Two types of voltage-dependent potassium channels in outer hair cells from the guinea pig cochlea

THIERRY VAN DEN ABEELE,1,2 JACQUES TEULONG,2 AND PATRICE TRAN BA HUY1

1Laboratoire de Neurobiologie des Systèmes Sensori-moteurs, Centre National de la Recherche Scientifique, Unité Propre de Recherche et de l’Enseignement Supérieur 7060, Faculté de Médecine Lariboisière and 2Institut National de la Santé et de la Recherche Médicale U426, Faculté de Médecine Xavier Bichat, Paris Cedex 18, France

Van Den Abbeele, Thierry, Jacques Teulon, and Patrice Tran Ba Huy. Two types of voltage-dependent potassium channels in outer hair cells from the guinea pig cochlea. Am. J. Physiol. 277 (Cell Physiol. 46): C913–C925, 1999.—Cell-attached and cell-free configurations of the patch-clamp technique were used to investigate the conductive properties and regulation of the major K⁺ channels in the basolateral membrane of outer hair cells freshly isolated from the guinea pig cochlea. There were two major voltage-dependent K⁺ channels. A Ca²⁺-activated K⁺ channel with a high conductance (220 pS, P_K/P_Na = 8) was found in almost 20% of the patches. The inside-out activity of the channel was increased by depolarizations above 0 mV and increasing the intracellular Ca²⁺ concentration. External ATP or adenosine did not alter the cell-attached activity of the channel. The open probability of the excised channel remained stable for several minutes without rundown and was not altered by the catalytic subunit of protein kinase A (PKA) applied internally. The most frequent K⁺ channel had a low conductance and a small outward rectification in symmetrical K⁺ conditions (10 pS for inward currents and 20 pS for outward currents, P_K/P_Na = 28). It was found significantly more frequently in cell-attached and inside-out patches when the pipette contained 100 μM acetylcholine. It was not sensitive to internal Ca²⁺, was inhibited by 4-aminopyridine, was activated by depolarization above −30 mV, and exhibited a rundown after excision. It also had a slow inactivation on ensemble-averaged sweeps in response to depolarizing pulses. The cell-attached activity of the channel was increased when adenosine was superfused outside the pipette. This effect also occurred with permeant analogs of cAMP and internally applied catalytic subunit of PKA. Both channels could control the cell membrane voltage of outer hair cells.

Most studies on K⁺ currents in mammalian outer hair cells (OHC) have used the whole cell patch-clamp technique. Earlier reports stated that Ca²⁺-dependent K⁺ currents were predominant in these cells (1, 13, 26), but recent studies indicate that Ca²⁺-independent types of K⁺ channels are important in isolated OHC (18, 23) and in the isolated whole organ of Corti (19). There is also controversy as to whether the acetylcholine-sensitive K⁺ currents are associated with metabotropic receptors (15) or ionotropic receptors (7, 12, 13), although the most recent electrophysiological (4, 23) and molecular studies (6, 10) are in favor of ionotropic receptors. The ion channels on the basolateral membrane of the OHC could play an important role by regulating the cell voltage, because the electromotive response of OHC depends on the membrane potential. Only two studies have examined individual K⁺ channels on the hair cell membrane of mammals. Ashmore and Meech (1) described two types of Ca²⁺-activated K⁺ channels: a high-conductance one (240 pS) and a low-conductance one (40 pS). The presence of high-conductance K⁺ channels was subsequently confirmed by Gitter et al. (9), although their sensitivity to internal Ca²⁺ and blockers was not confirmed.

This study describes the single-channel properties of two types of voltage-dependent K⁺ channels. The first is Ca²⁺ dependent, has a high conductance (near 220 pS) and the general properties of maxi-K⁺ channels or BKCa, and is regulated by intracellular Ca²⁺ and voltage. The second is Ca²⁺ insensitive, has a low conductance (20 pS), and has many features of delayed rectifier channels. It is more frequently found when acetylcholine is present in the pipette and seems to be regulated by adenosine, cAMP, and protein kinase A (PKA). These channels probably interact to control the membrane potential of OHC.

METHODS

Tissue preparation. Healthy pigmented guinea pigs (200–300 g, Preyer’s reflex positive) were killed with an overdose of pentobarbital sodium (30 mg/kg) and decapitated. Both bullas were quickly removed, the lateral wall of the bony cochlea was removed under a binocular microscope, and the second and third turns were dissected out into a Petri dish containing Leibowitz L-15 medium. The organ of Corti was gently removed using fine needles and transferred in a droplet of medium to a Petri dish. OHC were mechanically isolated by gentle refluxing through a 100-μl Gilson pipette tip.

Measurements. Isolated OHC were dissected out from the two middle turns of the cochlea and placed in a chamber on the stage of an inverted microscope (IM 35, Zeiss, Oberkochen, Germany) at room temperature (20–25°C). They were readily distinguished from inner hair cells by their cylindrical shape and height (40–70 μm). Any OHC that showed loss of tonotopic organization were discarded. Single-channel currents were recorded from cell-attached and excised, inside-out patches of basolateral membranes using the patch-clamp technique (11). Currents were measured with an RK-400 patch-clamp amplifier (Biologic, Claix, France) and stored on a digital audiotape recorder (DTR 1201, Biologic) for further analysis. The pipette voltage was monitored with an IBM-compatible computer using a
The Levenberg-Marquardt algorithm (Sigmaplot 2.0., Jandel) was used to fit the $P_o/V_{m}$ relationship to a Boltzmann distribution

$$P_o = P(0) + \frac{P_{max} - P(0)}{1 + e^{(V_{m} - V_{50})/d}}$$

where $P(0)$ is the resting $P_o$, $V_{50}$ is the half-maximal voltage, and $d$ is the slope constant.

Kinetic analyses were performed only on patches containing one level of activated channel, and current records were low-pass filtered (2 kHz) and digitized (sampling rate, 5 kHz). Idealized recordings were obtained by a half-amplitude threshold method, after analog filtering to avoid that spontaneous peaks of the baseline exceeded the detection threshold. Intervals were measured, binned, and fitted to the sum of exponentials by the maximum likelihood method (Biopatch Analysis Software, Biologic). The dwell-time distributions were fitted to the following function

$$N(t) = A \cdot e^{-t/t_1} + B \cdot e^{-t/t_2}$$

where $N$ is the number of events, $A$ and $B$ are the coefficients, and $t_1$ and $t_2$ are the fast and slow time constants, respectively.

Solutions. The OHC were initially bathed in a standard solution containing (in mM) 140 NaCl, 4.8 KCl, 1 CaCl$_2$, 1.2 MgCl$_2$, 10 HEPES, and 10 glucose, adjusted to pH 7.4 with NaOH. The pipette solution usually contained the same solution, except that 140 mM NaCl was replaced by 140 mM KCl and the pH was adjusted to 7.4 with KOH. In another protocol, 100 µM acetylcholine chloride was added to the pipette solution. Once a cell-attached patch had formed, the cell was superfused with a stream of solution from one of a series of pipette outlets. The cell-attached configuration was usually stable under these circumstances, and the flow of the liquid did not displace the cell. Tests with colored solutions showed that the cell was rinsed in <5 s. The same superfusion system was used after excision to produce the inside-out configuration, but the control solution was a standard solution at pH 7.2 plus 1 mM CaCl$_2$.

Ca$^{2+}$ concentrations below 10$^{-5}$ M were obtained by adding EGTA and Ca-EGTA (20). The free Ca$^{2+}$ concentration was calculated by an iterative method using stability constants for all reactions between Ca$^{2+}$, H$^+$, Mg$^{2+}$, and EGTA (10$^{-9}$ M was obtained by adding 2 mM EGTA and 0 mM Ca-EGTA). The concentration of Mg$^{2+}$ was increased to 0.1 mM MgCl$_2$ for 0.1 mM ATP to compensate for its chelation by ATP (24). Ca$^{2+}$ chelation was negligible at this ATP concentration.

The catalytic subunit of PKA (from pig heart; Sigma) was dissolved in NaCl standard solution with 10$^{-9}$ M CaCl$_2$ by adding 2 mM EGTA. The final solution contained 8 mM ATP (disodium salt), 8-bromoadenosine 3’5’-cyclic monophosphate (8-BrCAMP; sodium salt), PKA, 4-AP, and iberiotoxin were all obtained from Sigma (St. Louis, MO).

Statistics. Because the control and experimental values were both obtained for the same patches, data were analyzed by Student’s paired t-test and the $\chi^2$ test. The results are
expressed as means ± SE. The threshold of significance was P < 0.05.

RESULTS

Activity of the cell-attached patches. Table 1 summarizes the incidences of the various ion channels. The properties of each channel type are described below. Nonselective cation channels (CAN channels) were silent in 90% of the cell-attached patches, but they were found in one-half of the excised patches and have been described in previous studies (31, 32). Many cell-attached patches contained no spontaneously active ion channels, but they were significantly less frequent when 100 µM acetylcholine was in the pipette medium (38% silent cell-attached patches with acetylcholine vs. 64% without acetylcholine, P < 0.02). The frequency of high-conductance K⁺ channels was not significantly modified by the presence of acetylcholine (16% of cell-attached patches without acetylcholine vs. 12% with acetylcholine). On the contrary, significantly more low-conductance K⁺ channels were detected with acetylcholine inside the pipette (25% without acetylcholine vs. 56% with acetylcholine, P < 0.004). This property was used to study the low-conductance K⁺ channel. In

![Fig. 1. Properties of high-conductance K⁺ channel. A: typical inside-out recordings of high-conductance K⁺ channel at various membrane voltages (V_m). Note sensitivity of channel to voltage and activity of flickering bursts. CaCl₂ concentration on inner side of membrane was 10⁻² M. Note presence of smaller events with long openings that could correspond to another K⁺ channel or Ca²⁺-sensitive nonselective cation (CAN) channels. C and bars to left of each trace indicate closed-state current. B: current (I)-voltage (V) relationships in cell-attached and excised inside-out patches under various conditions. Pipette contained 145 mM KCl and 2 mM EGTA without CaCl₂. Command potential (V_c) was identical to V_m for inside-out patches but represents V_m − E_m for cell-attached patches, where spontaneous membrane potential (E_m) is unknown. Open triangles indicate mean ± SE current amplitudes of 6 cell-attached patches, and line represents least-squares fit to Goldman relationship. Intercept with abscissa was extrapolated to −57 mV, because there were no clear openings attributed to these channels at positive potentials. Open circles indicate mean ± SE currents of 5 excised inside-out patches, with bath containing 145 mM KCl, and line is linear regression of points weighted at each voltage by SE. Unit conductance was 216 ± 4 pS, and reversal potential was near 0 mV. Solid circles are mean ± SE currents of 6 excised inside-out patches, with bath containing 140 mM NaCl and 5 mM KCl. Line is least-squares fit to Goldman relationship. Reversal potential is shifted to an extrapolated value of −46 mV, close to Nernst equilibrium potential for K⁺, P_K/P_Na, relative permeabilities of K⁺ and Na⁺, was 8. Error bars are indicated when SE exceeded size of symbols. C: typical recordings of high-conductance K⁺ channels in cell-attached (top trace) and inside-out configurations (bottom trace). V_c is 0 mV, and C indicates closed level. Note typical behavior with burst of “flickering” activity with periods of silence. Activity is increased after excision with bath containing 1 mM CaCl₂.](http://ajpcell.physiology.org/doi/10.1152/ajpcell.00388.2016)
contrast, the superfusion of acetylcholine outside pipettes containing a control solution without acetylcholine did not modify the activity of spontaneously active K⁺ channels (n = 4) and did not stimulate K⁺ channels in inactive cell-attached patches (n = 4). Superfusion with 100 µM adenosine in the bath could activate low-conductance K⁺ channels in cell-attached patches when the pipettes did not contain acetylcholine.

High-conductance K⁺ channels. Figure 1C shows the typical activity of the high-conductance channels in the cell-attached and inside-out configuration. These channels were found in 22 of 102 patches (21%) and usually had a low resting Pₒ in the cell-attached configuration but could clearly be identified before excision in a majority of cases (15 of 22 patches, 68%) in negative potentials and low positive potentials. However, it was difficult to clearly identify openings in potentials greater than −60 mV, probably because the flickery activity of the channels could not be distinguished from the background noise. Their activity was immediately increased after excision into a bath containing 1 mM CaCl₂ (compare top and bottom recordings in Fig. 1C). No rundown was observed even several minutes after excision. These channels had a high unit conductance (216 ± 4 pS, n = 5) and a linear i-V relationship under symmetrical 145 mM K⁺ conditions (Fig. 1B). The i-V relationship showed a slight rectification when the K⁺ at the cytoplasm surface was reduced from 145 to 5 mM and could be fitted by a Goldman equation. The reversal potential of the i-V relationship was shifted to −46 ± 3 mV (Fig. 1B), indicating a preference for K⁺ over Na⁺ (Pₖ/Pₙa = 8). This channel, which is activated by depolarization (see Fig. 1A) and by an increase in internal Ca²⁺ (data not shown), had properties similar to those of the maxi-K⁺ channel described by Ashmore and Meech (2) in the guinea pig OHC. The channel was closed at all voltages with 10⁻⁹ M CaCl₂ in the bath solution (n = 8) and maximally activated for positive voltages at 10⁻⁵ M CaCl₂ (n = 8, Pₒ = 0.65 ± 0.10, Vₘ = −60 mV; see Fig. 1A). The sensitivity of high-conduc-

![Fig. 2. Effects of K⁺ channel blockers.](image-url)

A: single-channel recordings of an inside-out patch containing one level of high-conductance K⁺ channel at 0 mV. Top trace is control. Middle trace shows typical effect of internal 10 mM tetraethylammonium (TEA). Amplitude of current is reduced from 6 to 3 pA, but open probability (Pₒ) is unchanged. Bottom trace shows effect of 1 mM internal barium. Pₒ is greatly reduced. B: single-channel recordings of an inside-out patch containing low-conductance K⁺ channels at 0 mV. Top trace is control. Note flickering activity but at a slower rate than in A. Middle trace shows effects of 10 mM internal TEA. Amplitude of current is reduced from 1 to 0.6 pA. Bottom trace shows effect of barium. Pₒ is reduced, but amplitude of events remains unchanged. C: continuous single-channel recording of a patch containing one level of high-conductance K⁺ channel. Vₖ is 0 mV, and C indicates closed level. Arrow indicates excision from cell-attached to inside-out configuration. Tips of pipettes were filled with iberiotoxin-free solution and then back-filled with a solution containing 100 nM iberiotoxin.
tance channels to iberiotoxin, a specific blocker of maxi-K+ channels, was studied in excised inside-out patches. The tips of patch pipettes were filled with iberiotoxin-free solution and then back-filled with solution containing 100 nM iberiotoxin as described by Jackson and Blair (14). Blockade was complete 1–2 min after the gigaohm seal was obtained in all patches containing a spontaneous activity of high-conductance K+ channels after excision (n = 4, see Fig. 2C). The sensitivity of high-conductance channels on the internal side of the membrane to other blockers of K+ channels such as barium, tetraethylammonium (TEA), and quinine was studied in inside-out patches. Figure 2A shows a typical experiment in which the effects of barium (1 mM, n = 4) and TEA (10 mM, n = 3) were studied at 0 mV. Barium almost completely blocked the channel without altering the unit conductance, whereas the most notable effect of TEA was to reduce the conductance from 216 ± 4 pS (n = 5) to 166 ± 19 pS (n = 3). Quinine reduced the P0 from 0.60 ± 0.1 (n = 3) to 0.30 ± 0.08 (n = 3) and also caused a rapid flickering.

Low-conductance K+ channels. Low-conductance K+ channels were more frequently detected when there was acetylcholine in the pipette, and they opened spontaneously in the cell-attached configuration. This activity was usually bursts of openings separated by periods of silence lasting from several milliseconds to seconds (see Fig. 3, A and C). Excision was followed by a gradual rundown of the low-conductance K+ channels in most patches (34 of 44, 77%; see Fig. 3C). Acetylcholine in the pipette or 1 mM ATP (n = 3) in the bath did not prevent this phenomenon. Rundown was not observed for the high-conductance K+ channels and for the nonselective cation channels. The i-V relationship in the cell-attached configuration was curvilinear, with a slight inward rectification probably due to the low resting potential of isolated OHC and their low internal K+ concentration. However, the channel in excised

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**Fig. 3.** Single-channel recordings and conductive properties of low-conductance K+ channel. A: typical inside-out recordings of low-conductance K+ channel at various membrane voltages (Vm). Note low voltage sensitivity of channel and bursting activity. CaCl2 concentration on inner side of membrane was 10–9 M. C and bars to left of each trace indicate closed state current. B: i-V relationships in cell-attached and excised inside-out patches under various conditions. Pipette contained 145 mM KCl and 2 mM EGTA without CaCl2. Vm was identical to Vm for inside-out patches but represents Vm – ECl for cell-attached patches, where spontaneous membrane potential ECl is unknown. Open triangles indicate mean ± SE currents of 6 cell-attached patches. Intercept with abscissa was −64 mV. Open circles indicate mean ± SE currents of 6 excised inside-out patches, with bath containing 145 mM KCl. Slope conductance was significantly greater in positive Vm potentials (21.5 ± 2.4 pS, n = 6) than in negative Vm potentials (10.3 ± 1.7 pS, n = 6), and reversal potential was near 0 mV. Solid circles are mean ± SE currents of 6 excised inside-out patches, with bath containing 140 mM NaCl and 5 mM KCl. Reversal potential is shifted to −67.4 mV, close to Nernst equilibrium potential for K+. Pk/PNa, relative permeabilities of K+ and Na+, was 28. Error bars are indicated when SE exceeded size of symbols. Lines are least-squares fit to a third-order polynomial relationship. C: typical recordings in cell-attached (top trace) and inside-out configurations (top and bottom trace) of low-conductance K+ channels. Vm is 0 mV, and C indicates closed level. There is a gradual rundown after excision in bath containing 10–9 M CaCl2. Bottom trace shows activity of low-conductance channels with a shorter time scale.
Fig. 4. Sensitivity of low-conductance K⁺ channel to 4-aminopyridine (4-AP). A: typical recordings of an inside-out patch containing 3 levels of low-conductance channel and showing blockade by 4-AP at various concentrations. Bottom trace shows recovery from blockade. B: dose-effect relationship of inhibition of low-conductance K⁺ channel by 4-AP. Histograms are mean $P_o$ for 4 patches in control solution, $10^{-4}$ M 4-AP, and $10^{-3}$ M 4-AP. *$P < 0.02$ when compared with control. **$P < 0.001$ when compared with control (Student's paired t-test).

Fig. 5. Voltage dependence of two types of K⁺ channels. A: representative single-channel recordings obtained from an inside-out patch containing both high- and low-conductance K⁺ channel at various voltages. C and bars to left of each trace indicate closed state current. Internal concentration of CaCl₂ was $10^{-5}$ M. Note differences in voltage dependence of channels. High-conductance channel has a clear activity only at positive membrane potential ($V_m$). $P_o$ of low-conductance K⁺ channels is reduced at negative potentials, but openings are clearly identified. B: relationship between $P_o$ and $V_m$. Open circles are mean ± SE $P_o$ of 6 excised inside-out patches containing high-conductance K⁺ channels and exposed to $10^{-5}$ M CaCl₂ in bath, and solid circles indicate mean ± SE $P_o$ of 7 excised inside-out patches containing low-conductance K⁺ channels exposed to $10^{-9}$ M CaCl₂. Data points were fitted to a modified Boltzman equation using Marquardt method of least squares (see METHODS for details). Half-maximal voltages were −43 mV (high conductance) and −15 mV (low conductance).
patches had a slight outward rectification in symmetrical K\(^+\) concentrations (see Fig. 3B). The slope conductance was greater at positive \(V_m\) (21.5 ± 2.4 pS, \(n = 6\)) than at negative \(V_m\) (10.3 ± 1.7 pS, \(n = 6\)). The i-V relationship showed strong rectification with a NaCl-rich solution in the bath and the reversal potential could be extrapolated to -67.4 mV, a value very near the calculated equilibrium potential for K\(^+\) (−85 mV). Although this i-V relationship did not fit a Goldman relationship very well, it was assumed to be valid near the reversal potential. The \(P_o/P_{Na}\) was 28. Replacing the bath NaCl with RbCl showed no clearly identifiable openings in positive potentials. These results suggest a low permeability or blockade of the channel by Rb\(^+\) (data not shown, \(n = 3\)), as described in maxi-K\(^+\) channels of vestibular dark cells (30). The channel was blocked by internal barium (1 mM, \(n = 3\)) and TEA (10 mM, \(n = 4\)), as was the high-conductance channel. TEA also caused a “flickery” blockade of the channel with a smaller current amplitude (16 ± 8% of control) and decreased \(P_o\) (27 ± 6% of the control values, see Fig. 2B). Barium blocked the channel almost completely by decreasing the \(P_o\) to 11 ± 6% of the control value without altering the current amplitude. This type of channel was also blocked by 4-AP, which inhibits the voltage-gated delayed rectifier K\(^+\) channel (see Fig. 4A). The conductance of the channel remained unchanged, but the \(P_o\) was reduced with 10\(^{-4}\) M 4-AP (\(P_o = 72 ± 6\%\) of the control value, \(n = 4\), \(P < 0.02\)) and was almost completely blocked by 10\(^{-3}\) M 4-AP (\(P_o = 17 ± 4\%\) of the control value, \(n = 4\), \(P = 0.001\)).

Voltage dependence and kinetics of K\(^+\) channels. Both types of K\(^+\) channel were voltage dependent. Figure 5B shows their voltage and \(P_o\). The \(P_o\) did not exceed 0.8 for the high-conductance K\(^+\) channel and 0.6 for the low-conductance K\(^+\) channel, even when the patches were strongly depolarized, probably because of the flickering activity of the channels. The half-maximal activation potential was −43 mV for the high-conductance K\(^+\) channel (\(n = 6\)) with 10 \(\mu\)M internal Ca\(^{2+}\), and −15 mV (\(n = 7\)) for the low-conductance channel, indicating that the channels were sensitive over different ranges of voltage. We investigated the mechanism underlying this dependence by analyzing recordings containing only one level of channel. The kinetics of the two types of channels were best described by two time constants for the open state and two time constants for the closed state (see Figs. 6 and 7). The results are summarized in Tables 2 and 3. The voltage affected the long closed time constant in both channels, indicating that depolarization increased the \(P_o\) by reducing the interval between bursts of activity. However, depolarization increased both the open time constants only for high-conductance channels, indicating a modification of intraburst activity (Fig. 6). Analysis of the blockade by TEA revealed that the time constants were not affected. Blockade of

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**Fig. 6. Steady-state kinetic properties of high-conductance K\(^+\) channel.** A: histograms of closed (left) and open (right) times of an inside-out patch containing one level of high-conductance channel at a membrane potential of 0 mV. Recording lasted 60 s; total number of events was 1,811; and \(P_o\) was 0.06. Pipette contained 145 mM KCl and 2 mM EGTA, and bath contained 140 mM NaCl, 5 mM KCl, and 10\(^{-3}\) M CaCl\(_2\). Data were low-pass filtered at 2 kHz and digitized at 5 kHz, and idealized recordings were constructed. Curves were fitted to sum of 2 exponentials to dwell-time data by a least-squares method. Events shorter than 0.5 ms were excluded from fit. Time constants are given on each panel: \(\tau_1\) is fast time constant, and \(\tau_2\) is slow time constant. Line corresponds to fit of data with two exponentials. Inset in left panel shows analysis of long closures (longer than 10 ms) that were assumed to correspond to interval between bursts. Distribution of closures fits well with a single exponential, and number of closures of 10–400 ms was 473. B: closed-time constants (left) and open-time constants (right) are shown as a function of membrane potential. Plot shows mean time constants obtained from fits of 2–4 patches like those illustrated in A (see Table 3 for values). Open circles represent long time constants, and solid triangles represent short time constants.
the low-conductance K⁺ channel was of a different type, as it decreased the open time constant without altering the closed time constants.

We also investigated the properties of the low-conductance K⁺ channel by ensemble-averaged 2-s sweeps in response to pulses applied to −40 mV from a holding potential of −60 mV. The associated mean current produced by 20 steps is shown in Fig. 8. The current showed a very slow inactivation with a time constant of ~800 ms.

Effects of external purines. In a previous study (32), we demonstrated that superfusion with purinergic agonists modulated the activity of a Ca²⁺-sensitive nonselective cation channel (CAN) in the basolateral membrane of OHC. ATP increased the activity of CAN, probably by increasing intracellular Ca²⁺, and adenosine reduced the activity of CAN, probably by increasing intracellular cyclic AMP. We therefore investigated the effects of external ATP and adenosine on K⁺ channel activities using the following protocol. After obtain-

Table 2. Open probability and time constants for low-conductance potassium channel in response to depolarization, blockers, and activators

<table>
<thead>
<tr>
<th>Potential (mV)</th>
<th>n</th>
<th>P₀ (NP₀)</th>
<th>τ₁ (Closed), ms</th>
<th>τ₂ (Closed), ms</th>
<th>τ₁ (Open), ms</th>
<th>τ₂ (Open), ms</th>
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<tr>
<td>−60</td>
<td>3</td>
<td>0.08 ± 0.04</td>
<td>2.0 ± 0.1</td>
<td>54 ± 18</td>
<td>2.0 ± 0.3</td>
<td>4.6 ± 1.6</td>
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<td>−30</td>
<td>3</td>
<td>0.21 ± 0.05</td>
<td>1.9 ± 0.3</td>
<td>18 ± 4.7</td>
<td>1.4 ± 0.6</td>
<td>5.0 ± 0.5</td>
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<tr>
<td>+30</td>
<td>3</td>
<td>0.33 ± 0.02</td>
<td>1.0 ± 0.04</td>
<td>11.9 ± 2.9</td>
<td>0.9 ± 0.14</td>
<td>6.5 ± 1.4</td>
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<tr>
<td>+60</td>
<td>5</td>
<td>0.27 ± 0.07</td>
<td>1.5 ± 0.3</td>
<td>12 ± 2.6</td>
<td>1.0 ± 0.15</td>
<td>6.1 ± 1.2</td>
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<tr>
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<td>7.4</td>
<td>2.2</td>
<td>21</td>
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<td>4-AP (10⁻³ M)</td>
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<td>1.1</td>
<td>13.1</td>
<td>1.3</td>
<td>6.7</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>0.09 ± 0.02</td>
<td>1.7 ± 0.2</td>
<td>23 ± 3.6</td>
<td>1.2 ± 0.2</td>
<td>5.5 ± 0.6</td>
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<td>0.17 ± 0.02</td>
<td>1.5 ± 0.1</td>
<td>16 ± 1.8</td>
<td>1.4 ± 0.2</td>
<td>6.1 ± 0.7</td>
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<tr>
<td>Control</td>
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<td>0.19 ± 0.06</td>
<td>2.1 ± 0.1</td>
<td>21 ± 5</td>
<td>2.2 ± 0.3</td>
<td>13 ± 2</td>
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<tr>
<td>PKA (20 U/ml)</td>
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<td>0.78 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>7.0 ± 0.4</td>
<td>2.2 ± 0.3</td>
<td>20 ± 3</td>
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</table>

Values are means ± SE; n, no. of experiments. P₀, open probability; τ, time constant; V_m, membrane potential; 4-AP, 4-aminopyridine; PKA, protein kinase A; NS, not significant.
to depolarization, blockers, and activators

Table 3. Open probability and time constants for high-conductance potassium channel in response to depolarization, blockers, and activators

<table>
<thead>
<tr>
<th>V_m (mV)</th>
<th>n</th>
<th>P_o</th>
<th>( \tau_1 ) (Closed), ms</th>
<th>( \tau_2 ) (Closed), ms</th>
<th>( \tau_1 ) (Open), ms</th>
<th>( \tau_2 ) (Open), ms</th>
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<tr>
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</tr>
<tr>
<td>+40</td>
<td>3</td>
<td>0.48±0.13</td>
<td>0.71±0.35</td>
<td>11±4</td>
<td>1.9±0.4</td>
<td>8.5±3.2</td>
</tr>
<tr>
<td>+60</td>
<td>4</td>
<td>0.74±0.05</td>
<td>0.95±0.4</td>
<td>8.4±2.8</td>
<td>3.2±0.8</td>
<td>29±8</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>0.54</td>
<td>0.49</td>
<td>1.8</td>
<td>0.61</td>
<td>2.9</td>
</tr>
<tr>
<td>TEA (10 mM)</td>
<td>2</td>
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<td>0.61</td>
<td>2.65</td>
<td>0.45</td>
<td>1.75</td>
</tr>
<tr>
<td>Quinine (10 mM)</td>
<td>3</td>
<td>0.33</td>
<td>0.58</td>
<td>2.2</td>
<td>0.75</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. TEA, tetraethylammonium. Other definitions are as in Table 2.

Fig. 8. Low-conductance K⁺ current measured by cell-attached averaged single-channel recording. Pipette solution contained 145 mM KCl, and bath solution contained 140 mM NaCl and 5 mM KCl. Leak and capacitive currents were subtracted from all sweeps. Data were recorded with low-pass filtering at 2 kHz and digitized at 5 kHz. Cell-attached patch was clamped to a potential of -40 mV positive to holding potential (-60 mV). Five representative sample traces are shown below voltage pulse. Ensemble-averaged current obtained from 20 consecutive sweeps is shown below 5 sample traces. Bottom trace shows a single sweep before subtraction of leak and capacitive currents.

were recorded at the resting membrane potential (clamp potential = 0 mV) near the reversal potential for CAN to minimize their amplitude. ATP had no effect on maxi-K⁺ (n = 9) or low-conductance K⁺ channels (n = 3). ATP did not alter the activity of spontaneous opening maxi-K⁺ channels (n = 4) or stimulate maxi-K⁺ channels in inactive cell-attached patches having maxi-K⁺ channel activity after excision (n = 5). Adenosine also did not alter the activity of maxi-K⁺ channels (n = 5). Purines had no effect on the maxi-K⁺ channel as evaluated by the P_o or the kinetic analysis (data not shown, n = 5).

However, in the study where we reported the effects of adenosine on CAN (32), some patches frequently showed activation of small flickering channels, possibly the small K⁺ channels, but the ionic selectivity of these channels was not investigated because the pipette contained a low K⁺ concentration (4.8 mM). We therefore reinvestigated the effects of external adenosine in cell-attached patches; the pipette contained a control 145 mM KCl solution without acetylcholine to reduce the spontaneous activity of the low-conductance K⁺ channels. Eight of thirteen patches responded with a significant increase in NPS, from 0.36 ± 0.19 to 0.54 ± 0.19 (P < 0.005) with a delay of 51 ± 9 s (Fig. 9). This effect was reversed by washing out the activator in only four of eight cases. Kinetic analysis of the channel activity before and during the superfusion of adenosine revealed that the open time constants were not modified but that the slow closed time constant was increased.

Activation by 8-BrcAMP and PKA. Because the inhibition of CAN by adenosine is correlated with the effect of CAMP and PKA, we attempted to determine whether this possibility also held for the K⁺ channel. Of the 10 patches tested in the cell-attached configuration with 0.5 mM 8-BrcAMP, 5 clearly responded with a mean delay of 61 ± 15 s and increased their resting NPS significantly from 0.61 ± 0.27 to 0.92 ± 0.32 (P < 0.02). Eight of eleven inside-out excised patches increased their resting NPS significantly from 0.54 ± 0.04 to 0.89 ± 0.02 (P < 0.001) in response to PKA with a mean delay of 80 s (see Fig. 10). Kinetic analysis of four patches containing only one level of low-conductance K⁺ channel showed that only the slow closed time constant was altered. This effect was not significant.

Fig. 9. Averaged ensemble-averaged current obtained from 20 consecutive sweeps is shown below 5 sample traces.
DISCUSSION

We have shown that there are two voltage-sensitive $K^+$ channels in the basolateral membrane of OHC from the guinea pig cochlea. These channels are readily distinguished from each other by their conductance; one is ~200 pS and the other 20 pS. The high-conductance channel is Ca$^{2+}$-sensitive, blocked by iberiotoxin, and clearly belongs to the family of maxi-$K^+$ channels previously described in these cells. TEA reduced the conductance of the channel, suggesting a fast block, as described in other cells of the inner ear (30). The low-conductance channel is Ca$^{2+}$-insensitive and is blocked by barium, TEA, and 4-AP. There seem to be more low-conductance channels than maxi-$K^+$ channels, but the slow inactivation in cell-attached patches and the existence of a rundown phenomenon after excision makes them more difficult to study, and probably explains why they have not been reported previously in single-channel recordings of OHC.

Activity and regulation of the high-conductance $K^+$ channel. The high-conductance channel described here is very like the large Ca$^{2+}$-activated $K^+$ channel reported by Ashmore and Meech in 1986 (2) and the C-type $K^+$ channel found by Gitter et al. in 1992 (9). The channel opens for short periods in situ and was clearly identified in most cases, probably because it is very sensitive to Ca$^{2+}$, and the resting intracellular Ca$^{2+}$ concentration of isolated OHC is above the threshold of activation of the channel. The $P_0$ was greatly increased after excision in a Ca$^{2+}$-rich bath, indicating its Ca$^{2+}$ sensitivity. We tested only 10$^{-5}$, 10$^{-6}$, and 10$^{-7}$ M CaCl$_2$ concentrations, but the threshold of activation has not been precisely measured. Ashmore and Meech (2) found a low threshold near 10$^{-8}$ M in the guinea pig cochlea, Art et al. (1) examined turtle hair cells, and Sugihara (29) studied goldfish hair cells; they found that the $P_0/V_m$ relationship depended greatly on the intracellular Ca$^{2+}$ concentration. We find that the voltage dependence in inside-out patches with 10 µM
CaCl₂ bathing the inner side of the membrane is in the same range, which indicates a low but significant $P_o$ when the membrane potential has low negative values. Kinetic analysis demonstrates that the activity of these channels is made up of bursts and is well described with two open and two closed state constants. Depolarization decreases the long closed time constant, thus reducing the time between bursts, and increases the open time constants, indicating stabilization of the open state. Purines do not alter the $P_o$ or the intraburst behavior of the channels. These findings are compatible with the fact that voltage is the main regulator of these channels when intracellular Ca²⁺ level is kept constant.

Properties and regulation of the low-conductance K⁺ channel. The precise nature of the low-conductance channel is not yet clear. However, the voltage dependence, the small conductance of 10–20 pS, its insensitivity to internal Ca²⁺, the outward rectification, the bursts of activity, the inhibition by 4-AP, and the slow inactivation in averaged single traces all point to it being a delayed rectifier K⁺ channel. Lin et al. (18) and Nenov et al. (23) recently described a similar type of K⁺ current in whole cell recordings of guinea pig OHC and indicated that it could play an important part in generating the Ca²⁺-sensitive K⁺ channels, with the majority being the high-conductance maxi-K⁺ or BK Ca channels. The effects of K⁺ channel blockers, like barium, TEA, and cesium, suggested that Ca²⁺-activated channels carry most of the voltage-dependent outwardly rectifying K⁺ current. The voltage sensitivities of the high-conductance and low-conductance K⁺ channels in our study reveal that the low-conductance channel has a higher $P_o$ than the high-conductance channel when the membrane potential of OHC is more negative than –30 mV and the intracellular Ca²⁺ concentration is low. It could thus play an important role in resting conditions.

We found that the low-conductance K⁺ channel is regulated by several factors. The channel is significantly more frequent in patches obtained with an acetylcholine-filled pipette, suggesting local interaction between the mediator and the channel. In addition, external adenosine activates the channel when superfused outside the pipette. This implies activation by an internal second messenger, probably cAMP. This is reinforced by the fact that PKA significantly increases channel activity. Kinetic analysis points to adenosine and PKA acting via a common mechanism, as both decrease the slow closed time constant.

Putative role of these channels. The main function of basolateral K⁺ channels is to maintain the cell membrane potential under resting conditions and to cause hyperpolarization in response to activators, and thus reduce the excitability of the OHC. Most of the previous single-channel recordings of K⁺ channels in the basolateral membrane of guinea pig OHC reported Ca²⁺-sensitive K⁺ channels, with the majority being the high-conductance maxi-K⁺ or BK Ca channels. The effects of K⁺ channel blockers, like barium, TEA, and cesium, suggested that Ca²⁺-activated channels carry most of the voltage-dependent outwardly rectifying K⁺ current. The voltage sensitivities of the high-conductance and low-conductance K⁺ channels in our study reveal that the low-conductance channel has a higher $P_o$ than the high-conductance channel when the membrane potential of OHC is more negative than –30 mV and the intracellular Ca²⁺ concentration is low. It could thus play an important role in resting conditions.

Recent in vivo studies (33) showed that the superfusion of 5 mM 4-AP in the perilymph of guinea pig cochleas (on the basolateral membrane of hair cells) increased the summating potential at test frequencies below 4 kHz, where the major contribution is thought to come from the OHC. These results therefore suggest that...
4-AP-sensitive K\(^+\) channels like our low-conductance K\(^+\) channels are important under physiological conditions for clamping the membrane potential at more negative values.

Acetylcholine is thought to be the main neurotransmitter of the medial efferent system that sends many fibers directly to the base of the OHC (for a review, see Ref. 8) and may be the agent of neural control of the OHC by acting on basolateral K\(^+\) channels. Recent studies using whole cell recordings in guinea pig OHC indicate that acetylcholine triggers a brief cationic nonselective current that allows Ca\(^{2+}\) to permeate and thus immediately activate the neighboring Ca\(^{2+}\)-sensitive K\(^+\) channels, probably low-conductance ones that typically adapt rapidly (4, 12, 15, 21, 22). Although recorded at the base of the OHC, our low-conductance K\(^+\) channels are clearly distinct from these acetylcholine-related K\(^+\) channels, because they are insensitive to internal Ca\(^{2+}\) and are still activated after a few minutes of contact between the pipette containing acetylcholine and the membrane during the formation of the seal. Our low-conductance K\(^+\) channels could thus produce a long-lasting hyperpolarization of the OHC. Slow inhibitory effects of efferent stimulations with a latency of 10–50 s have been observed recently in the cochlea (5, 28). The authors suggest that these effects are caused by a more delayed intracellular mechanism, probably involving second messengers or phosphorylation. Acetylcholine was found to increase the activity of low-conductance K\(^+\) channels when present in the pipette, but not when added to the bath while the pipette contained control solution. This could indicate a mechanism distinct from the activation by adenosine and PKA, such as G proteins (15). Our low-conductance K\(^+\) channels, which are activated by acetylcholine and regulated by cAMP and PKA, could take part in this slow inhibition of OHC.

Purinergic receptors have been found in the apical and basolateral membranes of OHC, although their precise function is unclear. The organ of Corti itself could participate in the release of ATP (34). We previously studied the regulation by purines of a CAN that could depolarize the cell and thus increase its excitability. These channels were activated by ATP and inhibited by adenosine, its precursor and degradation product. The two types of basolateral K\(^+\) channels described here are activated by depolarization and/or adenosine. They could cooperate by balancing the effects of the nonselective cationic channels and so prevent prolonged depolarizations and injury to the cells.


