Voltage-Gated Outward K Currents in Frog Saccular Hair Cells

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Catacuzzeno, Luigi, Bernard Fioretti, and Fabio Franciolini. Voltage-gated outward K currents in frog saccular hair cells. J Neurophysiol 90: 3688–3701, 2003. First published September 10, 2003; 10.1152/jn.00308.2003. A biophysical analysis of the voltage-gated K (Kv) currents of frog saccular hair cells enzymatically isolated with bacterial protease VIII was carried out, and their contribution to the cell electrical response was addressed by a modeling approach. Based on steady-state and kinetic properties of inactivation, two distinct Kv currents were found: a fast inactivating I_A and a delayed rectifier I_DRK. I_A exhibited a strongly hyperpolarized inactivation V_1/2 (~83 mV), a relatively rapid single exponential recovery from inactivation (τ rec of ~100 ms at ~100 mV), and fast activation and deactivation kinetics. I_DRK showed instead a less-hyperpolarized inactivation V_1/2 (~48 mV), a slower, double-exponential recovery from inactivation (τ rec1 ~ 490 ms and τ rec2 ~ 4,960 ms at ~100 mV), and slower activation and deactivation kinetics. Steady-state activation gave a V_1/2 and 46.2 and 8.2 mV for I_A and ~48.3 and 4.2 mV for I_DRK. Both currents were not appreciably blocked by bath application of 10 mM TEA, but were inhibited by 4-AP, with I_DRK displaying a higher sensitivity. I_DRK also showed a relatively low affinity to linopirdine, being half blocked at ~50 μM. Steady-state and kinetic properties of I_DRK and I_A were described by 2nd- and 3rd-order Hodgkin–Huxley models, respectively. The goodness of our quantitative description of the Kv currents was validated by including I_A and I_DRK in a theoretical model of saccular hair cell electrical activity and by comparing the simulated responses with those obtained experimentally. This thorough description of the I_DRK and I_A will contribute toward understanding the role of these currents in the electrical response on this preparation.

INTRODUCTION

Sensory epithelia of inner ear organs transduce mechanical stimuli into electrical responses, a process involving the highly specialized hair cells. In many hair cells of lower vertebrates, injection of depolarizing currents evokes damped voltage oscillations, whose frequency and quality factor are tuned to the frequency selectivity and sharpness of the sensory organ (Crawford and Fettiplace 1981; Fettiplace and Fuchs 1999). The characteristics of the oscillatory response vary among tissues and are strictly dependent on the types of ion currents present in each specific hair cell. In general, the interplay between depolarizing currents (i.e., mechanotransducing non-selective cation currents and Ca currents) and repolarizing currents (i.e., Kv currents, inward-rectifying K and large-conductance Ca-activated K (BK) currents) shapes the oscillatory response. At least 2 types of Kv current have been described in hair cells, differing primarily in their inactivation properties.

The transient K_+ current (I_A) exhibits relatively fast inactivation kinetics, and a steady-state inactivation midpoint V_1/2 of ~80 to ~90 mV (Griguer et al. 1993; Hudspeth and Lewis 1988a; Lang and Correia 1989; Lewis and Hudspeth 1983; Masetto et al. 1994; Murrow and Fuchs 1990; Smotherman and Narins 1999a, b). At the normal resting potential of the hair cell I_A will be mostly unavailable, and its relevance to the cell’s electrical behavior has been questioned (Hudspeth and Lewis 1988a; Murrow and Fuchs 1990; Smotherman and Narins 1999a; but see Masetto et al. 1994). The other Kv conductance, reported for several hair cell preparations, displays slow and sometimes incomplete inactivation. Its inactivation V_1/2 varies among hair cells by as much as 40 mV (range from ~50 to ~90 mV), suggesting that the molecular counterpart of this current is not uniform among different hair cells. This current is usually referred to as delayed rectifier K current (I_DRK; Fuchs and Evans 1990; Goodman and Art 1996; Lang and Correia 1989; Marcotti et al. 1999; Masetto et al. 1994; Smotherman and Narins 1999a; Sugihara and Furukawa 1995). In the tissues where this current is available at physiologically relevant membrane potentials, its role in the control of the electrical response has been proposed and supported by pharmacological tests (Armstrong and Roberts 1998; Goodman and Art 1996).

The frog sacculus has been extensively used as an experimental model to investigate the role of basolateral ion channels in the electrical response of hair cells (Ashmore 1983; Holt and Eaton 1995; Hudspeth and Lewis 1988a, b; Lewis and Hudspeth 1983). Most studies have used papain-dissociated frog saccular hair cells, where 2 ion conductances are activated in response to depolarizing pulses from the resting potential: a voltage-gated Ca current and a BK current (Holt and Eaton 1995; Hudspeth and Lewis 1988a; Lewis and Hudspeth 1983; Roberts et al. 1990). An additional fast-inactivating I_A was also reported, but was recruited only if depolarizations were preceded by hyperpolarizing conditioning pulses, indicating that this current was normally not functional at the cell’s resting potential (Hudspeth and Lewis 1988a; Lewis and Hudspeth 1983). There was no evidence for I_DRK in these papain-dissociated hair cells. Accordingly, both experimental data and modeling studies indicated that the interplay between voltage-gated Ca currents and BK currents was sufficient to account for the electrical responses recorded from these cells (Holt and Eaton 1995; Hudspeth and Lewis 1988b). The frequency response of these papain-dissociated hair cells (80–160 Hz; Armstrong and Roberts 1998; Holt and Eaton 1995; Lewis...
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and Hudspeth 1983) was, however, markedly higher than the frequency range the organ in situ is tuned for (20–100 Hz; Koyama et al. 1982; Lewis 1988; Yu et al. 1991). This discrepancy led a number of investigators to conclude that the electrical characteristics of these cells were not major determinants in frequency discrimination (Eatock et al. 1993; Lewis 1988).

Recently it was found that papain alters the electrophysiological properties of saccular hair cells. Frog saccular hair cells in undissociated (in situ) epithelial preparations, subjected to neither enzymatic treatment nor mechanical cell dissociation, possess a transient IBTX-sensitive BK current and a sustained (or slowly inactivating) 4-AP-sensitive Kv current, in addition to the A- and sustained BK currents (Armstrong and Roberts 1998). In situ hair cells display in addition a resonant frequency response matching the tuning frequency range of the organ in vivo (Armstrong and Roberts 1998), stimulating intense interest in these newly reported K currents. However, whereas the partially inactivating BK current has been the subject of extensive investigation (Armstrong and Roberts 2001), the sustained, 4-AP–sensitive Kv current has, to our knowledge, been neglected.

We recently reported that the isolation of frog saccular hair cells with the bacterial protease VIII instead of papain preserves the pattern of outward K currents and the electrical response of the undissociated (in situ) hair cells (Catacuzzeno et al. 2003). This isolation procedure has allowed us to investigate the biophysical properties of the Kv currents of frog saccular hair cells to a level of detail required to model the electrical response of these cells.

METHODS

Hair cell preparation

Frogs (Rana esculenta) obtained from local suppliers were chilled and decapitated according to the Animal Experimentation guidelines of the University of Perugia. The dissociation of hair cells was described previously (Catacuzzeno et al. 2003; Holt et al. 2001). Briefly, the saccular epithelium was removed from the organ, and incubated for 3 min in a low-Ca solution containing 0.25 mg/ml protease VIII (P-5380, Sigma). The epithelium was then transferred to a low-calcium solution containing 0.5 mg/ml BSA for 15 min to stop the enzymatic reaction, and subsequently into a petri dish where the hair cells were mechanically dissociated by gently rubbing the saccular epithelium with a fine tungsten filament. For electrophysiological recordings hair cells were transferred to concanavalin A–coated petri dishes to allow cell adhesion. The experiments described in this study were all carried out on cylindrical hair cells, the most abundant cell type in this preparation (Chabbert 1997).

Electrophysiology

Macroscopic currents were recorded using the perforated-patch method (Horn and Marty 1988). Borosilicate pipettes (Hilgenberg GmbH, Malsfeld, Germany), pulled with a programmable puller (PUL-100; WPI, Sarasota, FL) were used. Their resistance ranged between 3 and 6 MΩ when filled with standard pipette solution. Electrical access to the cytoplasm was obtained by adding amphotericin B to the pipette solution. Stock solutions of amphotericin B (A-4888, Sigma; 50 mg/ml in DMSO) were stored at −20°C for a maximum of 8 h. The working solution of amphotericin B (4 μl of stock per ml of pipette solution) was prepared about every 40 min and kept at 0°C in the dark. A series resistance Rs of 20–30 MΩ (measured using the Membrane Test routine of the pClamp software) was usually achieved within 15 min of attaining the cell-attached configuration. Although at least 50% of the Rs was compensated, a significant uncompensated Rs component remained (ranging between 10 and 15 MΩ), which would introduce significant errors in the applied (command) voltage, Vcom, when large currents were recorded. Vcom was thus always corrected for errors attributed to Rs by subtracting IRs (i.e., the amount of voltage drop across Rs, where I is the current being measured): Vreal = Vcom − IRs. The voltage applied was also corrected for the liquid junction potential, estimated to be −13 mV under our recording conditions using the method developed by Neher (1992). Currents were amplified with a List EPC7 amplifier (List Medical Instruments, Darmstadt, Germany), and digitized with a 12-bit A/D converter (DigiData 1200 interface; Axon Instruments, Union City, CA). The pClamp software package (version 7.0; Axon Instruments) was used on a Compaq Pentium PC for generating the command voltage pulses, recording and archiving the currents, and preliminary analysis of the data. For on-line data collection, current signals were normally filtered at 5 Khz and sampled at 25–50 μs/point. All recordings and procedures were performed at room temperature (18–22°C).

Solutions and pharmacological agents

The low-Ca solution used for the cell dissociation procedure contained (in mM): 110 Na, 2 K, 0.05 Ca, 110 Cl, 3 d-glucose, 5 HEPES. The physiological salt solution (PSS) used for current clamp experiments contained (in mM): 112 Na, 2 K, 1.8 Ca, 0.7 Mg, 59 Mg, 119 Cl, 3 d-glucose, 5 MOPS. Frog saccular hair cells possess a variety of ion currents, including voltage-gated K and Ca currents, Ca-activated K (mainly BK) currents, and hyperpolarization-activated currents (Armstrong and Roberts 1998, 2001; Catacuzzeno et al. 2003; Holt and Eatock 1995; Hudspeth and Lewis 1988a; Lewis and Hudspeth 1983; Roberts et al. 1990). To isolate the Kv current from the Ca current and the coupled BK current under voltage-clamp the external Ca was lowered to 100 μM (replaced with Mg), and the Ca channel blocker Cd (100 μM) was added to the bath solution. Under these conditions, the Ca current and the BK current were fully suppressed, as indicated by the lack of effect on the macroscopic outward current of the BK channel blocker IBTX (200 nM; n = 4), and reduction of external Ca to 10 μM (n = 4). Previous studies on hair cells have demonstrated that millimolar concentrations of Cd significantly affected steady-state properties of Kv currents (Smotherman and Narins 1999a, b). Although we also observed an effect of millimolar Cd on Kv current gating on our preparation, dedicated experiments clearly indicated that the low-Cd concentration we used (100 μM) had no effect on the steady-state current parameters (n = 6). To exclude the possibility that hyperpolarization-activated currents could make a significant contribution in the voltage range tested only hair cells with high membrane resistance at −90 mV (Rs > 1 GΩ) were used. Solutions containing 4-AP and TEA were prepared by equimolar substitution for NaCl. The standard pipette solution contained (in mM): 114 K, 114 aspartate, 0.08 Ca, 4 Cl, 2 Mg, 5 MOPS, 1 EGTA. All solutions were adjusted to a pH of 7.25. All reagents were from Sigma (St. Louis, MO), with the exception of IBTX, which was obtained from Alomone Labs (Israel).

Data analysis

The time course of the current, as well as kinetic and steady-state parameters were fitted with the indicated equations by using the Simplex algorithm incorporated in Microcal Origin v 4.1. The χ² statistic was used as an indicator of the quality of the fit (Dempster 1993). Unless otherwise indicated, χ² values for the fits to the experimental data shown in the RESULTS section correspond to levels of significance probability lower than 0.05 (the degrees of freedom being given by nobs − np, where nobs is the number of experimental points.
used in the fitting procedure and $n_i$ is the number of free parameters). A comparison of the $\chi^2$ values was used to ascertain the order of the Hodgkin–Huxley model ($N$) chosen to describe $I_A$ and $I_{DARK}$. The number of exponential components necessary to fit the inactivation time courses was determined by comparing the sums of squares generated for the different fits, using the quantity $F = (S_p - S_g)(n - k_s)/(S_gk_s)$, where $S_p$, $S_g$, and $k_s$, $k_g$ are, respectively, the residual sum of squares and number of parameters for each consecutive model (i.e., $f$ and $g$), and $n$ is the number of points in the data set (cf. Dempster 1993). The higher-order model $g$ was accepted if the quantity $F$ was higher than the value of the $f$-distribution with $k_f$ and $n - k_g$ degrees of freedom for a level of significance probability of 0.05.

Results are expressed as means ± SE. Statistical differences between means were analyzed using the $t$-test, which does not assume equal variances. Where appropriate the significance level of probability ($P$) for the difference between mean values are given.

**Hodgkin–Huxley modeling of the $K_v$ currents**

In this study we describe the 2 $K_v$ currents, $I_A$ and $I_{DARK}$, with the Hodgkin–Huxley model (Hodgkin and Huxley 1952). This formalism assumes that an ion channel has one or more independent gates, each of which can reside in a permissive ($m$) or in a nonpermissive (“$1 - m$”) state, according to the following kinetic scheme


$$
\frac{dm(V,t)}{dt} = \alpha [1 - m(V,t)] - \beta m(V,t)
$$

or, alternatively


$$
\frac{dm(V,t)}{dt} = \frac{m(V,\infty) - m(V,t)}{\tau(V)}
$$

where $m(V,\infty)$, the steady-state probability of the $i$th gate being in the $m$ state, and $\tau(V)$, the relaxation time constant of the kinetic scheme, are related to $\alpha$ and $\beta$ by the relationships

$$
m_i(V,\infty) = \frac{\alpha}{\alpha + \beta} \quad \text{and} \quad \tau(V) = \frac{1}{\alpha + \beta}
$$

If membrane voltage does not change with time (i.e. under voltage-clamp conditions) Eq. 2 can be analytically solved to give (Connor and Stevens 1971)

$$
m_i(V,t) = m_i(V,\infty) + [m_0 - m_i(V,\infty)] e^{-[\alpha[V(t)]/\tau(V)]}
$$

where $m_0$ is the probability of $m$ at $t = 0$.

**MODELING THE $I_A$**. $I_A$ was described by a combination of 3 activation gates and 2 inactivation gates, according to the following relationship

$$
I_A(V,t) = m_1(V,t)[a_1(V)h_1(V,t) + [1 - a_1(V)]h_2(V,t)]
$$

$$+ [1 - a_1(V)]h_2(V,t)]P_A \frac{F^2}{RT} V \frac{K_e - K_o e^{-\psi(V)}}{1 - e^{-\psi(V)}}
$$

where $P_A$ is the maximal $K$ permeability through $A$ channels, $m_1(V,t)$, $h_1(V,t)$, and $h_2(V,t)$ were computed according to Eq. 2, with

$$
m_1(V,\infty) = [1 + e^{-[\psi(V)/k_2]}]^{-1}
$$

$$
\tau_2(V) = a_2 e^{[\psi(V)/k_3]}
$$

$\rho_2(V) = h_2(V,\infty) = [1 + e^{-[\psi(V)/k_3]}]^{-1}

and $\tau_{3a1} = 300$ ms at all membrane potentials.

$a_1(V)$ was found to follow a Boltzmann-like relationship

$$
a_1(V) = \frac{1 - C_a e^{-[\psi(V)/k_4]}}{1 + e^{-[\psi(V)/k_4]}} + C_a
$$

All the parameters in the right-hand side of Eqs. 5, 6, and 8–12 were determined experimentally, and are presented in Table 1.

**Modeling the electrical response of hair cells**

The electrical (voltage) response in the absence of BK currents (i.e., in the presence of IBTX) was modeled by solving the following current-clamp equation, that includes all the other major ion currents of this preparation

$$
-C_m \frac{dV(t)}{dt} = I_A(V, t) + I_{DARK}(V,t) + I_{K1}(V,t) + I_{K2}(V,t)
$$

$$+ I_{L}(V, t) + I_{L}(V, t) - I_{con}(t)
$$

where $I_{con}(t)$ is the current flowing through the model circuit; $C_m$ is the cell membrane capacitance; $I_{Ca}(V, t)$ is the voltage-gated $Ca^{2+}$ current; $I_{K1}(V,t)$ and $I_{K2}(V,t)$ are the hyperpolarization-activated K and cationic currents, respectively; and $I_L(V)$ is the leakage current. $I_A(V,t)$ and $I_{DARK}(V,t)$ were modeled according to Eqs. 4–12 described above.
The other currents were modeled using the parameters taken from published results for this preparation (Armstrong and Roberts 1998; Holt and Eatock 1995; Hudspeth and Lewis 1988a). A brief description of these currents can be found in the APPENDIX.

Modeling of membrane potential changes was performed with programs implemented in C, solving Eqs. 2, 4–13, and A1–A7 by a 4th-order Runge–Kutta algorithm (Press et al. 1992) with a fixed step size of 10 μs. A 10 times reduction in the time step used for the computation did not appreciably change the simulated curves. The current parameters reported in Table 1 were used. \( P_{\text{DRK}}, P_{\text{A}}, g_c \), and \( C_m \) were estimated from the same saccular hair cell used to compare the simulated versus experimental experimental response. In particular \( P_{\text{DRK}} \) and \( P_{\text{A}} \) were estimated by assessing the early and late inactivating current by applying inactivation protocols similar to that shown in Fig. 1, and \( g_c \) was assessed from the current amplitude at the K equilibrium potential. Finally \( K_c \) and \( g_s \) were adjusted by visual inspection until a well reproducible voltage response was obtained. For the assessment of \( I_{\text{DRK}} \) and \( I_A \) activity during electrical (oscillatory) response of a saccular hair cell (cf. Fig. 11), Eqs. 4 and 7 were solved using a voltage trajectory experimentally recorded from a saccular hair cell bathed in PSS.

RESULTS

Two distinct \( K_v \) currents are present in frog saccular hair cells

Under the conditions used to isolate the voltage-gated \( K_v \) current (cf. METHODS), 1.2-s depolarizing pulses from \(-70 \) to \(+30 \) mV, from a holding potential of \(-90 \) mV, evoked voltage-gated outward currents characterized by a relatively fast but incomplete inactivation (Fig. 1, A and B), suggesting the presence of multiple current components. To explore this possibility, steady-state inactivation protocols, consisting of a series of 30-s prepulses from \(-120 \) to \(-20 \) mV, in steps of 10 mV, followed by a test pulse to \(-10 \) mV were applied (Fig. 1C). With very negative prepulses (< \(-90 \) mV), the test current at \(-10 \) mV rapidly reached a peak and then declined to about 50% by the end of the pulse. With less negative prepulse voltages the evoked current would progressively decrease its inactivating component, until at a prepulse voltage of \(-60 \) mV no transient component could be recorded. In contrast, within the prepulse range \(-120 \) to \(-60 \) mV, the sustained component was unaltered (Fig. 1C). Further depolarization of the prepulse now resulted in a progressive decrease of the noninactivating component that reached about 10% of the peak current with a prepulse of \(-20 \) mV. Figure 1D shows current density versus conditioning voltage for the cell shown in Fig. 1C. Current densities were measured at the peak, and after 1.2 s from the beginning of the depolarizing pulse, when current inactivation had stabilized. The decrease in peak current versus prepulse voltage was described by 2 distinct phases (closed symbols, Fig. 1D), indicating the presence of at least 2 inactivation processes with different stabilities. Data were well fitted (solid line) by the following double Boltzmann function

\[
I_p = \frac{a}{1 + e^{V/V_{1/2a}}} + \frac{b}{1 + e^{V/V_{1/2b}}} + c
\]

This behavior was observed in all cells tested (\( n = 10 \)), with mean values for half inactivation voltage and voltage steepness for the 2 Boltzmann components: \( V_{1/2a} = -83 \pm 1.7 \) mV, \( V_{1/2b} = -48.6 \pm 2.0 \) mV, \( k_a = 3.9 \pm 0.2 \) mV, and \( k_b = 4.5 \pm 0.3 \) mV \((\text{mean} \pm \text{SE})\). \( a, b, \) and \( c \), the current density amplitudes of the 2 inactivating components, and of the noninactivating portion of the current had mean values of \( 39 \pm 5, 40 \pm 8, \) and \( 10.1 \pm 1.5 \) pA/pF, respectively. Current densities taken at 1.2 s from the beginning of the depolarizing pulse inactivated with a single phase (open symbols, Fig. 1D). The data points were well fitted by a single Boltzmann component (dotted line) with a mean \( V_{1/2a} \) of \(-48.7 \pm 1.9 \) mV and \( k \) of \( 4.8 \pm 0.4 \) mV, values not significantly different from those of the inactivating component with more depolarized \( V_{1/2a} \) as measured at the peak current \((P > 0.1)\). The noninactivating component was \( 11.2 \pm 1.8 \) pA/pF. These results indicate the presence of 2 outward \( K_v \) currents principally distinguished by their inactivation parameters. Specifically, a fast inactivating \( K_v \) current \( I_A \), already described in papain-dissociated saccular hair cells, and in other hair cell preparations (Griguer et al. 1993; Hudspeth and Lewis 1988a; Lang and Correia 1989; Lewis and Hudspeth 1983; Masetto et al. 1994; Murrow and Fuchs 1990; Smotherman and Narins 1999a, b), and a slowly activating and inactivating \( K_v \) current \( I_{\text{DRK}} \), similar in appearance to the delayed rectifier \( K_v \) current observed in undissociated (in situ) saccular hair cells (Armstrong and Roberts 1998) and in chick and turtle basilar papilla (Fuchs and Evans 1990; Goodman and Art 1996).

All hair cells probed with the above protocol exhibited a significant noninactivating residual current that persisted even at the most depolarized conditioning pulses \((c \in \text{Eq. 14})\). Most of this residual current appeared to originate from incomplete
inactivation of $I_{\text{DRK}}$, attributed to insufficient prepulse duration. Cells held at $-10$ mV for more than 30 s displayed a residual, holding current smaller than that observed in the inactivation protocol shown above (cf. Fig. 5A). In addition the inactivation time course of $I_{\text{DRK}}$ revealed a very slow exponential component ($\tau \approx 20$ s, at $-30$ mV; cf. Fig. 4, D and E), that would be consistent with an incomplete inactivation for 30-s conditioning pulses used in the inactivation protocol.

**RECOVERY FROM INACTIVATION.** Further evidence for the presence of 2 distinct $K_v$ current components in saccular hair cells comes from their different rate of recovery from inactivation. Figure 2A shows current traces obtained from a voltage protocol that allows measurement of recovery from inactivation of the total $K_v$ current. The current was first inactivated by holding the cell at $-10$ mV. A subsequent repolarizing step to $-100$ mV of variable duration was then applied to allow recovery of the $K_v$ current, the amount of which was assessed by a final 1.2-s depolarizing test pulse. For short repolarizing pulses ($\leq 300$ ms) the recovered current evoked by the test pulse inactivated almost completely (Fig. 2A). For short repolarizing pulses ($\leq 300$ ms) the recovered current evoked by the test pulse inactivated almost completely (Fig. 2A). Increasing the duration of the repolarizing pulse revealed the presence and increased the amplitude of the sustained current. This complex behavior of recovery from inactivation of the $K_v$ current provides further evidence for the presence of 2 distinct $K_v$ current components, the $I_A$ and $I_{\text{DRK}}$.

The rates of recovery from inactivation of the $I_A$ and $I_{\text{DRK}}$ shown in Fig. 2A were assessed by measuring the current amplitudes at the peak and at 1.2 s, respectively (Fig. 2B). Recovery of the $I_{\text{DRK}}$ was measured at 1.2 s, when $I_A$ had fully inactivated, was fitted by a double-exponential function, with mean time constants of 491 ± 22 and 4,964 ± 1,183 ms, with the fractional contribution of the fast component being of 0.53 ± 0.09 ($n = 3$). The rate of recovery of the peak current, used to assess the recovery of $I_A$, but also containing the $I_{\text{DRK}}$, was described by the sum of 3 exponentials, 2 of which are associated with the sustained $I_{\text{DRK}}$. The additional 3rd exponential component resulting from the rate of recovery of $I_A$ is much faster, having a time constant of $90 \pm 15$ ms ($n = 3$). The fast, single-exponential recovery from inactivation of $I_A$ was confirmed by applying the stimulation protocol illustrated in Fig. 2C. Cells were held at $-60$ mV, a potential that will fully inivate $I_A$, but not alter $I_{\text{DRK}}$ (cf. Fig. 1). Recovery of $I_A$ was achieved by subjecting the cells to a $-100$-mV hyperpolarizing pulse of variable duration, and assessed by applying a depolarizing pulse to $+40$ mV. As shown in Fig. 2D, peak current versus hyperpolarizing pulse duration could be described by a single-exponential function (solid line) with a time constant of 102.6 ms ($n = 3$), a value close to that determined in Fig. 2, A and B. Based on the observed differences on steady-state and recovery from inactivation, the 2 outward $K_v$ current components can be isolated and their kinetic and pharmacological features studied in detail.

**Fig. 2.** Recovery from inactivation of $I_{\text{DRK}}$ and $I_A$: A: family of currents evoked with pulse protocol illustrated below. Cell was held at holding potential of $-10$ mV to fully inactivate $K_v$ current. Membrane was then repolarized to $-100$ mV for varying times (30, 100, 300, 1,000, and 3,000 ms; indicated in figure), and finally depolarized to $-10$ mV to evaluate amount of $K_v$ current recovered from inactivation. B: plot of recovery from inactivation of $K_v$ current vs. repolarizing pulse duration for peak current (squares) and current at 1.2 s from beginning of pulse at $-10$ mV (circles). Data points for recovery at 1.2 s were fitted with a double-exponential function as follows: $I_{\text{rec}(t)} = a[1 - \exp(-t/\tau_1)] + (1 - a)[1 - \exp(-t/\tau_2)]$. Best-fit parameters are $a = 0.62, \tau_1 = 527$ ms, and $\tau_2 = 6,147$ ms. Data points of peak current recovery could be fitted with triple-exponential function: $I_{\text{rec}(\text{peak})} = a[1 - \exp(-t/\tau_1)] + b[1 - \exp(-t/\tau_2)] + (1 - a - b)[1 - \exp(-t/\tau_3)]$, where $\tau_1$ and $\tau_2$ were held fixed to values found from fit of recovery data at 1.2 s. Best-fit free parameters are $a = 0.33, b = 0.059$, and $\tau_3 = 87$ ms. Inset: plot showing recovery functions over 0- to 50-ms interval for $I_{\text{DRK}}$ and $I_A$ obtained from fit described above. C: current traces evoked by 1.2-s depolarizations to $+40$ mV, after hyperpolarizing cell to $-100$ mV for varying times, from holding potential of $-60$ mV (which inactivated $I_A$). D: $I_A$ and $I_{\text{DRK}}$ fitted by single-exponential function having time constant of 102.6 ms (solid line).

**STEADY-STATE ACTIVATION.** The properties of $I_{\text{DRK}}$ were studied by stepping from a holding potential of $-70$ mV (where virtually all the $I_A$ is inactivated; cf. Fig. 1, C and D). Under these conditions depolarizing pulses from $-70$ to $+30$ mV evoked currents showing little or no inactivation during the first few hundred milliseconds (Fig. 3A) as expected for $I_{\text{DRK}}$. The voltage dependency of steady-state $I_{\text{DRK}}$ activation was determined from tail currents measured immediately after repolarization to $-60$ mV (Fig. 3A). Tail current amplitudes increased with depolarization up to about $-30$ mV (Fig. 3B). For depolarizations more positive than $-30$ mV, tail current amplitudes tended to decrease. In the voltage range $-70$ to $-30$ mV experimental data could be fitted by a Boltzmann relationship (solid line, Fig. 3B) with an activation $V_{1/2}$ of $-48.3$ mV and a slope factor $k$ of $4.19$ mV. Possible explanations for the observed tail current reduction at the more depolarized potentials include the presence of a fast, voltage-dependent inactivation gating or a voltage-dependent block of $I_{\text{DRK}}$ by an unknown intracellular component. We did not investigate this aspect further. Superimposing the steady-state $I_{\text{DRK}}$ activation and inactivation curve (Fig. 3B; dashed line, from Fig. 1D) reveals that the 2 processes develop over the same voltage range.

**ACTIVATION AND INACTIVATION KINETICS.** The activation time course of the current in response to depolarizing steps was sigmoidal, and could be well described by a Hodgkin–Huxley model with 2 activation gates over the voltage range examined (Fig. 4A, smooth lines). Deactivation time courses followed a single-exponential function (Fig. 4B). The calculated time con-
FIG. 3. Steady-state activation of $I_{\text{DRK}}$: A: family of currents evoked by depolarizing steps from $-70$ to $+30$ mV, from holding potential of $-70$ mV. Holding potential was chosen to obtain a nearly complete steady-state inactivation of $I_A$. B: plot of mean normalized tail currents (taken 250 µs after beginning of repolarizing pulse) vs. voltage obtained from 8 different experiments similar to that shown in A. Solid line represents best fit of experimental data (in voltage range $-70$ to $-30$ mV) with Boltzmann relationship $I_{\text{tail}} = I_{\text{tail(max)}}/[1 + \exp(-(V - V_{1/2})/k)] + b$. Parameters obtained are $I_{\text{tail(max)}} = 1.002, V_{1/2} = -48.3$ mV, $k = 4.19$ mV, and $b = -0.0133$. Dashed line represents mean steady-state inactivation curve of $I_{\text{DRK}}$ assessed as shown in Fig. 1D.

Inactivation of $I_{\text{DRK}}$ was very slow, requiring long duration depolarizing pulses to chart its progress (Fig. 4D). In all cells tested ($n = 8$) the inactivation time course approximated a double-exponential function (Fig. 4D, smooth lines). The fast time constant was voltage independent over the voltage range $-30$ to $+10$ mV, and had a mean value of $3.6 \pm 0.3$ s (Fig. 4E). The slow component had a small, but significant, voltage dependency, increasing from $20 \pm 2$ s at $-30$ mV to $29 \pm 3$ s at $+10$ mV ($P < 0.05; n = 8$; Fig. 4E). The amplitudes of the 2 fitted exponentials were also weakly, but not significantly, voltage dependent ($P > 0.1$; Fig. 4F), with the fast exponential component giving a fractional contribution of $0.53 \pm 0.12$ at $-30$ mV and of $0.41 \pm 0.13$ at $+10$ mV.

The $I_A$

$I_A$ was isolated from $I_{\text{DRK}}$ by 2 different methods, both giving similar results. The 1st method takes advantage of the faster time course of $I_A$ recovery from inactivation, as compared to $I_{\text{DRK}}$ (cf. Fig. 2). Hair cells were held at a holding potential of $-10$ mV at which both $K_v$ currents were fully inactivated. $I_A$ could be essentially isolated by applying depolarizing test pulses, preceded by a 100-ms conditioning pulse to $-100$ mV, sufficient to recover almost exclusively the $I_A$ (cf. inset of Fig. 2B). This protocol is shown in Fig. 5A, where a family of outward currents showing a high degree of inactivation were recorded. The 2nd method was to evoke outward $K$ currents from 2 different holding potentials, $-70$ and $-100$ mV, and revealed $I_A$ by subtraction of the resulting current traces. A pharmacological isolation was not available because of the lack of selective $I_{\text{DRK}}$ inhibitors (cf. next section).

STeady-state activation. The voltage dependency of $I_A$ activation was obtained by plotting the peak $K$ permeability at different voltages, as estimated from experiments similar to that shown in Fig. 5A ($n = 3$), or alternatively using the subtraction protocol ($n = 2$). $I_A$ activated over a wider voltage range than that needed for $I_{\text{DRK}}$, indicating a smaller voltage dependency of gating (Fig. 5B). Data points fitted by a Boltzmann relationship gave a $V_{1/2}$ of $-46.2$ mV and a voltage steepness $k$ of $8.2$ mV ($n = 5$, solid line). The dotted line in

FIG. 4. Activation and inactivation kinetics of $I_{\text{DRK}}$: A: family of currents evoked by depolarizing steps from $-50$ to $+30$ mV, from holding potential of $-60$ mV. Smooth, black lines are best fits to data with a 2nd-order Hodgkin-Huxley model. B: tail (deactivating) currents obtained by repolarizing membrane at varying voltages (from $-50$ to $-85$ mV, in 5-mV steps), after $I_{\text{DRK}}$ had been activated with a 400-ms voltage step to $-10$ mV. Holding potential was $-60$ mV. Smooth lines are best fits to data with a single-exponential function. C: plot of mean activation (closed symbols) and deactivation time constants ($\tau_A$, open symbols) vs. membrane potential, obtained from 8 experiments similar to those shown in A and B. Solid line represents best fit of experimental data with Eq. 6. Best-fit parameters are $P_0 = 0.00323$ s$^{-1}$; $P_1 = -20.9209$ mV; $P_2 = 0.003$ s$^{-1}$; $P_4 = 1.46674$ s$^{-1}$; $P_5 = 5.96571$ mV; $P_6 = 0.00928$ s$^{-1}$. $D$: family of current traces evoked by long (60-s) depolarizing steps at $-30$, $-10$, and $+10$ mV, from holding potential of $-90$ mV. Current decay in time interval 1–60 s was fitted with double-exponential function $I = A_f \exp(-\tau_f) + A_i \exp(-\tau_i) + A_{\text{tail}}$ (superimposed smooth lines). Best-fit parameters are as follows: trace at $-30$ mV: $\tau_f = 4.421$ ms, $A_f = 0.147$ nA, $\tau_i = 20.424$ ms, $A_i = 0.395$ nA, $A_{\text{tail}} = 0.013$ nA; trace at $-10$ mV: $\tau_f = 4.106$ ms, $A_f = 0.264$ nA, $\tau_i = 34.305$ ms, $A_i = 0.575$ nA, $A_{\text{tail}} = 0.119$ nA; trace at $+10$ mV: $\tau_f = 5.966$ ms, $A_f = 0.337$ nA, $\tau_i = 34.305$ ms, $A_i = 0.575$ nA, $A_{\text{tail}} = 0.119$ nA. $E$: plot of mean inactivation time constant vs. voltage obtained from 8 experiments similar to that shown in D. Fast component vs. voltage could be fitted by constant function of 3,623 ms. Slow component was fitted with relationship: $\tau_i = \tau_i(0) \exp(V/k)$. Best-fit parameters are $\tau_i(0) = 24.252$ ms and $k = 103$ mV. $F$: plot of fractional amplitude of fast exponential component vs. voltage. Data were fitted with relationship $a_i/I_{\text{tail}} + a_i = A_0 \exp(V/k) + A_i$. Best-fit parameters are $A_0 = 0.288$, $A_i = 0.242$, and $k = 85.6$ mV.
Fig. 5. Steady-state activation of $I_A$: A family of $I_A$ currents isolated by applying pulse protocol illustrated below that allowed isolation of $I_A$. B: plot of mean K permeability vs. voltage obtained from 5 different experiments, 3 of which were similar to that shown in A and 2 obtained by subtracting current traces obtained from holding potential of −70 and −100 mV. Permeability of $I_A$ was obtained as ratio between peak current and driving force for K ions, taken as $\frac{V}{\sqrt{2RT}}(K_e - e^{FV} / (1 - e^{FV}))$. Solid line represents best fit of experimental data with Boltzmann relationship $P_A = P_{A\text{max}} / (1 + \exp(-(V - V_{1/2})/k))$. Parameters obtained are $P_{A\text{max}} = 1.07 \times 10^{-13}$ L/s, $V_{1/2} = -46.2$ mV, and $k = 8.2$ mV. Dotted line represents best fit of experimental data with Boltzmann relationship $P_A = P_{A\text{max}} / (1 + \exp(-(V - V_{1/2})/k))^2$, with best-fit parameters $P_{A\text{max}} = 1.08 \times 10^{-13}$ L/s, $V_{1/2} = -61$ mV, and $k = 10.7$ mV. Dashed line represents steady-state inactivation of $I_A$ assessed in Fig. 1 (scaled to match $P_{A\text{max}}$).

Fig. 5B shows a fit of the experimental data with a 3rd-power Boltzmann relationship, giving a $V_{1/2}$ of −61 mV and a $k$ of 10.7 mV. Comparison of the $I_A$ activation and inactivation (dashed line, from Fig. 1D) curves shows that no significant overlap rate is observed, indicating that $I_A$ is not available within its activation voltage range.

**Activation and Inactivation Kinetics.** The time course of $I_A$ activation was fitted by a 3rd-order Hodgkin–Huxley model (Fig. 6A, smooth lines). The goodness of fit was poor for the higher voltage range examined ($P > 0.05$, $\chi^2$ test), and was not improved by increasing the order, suggesting that current activation at high voltages deviates from a simplified independent particle model. The resulting time constants were well described by single-exponential functions of voltage with values decreasing from 5.2 ± 1.8 ms at −50 mV to 1.5 ± 0.4 ms at +10 mV (open symbols and solid line in Fig. 6C). Deactivation kinetics were assessed from the time course of the tail currents obtained in response to varying repolarizing voltages after a 20-ms depolarization to +40 mV (the remainder of the pulse protocol was as outlined above). Tail currents had a single-exponential time course (Fig. 6B), with time constants increasing with voltage from 8.2 ± 1.3 ms at −75 mV to 13.3 ± 1.9 ms at −55 mV (Fig. 6C, closed symbols).

Inactivation of $I_A$ during depolarizing pulses followed a double-exponential time course (Fig. 7A, smooth lines). Both time constants were voltage independent over the voltage range examined, and had mean values of 75 ± 10 and 322 ± 48 ms (Fig. 7C, open symbols). The relative contribution of the fast exponential component changed with voltage, increasing at negative voltages (inset of Fig. 7C). At membrane potentials lower than −30 mV, current decay was described by a single-exponential function in all cells examined (applying a further component did not improve the fit). Inactivation rates at membrane potentials lower than the threshold for activation of $I_A$ were determined by evaluating the changes in peak current at +30 mV, following a conditioning prepulse of variable duration (50, 100, 300, 1,000 ms) at different subthreshold voltages (from −120 to −60 mV), from a holding potential of −90 mV. Figure 7B illustrates a typical experiment. Peak currents versus prepulse duration plots were described by a single-exponential function at all voltages examined (Fig. 7D), with the time constants showing a bell-shaped dependency on voltage (Fig. 7C, closed symbols).

**Pharmacology of the $K_e$ Currents.** Pharmacological tests on $K_e$ currents used a voltage protocol consisting of 2 successive depolarizing steps, the 1st from a holding potential of −60 mV that would activate only the $I_{DRK}$, the 2nd preceded by a 100-ms hyperpolarization to −120 mV, allowing $I_A$ to recover from inactivation (Fig. 8A). The effects of the blocking agents on $I_{DRK}$ were evaluated from the steady-state current activated by both depolarizing steps to +40 mV, whereas the effects on $I_A$ were evaluated from the peak current recovered after the hyperpolarizing step. Both current components were insensitive to 10 mM TEA ($n = 5$; $P > 0.1$; Fig. 8, A and E, top traces). In contrast, 4-AP blocked both $K_e$ currents (Fig. 8A, bottom traces), but with different affinities. 4-AP at 1 mM blocked most of the $I_{DRK}$ (the fractional inhibition of the steady-state current being 0.78 ± 0.06 at a membrane potential of −20 mV; $n = 3$; Fig. 8B), whereas it did not alter $I_A$. This is clearly shown by point-by-point subtraction of current traces, in control conditions and in the presence of 1 mM 4-AP (Fig. 8C), showing that 1 mM 4-AP-sensitive current does not possess fast inactivation. A 10-fold higher concentration of 4-AP was required to block $I_A$ (Fig. 8B, bottom traces and Fig. 8E). The mean fractional inhibition of the peak current by 10 mM 4-AP was 0.54 ± 0.06 ($n = 3$). This higher concentration

![Activation and deactivation kinetics of $I_A$. A: family of currents obtained in response to following pulse protocol, devised to isolate $I_A$. Cell was held at holding potential of −10 mV to fully inactivate both $I_A$ and $I_{DRK}$. Cell was then hyperpolarized to −100 mV for 50 ms to allow sole recovery of $I_A$, which was subsequently activated by depolarizing pulses from −50 to +10 mV. Solid lines represent a 3rd-order Hodgkin–Huxley model fit to currents. B: family of currents obtained by repolarizing membrane from −55 to −75 mV, in 5-mV steps, after activation of $I_A$ with a depolarizing pulse to +40 mV. Isolation of $I_A$ was obtained with a pulse protocol as described in A. C: plot of mean activation (open symbols) and deactivation (×3, closed symbols) time constant vs. voltage obtained from 3 different cells by applying activation and deactivation protocols as described in A and B. Solid lines represent best fits to experimental data with Eq. 9. Best-fit parameters are $a = 0.48$ ms, $b = −23.1$ mV, and $c = 1.14$ ms for activation time constants, and $a = 173.6$ ms, $b = 17.9$ mV, and $c = 5.4$ ms for deactivation time constants.](http://jn.physiology.org/content/90/6/3694)
of 4-AP also increased the rate of inactivation of \( I_A \) (Fig. 8B). In 2 of the 3 cells tested a substantial steady-state current remained even at 10 mM 4-AP (the mean fractional inhibition of the steady-state current with 1 mM 4-AP was not significantly different from that observed with 10 mM 4-AP; \( P > 0.1; \) cf. Fig. 8B and E), suggesting that a small 4-AP-insensitive, sustained current may contribute to \( I_{DRK} \).

We also tested the inhibitory effect of linopirdine on \( I_{DRK} \). This agent, a relatively selective inhibitor of the KCNQ channel family, has been reported to block both a delayed rectifier

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**FIG. 7.** Inactivation kinetics of \( I_A \): A: family of currents obtained by depolarizing membrane from \(-50\) to \(+30\) mV, in 10-mV steps. To isolate \( I_A \), cells were held at holding potential of \(-10\) mV to fully inactivate both \( I_A \) and \( I_{DRK} \). Membrane was then hyperpolarized to \(-100\) mV for 50 ms to allow sole recovery of \( I_A \). Superimposed solid lines are single-exponential (\(-50\) and \(-40\) mV) or double-exponential (from \(-30\) to \(+30\) mV) fits of decaying portion of current traces. B: families of currents obtained with 1.2-s depolarizing pulses to \(+30\) mV, after a conditioning pulse to varying voltages (from \(-120\) to \(-60\) mV, in 10-mV steps) and for variable durations (50, 100, 300, and 1,000 ms, as indicated in figure). Holding potential was \(-90\) mV. C: plot of mean inactivation time constants vs. test voltage obtained from 8 different cells by applying pulse protocols described in A (open symbols; \( n = 5 \)) and B and D (closed symbols; \( n = 3 \)). Solid line represents best fit of data points with Eq. 11, with \( C_1 = 74 \) ms; \( C_2 = 321.5 \) ms; \( C_3 = -82 \) mV; \( C_4 = 15.5 \) mV. Inset: plot of mean fractional amplitude of \( t_{inact} \) vs. voltage. Smooth line represents fit of data points with Eq. 12, with \( C'_1 = 0.54 \), \( V_{1/2} = -21.5 \) mV, and \( I_\infty = 15.6 \) mV. D: plot of peak current at \(+30\) mV vs. conditioning pulse duration for varying conditioning voltages. Cell is same as that used in B. Solid lines represent best fit of data points with single-exponential functions. Resulting time constants at varying conditioning voltages are: 61.6 ms at \(-120\) mV; 85 ms at \(-110\) mV; 127.4 ms at \(-100\) mV; 388 ms at \(-80\) mV; 169.4 ms at \(-70\) mV; 103 ms at \(-60\) mV.

**FIG. 8.** Pharmacology of the \( K_\alpha \) currents. A, B, D: representative current records showing effect of 10 mM TEA (A), 1 and 10 mM 4-AP (B), and 5, 10, and 50 \( \mu \)M linopirdine (D) on \( K_\alpha \) current of frog saccular hair cells. Stimulation protocol consisted of repeated stimuli of 2 depolarizing steps separated by a 100-ms repolarization pulse to \(-120\) mV to selectively recover \( I_A \) (A and B) or of a single depolarizing step to 0 mV (D). Holding potential was \(-60\) mV. C: families of currents obtained by applying depolarizing steps from \(-80\) mV to \(+30\) mV, in 10-mV steps, from holding potential of \(-90\) mV, in control conditions (top panel), and after addition of 1 mM 4-AP to bath (middle panel). Family of currents in bottom panel is point-by-point subtraction of current traces in 1 mM 4-AP from current traces in control conditions. E: bar histogram showing mean blocking efficacy of TEA, 4-AP, and linopirdine on \( I_{DRK} \) and \( I_A \). Blocking efficacy on \( I_{DRK} \) was assessed from current amplitude at end of 1.2-s-long depolarizing pulse before and after addition of agent. Blocking efficacy on \( I_A \) was evaluated from difference in \( I_A \) before and after addition of drug. \( I_A \) in control and test conditions was assessed from current amplitudes of 2nd and 1st depolarizing pulses, measured 20 ms from beginning of each pulse.
current component of gerbil and pigeon type II hair cells (Rennie et al. 2001), and mammalian inner and outer hair cells (Marcotti and Kros 1999; Oliver et al. 2003) with high affinity (IC_{50} \leq 5 \mu M). At 5 \mu M, linopirdine did not inhibit the I_{DRK} significantly (Fig. 8, D and E), whereas higher concentrations (10 and 50 \mu M) produced a significant block (Fig. 8, D and E).

**Model results**

The validity of the quantitative description of the I_{A} and I_{DRK} was tested by modeling both the macroscopic K_{s} currents and the electrical response of saccular hair cell. Figure 9 compares the experimental K_{s} currents obtained by depolarizing steps from \(-50\) to \(-10\) mV, in 10-mV steps, from holding potentials of \(-90\) mV (top traces), or \(-60\) mV (bottom traces). I_{A} and I_{DRK} were modeled by assuming a 2nd- and 3rd-order Hodgkin-Huxley model, respectively (cf. METHODS).

## Table 1. Kinetic and steady-state parameters of the ionic currents used to model the electrical response of frog saccular hair cells

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**FIG. 9.** Modeling of the K_{s} current of frog saccular hair cells. Experimental (left) and modeled (right) currents obtained by applying depolarizing steps from \(-50\) to \(-10\) mV, in 10-mV steps, from holding potentials of \(-90\) mV (top traces), or \(-60\) mV (bottom traces). I_{A} and I_{DRK} were modeled by assuming a 2nd- and 3rd-order Hodgkin-Huxley model, respectively (cf. METHODS).
Contribution of \( K_v \) currents to the electrical response of frog saccular hair cells

The contribution of \( I_{\text{DRK}} \) to the electrical response of these cells was tested using 4-AP. 4-AP at 1 mM blocks most of the \( I_{\text{DRK}} \) and voltage-gated Ca currents \( (I_{\text{Ca}}) \), modeled according to the experimental data obtained previously in these cells (Armstrong and Roberts 1998; Holt and Eatock 1995; Lewis and Hudspeth 1988a; cf. APPENDIX). These results indicate that a Hodgkin–Huxley formalism of the \( I_A \) and \( I_{\text{DRK}} \) can be used in future investigation of saccular hair cell electrical activity.

**DISCUSSION**

**General**

In spite of the functional relevance of \( K_v \) currents in the electrical response of frog saccular hair cells, their major biophysical properties have remained largely unexplored. This situation has most likely arisen from the distortion of the \( K_v \) currents associated with the commonly used papain-based enzymatic isolation procedure. Using an isolation method that we showed to preserve the in situ properties of the \( K_v \) currents (Catacuzzeno et al. 2003), we have attempted to provide a quantitative description of these currents, a prerequisite to understanding their role in shaping the electrical response of hair cells. Based on steady-state and kinetic properties of inactivation, 2 distinct \( K_v \) currents could be discerned: a fast inactivating \( I_A \), and a delayed rectifier \( I_{\text{DRK}} \). \( I_A \) exhibited a strongly hyperpolarized inactivation \( V_{1/2} = -83 \text{ mV} \) (Fig. 1), a relatively rapid single-exponential recovery from inactivation \( (\tau_{\text{rec1}} \approx 490 \text{ ms}) \), and fast activation and deactivation kinetics (Fig. 6). \( I_{\text{DRK}} \) showed instead a less-hyperpolarized inactivation \( V_{1/2} = -48 \text{ mV} \) (Fig. 1), a slower, double-exponential recovery from inactivation \( (\tau_{\text{rec1}} \approx 490 \text{ ms} \) and \( \tau_{\text{rec2}} \approx 4960 \text{ ms} \) at \(-100 \text{ mV} \)), and slower activation and deactivation kinetics (Fig. 4). The inactivation time course of both \( I_A \) and \( I_{\text{DRK}} \) was double exponential, suggesting that multiple channel types participate in each current component, or alternatively that these currents have complex inactivation gating. Both current components were not significantly inhibited by TEA up to a concentration of 10 mM, but were blocked by 4-AP, with \( I_{\text{DRK}} \) being more sensitive than \( I_A \) to this agent (Fig. 8).

Both these currents have already been reported in a variety of auditory and vestibular hair cell preparations. Slowly activating and inactivating \( I_{\text{DRK}} \) have been studied in hair cells of many inner ear organs, including turtle and chick cochleas, goldfish saccule, frog semicircular canals (SCC) and basilar papilla, and pigeon semicircular canals (Fuchs and Evans 1990; Goodmann and Art 1996; Lang and Correia 1989; Masotto et al. 1994; Smotherman and Narins 1999a; Sugihara and Furukawa 1995). In frog saccule, the \( I_{\text{DRK}} \) has only been reported to be present, no investigation being carried out to define its major properties (Armstrong and Roberts 1998; Catacuzzeno et al. 2003). The \( I_{\text{DRK}} \) we studied here shares several properties with the slowly inactivating \( K_v \) currents of goldfish oscillatory type saccular hair cells (Sugihara and Furukawa 1995) and turtle and chick cochlear hair cells (Fuchs and Evans 1990; Goodmann and Art 1996), that is, high sensitivity to 4-AP, slow inactivation rate, activation range –60 to –20 mV,
and inactivation $V_{1/2} \sim -50$ mV. It differs from the $I_{DRK}$ of frog SCCs and from frog basilar papilla (another auditory organ of the frog). In both preparations it has been reported to have an inactivation $V_{1/2} \sim -90$ mV, that is, about 40 mV more hyperpolarized than the $I_{DRK}$ studied here (Masetto et al. 1994; Smotherman and Narins 1999a). The $I_{DRK}$ of frog SCC hair cells differed also for being insensitive to 4-AP concentrations as high as 10 mM (Marcotti et al. 1999; Masetto et al. 1994). Notably, this comparison shows that hair cells deriving from functionally similar and spatially very close inner ear organs from the same species (such as frog basilar papilla and saccus) possess different $K_v$ channels underlying $I_{DRK}$.

Fast transient $I_A$ has been found in many hair cells, including frog SCCs, basilar and amphibian papilla, chick cochleas, mouse utricle and pigeon SCCs (Griguer et al. 1993; Lang and Correia 1989; Lennan et al. 1999; Masetto et al. 1994; Murrow and Fuchs 1990; Smotherman and Narins 1999a, b). $I_A$ has also been reported in frog saccular hair cells isolated with papain (Hudspeth and Lewis 1988a; Lewis and Hudspeth 1983), indicating that this current, unlike the $I_{DRK}$, is not sensitive to the proteolytic action of this agent. We found a good match between the properties of frog saccular $I_A$ reported here, and those of hair cells from other inner ear organs. Hair cell $I_A$ always shows a relatively hyperpolarized inactivation $V_{1/2}$, and the major inactivation time constant usually less than a few hundred milliseconds. The voltage dependency of steady-state activation is also low. The $I_A$ of frog saccular hair cells appeared to have a relatively low sensitivity to 4-AP, with 10 mM giving a fractional peak current block of 0.54. In this respect it differs from $I_A$ in other frog auditory organs, such as the amphibian papilla where 1 mM 4-AP suppressed nearly all the $I_A$ (Smotherman and Narins 1999b).

Because very few studies have addressed the expression of cloned $K$ channel subunits in hair cells, it is not possible to identify the molecular counterparts of the $K_v$ currents we have reported here. Recent pharmacological and biochemical evidence has suggested that the $I_{DRK}$ found in several hair cells belongs to the KCNQ family (Kharkovets et al. 2000; Kubisch et al. 1999; Marcotti and Kros 1999; Oliver et al. 2003; Rennie et al. 2001), whose general electrophysiological properties include a relatively slow activation kinetics ($\tau_{act} \sim 12$ ms at $+40$ mV), a double-exponential inactivation time course ($\tau_{act} \sim 300$ ms and $\sim 4$ s), and inactivation $V_{1/2} \sim -90$ mV (Rennie et al. 2001). $I_{DRK}$ showing all the properties of the mammalian KCNQ current was reported in frog SCC hair cells (Marcotti et al. 1999), providing electrophysiological evidence of the association of this hair cell $I_{DRK}$ with the KCNQ family. The $I_{DRK}$ we described here displays biophysical properties not matching those possessed by hair cell KCNQ current. Our pharmacological tests, giving a half block for $I_{DRK}$ by linopirdine of about $50 \mu M$, seem to further support the notion that neither of the $K_v$ currents described here belongs to the KCNQ channel family. The $IC_{50}$ values reported for linopirdine block of cloned KCNQ channels (KCNQ1–3) are in fact markedly lower ($<10 \mu M$; Schne and Brown 1998; Wang et al. 2000) than our value, and $IC_{50}$ even lower ($0.6–5 \mu M$) were reported on hair cells for $K_v$ channels identified as KCNQ (Marcotti et al. 1999; Oliver et al. 2003; Rennie et al. 2001). In contrast, expressed KCNQ4 channels have been found to be not very sensitive to block by linopirdine (Kubisch et al. 1999; Sogaard et al. 2001). Our results with linopirdine therefore cannot be conclusive. Future tests with the more selective KCNQ channel antagonist XE991 (Wang et al. 1998) will provide more definitive evidence concerning the identity of the channel.

**Kinetic modeling**

We have provided a quantitative description of the $K_v$ currents of frog saccular hair cells using a Hodgkin–Huxley formalism (Figs. 4, 6, and 7). Hodgkin–Huxley modeling of $I_A$ in other preparations usually assumed 4 independent gating particles (Huguenard and McCormick 1992; Lockery and Spitzer 1992; but see Bekkers 2000), as was done in the original description of this current (Connor and Stevens 1971). In our study a 3rd-order model seemed to provide a better description of the data (Figs. 5 and 6). With regard to the $I_{DRK}$, a 2nd-order Hodgkin–Huxley model seemed to provide a good description of its main features, as also found for other hair cells $I_{DRK}$ currents (Wu et al. 1995).

The Hodgkin–Huxley approach has been shown to have limitations in describing subtle properties of $K_v$ current gating (Horrigan et al. 1999; Koren et al. 1990; Stefani et al. 1994; Zagotta and Aldrich 1990; Zagotta et al. 1994). Accordingly we found that at relatively depolarized potentials the fit of $I_A$ activation time course deviated significantly from the experimental data (Fig. 6). In addition, the voltage dependency of the activation time constant required a segmental approach (Fig. 6).

The Hodgkin–Huxley formalism, however, proved adequate for modeling the electrical response in a large number of cell preparations, including hair cells (Holt and Eatock 1995; Hudspeth and Lewis 1988a; Wu et al. 1995). Accordingly, our modeling results indicate that the electrical response of frog saccular hair cells could be well reproduced based on the Hodgkin–Huxley description of the $K_v$ currents, under experimental conditions where these currents represented the main repolarizing currents (Figs. 9 and 10).

**Functional role of the $K_v$ currents**

Several biophysical properties of the $I_{DRK}$ we described here will assign an important role to this current in the electrical responses of frog saccular hair cells; that is, the $I_{DRK}$ inactivation $V_{1/2}$ and $k$, found to be about $-50$ and $4.5$ mV, respectively, indicate that a large fraction of the total
$I_{DRK}$ is available at the resting potential of these cells ($-60$ to $-70$ mV; Armstrong and Roberts 1998; Catacuzzeno et al. 2003), and thus may well contribute in shaping the receptor potential of saccular hair cells. Accordingly, relatively low concentrations of 4-AP that would selectively block this current, markedly altered the electrical response in many cells (cf. Fig. 11; Armstrong and Roberts 1998). Another feature of the $I_{DRK}$ described here that would speak for an important role of this current in generating the electrical response is its voltage dependency of activation. We estimated a voltage steepness of activation $k$ of 4.2 mV, a value unusually high for a sustained $K_\alpha$ current, which would translate into a significant change in $I_{DRK}$ activation upon only a few millivolts change of the cell membrane potential. The frequency of hair cell electrical resonance has been usually found to be strictly dependent on the kinetics of the outward $K$ current, with faster currents giving high frequencies of hair cell resonance (Lewis 1988b; Lewis and Hudspeth 1983). By contrast, the undissociated in situ preparation or hair cells subjected to isolation procedures that preserve the $I_{DRK}$ resonate at lower frequencies, between 20 and 100 Hz (Armstrong and Roberts 1998; Catacuzzeno et al. 2003).

A contribution of $I_\alpha$ to the electrical response is unlikely because of its highly hyperpolarized inactivation $V_{1/2}$. This is suggested by our modeling results (cf. Fig. 11), which indicate a very low activity of $I_\alpha$ attributed to its high degree of inactivation at the membrane potentials encountered by the cell during the electrical response. These results suggest that of the 2 $K_\alpha$ currents, only $I_{DRK}$ could significantly contribute to the electrical activity observed in saccular hair cells. However, efferent stimulation has been shown to hyperpolarize frog saccular hair cells by as much as 20 mV (Holt et al. 2001). Such a hyperpolarization could release a significant fraction of $I_\alpha$ inactivation, and the resulting increase in outward $K$ current would diminish the amplitude of the receptor potential. Similar to what has been proposed for $I_\alpha$ in the chick cochlea and amphibian papilla (Murrow and Fuchs 1990; Smotherman and Narins 1999b), we think that this current may act in conjunction with efferent stimulation to modulate the receptor potential under inhibitory conditions.

### APPENDIX

Modeling of the electrical response of frog saccular hair cells required the inclusion of the major ion currents operating in these cells under our recording conditions. In addition to the $I_{DRK}$ and $I_\alpha$ described in this study the model included several other currents whose kinetic description and parameters were taken from the literature. A brief description of these currents follows.

#### Leakage current

The leakage current was modeled as $I_L(V) = g_L(V - E_L)$, where $g_L$ is the leakage conductance and $E_L$ is its reversal potential.

#### $K_1$ current

The $K_1$ current was modeled using a single activation gate (Holt and Eatock 1995)

$$I_{k1}(V, t) = g_{k1}(V - E_{k1})m_{k1}(V, t)$$

where $g_{k1}$ is the maximal conductance for this current and $m_{k1}(V, t)$ was assessed from Eq. 2 with

$$
\tau_{k1}(V) = \tau_{k1}(0) e^{(V - \nu_{k1})/\gamma_k} + K_f
\tag{A2}
$$

and

$$m_{k1}(V, \infty) = \frac{P_{max} - P_{min}}{1 + e^{(V - V_{1/2})/\delta_{k1}}} + P_{min} \tag{A3}
$$

#### The $h$ current

The $h$ current was modeled using a modified Hodgkin–Huxley model with 3 independent activation gates, assuming that only 2 need to enter the $m$ state to open the channel (Holt and Eatock 1995). This gives

$$I_h(V, t) = g_h(V - V_h)[m_h(V, t)]^3[1 - m_h(V, t)]^2 + m_h(V, t)]$$

where $g_h$ is the maximal $h$ conductance. $m_h(V, t)$ was assessed from Eq. 2 with $m_h(V, \infty)$ and $P_h(V)$ given by relationships having the form of Eqs. A2 and A3.

#### Ca current

The Ca current has been shown to require a model with 3 independent activation gates (Armstrong and Roberts 1998; Hudspeth and Lewis 1988), giving

$$I_{cA}(V, t) = g_{cA}(V - E_{cA})m_{cA}(V, t)^3$$

where $g_{cA}$ is the maximal Ca conductance and $E_{cA}$ is the reversal potential for Ca ions. As for the other currents, $m_{cA}(V, t)$ can be assessed from Eq. 2. Model parameters of this current have already been published for papain-dissociated saccular hair cells (Holt and Eatock 1995; Hudspeth and Lewis 1988a). However, the papain-based cell dissociation has been demonstrated to alter the steady-state and kinetic properties of this current (Armstrong and Roberts 1998). For this reason we assessed $m_{cA}(V, \infty)$ and $P_{cA}(V)$, as well as $E_{cA}$, from the $I-V$, and $P_{cA}$ versus voltage data reported by Armstrong and Roberts (1998) for undissociated saccular hair cell Ca current (symbols in Fig. A1, A and B). The experi-

![Fig. A1](http://jn.physiology.org/Downloadedfrom)
motional $I$–$V$ data were well fitted (solid line in Fig. 1A1) using the relationship: $I_{Ca}/I_{max} = g_{Ca}(V - E_{Ca})m_{Ca}(V, \infty)^{3}$, with

$$m_{Ca}(V, \infty) = \left[1 + e^{-(V - V_{1/2})/\sigma_{m}}\right]^{-1}$$  \hspace{1cm} (A6)

and the $\tau_{m}$ versus voltage data were well fitted (solid line in Fig. 1B) when $\tau_{m}(V)$ had the following form

$$\tau_{m}(V) = \tau_{0} + \tau_{e} e^{-(V - V_{1/2})/\sigma_{e}}$$  \hspace{1cm} (A7)

The parameters of the fit can be found in Table 1.

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DISCLOSURES

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