Effects of KCNQ channel blockers on K⁺ currents in vestibular hair cells

KATHERINE J. RENNIE,1 TIANXIANG WENG,1 AND MANNING J. CORREIA1,2

Departments of 1Otolaryngology and 2Physiology and Biophysics,
The University of Texas Medical Branch, Galveston, Texas 77555-1063

Received 29 March 2000; accepted in final form 3 October 2000

Rennie, Katherine J., Tianxiang Weng, and Manning J. Correia. Effects of KCNQ channel blockers on K⁺ currents in vestibular hair cells. Am J Physiol Cell Physiol 280: C473–C480, 2001.—Linopirdine and XE991, selective blockers of K⁺ channels belonging to the KCNQ family, were applied to hair cells isolated from gerbil vestibular system and to hair cells in slices of pigeon cristae. In type II hair cells, both compounds inhibited a slowly activating, slowly inactivating component of the macroscopic current recruited at potentials above −60 mV. The dissociation constants for linopirdine and XE991 block were <5 μM. A similar component of the current was also blocked by 50 μM capsaicin in gerbil type II hair cells. All three drugs blocked a current component that showed steady-state inactivation and a biexponential inactivation with time constants of ~300 ms and 4 s. Linopirdine (10 μM) reduced inward currents through the low-voltage-activated K⁺ current in type I hair cells, but concentrations up to 200 μM had little effect on steady-state outward K⁺ current in these cells. These results suggest that KCNQ channels may be present in amniote vestibular hair cells.

vestibular hair cells express a wide variety of K⁺ channels that are thought to confer distinct filtering properties on specific cell types. Although the biophysical properties of these K⁺ channels have been characterized in type I and type II vestibular hair cells, little is known about their molecular structure (2, 4, 7, 11, 12, 15, 17, 18–21, 25–27, 36). All mature type I hair cells express a delayed rectifier conductance (g_KI or g_K1L), which can be active at unusually negative membrane potentials and shows little inactivation (2, 12, 20, 25–27). Type II cells form a more heterogeneous population and exhibit a number of K⁺ conductances with different activation and inactivation properties. Outward K⁺ currents in pigeon type II hair cells have been shown to consist of a rapid A-type conductance (g_KA) sensitive to 4-aminopyridine (4-AP), a Ca²⁺-activated conductance [g_K(Ca)], and a slowly activating, slowly inactivating delayed rectifier conductance (g_KII) sensitive to external tetraethylammonium (11). The main outward conductance in mammalian type II hair cells is a 4-AP-sensitive delayed rectifier g_K, but g_K(Ca) and g_KA have also been reported (4, 12, 19, 27). In pigeon vestibular epithelia, K⁺ conductances have a regional distribution: g_KA predominates in type II cells of the peripheral crista and extrastriolar regions of the utricule, whereas cells in more central regions have slower kinetics owing to g_KII (16, 36). A similar expression pattern was reported in the frog crista, where a delayed rectifier g_K was the predominant conductance in central regions, and both g_KA and g_K were present in peripheral locations (17).

Recent studies have implicated the involvement of KCNQ channels in inner ear function (9, 10, 33). KCNQ1 subunits, together with the smaller subunit “minK,” form a K⁺ channel in vestibular dark cells and marginal cells of the stria vascularis, which appears to be important for the maintenance of K⁺ levels in endolymph (33). KCNQ4 channels are expressed in outer hair cells of the mammalian cochlea, and a mutation in the KCNQ4 gene gives rise to a form of nonsyndromic deafness (10). Most recently, KCNQ4 channels have also been shown in type I hair cells and their associated calyx fibers with the use of antibodies to KCNQ4 (9). To investigate the functional presence of channels encoded by KCNQ genes in vestibular hair cells, we tested the effects of two known blockers of KCNQ channels on type I and type II hair cells from the pigeon and gerbil. Although we found a component of the macroscopic K⁺ current in type II hair cells with high sensitivity to KCNQ channel blockers and capsaicin, the kinetics of this current did not resemble those of previously described KCNQ currents. In type I hair cells, the resting conductance g_KI was partially blocked by KCNQ channel blockers.

We conclude that the high sensitivity of a type II hair cell current to linopirdine and XE991 suggests the presence of KCNQ channels in these cells. The low sensitivity of the type I hair cell conductance to these blockers suggests that KCNQ1–3 are not involved but, together with recent molecular evidence, strongly suggests that KCNQ4 contributes to the resting current in type I hair cells.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Experimental procedures for animal treatment were approved by The University of Texas Medical Branch Animal Care and Use Committee and were within American Physiological Society and National Institutes of Health guidelines.

Isolation of gerbil vestibular hair cells. Hair cells were nonenzymatically dissociated as described previously (21). The semicircular canals and utricles were removed from Mongolian gerbils (35–65 g) under deep anesthesia [pentobarbital sodium (Nembutal), 50 mg/kg ip, and ketamine, 40 mg/kg im] and placed in a high-Mg2+-containing solution of Leibovitz’s L-15 medium (Life Technologies) containing bovine albumin (1 mg/ml) for ≥50 min at room temperature. Hair cells were mechanically dissociated by drawing a probe across the surface of the epithelium immersed in L-15 medium in the recording chamber and viewed on a Nikon inverted microscope. Type I and type II vestibular hair cells were identified by their neck-to-plate and neck-to-body ratios (24).

Preparation of pigeon vestibular slices. Slices were prepared as described previously (16, 36). Briefly, the semicircular canals and utricles were removed from deeply anesthetized (pentobarbital sodium, 40 mg/kg iv, supplemented with ketamine, 60 mg/kg im) white king pigeons (200–350 g) and kept at 37°C in DMEM (Life Technologies) supplemented with 24 mM NaHCO3, 15 mM PIPES, 50 mg/l ascorbate, and 5.5 mM glucose and 50 mg/l ascorbate, with pH adjusted to 7.4 with NaOH.

Recording conditions. Electrodes were fabricated from capillary tubing (model PG165T, Warner Instruments, and model 1B150F-3, World Precision Instruments) using a microelectrode puller (model P-87; Sutter Instruments). Electrodes were coated with silicone elastomer (Sylgard, Dow Corning) or 5% silanizing solution, and the tips were polished on a microforge (model MF 83; Narashige). The electrode solution for recording from gerbil hair cells contained (in mM) 145 NaCl, 5 KCl, 10 MgCl2, 0.02 CaCl2, 2 NaH2HPO4, 8 Na2HPO4, and 3 glucose for 32 min at 37°C. Tissue was then transferred to a solution of Leibovitz’s L-15 medium (Life Technologies) containing bovine albumin (1 mg/ml) for ≥50 min at room temperature. Hair cells were mechanically dissociated by drawing a probe across the surface of the epithelium immersed in L-15 medium in the recording chamber and viewed on a Nikon inverted microscope. Type I and type II vestibular hair cells were identified by their neck-to-plate and neck-to-body ratios (24).

RESULTS

KCn7 channel blockers reduce current in type II hair cells. Outward currents in vertebrate type II hair cells have previously been characterized as K+ selective with delayed rectifier, A-type, and Ca2+-activated K+ channel components (4, 11, 12, 15, 17, 18, 19, 27, 36). Figure 1 shows whole cell currents recorded from a dissociated type II gerbil semicircular canal hair cell in response to a series of depolarizing steps during control (Fig. 1A) and during superfusion of 2 μM linopirdine (Fig. 1B). This concentration was chosen initially, since previous results showed that linopirdine was a selective blocker of the M current in hippocampal neurons at <3 μM (30). Linopirdine reduced outward currents, unmasking currents with activation and inactivation kinetics that were faster than those of control (Fig. 1B). The currents blocked by linopirdine, obtained by subtraction of currents in the presence of linopirdine from control, are shown for three depolarizing steps in Fig. 1C. Peak currents and currents measured at the end of the 4-s pulse are shown in the current–voltage plot of Fig. 1D for control and in the presence of linopirdine. Current at 0.5 s into the pulse was reduced by an average of 44.1 ± 7.9% (n = 8) by 2 μM linopirdine in gerbil type II hair cells.

A slowly activating, slowly inactivating component of the whole cell current was also blocked by linopirdine in pigeon type II hair cells, as shown in the currents of Fig. 2. In the presence of 200 μM linopirdine, all currents above −60 mV were substantially reduced and a rapidly activating, rapidly inactivating current with characteristics of gKA remained. This was further examined as shown for the cell in Fig. 3. This cell had

ATTEMPT was made to compensate for series resistance, and the maximum voltage error due to the series resistance was <10 mV. Corrections were made for errors due to junction potentials.

Data analysis. Data were analyzed using the patch-clamp acquisition software described above and Sigmaplot (version 5.0; Jandel Scientific) and Origin (version 5.0; Microcal). No leak subtraction of records was performed. Values are means ± SD.

Chemicals and drugs. Stock solutions of linopirdine (Research Biochemicals) and XE991 (Dupont Pharmaceuticals, Wilmington, DE) were prepared in DMSO and 0.1 M HCl, respectively. Capsaicin was purchased from Sigma Chemical and dissolved in ethanol. The final concentration of DMSO or ethanol did not exceed 0.1%, and at this concentration, vehicles alone produced no significant change in the amplitude of outward currents in isolated type I and type II vestibular hair cells. 4-AP (Fluka) was added directly to the external solution. Solutions were applied to isolated gerbil cells via a series of flow pipes (380 μm ID) positioned close to the cell under study and removed by aspiration with a peristaltic pump. Solutions were applied to pigeon hair cell slices using a DAD superfusion system (ALA Scientific Instruments). The tip (100 μm ID) of the delivery electrode was placed ~50 μm from the target cell. Drugs were superfused onto the cell for 60 s before and during recordings. Drugs were washed off the cell by 180 s of superfusion with bath solution and exchange of the bath solution using a peristaltic pump (1 ml/min, bath volume 2 ml).

C474 KCN7 CHANNEL BLOCKERS AND VESTIBULAR HAIR CELL CURRENTS

Downloaded from http://ajpcell.physiology.org/ by 10.220.246.101 on October 13, 2017
a prominent $g_{K_A}$, as evidenced by the rapidly activating and rapidly inactivating current present after 250-ms prepulses to $-110$ mV. The $g_{K_A}$ was not blocked by linopirdine but could be inactivated by prepulses to $-30$ mV (Fig. 3A). The linopirdine-sensitive current after the prepulse to $-30$ mV is shown in the inset (C-L, Fig. 3A). During a 100-ms pulse from $-60$ to $-10$ mV, the current blocked by 200 $\mu$M linopirdine activated with a mean time constant ($\tau$) of $5.0 \pm 0.4$ ms ($n = 6$) and inactivated slowly ($\tau > 100$ ms when fitted with a single exponential). In a separate series of experiments, with $g_{K_A}$ inactivated, mean dose-response data values were fitted with a Hill equation, and the dissociation constant ($K_D$) for linopirdine was determined to be 2 $\mu$M (Fig. 3B).

The slow inactivation of the linopirdine-sensitive current was further studied in gerbil type II hair cells using pulses of several-seCONDS duration. In Fig. 4, the cell membrane potential was stepped to various prepulses for 10 s before it was stepped to a test potential of $-18$ mV for 8 s. Linopirdine-sensitive currents obtained during the test pulse are shown in Fig. 4A. The inactivation of currents during the test pulse following prepulses between $-136$ and $-77$ mV could be fitted well by the sum of two exponentials according to the following equation

$$I(t) = A + B \exp(-t/\tau_1) + C \exp(-t/\tau_2)$$

where $I$ is the current at time $t$, $A$, $B$, and $C$ are current amplitudes, and $\tau_1$ and $\tau_2$ are the decay time constants. Values for $\tau_1$ and $\tau_2$ were $-400$ ms and 3 s, as shown in the inset (Fig. 4A), and were relatively insensitive to voltage. The linopirdine-sensitive current activated with $\tau$ of 36.7 ± 3.2 ms at $-18$ mV ($n = 5$, not shown). The linopirdine-sensitive current also showed steady-state inactivation, and peak currents as a function of prepulse potential are shown in Fig. 4B. The solid line represents the fit to the data with the Boltzmann equation

$$I = I_{\max} \left[1 + \exp(V_{\text{half}} - V_m/S)\right]^{-1}$$

where $I$ is the peak current measured during a given test potential, $I_{\max}$ is the maximum current obtained, $V_{\text{half}}$ is the potential at which half-maximal activation occurred, and $S$ represents the voltage sensitivity of activation. The linopirdine-sensitive current in this cell was half-activated at the cell’s zero-current potential.

When screened against a range of K$^+$ channel types expressed in Xenopus oocytes, 10,10-bis(4-pyridinylmethyl)-9(10 H)-anthracenone (XE991) was found to be a more potent and selective blocker of KCNQ channels than linopirdine (35). The effects of three different concentrations of XE991 on gerbil type II hair cell currents are shown in Fig. 5A. This cell showed a slowly activating current with little inactivation over the 500-ms test pulse. Dose-response data indicated a mean $K_D$ of 4.8 $\mu$M for block of the current by XE991 (Fig. 4B). The effects of XE991 were reversible in four cells tested. When studied with long-duration pulses, the XE991-sensitive current inactivated with two time constants of $-0.3$ and 2 s. In addition, 5 $\mu$M XE991 reduced current in pigeon type II hair cells by 37.7 ± 30.0% ($n = 4$, range 16.5–58.9%) at the end of a 100-ms pulse to 0 mV (not shown).

Linopirdine and XE991 unmasked a rapidly activating and rapidly inactivating current and reduced the time to peak ($T_{\text{peak}}$) of outward currents in gerbil type II hair cells. $T_{\text{peak}}$ at $-8$ mV was reduced from 71.9 ± 24.2 to 19.7 ± 13.3 ms ($n = 10$) in the presence of linopirdine or XE991, and the difference was statisti-
Results from the prominent faster activation and inactivation. This probably re-
geon type II hair cells than in the gerbil, showing a
Macroscopic control currents were much faster in pi-
A were generally more responsive to blockers (Fig. 5
currents that showed the least amount of inactivation
y
pigeon type II hair cells. Data were fitted with the Hill equation:
concentration of blocker, 
K
at
(y
p,
\frac{1}{n}
D is the dissociation constant, and 
n is the
Hill coefficient, which was set to 1.0, resulting in 
K_D = 2.03 \mu M.
Currents were measured at 100 ms after a prepulse to −30 mV in 5 cells.

cally significant (paired \( t \)-test, \( P < 0.005 \)). Control
currents that showed the least amount of inactivation
were generally more responsive to blockers (Fig. 5A).
Macroscopic control currents were much faster in pi-
gon type II hair cells than in the gerbil, showing a
faster activation and inactivation. This probably re-
results from the prominent \( g_{KA} \) in pigeon cells, which
have previously been classified as “fast” (\( T_{peak} \) for a
step from −60 to 0 mV < 3.83 ms) or slow (\( T_{peak} > 3.83 \)
ms) (36). \( T_{peak} \) in pigeon cells was 6.1 ± 5.8 ms for
control currents and 3.3 ± 1.6 ms in the presence of
drugs (\( n = 13 \)), but the difference was not statistically
signiﬁcant.

Capsaicin block of current in gerbil type II hair cells.
Capsaicin is reported to block a component of \( I_K \) with
slow activation and inactivation kinetics in frog semicir-
cular canal hair cells (15). We also tested the effect
of capsaicin on currents in gerbil type II hair cells and
found a block similar to that produced by linopirdine
and XE991 (Fig. 6A). As shown in Fig. 6A, the capsai-
icin-sensitive current, like the linopiridine-sensitive
current, inactivated with a double-exponential time
course. Values for \( \tau_1 \) ranged from 330 to 664 ms and
values for \( \tau_2 \) from 1.9 to 7.3 s over a range of voltages
in four cells studied. Figure 6B shows that a combina-
tion of linopirdine and capsaicin produced a reduction
in current that was no greater than that produced by
10 \mu M linopirdine alone. Similarly, no additional re-
duction in current was observed when both capsaicin
and linopirdine were applied to a cell after capsaicin
alone, suggesting that the two drugs blocked the same
set of channels.

The dose dependence of the capsaicin block is shown
in Fig. 7. Figure 7A shows control current (unmarked
solid trace), currents during perfusion with 1 and 100
\mu M capsaicin, and recovery after washout of capsaicin.
Figure 7B shows dose-response data for peak currents,
and Fig. 7C shows dose-response data for currents at the end of a 500-ms pulse in response to a range of concentrations of capsaicin. The \( K_D \) was 10.1 \( \mu \text{M} \) for current measured at 0.5 s and 18.9 \( \mu \text{M} \) for current measured at peak. As was typically seen after linopirdine or XE991 application, a residual rapidly activating, rapidly inactivating current was evident after capsaicin application (Figs. 6 and 7 A).

Effects of KCNQ channel blockers on currents in type I vestibular hair cells. Figure 8 shows typical currents from a type I gerbil hair cell before and during application of 10 \( \mu \text{M} \) linopirdine. The large resting conductance is due to the presence of the signature type I hair cell current, \( I_{\text{KI}} \) (20, 26). Linopirdine reduced inward currents and instantaneous outward currents but had little effect on steady-state outward currents (Fig. 8, B and C). The effects of linopirdine on control currents are compared with those of 0.5 mM 4-AP, a known blocker of \( I_{\text{KI}} \) in Fig. 8C. At 200 \( \mu \text{M} \) linopirdine only slightly reduced steady-state outward currents measured at \(-50 \text{ mV}\) in pigeon and gerbil type II hair cells (Fig. 8D). \( I_{\text{KI}} \) is the main contributor to the outward current at this potential (20, 26). In pigeon and gerbil type I hair cells, inward currents were reduced by an average of 17.3 \( \pm \) 16.6\% (\( n = 4 \)) and 37.0 \( \pm \) 23.9\% (\( n = 6 \)), respectively, in the presence of 200 \( \mu \text{M} \) linopirdine. XE991 had no effect on steady-state outward currents measured between \(-10 \text{ and } 0 \text{ mV}\) in gerbil type I hair cells at concentrations up to 10 \( \mu \text{M} \) in six cells tested but reduced steady-state inward currents by an average of 55 \( \pm \) 30.9\% (data not shown). Linopirdine and XE991 were therefore not effective blockers of outward type I hair cell \( I_{\text{K}} \) but reduced inward currents through \( I_{\text{KI}} \) at hyperpolarized potentials. In addition, capsaicin (50 \( \mu \text{M} \)) had no effect on voltage-dependent currents in two gerbil type I hair cells tested.

DISCUSSION

Are KCNQ channels expressed in type II vestibular hair cells? In this work, we show that low concentrations of linopirdine and XE991 block a slowly inactivating component of the delayed rectifier current (\( I_{\text{K}} \)) in gerbil and pigeon type II vestibular hair cells. At comparable concentrations, these drugs have previously been characterized as selective inhibitors of K\(^+\) channels belonging to the KCNQ family (30, 35). Five members of this gene family have been described: KCNQ1, which associates with minK to form a slowly activating \( I_{\text{K}} \) (29, 33), KCNQ2 and KCNQ3, which coassemble to form the M channel (35), KCNQ4, which underlies an \( I_{\text{K}} \) in outer hair cells of the mammalian cochlea (10, 14), and KCNQ5, which coassembles with KCNQ3 and may also underlie certain types of M current (13). The IC\(_{50}\) values for XE991 blockade of expressed KCNQ1, KCNQ2, and KCNQ2 + KCNQ3 channels were reported to be <1 \( \mu \text{M} \) (35). This was at least an order of magnitude less than values for a variety of other K\(^+\) channels expressed in oocytes (35). However, regulatory \( \beta \)-subunits can alter current kinetics as well as their sensitivity to drugs. For example, coexpression of minK subunits with KCNQ1 produces channels that are less sensitive to drugs such as clofilium, XE991,
and the benzodiazepine R-L3 compared with KCNQ1 alone (28, 34, 37). Furthermore, species differences in the sensitivity of the slowly activating IsK to lanthanum have been reported (6).

IC$_{50}$ values for linopirdine, which blocks M channels directly (3), were >15 $\mu$M for non-KCNQ channel types and <10 $\mu$M for KCNQ1–3 (30, 35). The recently cloned KCNQ4 was not very sensitive to block by li-

Fig. 7. Dose dependence of capsaicin block in gerbil type II hair cells. A: currents after a test pulse to −8 mV for control, in the presence of 1 and 100 $\mu$M capsaicin, and after washout of the drug (thin trace). The inward current at −126 mV is due to the presence of an inward rectifier conductance. Dashed line, zero current. B: dose-response curve for capsaicin block of type II hair cell current measured at peak. Averaged data at 1 $\mu$M ($n = 3$), 5 $\mu$M ($n = 3$), 10 $\mu$M ($n = 4$), 20 $\mu$M ($n = 5$), 50 $\mu$M ($n = 6$), and 100 $\mu$M ($n = 5$) are shown; $K_D = 18.9$ $\mu$M and Hill coefficient (nonconstrained) = 0.9. C: dose-response data for current at the end of a 500-ms pulse; cells are the same as in $B$. $K_D = 10.1$ $\mu$M, and Hill coefficient (nonconstrained) = 1.0. Voltage protocol in $A$ was used.

Fig. 8. Effect of linopirdine on type I hair cell currents. Currents were recorded from a gerbil type I hair cell before ($A$) and after ($B$) application of 10 $\mu$M linopirdine. Linopirdine reduced inward currents and instantaneous outward currents. Cell was held at its zero-current potential of −78 mV and stepped through a range of potentials in 10-mV increments. $C$: current-voltage plots showing effects of linopirdine on steady-state outward currents (measured at the end of 100-ms pulses) for control (○) and linopirdine (●). For comparison, effects of 500 $\mu$M 4-AP, applied after removal of linopirdine, are also shown (●). Effects of linopirdine did not reverse after washout. Dashed line, zero current. $D$: effects of 200 $\mu$M linopirdine on outward currents in gerbil and pigeon type I hair cells. Cells were held at or close to zero current (typically −70 mV), and steady-state currents were measured after a step to −50 mV. Currents in the presence of linopirdine expressed as a percentage of control values for gerbil (G, $n = 6$) and pigeon cells (P, $n = 2$) are shown.
n opinidine when the homomeric channel was expressed, but a heteromeric channel formed by a combination of KCNQ3 + KCNQ4 was more sensitive to linopirdine block (10).

The linopirdine- and XE991-sensitive current in type II vestibular hair cells described here was recruited at potentials depolarized to −60 mV, activated relatively slowly, and showed a slow inactivation. Although \( I_K \) carried by KCNQ channels do not typically inactivate, a small inactivation was evident during long depolarizations for KCNQ2 and KCNQ3 channels expressed in Chinese hamster ovary cells (32). These channels are therefore possible candidates for the slowly inactivating current in type II hair cells. Alternatively, type II hair cells may express a new type of KCNQ channel or heteromers comprising a KCNQ family member with another class of \( K \) channel. Different members of the KCNQ family may coassemble to form functional ion channels, and a recent study suggests that the M-like current in NG108-15 cells may result from an association of KCNQ2/3 and mrg1a channels (31). In summary, the molecular species underlying the type II hair cell linopirdine-sensitive current remains to be described, but a KCNQ component is likely involved.

Capsaicin-sensitive current in type II hair cells. The effect of capsaicin on gerbil type II hair cells was similar to that of linopirdine and XE991; i.e., it blocked an inactivating component of the outward current in gerbil type II hair cells. Capsaicin blocks a variety of \( K \) channels, including \( K_v1.1, 1.2, 1.3, 1.5, \) and \( 3.1 \) (5), and was recently reported to block \( I_C \), the slowly inactivating component of \( I_K \) in frog hair cells (15). Capsaicin activates a nonselective cation channel in sensory neurons that mediates a sustained inward current at hyperpolarized potentials (1). The properties of the capsaicin-sensitive current reported here are inconsistent with those of a cation current. Furthermore, the observation that linopirdine and a combination of linopirdine and capsaicin block the same current strongly suggests that the capsaicin effect is specific to the \( K^+ \) channel in type II hair cells.

At least two channel types can now be defined as underlying the previously described 4-AP-sensitive \( I_K \) in mammalian type II hair cells (27), one of which is sensitive to linopirdine, XE991, and capsaicin. \( I_C \) in frog hair cells activated slowly (\( \tau = 12 \) ms at +40 mV), inactivated with two time constants of 300 ms and 4 s, and showed steady-state inactivation (15). The linopirdine- and capsaicin-sensitive current described here therefore closely resembles \( I_C \). The linopirdine-insensitive current has more rapid kinetic properties and is likely composed of \( g_K \).

Regional variations and the linopirdine-sensitive current. Across species, macroscopic currents in type II vestibular hair cells have been broadly divided into two classes: those with rapid activation and inactivation kinetics ("fast" cells) with a dominant \( I_A \) and those with slower kinetics ("slow" cells) with a dominant \( I_K \) (4, 11, 12, 17, 18, 23, 36). The different \( K^+ \) channels underlying these responses can be ascribed specific roles in the filtering of vestibular signals. For example, in response to current injections, the membrane potential of pigeon slow cells oscillated at lower frequencies than the membrane potential of fast cells (36). Furthermore, the quality of membrane resonance varied linearly with frequency only in type II hair cells with slow inactivation in the pigeon lagena (23). Our results suggest that the linopirdine-sensitive current contributes preferentially to the properties of slow cells. Because the zero-current potential for pigeon and mammalian type II hair cells averages about −55 mV (11, 12), this current will only become substantially activated after periods of hyperpolarization. The significance of the predominance of the slower conductance in central locations of the crista and utricle remains to be explored.

KCNQ channel blockers and type I hair cells. Type I hair cells have a low-voltage-activated \( K^+ \) conductance, \( g_K^{II} \), which bears some similarity to \( g_K^{III} \) in outer hair cells. Both conductances are active at rest, show a characteristic deactivation on stepping to hyperpolarized potentials, are relatively insensitive to externally applied tetraethylammonium, and in mouse are first expressed a few days after birth (2, 8, 14, 20, 27). KCNQ4 subunits are required for \( I_K^n \), which was reported to be very sensitive to block by linopirdine (\( K_D = 0.7 \) μM) (14). However, KCNQ4 channels expressed in oocytes were reportedly much less sensitive, with 200 μM linopirdine producing <40% block of the current (10). This discrepancy could be explained if KCNQ3, together with KCNQ4, underlies \( I_K^n \) in outer hair cells. Although we observed a reduction in inward currents carried through \( I_K^n \) in type I hair cells in response to linopirdine, outward currents were relatively resistant to block by the drug at concentrations as high as 200 μM. Coupled with the recent localization of KCNQ4 in the membranes of type I hair cells and calyces (9) and the relative insensitivity of KCNQ4 to linopirdine, these results suggest that KCNQ4 channels contribute to the resting conductance in type I vestibular hair cells.

We thank Brett Pirtle for technical assistance and Dr. Barry S. Brown for the gift of XE991. This work was supported by National Institutes of Health Grants DC-03287 (to K. J. Rennie) and DC-01273 (to M. J. Correia). A preliminary account of this work has been published in abstract form (22). Present address of T. Weng: Dept. of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77555-0641.

REFERENCES


