oxide (NO) is an ion channel modulator that is present in vestibular epithelia. Four different NO donors, applied externally, inhibited the IK,L conductance at −67 mV, with mean effects ranging from 33 to 76%. The NO donor sodium nitroprusside inhibited channel activity in patches when they were cell-attached but not excised, suggesting an intracellular cascade. Consistent with an NO-cGMP cascade, 8-bromo-cGMP also inhibited whole cell IK,L. Ca2+-dependent NO synthase is reported to be in hair cells and nerve terminals in the vestibular epithelium. Excitatory input to vestibular organs may lead, through Ca2+ influx, to NO production and inhibition of IK,L. The resulting increase in Rm would augment the receptor potential, a form of positive feedback.

INTRODUCTION

The type I hair cell is a relatively recent arrival found only in the vestibular sensory epithelia of reptiles, birds, and mammals. Its distinctive properties are just beginning to be understood. Type I hair cells were first described by Wersäll, who distinguished them from other hair cells in the vestibular epithelia (type II hair cells) by cell shape and synaptic contacts (Wersäll 1956). Type I cells have a flask shape and are engulfed by cup-like calyx afferent terminals. Type II cells come in diverse shapes but tend to be more cylindrical and receive bouton afferent contacts. Efferent neurons form bouton terminals on type II hair cells and on the calyx endings around type I cells. More subtle differences are evident at the ultrastructural level (Favre and Sans 1983; Rüschi et al. 1998).

Recent work has shown that type I and II hair cells also differ electrophysiologically. Type I cells alone have a large outwardly rectifying K+ current, IK,L (also called IK1), that is unusual in several respects (Correia and Lang 1990; Rennie and Correia 1994; Rüschi and Eatock 1996a). Most outward rectifiers in hair cells activate positive to the resting potential, Vr, which is typically between −70 and −50 mV. The IK,L conductance (gK,L), however, is frequently activated at Vr; voltages corresponding to half-maximal activation (V1/2) can be as negative as −100 mV. Another unusual property of the activation range is that it varies widely between cells, by as much as 50 mV (Rüschi and Eatock 1996a). Here we present properties of IK,L in hair cells isolated from the rat crista, including single-channel properties, that have not previously been described.

The size of gK,L at Vr largely determines the type I cell’s input resistance, and therefore the gain and time course of its voltage response to the current through the mechanosensitive transduction channels. As shown here and previously for other type I hair cells (Rennie et al. 1996; Rüschi and Eatock 1996b), when gK,L is appreciably activated at Vr, currents comparable with the largest transduction currents (hundreds of picamperes) depolarize the cell by <10 mV. If similar conditions hold in vivo, even large transduction currents (<1 nA) (Géléc et al. 1997; Holt et al. 1997) will not depolarize the cells into the activation range of the voltage-gated calcium channels that mediate transmitter release (positive to −60 mV in type I cells as in other hair cells) (Bao et al. 1999; Chen and Eatock 1993). As a possible solution to this problem, it has been proposed...
membranes, activating soluble guanylate cyclase to raise cGMP, which can then have diverse effects (Garthwaite and Boulton 1995). Here we show that \( I_{K,L} \) is inhibited near resting potential both by NO-producing agents and by a cGMP analog.

**Methods**

**Isolation of hair cells**

The medium used for preparing isolated hair cells was Leibovitz’s L-15 medium (GIBCO BRL, Gaithersburg, MD) with a modified Ca\(^{2+} \) concentration: either 100 \( \mu \)M (low-Ca\(^{2+} \) L-15) or 3.3 mM (high-Ca\(^{2+} \) L-15). Long-Evans rats (age 10–45 days, 16–200 g body wt) were deeply anesthetized with pentobarbital sodium (Nembutal, 100 ml/kg ip) and decapitated. All procedures for handling animals have been approved by institutional animal care review committees. The temporal bones were rapidly dissected out and placed in chilled, oxygenated high-Ca\(^{2+} \) L-15. The ampullae of the semicircular canals were excised and treated for 10–12 min at 37°C with protease XXVII (Sigma, St. Louis, MO; 500 \( \mu \)g/ml in low-Ca\(^{2+} \) L-15) to loosen the bonds between hair bundles and cupulae. They were then immersed in low-Ca\(^{2+} \) L-15 containing papain (Sigma, crude; 500 \( \mu \)g/ml and L-cysteine (Sigma; 300 \( \mu \)g/ml) for 40 min at 37°C, following which they were left in bovine serum albumin (Sigma; 500 \( \mu \)g/ml in low-Ca\(^{2+} \) L-15 medium) for 40 min at room temperature. One crista (sensory epithelium) was then brushed with a fine probe. The dissociated hair cells were allowed to settle onto the glass floor of the recording chamber. The other cristae were stored in high-Ca\(^{2+} \) L-15 at 6–8°C for later dissociation. All cristae were used within 36 h. No difference was found in the resting potentials and the current amplitudes of cells studied immediately or after storage at 6–8°C. Data from these two groups were pooled. The dissociated cells were viewed at \( \times 600 \) with Nomarski optics on an inverted microscope (Olympus IMT-2, Olympus Corporation, Lake Success, NY). The recording chamber was continuously perfused with oxygenated high-Ca\(^{2+} \) L-15.

**Electrophysiology**

**Whole Cell Recordings.** All recordings were done at room temperature (23–25°C). Whole cell currents were recorded using the conventional ruptured-patch method. Borosilicate pipettes were pulled and heat-polished to a final pipette resistance of between 3 and 5 M\( \Omega \) in our standard solutions and coated with silicone elastomer (Sylgard; Dow Corning, Midland, MI). The pipettes were filled with a solution containing (in mM): 130 KCl, 0.1 CaCl\(_2\), 2 MgATP, 11 EGTA, 10 HEPES, and 0.2 Li\(_2\)GTP. The pH was titrated to 7.3 and the osmolality was \( \sim 300 \) mmol/kg as measured with a vapor-pressure osmometer (Wescor, Logan, UT). The intracellular calcium concentration was estimated with Eqcal software (Biosoft, Cambridge, England) to be 900 pM. pH was titrated to 7.3 with 1 N KOH for a final K\(^+\) concentration of 159 mM. The standard bath solution was high-Ca\(^{2+} \) L-15 (330 mmol/kg; pH 7.3). The junction potential (7 mV) was subtracted from voltages off-line.

**Single-Channel Recordings.** All recordings were done at room temperature (23–25°C). Single-channel currents were recorded from membrane patches in either the inside-out or the cell-attached configuration. Inside-out patches were used to characterize \( I_{K,L} \), and cell-attached patches were used to study modulation of \( I_{K,L} \) by NO. The external solution was (in mM): 145 K gluconate, 1.2 MgSO\(_4\), 5 HEPES, and 10 EGTA. For cell-attached patches, we assume that the resting membrane potential is 0 mV in this external solution. Recording pipettes (5–8 M\( \Omega \)) contained a solution similar to the external solution but with 1 mM EGTA and 10 \( \mu \)M Ca\(^{2+}\), for a final estimated Ca\(^{2+}\) concentration of 1.5 mM (calculated with Eqcal software).

**Local Solution Exchange.** Chemicals such as 4-aminopyridine (4-AP, Sigma) or NO donors (see Nitric oxide experiments) were
dissolved in high-Ca²⁺ L-15 and applied to individual cells via wide-bore pipettes (≈ 100 μm tip diameter). Test and control solutions were applied by separate pipettes fed by separate lines.

Data acquisition and analysis
WHOLE CELL DATA. Whole cell currents were recorded with an amplifier (L/M EPC-7, Adams and List Associates, Great Neck, NY; or Axopatch-1D, Axon Instruments, Foster City, CA) and 12-bit A/D and D/A converters (Scientific Associates, Rochester, NY), controlled by data acquisition software (DATAQ, JPM Programming, Rochester, NY). The sampling interval varied from 125 μs to 1.9 ms. Data were low-pass filtered on-line at a cutoff frequency (≤ 3 dB) of 3 kHz (using the 3-pole Bessel filter of the EPC-7) or 5 kHz (using the 4-pole Bessel filter of the Axopatch-1D).

Membrane capacitance ($C_m$) and series resistance were usually estimated with the intrinsic compensation circuitry of the amplifier, from the settings that nullled capacitive transients evolved by 10-ms, −10-mV, steps from the holding potential ($V_h$). For most voltage protocols, $V_h$ was −67 mV. Because $I_{K,L}$ can be large at −67 mV, we measured $C_m$ in type I cells from a holding potential of −97 or −107 mV, where $I_{K,L}$ is largely deactivated. The values taken from the amplifier dials agree within 10% with values obtained from single exponential fits of the capacitive transients. Series resistance was compensated 50–80%, leaving residual series resistances ($R_s$; after compensation) of between 1 and 8 MΩ. Activation curves were corrected off-line for voltage errors introduced by $R_s$. Type I cells were selected for further data acquisition if the linear leak conductance ($G_m$) at −97 mV or −107 mV was <1 nS. The mean value in the data set was 0.6 ± 0.1 (SE) nS (n = 44). Leak subtraction was not done, either during recording or off-line. Resting potential, $V_R$, was frequently checked during recording; cells with $I_{K,L}$ were abandoned if $V_R$ was positive to −60 mV. $G_m$ values below 1 nS at −97 or −107 mV correlated well with stable resting potentials.

Data were stored digitally on disk and analyzed off-line. Data values were extracted (Datanal, JPM Programming, Rochester, NY) and imported to Origin (Versions 3–6, MicroCal Software, Northampton, MA) for fits of Boltzmann functions (Marquardt-Levenberg method). Values are presented as means ± SE.

SINGLE-CHANNEL DATA. Single-channel data were recorded with the Axopatch 1D or 200A amplifier (Axon Instruments) and a 12-bit acquisition board (Digidata, Axon Instruments), controlled by pClamp. The sampling interval varied from 50 μs to 1 ms. Data were low-pass filtered on-line at a cutoff frequency of 2 kHz and in some cases were then digitally filtered off-line at 1 kHz. Data were analyzed off-line with pClamp and plotted with Origin.

Nitric oxide experiments

The following NO donors were dissolved in high-Ca²⁺ L-15 medium: sodium nitroprusside (SNP, Sigma), nitroglycerin (NTG, Tridil, Du Pont Pharmaceutical, Wilmington, DE), 3-morpholinosydnonimine (SIN-1, Molecular Probes, Eugene, OR) and S-nitroso- N-acetylpenicillamine (SNAP, Molecular Probes). These donors are all light sensitive and probably employ photolytic degradation as one mechanism of NO release. All solutions were prepared under dim light and used within 6 h after they were made. To slow photodegradation, we darkened the room and wrapped aluminum foil around the perfusion apparatus. We used the microscope light to facilitate NO release near the cell of interest. To avoid unwanted effects of leak NO on control recordings, we introduced the local perfusion pipette into the recording chamber after the last control recording was done. Preparations were generally exposed to NO donors or 8-bromo-cGMP just once.

SNP dissolves in solution into Na⁺² and nitroprusside ([Fe(CN)₆]NO⁻²) (Arnold et al. 1984; Bates et al. 1991). Nitroprusside breaks down into NO and other products that may release minute amounts of cyanide and iron. To control for effects by these other breakdown products, we added the NO scavenger, carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl (carboxy-PTIO, Calbiochem, La Jolla, CA) (Yoshida et al. 1993). As shown in RESULTS, carboxy-PTIO largely eliminated the SNP effect.

NTG (C₅H₅N₃O₇) releases NO when exposed to thiol-containing compounds (Harrison and Bates 1993), such as cysteine and dithiothreitol. The L-15 solution in which NTG was dissolved contains cysteine. Because the NTG stock solution contained ethanol, which might affect channel activity, we added anhydrous ethanol to control solutions at the same concentrations as in the NTG test solutions: 1.3% in 1 mM NTG and 0.4% in 0.3 mM NTG.

SNAP (C₇H₁₂N₂O₄S) and SIN-1 (C₆H₁₀N₄O₂.HCl) probably release NO via mechanisms similar to those for NTG (Lincoln et al. 1997).

NEUROTOXICITY. One of the oxidation-reduction states of NO (NO⁻) reacts with superoxide anion to form peroxynitrite, a neurotoxic agent. Superoxide dismutase (SOD) and catalase (CAT) can prevent the formation of peroxynitrite and H₂O₂, respectively. In most but not all of the SNP experiments, SOD (50 IU/ml, Sigma) and CAT (50 IU/ml, Sigma) were added. There was no obvious difference from SNP data obtained without SOD and CAT. Also, resting potentials were stable during SNP treatments without SOD and CAT. These observations are consistent with the report that SNP releases the neural protective redox state (NO⁺) (Lipton et al. 1994). SNP data obtained with and without SOD and CAT are pooled.

RESULTS

$I_{K,L}$ is strongly correlated with type I morphology

Hair cells were classified before recording as type I if they had flask-shaped bodies and as type II if they had more cylindrical shapes. Rounded cells, which are likely to have lost their shape during the preparation, were not classified.

The morphological and whole cell electrophysiological profiles of hair cells were correlated. The mean resting potentials, $V_R$, were −81.3 ± 0.2 mV for 144 type I hair cells and −71.3 ± 2.3 mV for 36 type II hair cells. Mean $C_m$ values, which are proportional to membrane surface area, were 2.6 ± 0.1 pF for 142 type I hair cells and 3.7 ± 0.2 pF for 42 type II hair cells. These numbers are presumably low as many hair cells had lost their hair bundles during preparation.

Morphological cell type correlated strongly with the whole cell currents evoked by our standard voltage protocol: an iterated series of 50-ms steps from $V_h$ = −67 mV. In type I cells, the voltage steps evoked currents with a large instantaneous component (see Figs. 6B, 9A, and 10A for examples). The properties of this instantaneous current, described in the following text, show that it is $I_{K,L}$. In the NO experiments, we interpret effects of the NO donors on the instantaneous current as effects on $I_{K,L}$. Voltage steps positive to −60 mV evoked sigmoidally activating outward currents (Fig. 2A). It is not known whether these currents are through $I_{K,L}$ channels or other outwardly rectifying channels, as described in type I hair cells of the mouse utricule (Rüscher and Eaton 1996a; Rüscher et al. 1998).

In type II cells, in contrast, voltage steps from −67 mV typically evoked very small instantaneous currents, reflecting the absence of $g_{K,L}$ or other large conductances at −67 mV (data not shown). Voltage steps positive to −60 mV evoked sigmoidally activating outwardly rectifying currents as is typical of type II hair cells from the vestibular organs of other amniotes (Eatock et al. 1998; Lang and Correia 1989).
The presence of a large conductance ($g_{K,L}$) at $V_R$ dramatically affects the voltage responses of type I hair cells to injected currents (Fig. 2C). The median input resistance ($R_m$) of 27 type I hair cells at $-67$ mV was only 25 MΩ (mean 60 ± 16 MΩ; Fig. 2D) compared with 1.3 GΩ (mean 1.5 ± 0.2 GΩ, data not shown) in 27 type II hair cells. Thus a given input current at $-67$ mV will evoke a voltage change in the median type I cell that is just one-fiftieth that of the median type II cell. Median $R_m$ and mean $C_m$ values yield membrane time constants ($\tau_m = R_mC_m$) for type I and type II hair cells of 64 ms and 3.8 ms, respectively, at $-67$ mV. The rise time of the receptor potential will therefore be much faster for type I cells than for type II cells. Differences will be smaller when values at resting potential are compared because fewer $I_{K,L}$ channels are open at the average $V_R$ ($-81$ mV) than at our usual holding potential of $-67$ mV. Nevertheless even from $V_R$, large current injections (360 pA), comparable with the largest transduction currents measured in mammalian vestibular hair cells (Géléczy et al. 1997; Holt et al. 1997), may depolarize the type I cells by $<10$ mV (Fig. 2C). Similar observations have been made in type I hair cells from gerbil semicircular canals (Rennie et al. 1996) and mouse utricles (Rüsch and Eatock 1996b).

**Properties of $I_{K,L}$**

**Isolation of $I_{K,L}$.** Several lines of evidence indicate that negative to $-50$ mV, most of the current in type I cells is $I_{K,L}$. It is not leak or inwardly rectifying current because it deactivate with hyperpolarization and is almost completely blocked by 4-AP (see following text). The criterion used to select cells for analysis ($G_m \approx 1 nS$ at $-97$ or $-107$ mV) ensures that any contaminating leak or inwardly rectifying currents are at most 5% of the total conductance (1 in $20 nS$, the minimum $G_{\infty}$ estimated for $I_{K,L}$; see ACTIVATION). As shown next, activation curves in this voltage range are consistent with a single conductance. Although other outwardly rectifying K+ currents have been described in type I hair cells (Rennie and Correia 1994; Rüsch and Eatock 1996a), they activate positive to $-60$ mV (typical half-maximal activation voltages of $-30$ mV).

**Reversal potential.** The reversal potential of $I_{K,L}$, $V_{REV}$, was estimated from a linear regression of the instantaneous currents evoked by steps from $-67$ mV to voltages on either side of the current reversal. The mean $V_{REV}$ in 10 cells was $-80.2 \pm 0.5$ mV. The proximity of $V_{REV}$ to the estimated equilibrium potential for K+ ($-85$ mV) shows that the instantaneous current was very K+ selective and had little contribution from nonselective leak.

**Activation.** Figure 3A illustrates the slow sigmoidal activation of $I_{K,L}$ in response to depolarizing 600-ms steps from $V_H = -97$ mV. $I_{K,L}$ look in excess of 600 ms to reach steady state at the very negative voltages at which it started to activate, consistent with data from the mouse utricle (Rüsch and Eatock 1996a). The activation curve of $I_{K,L}$ (Fig. 3B) was generated from the tail currents at $-37$ mV following the iterated steps. The curve was fitted with a first-order Boltzmann function

$$I(V) = \frac{I_{\max} - I_0}{1 + e^{[V_h - V_{1/2}] / S}} + I_0$$

where $I_{\max}$ and $I_0$ are the maximum and minimum currents, $V$ is the prepulse voltage, $V_{1/2}$ is the voltage at half-maximal activation, and $S$ is the voltage change per e-fold increase of $I(V)$. In the example in Fig. 3, with $V_{1/2} = -73$ mV, there is no hint of a second, more positively activating outward rectifier. In some cells, a second, separate Boltzmann could be fit at potentials positive to $-50$ mV, consistent with a second outward rectifier as described in other type I hair cells. In other cells, the activation curve did not have an upper asymptote, consistent with overlapping activation curves from more than one conductance. Such curves were not fitted. Activation

**Effects of $I_{K,L}$ on membrane voltage response to injected currents**

We demonstrated the correlation between $I_{K,L}$ and type I morphology in a subset of 88 sequentially recorded cells. Thirty-one of 36 type I hair cells had $I_{K,L}$ (86%), compared with 4 of 29 (11%) of type II cells and 11 of 23 (48%) of the unclassified cells. Whether the few type I cells without $I_{K,L}$ and type II cells with $I_{K,L}$ represent true variation in the sample or misclassification of cell type is not known. The necks of dissociated type I cells sometimes relax; this could lead to their misidentification as type II.

![Image](https://example.com/image.png)

**FIG. 2.** A low-voltage-activating potassium current, $I_{K,L}$, in type I hair cells. A: whole cell currents in response to voltage steps from a holding potential, $V_H$, of $-81$ mV, near the cell’s resting potential, $V_R = -83$ mV. Voltage commands were incremented from $-141$ to $+19$ mV (values not corrected for series resistance) in steps of 20 mV. The large instantaneous currents evoked by the voltage steps were largely through $I_{K,L}$ channels that were open at $V_H$. These channels were deactivated by stepping to more negative potentials; see trace at $-141$ mV and data from the same cell in B. B: a 100-ms prepulse to $-141$ mV turned off the outward current that was present at $V_H$ and virtually all of the instantaneous current evoked by the voltage steps. C: current clamp records showing the voltage response of a different type I cell to 50-ms current steps from holding current. Current steps: $-20$, 0, 100, 200, 300, and 360 pA. Because $I_{K,L}$ was activated at $V_R$ ($-84$ mV), $R_m$ was just $18 M\Omega$. D: distribution of input resistances in 27 type I hair cells, measured from current clamp records as in C. Median value: 25 MΩ. Mode: 12.5 MΩ.
ions can be reconciled by assuming that the channel occupies multiple closed states e.g., $C_i \rightarrow C \ldots \rightarrow O$, but that only one of the transitions is strongly voltage dependent.

**K CHANNEL BLOCKERS.** $I_{K,L}$ channels in type I hair cells from other organs are blocked by 4-AP (Rennie and Correia 1994; Rüsch and Eaton 1996a) and are permeable to Cs$^+$ (Rüsch and Eaton 1996a). Figure 5 shows that the instantaneous current evoked by steps from $-67$ mV to potentials between $-97$ and $-7$ mV was blocked by external application of 5 mM 4-AP. In three cells, the mean percentage inhibition of instantaneous currents was $92 \pm 1\%$. $I_{K,L}$ persisted when Cs$^+$ replaced K$^+$ in the internal solution and was added at 5 mM to the external solution ($n = 5$; Fig. 5, D and E). These results indicate that the conductance at $-67$ mV is principally $g_{K,L}$. Later we exploit this observation to study the effects of NO on $g_{K,L}$.

**SINGLE-CHANNEL CURRENTS.** Ion channel activity was recorded in inside-out membrane patches excised from type I hair cells, in symmetrical K gluconate solutions (145 mM; $n = 10$). A good candidate for the $I_{K,L}$ channel was identified on the basis of its prevalence and the following properties shared with whole cell $I_{K,L}$: voltage range of activation, $K^+$ selectivity, deactivation kinetics, sensitivity to 4-AP, and sensitivity to sodium nitroprusside. These properties are demonstrated in Figs. 6–8 and 12. Figure 6A shows channel activity at $-60$ mV that was inhibited by stepping to $-130$ mV. The ensemble average of 17 such traces revealed an inward current that deactivated with a time constant of 4.4 ms. Whole cell $I_{K,L}$ recorded from the same cell later deactivated with a similar time course (Fig. 6B).

In 145 mM symmetrical K$^+$ solutions, the channels had a conductance of $30.9 \pm 1.5$ pS at $-60$ mV, estimated from patches with fewer than two channels concurrently open ($n = 6$). We used data from a patch that seemed to have just one channel to estimate the conductance over different voltage ranges (Fig. 7A). For each trace in Fig. 7A, an all-data-point amplitude histogram was made and fitted with a two-mode Gaussian function (Fig. 7B). The second (open-state) mode was plotted against voltage (Fig. 7C). Linear regressions of the

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**FIG. 3.** Activation curve for $I_{K,L}$. A: $I_{K,L}$ was deactivated by holding at $-97$ mV. During 600-ms test steps, membrane potential was iterated in 5-mV increments from $-102$ mV (command voltages shown, uncorrected for $R$). $I_{K,L}$ activated with slow kinetics when voltage was stepped positive to $-97$ mV. Following the 600-ms step, tail currents were evoked at $-37$ mV. The first point (*) at $-37$ mV is plotted in B against the voltage of the preceding step (corrected for $R$ error) and the points are fitted with Eq. 1. $V_{1/2} = -73$ mV, $S = 7$ mV, $I_{max} = 2.21$ nA; $G_{max} = 51$ nS.

The 35 activation curves just described were from data recorded within 10 min of the onset of whole cell recording. In some cases, the activation curve of individual cells shifted positively with time after the start of recording. In the example in Fig. 4, $V_{1/2}$ shifted from $-63$ mV at 5 min to $-39$ mV at 80 min, a 24-mV shift with a time constant of 26 min. A plausible explanation is that intracellular proteins that regulate the activation range wash out with time following patch rupture.

The sigmoidal activation kinetics of $g_{K,L}$ suggest that there are multiple closed states available to the channels, but the sigmoidal voltage dependence of activation is consistent with a two-kinetic-state model ($C \rightarrow O$, where $C$ and $O$ stand for closed and open states). These seemingly disparate observa-

**FIG. 4.** Positive shift with time in the activation curve of $I_{K,L}$ from a single cell. A: 3 activation curves from 1 cell, obtained as shown in Fig. 3, at 5, 24, and 58 min after the start of whole cell recording. Data (●, ●, ●) were fitted with Eq. 1 (●). The fits for the 3 curves yielded $V_{1/2}$ values of $-63$, $-52$, and $-42$ mV, and $S$ values of 6.9, 7.9, and 9 mV, respectively. B: plots of $V_{1/2}$ (●) and $S$ (○) for the cell in A, against time of recording. $S$ values fluctuated between 6.9 and 9.5 mV. $V_{1/2}$ depolarized from $-63$ to $-39$ mV with a time constant of 26 min as determined from the single exponential fit (●). Over the same time period, the resting potential depolarized from $-80$ to $-59$ mV, reflecting the positive shift of the $I_{K,L}$ conductance, which dominates the resting membrane.
The Cs steps (and additional traces). 4-AP blocked 70% of the current obtained in B respectively (Fig. 8). The low total probability reflects long closed potentials (60 to 0 mV) and positive potentials (0 to 2 mV), yielded slopes of 23, 29, and 20 pS, respectively.

This current was substantially reduced by adding 5 mM 4-AP to the standard external solution (fine line). A Boltzmann function fitted to the P-V curve (Fig. 8B) yielded $V_{1/2}$ and $S$ values of $-72.5$ and 2.4 mV. The $V_{1/2}$ value is compatible with activation curves for whole cell $I_{K,L}$, but the $S$ value is significantly smaller than the mean value of 4.8 ± 0.9 mV for the whole cell current. Differences in the recording conditions might account for the discrepancy. The single-channel data in Figs. 6 and 7 were obtained from inside-out patches in very simple solutions containing symmetrical K+ and no ATP; recent data show variation in $g_{K,L}$ with K+ concentration (Rennie and Correia, 2000) and ATP (see discussion). Also, the patches were held at the voltages indicated for long periods (>60 s), while in whole cell recordings, voltage was stepped positively for <1 s from a holding potential between −60 and −80 mV. Thus long-term processes such
data over the whole range (−60 to +80 mV), at negative potentials (−60 to 0 mV) and positive potentials (0 to +80 mV), yielded slopes of 23, 29, and 20 pS, respectively.

The single-channel open probability of $I_{K,L}$ was calculated using the equation

$$p = \frac{i}{i \times n}$$

$p$ is the open probability of a single channel in a patch, $i$ is the mean current over the interval used for the calculation, $i$ is the single-channel current amplitude, and $n$ is the number of channels in the patch. At negative voltages the channels showed bursts of openings separated by silent periods of up to several seconds (Figs. 7 and 8). Therefore continuous recordings of ~60-s duration were used to estimate $p$ at each voltage (Fig. 8A). In an excised patch that probably included four channels, $p$ increased from 0 at −100 mV to ~0.08 at −60 mV, respectively (Fig. 8B). The low total probability reflects long closed times between bursts. Within bursts at −60 mV (Figs. 7A and 8A), $p$ was ~0.7.

We do not have 50-s recordings at positive potentials, but the data in Fig. 7A were taken from 7.5-s records. The 600-ms records shown in Fig. 7A for +40, +60, and +80 mV are representative of the entire 7.5 s: there were neither bursts nor long silent intervals, and open probabilities were again quite low (~5–10%). At these holding potentials, the channel may be inactivated much of the time.

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Pharmacology shows that the conductance at $V_H = -67$ mV is principally $g_{K,L}$. Previous studies have shown that $g_{K,L}$ is blocked by 5 mM 4-aminopyridine (4-AP) and permeant to Cs+ (see text). A–C: addition of 5 mM 4-AP to the external solution suppressed the instantaneous currents evoked by steps from $V_H = -67$ mV, as shown for steps to −87 (A) and −7 mV (B). The suppression was partly reversible (wash). In C, the instantaneous I-V relations are plotted from the 1st data points after the step (at 125 μs); data from (A) and (B) and additional traces. D and E: current through $I_{K,L}$ channels recorded with a Cs+ internal solution and with 5 mM Cs+ added to the standard external solution. In these solutions $V_{rev}$ was approximately −30 mV. A step from −100 to −70 mV (D) activated a large slow inward current through $I_{K,L}$ channels, which deactivated on returning to −100 mV (thick line). This current was substantially reduced by adding 5 mM 4-AP to the standard external solution (fine line). E: I-V relations taken near the end of the 600-ms steps (D and additional traces). 4-AP blocked 70% of the current obtained in the Cs+ solutions. The inward current ($I_{K,L}$) activated above −90 mV and declined positive to −60 mV as it approached $V_{rev}$. Pipette solution contained (mM) 24 CsCl, 24 CsF, 96 d-glucose, 1 CaCl2, 0.04 Li3GTP, 5 MgATP, 20 di-Tris creatine phosphate, 10 EGTA, and 10 HEPES; creatine phosphokinase, 50 U/ml. External solution contained (mM) 5 CsCl, 146 NaCl, 5.8 KCl, 3.3 CaCl2, 5 HEPES, 1.8 MgCl2, and 11 d-glucose. 5 mM 4-AP was added to the external solution.

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Patch recordings reveal a candidate $I_{K,L}$ channel. A: inside-out patch recordings from a type I hair cell show channels that are open at −60 mV and that deactivate rapidly on stepping to −130 mV (top 4 traces). Bottom: the average of 17 traces with channel openings, including the top 4 traces. All traces were subtracted from averages of 12 traces without channel openings at the same potential. The deactivation in the bottom trace was fitted with a single exponential function, $\tau = 4.4$ ms. Symmetrical K+ gluconate solutions. Sampling interval: 50 μs, filtered at 2 kHz. B: whole cell recording from the same cell, taken after the patch recordings in A; standard solutions. The cell clearly had $I_{K,L}$; its deactivation at −147 mV was fitted by a single exponential function with $\tau = 3.5$ ms.
Addition of NO donors to the external solution reduced the instantaneous currents evoked by voltage steps from $-67 \text{ mV}$, which as shown in the preceding text are principally carried by $I_{K,L}$ channels. Figure 9, A–D, shows reversible suppression of the instantaneous current by 1 mM SNP. The residual outward current in 1 mM SNP (Fig. 9B) is similar in size and kinetics to the time-varying component of the outward current in control conditions (Fig. 9A). Whether this residual current was through $I_{K,L}$ channels or through a second, more positively activating outward rectifier is not known. We quantified the inhibition of the instantaneous current by subtracting a fit of the instantaneous current-voltage ($I$-$V$) relation in SNP from a fit of the instantaneous $I$-$V$ relation in control conditions (Fig. 9E), then normalizing the subtracted values by dividing by the control data. The suppression shown in Fig. 9 was the most complete in a series of experiments in which we varied the NO donor or the donor concentration. The concentration of NO is not known; it is a function of the donor concentration, time since the solution was made, temperature and light exposure.

If the NO effect is mediated by a second-messenger cascade, then it may increase gradually. The reversible inhibition of $I_{K,L}$ by another NO donor, NTG, is shown in Fig. 10. After 1 min of NTG treatment, the instantaneous current ($I_{K,L}$) was sub-

**Fig. 7.** Single-channel conductance of candidate $I_{K,L}$ channel. A: inside-out patch recording of $I_{K,L}$; probably 1 channel, at different holding potentials. At negative potentials, the channel switched between long silent periods, up to several seconds at $-60 \text{ mV}$, and bursts of openings; the data shown are selected to show bursts. Symmetrical $K^+$ gluconate solutions. Sampling interval at 50 $\mu$s, filtered at 2 kHz, off-line digital filtered at 1 kHz. B: all-data-point amplitude histograms were generated and fitted with 2-mode Gaussian functions at various voltages. The histogram at $-60 \text{ mV}$ is shown. C: center values of the Gaussian fits to the amplitude histograms are plotted against voltage.

**Fig. 8.** Open probability for candidate $I_{K,L}$ channels. A: inside-out patch recordings of $I_{K,L}$ channel activity. Each trace is a continuous recording of 51.2 s. Four opening levels are evident in the current traces at $-50$ and $-60 \text{ mV}$. Symmetrical $K^+$ gluconate solutions. The sampling and analog filter frequencies were both 1 kHz, which may produce aliasing. This is not likely to have affected the measurement of open probabilities, given that similar values were obtained from a patch from another cell with records that were 7.5 s long, filtered at 2 kHz, and sampled at a resolution of 50 $\mu$s. B: the single-channel open probability, $P_o$, as a function of voltage, calculated from the data in A assuming 4 channels (see text), then plotted against $V_{1/2}$ and fitted with a Boltzmann function ($-1$, $V_{1/2} = -72.5 \text{ mV}$, $S = 2.4 \text{ mV}$). This cell was exposed to 5 mM 4-AP before the patch was excised.

**As inactivation might occur in the patches but not in whole cell conditions.**

The number of $I_{K,L}$ channels in a type I hair cell can be estimated from the equation

$$n = \frac{G_{\text{max}}}{\gamma \times P_{\text{max}}}$$

$n$ is the number of channels, $G_{\text{max}}$ is the maximal whole cell conductance of $I_{K,L}$, $\gamma$ is the single channel conductance of $I_{K,L}$, and $P_{\text{max}}$ is the maximal single channel open probability. If we assume that $P_{\text{max}}$ is similar in whole cell and patch recording (i.e., that the estimated $P_{\text{max}}$ is not depressed), we can estimate the density of $I_{K,L}$ channels. For the average whole cell $G_{\text{max}}$ of 91 nS, $\gamma = 29 \text{ pS}$ at negative potentials and $P_{\text{max}} = 0.08$ ($-60 \text{ mV}$), $n = 39,000$ channels per cell. The membrane surface area can be estimated from the specific capacitance of membrane, 1 $\mu\text{F/cm}^2$, and the average cell capacitance, 2.6 $\mu\text{F}$, to be 260 $\mu\text{m}^2$. The average $I_{K,L}$ channel density in a type I hair cell is then $\sim 150$ channels/$\mu\text{m}^2$. 

**Modulation of $I_{K,L}$ by NO donors**
stantially reduced, but the time-varying component was still present (Fig. 10B). After wash and recovery of the instantaneous current (Fig. 10C), the cell was treated with NTG for 4.5 min before the recording shown in Fig. 10D, in which the instantaneous current was almost completely suppressed and the time-varying component was substantially reduced. Thus the full effect of NTG took minutes to develop. The mean durations of exposure to the various NO donors ranged from 1 to 4 min. The results of Fig. 10 suggest that mean inhibitory effects might have been greater had exposure durations been longer.

NO donors may affect channels by mechanisms other than NO release (see METHODS). As one approach to this problem, we tested four different NO-producing agents. The fact that all four inhibited the instantaneous current argues that the effect was mediated by the common release product, NO. Figure 11 summarizes the inhibition of instantaneous current by four different NO donors: SNP, NTG, SIN-1, and SNAP, some at more than one concentration. As another form of control for effects not mediated by NO, we added the NO scavenger, carboxy-PTIO (200 μM), to solutions containing 1 mM SNP or 250 μM SNAP (Fig. 11, ■). Carboxy-PTIO was very effective at blocking the inhibitory effects of SNP and SNAP. The mean inhibitory effects of 1 mM SNP without and with carboxy-PTIO were 52 ± 8% (n = 9) and 7 ± 1% (n = 3), respectively. The mean inhibitory effects of 250 μM SNAP without and with carboxy-PTIO were 33 ± 6% (n = 8) and 5 ± 3% (n = 4), respectively.

**Mechanism of NO effect**

The effects of SNP on channel activity recorded from patches suggest that the NO effect involves intracellular second messengers. \( I_{K,L} \) channels were inhibited by 1 mM SNP in cell-attached-patch mode (n = 5 patches from 5 cells) but not in excised inside-out patches (n = 10 patches from 10 cells). Figure 12 shows a patch from which both kinds of recording were obtained. The patch appeared to have at least two such channels, plus other channels with much smaller conductances (Fig. 12A). Perfusion of the cell with 1 mM SNP greatly reduced all channel activity when the patch was cell-attached (Fig. 12B). Excision of the patch restored channel activity, despite continued perfusion with SNP (Fig. 12C). This suggests that the SNP effect is mediated by soluble second messengers that are no longer present in excised patches. It provides further evidence that the SNP effect is mediated by an NO cascade and not another byproduct of the breakdown of SNP. The 29-pS channels in the excised patch were reversibly blocked by 5 mM 4-AP (Fig. 12D), consistent with their being \( I_{K,L} \) channels. The small-conductance channels were more visible after the 29-pS channels were blocked (Fig. 12D). From their size (~10-pS) and the fact that they were appreciably activated at ~60 mV, these may have been inwardly rectifying channels. Their absence in Fig. 12B suggests that they too may be inhibited by NO.

In some systems, NO effects are mediated by cGMP, produced as a consequence of NO activation of guanylate cyclase.
**DISCUSSION**

$I_{K,L}$ is a feature of type I hair cells from all kinds of amniote vestibular organs: the gerbil crista (Rennie and Correia 1994), mouse utricle (Rüsch and Eatock 1996a), all vestibular organs of the pigeon (Ricci et al. 1996), and the turtle crista (Brichta et al. 1996). $I_{K,L}$ is $K^+$-selective, activates sigmoidally and relatively slowly with depolarization, and inactivates slowly. It is independent of external $Ca^{2+}$ (Rüsch and Eatock 1996a). Here we show that its single-channel conductance is between 20 and 30 pS. In all of these respects, $I_{K,L}$ resembles a delayed rectifier, but it is unlike most delayed rectifiers in that it has an appreciable permeability to Cs$^+$ and an activation range that is unusually negative and variable.

In its activation range and sensitivity to phosphorylation, $I_{K,L}$ resembles a major conductance, $I_{K,n}$, found in some outer hair cells of the mammalian cochlea (Jagger and Ashmore 1999; Mammano and Ashmore 1996). It has recently been suggested that $I_{K,n}$ channels contain the KCNQ4 subunit (Marcotti and Kros 1999). C. Chen et al. (1995) found that 10 mM SNP reduced the outward current of outer hair cells by 40%; whether the current that they studied included $I_{K,n}$ is not known. Another member of the KCNQ family is the neuronal M current, which is a heteromultimer of KCNQ2 and KCNQ3 (Wang et al. 1998). Like $I_{K,n}$ and $I_{K,L}$, M current dominates the resting membrane conductance and is sensitive to modulation.

Type I hair cells express KCNQ4 subunits (Hurley et al., 2000), but whether these contribute to $I_{K,L}$ channels is not known.

From a functional point of view, the activation range of $I_{K,L}$ is its most intriguing property. The relatively negative range means that many type I hair cells have unusually low input resistances. As a consequence, their voltage responses to injected current have relatively fast rise times and low gains (voltage output per current input). A powerful way to control the gain and time course of the receptor potential, therefore, is to modulate factors that control the number of $I_{K,L}$ channels that are open at the cell’s resting potential. The effect of NO donors on $I_{K,L}$, in combination with anatomical data showing NOS at various sites in vestibular epithelia, show that NO is a potential endogenous modulator.

**Spontaneous shifts of the activation curve**

Positive spontaneous shifts of the voltage activation range (Fig. 4) are also seen when $I_{K,L}$ is recorded in ruptured-patch whole cell mode from hair cells isolated from the rat utricle (Hurley and Eatock 1999) and from hair cells in situ in the epithelium of the mouse utricle (Rüsch and Eatock 1996a). These shifts suggested to us that the activation range is under the control of an intracellular factor that diffuses into the recording pipette rather slowly during whole cell dialysis. In agreement with this hypothesis, no shift occurs in perforated patch experiments, which largely preserve the intracellular milieu (Hurley and Eatock 1999). Negative shifts in activation or inactivation curves of other ionic currents, with similar time courses, have been attributed to changes in the junction potential as large intracellular anions slowly wash out (Marty and Neher 1983). A similar trivial mechanism does not explain the positive shift in $V_{1/2}$ of $g_{K,L}$, however, given that the $V_{1/2}$ value of the delayed rectifier in type II cells does not shift with time (Hurley and Eatock 1999).

In a recent report on type I cells in the rat utricle, ruptured-patch recordings of the outwardly rectifying current had more positive $V_{1/2}$ values when ATP was omitted from the pipette solution (Lennan et al. 1999). Preliminary data from our laboratory suggest that phosphorylation inhibitors positively shift the activation curve of $I_{K,L}$ (Hurley and Eatock 1999). The absence of internal ATP in the experiments of Lennan et al. may also shift the activation curve positively by preventing phosphorylation. A comparable effect is seen in cochlear outer hair cells, where the activation range of $I_{K,n}$ is negatively shifted by cAMP-dependent phosphorylation (Jagger and Ashmore 1999). Phosphorylation induces shifts in the activation curves of other kinds of $K^+$ channels, although in some cases

**FIG. 12.** SNP inhibits the activity of candidate $I_{K,L}$ channels recorded from cell-attached patches (CAP) but not inside-out patches (IOP). Traces B–E were recorded at 4, 14, 18, and 29 min relative to trace A. This patch appeared to have $\approx 2$ $I_{K,L}$ channels; the current levels for 1 and 2 29-pS channels. Channel activity at $-60$ mV (A) was inhibited by 1 mM SNP in cell-attached recordings (B; trace recorded 2 min after start of SNP perfusion) but not when the patch was excised (C; trace recorded 7.5 min after patch excision). The channels are likely to be $I_{K,n}$ channels because they were activated at $-60$ mV, had conductances of $\approx 29$ pS $\cdot \cdot \cdot$ and were reversibly blocked by 1 mM SNP (B and C) and 5 mM 4-AP (D and E). Symmetric K gluconate solutions.

We therefore tested the effect of 8-bromo-cGMP, a nonhydrolyzable membrane-permeant cGMP analogue, on the instantaneous currents recorded in whole cell mode with the standard voltage protocol. 8-bromo-cGMP (1 mM) in the external solution inhibited the instantaneous current by $45 \pm 7\%$ ($n = 10$; Fig. 11), consistent with a role for cGMP in the inhibition produced by NO donors.
the shifts are in the opposite direction (Hoffman and Johnston 1998; Thomas et al. 1999).

Lennan et al. argue that the $V_{1/2}$ values reported by us and others for $I_{K,L}$ are an artifact induced by pipette ATP. ATP is, however, normally present in cells and is essential to prevent rundown of some ion channels (Forscher and Oxford 1985). Moreover, the range of $V_{1/2}$ values that we obtain in whole cell recordings from rat utricular type I cells is the same ($\sim$90 to $\sim$40 mV) whether we record with the perforated-patch method, which preserves endogenous ATP levels, or the ruptured-patch method (Hurley and Eaton 1999).

$I_{K,L}$ channels

MULTIPLE CLOSED STATES. Several features of the whole cell and single-channel data are consistent with a multi-state kinetic model, of the form: $C_1 \rightleftharpoons \ldots \rightleftharpoons C_n \rightleftharpoons O$, in which just one transition is strongly voltage dependent. The activation kinetics are sigmoidal, consistent with multiple closed states (Rüsch and Eaton 1996a). The $G$-$V$ curve, however, is well fitted by a first-order Boltzmann function, suggesting that only one transition is strongly voltage dependent. When recorded at high temporal resolution, deactivation follows a double-exponential function (Rüsch and Eaton 1996a). This can indicate the contribution of more than one channel type or open state but also can occur for a single channel type with multiple closed states if the $C_n \rightleftharpoons O$ transition has a weak voltage dependence (see discussion in Bezanilla et al. 1994). Finally, the single-channel behavior shows long inter-burst intervals and within bursts both very short closures and intermediate ones (3–40 ms in trace at $\sim$60 mV in Fig. 7A), possibly corresponding to different closed (and/or blocked or inactivated) states.

$I_{K,L}$ CHANNEL DISTRIBUTION. Our estimates of single-channel and maximum whole cell conductances suggest that there are tens of thousands of $I_{K,L}$ channels per hair cell at a density of 100–200/μm². How $I_{K,L}$ channels are distributed on the type I hair cell membrane surface is not known. In a freeze-fracture study of type I hair cells from the guinea pig, Gulley and Bagger-Sjöbäck (1979) described medium-sized intramembrane particles that are diffusely distributed over the type I cell membrane and irregular patches of larger particles (12–14 nm diam). Either set of particles might include $I_{K,L}$ channels. The active zones of frog saccular hair cells display elongate clusters of particles, also $\sim$12 nm diam in freeze fracture replicas, which may be co-localized Ca²⁺ and Ca²⁺-dependent K⁺ channels (Roberts et al. 1990). In type I cells, the patches of large particles surround “invaginations” (Gulley and Bagger-Sjöbäck 1979): zones where the calyx invaginates the hair cell and where the hair cell and calyx membranes are unusually close together. The function of the invaginations is not known. Although it is attractive to speculate that they are involved in afferent transmission, only one-quarter of presynaptic ribbons are in close proximity to invaginations (Lysakowski and Goldberg 1997). Inspection of micrographs in the Gulley and Bagger-Sjöbäck paper suggests that some particle clusters form annuli of $\sim$1,000 particles around an invagination. In the mature chinchilla crista, the number of invaginations per type I cell varies from $\sim$10 for peripheral cells to $\sim$50 for central cells (Lysakowski and Goldberg 1997). Dividing the estimated mean number of $I_{K,L}$ channels in our cells (39,000) by the mean number of invaginations in chinchilla type I cells ($\sim$30) yields $\sim$1,300 channels per invagination, consistent with the number of large particles per invagination in the guinea pig type I cells. If the particles were the $I_{K,L}$ channels, the variance in number of invaginations could account for a good fraction of the variance in number of channels per cell ($G_{\text{max}}$, 10-fold range in our sample).

NO-cGMP cascade in the inner ear?

In one scheme by which NO is proposed to mediate retrograde neurotransmission (reviewed by Dawson and Snyder 1994; Garthwaite and Boulton 1995), glutamate released from the presynaptic neuron binds to postsynaptic N-methyl-D-aspartate (NMDA) receptors, which open and admit Ca²⁺. The incoming Ca²⁺ activates NOS to make NO, which diffuses out of the postsynaptic terminal to act on neighboring cells. A major target of NO is believed to be soluble guanylate cyclase (sGC). NO activates sGC, thereby increasing the production of cGMP, which can have many actions. In the next paragraphs we review some of the evidence that the inner ear has the elements of the NO-cGMP cascade.

NMDA RECEPTORS. The afferent transmitter is either glutamate or another substance that can activate glutamate receptors (Kataoka and Ohmori 1994). Although fast excitatory transmission is likely to involve AMPA receptors (see Sewell 1996 for review), NMDA receptors are found on cultured chick cochlear ganglion neurons (Yamaguchi and Ohmori 1990) and appear to participate in the background discharge of vestibular afferents in amphibians (Soto et al. 1994; Zucca et al. 1993). According to a preliminary report, both calyx endings and type I hair cells are immunoreactive for NMDA receptor subunits (Ishiyama et al. 2000).

NITRIC OXIDE SYNTHASE. Various groups have found evidence for NOS in vestibular and cochlear epithelia. Lysakowski and colleagues have found that efferent terminals and some hair cells in the rodent vestibular epithelium stain positively for NADPH diaphorase (Lysakowski and Singer 2000; Singer and Lysakowski 1996), which in formaldehyde-fixed neural tissue correlates well with bNOS (reviewed in Lincoln et al. 1997). Consistent with the localization to efferent terminals in the epithelium, a subset of efferent neuron cell bodies in the brain stem stain for diaphorase and is immunoreactive for bNOS.

While the diaphorase method did not label calyx endings, Lysakowski and colleagues have recently found them to be immunoreactive for bNOS (A. Lysakowski, personal communication). Hess and colleagues reported that both calyx endings and vestibular ganglion cell bodies are immunoreactive for bNOS and that calyx endings are also immunoreactive for eNOS (Hess et al. 1998a,b). Both hair cells and afferent cells stain with the diaphorase method in vestibular organs of the axolotl (Flores et al. 1996); whether efferents also stain was not established. In the cochlea, afferent and efferent nerve fibers and possibly hair cells contain NOS (Fessenden and Schacht 1998; Michel et al. 1999; Rieman and Reuss 1999).

SOLUBLE GUANYLATE CYCLASE. For NO’s effect on $I_{K,L}$ to be mediated by cGMP, sGC must be present in type I hair cells; this remains to be determined, although sGC immunoreactivity has been localized to vestibular maculae (Hess et al. 1998a). Whether sGC is present in cochlear hair cells is in dispute.
NO MODULATES A K CONDUCTANCE IN VESTIBULAR HAIR CELLS

In type I hair cells from a variety of organs, either enzymatically dissociated or in situ in semi-intact epithelia, \( I_{\text{K,L}} \) produces low input resistances and unusually negative resting potentials. Preliminary data indicate that the activation range of voltage-gated \( \text{Ca}^{2+} \) channels in type I hair cells is positive to \(-67 \text{ mV} \) (Bao et al. 1999) as it is in other hair cells. Therefore it is not clear how type I cells generate receptor potentials large enough to activate conventional chemical transmitter release in vivo. Our data suggest that NO released within the vestibular epithelium would reduce \( I_{\text{K,L}} \) and consequently would both move the resting potential positive and enhance the receptor potential.

NO may be active as far as \(-300 \mu \text{m} \) from the source (Garthwaite and Boulton 1995), a distance that would include much of the crista. Thus NO produced by hair cells or afferent or efferent terminals might affect \( I_{\text{K,L}} \) in any given type I hair cell. It has been argued that NO produced in one cell acts principally on NO targets in other cells (Lincoln et al. 1997) because \( \text{Ca}^{2+} \) influx that stimulates NOS in a cell simultaneously inhibits SG and stimulates cGMP phosphodiesterase in that same cell. In that case, \( I_{\text{K,L}} \) channels would be most affected by NO produced outside the hair cell, in neighboring hair cells or in nerve terminals. Excitatory head movements would produce \( \text{Ca}^{2+} \) flow into hair cells and afferent terminals through multiple routes, which might then activate NO in either or both sites, producing NO and inhibiting \( I_{\text{K,L}} \) in type I hair cells in the vicinity.

The evidence that NOS is present in vestibular efferent neurons is particularly intriguing, as it suggests a way for efferents to modulate type I cells without physically contacting them (Fig. 1). Activation of efferent neurons would cause \( \text{Ca}^{2+} \) influx through voltage-gated \( \text{Ca}^{2+} \) channels in the efferent terminals. The influx would promote the release of conventional neurotransmitter, chiefly acetylcholine, from the efferent terminal onto the calyx ending but would also stimulate NO production by NOS in the efferent terminals. The NO would readily cross the calyx to the type I cell and inhibit \( I_{\text{K,L}} \), making the resting potential more positive (enhancing background transmitter release) and increasing the receptor potential (enhancing response gain). These effects are consistent with efferent actions in the mammalian vestibular system. Shocking the vestibular efferents has predominantly excitatory effects on vestibular afferents, increasing background discharge rates and in some cases increasing the gain of the response (spike rate per unit of stimulus) (Goldberg and Fernandez 1980). In the axolotl inner ear, there is evidence for an excitatory effect on afferent discharge by endogenous NO. Inhibition of NO production reduces both the background and evoked discharge of vestibular afferents (Flores et al. 1996).

In summary, our results show that NO could substantially affect the excitability of type I hair cells by inhibiting the dominant conductance near resting potential. But in the intact epithelium, NO actions may be more complex. As a simple example, NO activates L-type \( \text{Ca}^{2+} \) channels in some cell types (Kurenny et al. 1994) but inhibits them in other cell types (Summers et al. 1999). A preliminary report suggests that NO reduces the open probability of \( \text{Ca}^{2+} \) channels in hair cells of the frog sacculus (Rodriguez-Contreras et al. 2000). In type I hair cells, NO activation of \( \text{Ca}^{2+} \) channels would act synergistically with NO suppression of \( I_{\text{K,L}} \) to enhance afferent transmission, whereas NO inhibition of \( \text{Ca}^{2+} \) channels would antagonize the \( I_{\text{K,L}} \) effect.

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REFERENCES


Marcotti W and Kros CJ. Developmental expression of the potassium current I_{K,0} contributes to maturation of mouse outer hair cells. J Physiol (Lond) 520: 653–663, 1999.


