Voltage-dependent outward $K^+$ current in intermediate cell of stria vascularis of gerbil cochlea

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Takeuchi, Shunji, and Motonori Ando. Voltage-dependent outward $K^+$ current in intermediate cell of stria vascularis of gerbil cochlea. Am. J. Physiol. 277 (Cell Physiol. 46): C91–C99, 1999.—A voltage-dependent outward $K^+$ ($K_V$) current in the intermediate cell (melanocyte) of the cochlear stria vascularis was studied using the whole cell patch-clamp technique. The $K_V$ current had an activation threshold voltage of approximately $-80$ mV, and $50\%$ activation was observed at $-42.6$ mV. The time courses of activation and inactivation were well fitted by two exponential functions: the time constants at 0 mV were 7.9 and 58.8 ms for activation and 0.6 and 4.3 s for inactivation. The half-maximal activation time was 13.8 ms at 0 mV. Inactivation of the current was incomplete even after a prolonged depolarization of 10 s. This current was independent of intracellular Ca$^{2+}$, Quinine, verapamil, Ba$^{2+}$, and tetraethylammonium inhibited the current in a dose-dependent manner, but 4-aminopyridine was ineffective at 50 mM. We conclude that the $K_V$ conductance in the intermediate cell may stabilize the membrane potential, which is thought to be closely related to the endocochlear potential, and may provide an additional route for $K^+$ secretion into the intercellular space.

patch clamp; melanocyte; endocochlear potential

Introduction

It has been generally accepted that the stria vascularis in the cochlea is responsible for the production of the positive endocochlear potential (EP) and the $K^+$-rich endolymph. Both the EP and the high $K^+$ concentration in the endolymph are essential for sound transduction by hair cells. The meanoelectrical transducer channels in the cilia of hair cells make contact with the endolymph, and $K^+$ flows into hair cells when the transducer channels open. The driving force for the $K^+$ flux through the transducer channels is the electrical gradient across the cilary membrane produced by the sum of the EP ($+80$ to $+90$ mV) and the resting membrane potential of hair cells ($-40$ to $-50$ mV for inner hair cells) (7). Accordingly, the cochlear microphonnic potential decreases when the EP is suppressed (21).

The stria vascularis consists of several types of cells: marginal cells, intermediate cells, basal cells, capillary endothelial cells, and pericytes. Figure 1 shows major ionic pathways in cells constituting the stria vascularis and the underlying spiral ligament. Among several cell types, intermediate cells are melanocytes, which migrate from the neural crest during ontogeny (12) to become located between the epithelial marginal cell layer and the mesodermal basal cell layer. Intermediate cells play an essential role in the development and/or in the physiological function of the stria vascularis, because a congenital deficiency in the intermediate cell causes low EP (4, 34) and an increase in the threshold of sound pressure levels to elicit compound action potential responses (4).

$K^+$ channels in the intermediate cell and/or the basal cell are thought to play an important role in the generation of EP and in the recycling of $K^+$ from the perilymph to the endolymph (30, 32, 43). We have succeeded in obtaining dissociated intermediate cells and we reported an inwardly rectifying $K^+$ ($K_{IR}$) current in the intermediate cell (37). The similarity between the drug sensitivity of the $K_{IR}$ current and that of the EP suggests a direct contribution of the $K_{IR}$ conductance to the generation of the EP (37, 40). In addition to the $K_{IR}$ current, an outward current in the intermediate cells was observed when the $K_{IR}$ current was inhibited by Ba$^{2+}$ (37). However, the nature of the outward current has not yet been investigated. The aim of this study was to characterize the voltage-dependent outward $K^+$ ($K_V$) current and to consider its relevance to the physiological function of the intermediate cell.

Methods

Cell preparation and electrophysiological recordings were performed in essentially the same way as described previously (37). The care and the use of animals were approved by the Kochi Medical School Animal Care and Use Committee. Cochleae of gerbils were obtained under anesthesia with pentobarbital sodium (50 mg/kg ip). Tissue strips of the stria vascularis were incubated for 30 min at 24–26°C in the control solution containing 0.2% trypsin and kept for up to 4 h in the cold (4°C) control solution until use. These strips were dissected with fine needles under visual control. Single intermediate cells that displayed the characteristic morphology (i.e., densely packed pigment in the cell and dendritelike projections) were selected and separated from other dissociated cells.

The whole cell configuration of the patch-clamp technique was employed. Pipette resistance was 2.2–3.6 MΩ when filled with the pipette solution. Recordings were made with an amplifier (3900A with 3911A; Dagan, Minneapolis, MN). Current signals were filtered at 5 kHz through a four-pole Bessel low-pass filter, digitized at frequencies of 0.5–5 kHz, and stored on the hard disk of a computer. Voltage command generation, data storage, and analyses were performed using pCLAMP (version 6.0.3; Axon, Foster City, CA). Membrane capacitance, estimated using the circuitry of the amplifier, was $23.5 \pm 0.6 \mu F/cell$ ($n = 157$). Series resistance ($4–15$ MΩ) was compensated as much as possible (by $50–90\%$) within a range that did not provoke oscillations of the current, whereas voltage errors might be significantly large when currents were large. Membrane potentials were corrected for voltage errors derived from uncompensated series resistance (current $\times$ uncompensated resistance) when activation curves were made [see Figs. 4 (C and D) and 8D]. Liquid junction

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potentials were measured against a flowing 3 M KCl electrode and corrected. Leak conductance was estimated from the slope of current-voltage relations of steady-state currents between 2110 and 290 mV in the presence of 0.5 mM Ba2+ (see RESULTS) and subtracted linearly unless indicated otherwise, since current-voltage relations of residual currents at membrane potentials below ~90 mV could be regarded as linear. Experiments were performed at room temperature (24–26°C). Data were expressed as means ± SE, where n is the number of cells.

The pipette solution contained (in mM) 110 KCl, 15 potassium aspartate, 10 KOH, 5 EGTA, 6 HEPES, 6 Tris, 1.1 MgCl2, and 2 MgATP (pH 7.2). The control bath solution contained (in mM) 110 NaCl, 40 sodium aspartate, 3.6 KCl, 6 HEPES, 2.6 Tris, 0.7 CaCl2, 1 MgCl2, and 5 glucose. When tetraethylammonium (TEA) or 4-aminopyridine (4-AP) was added to the bathing solution, equimolar Na+ was replaced by these inhibitors. Because 4-AP is strongly basic, it was neutralized by equimolar HCl. The pH values of all bath solutions were finally adjusted to 7.4. Unless otherwise noted, Kv currents were recorded when the Kir currents were inhibited by 0.5 mM Ba2+. Higher concentrations of Ba2+ were required for a complete inhibition of Kir currents when the extracellular K+ concentration was raised (see Fig. 3), probably because of the competition between K+ and Ba2+ (3).

RESULTS

Separation of Kv current from Kir current. Both inward and outward currents were observed in all the cells investigated. Ba2+ at a concentration of 0.5 mM was able to inhibit the inward current almost completely (Fig. 2). Inhibition of the inward current is incomplete at lower concentrations (37). The reversal potentials of the Ba2+-sensitive current were −84.4 ± 0.8 mV and −85.7 ± 0.7 mV (n = 26) for instantaneous and steady-state currents, respectively. In addition, the steady-state current-voltage relationship of the Ba2+-sensitive current showed inward rectification (c in Fig. 2B). The above characteristics of the current sensitive to 0.5 mM Ba2+ suggest that it belongs to the category of Kir currents. The outward current sensitive to 0.5 mM Ba2+ was regarded as being mediated by Kir channels because of its time course on depolarization from a holding potential of ~90 mV. More specifically, the current reached its peak value immediately after depolarization, indicating that the conductance sensitive to 0.5 mM Ba2+ was already activated at 290 mV. The above property is not likely to be the case for Kv currents. The instantaneous peak current disappeared in the presence of 0.5 mM Ba2+ (Fig. 2A). Similar
outward currents mediated by KIR channels have been reported for other types of cells (18, 22). The slope conductance between −100 and −60 mV, around the reversal potential of the KIR current, was 93.0 ± 8.0 nS (n = 26) for the steady-state KIR current (Fig. 2B).

The activation of the current resistant to 0.5 mM Ba2+ was time and voltage dependent (Fig. 2A). Both the voltage dependency and time course of the current were apparently different from those of the KIR current. The slope conductance between −100 and −60 mV was 27.7 ± 2.5 nS (n = 26) for the KV current (Fig. 2B). Further characterization of the depolarization-activated outward current follows.

K+ selectivity, deactivation, and voltage-dependent activation. Selectivity for K+ was examined using the reversal potential (Erev) of the instantaneous tail current recorded after activation at 0 mV for 150 ms (Fig. 3). Erev was −85.3 ± 0.6 mV (n = 5) under control conditions (extracellular K+ concentration, [K+]o = 3.6 mM) and shifted in a positive direction when [K+]o was raised to 10 and 36 mM. The slope of the fitted line in Fig. 3C was 52.9 mV per 10-fold change in [K+]o. The above result indicates a high selectivity for K+ in the KV current.

The time course of deactivation was apparently accelerated when both [K+]o and [Ba2+]o were elevated (Fig. 3A). To find the reason for the above observation, the effect of Ba2+ on deactivation under a constant [K+]o condition was examined. As shown in Fig. 3, D and E, elevation of [Ba2+]o from 0.5 to 2 mM caused faster deactivation. Inhibition of full activation by Ba2+ at this concentration (see Fig. 8, B and C) and/or a direct effect of Ba2+ on the deactivation process could be the mechanisms underlying the faster deactivation.

The activation of the outward K+ current was voltage dependent (Fig. 4). The rising phase of the current was well fitted by two exponential functions (Fig. 4B). The two time constants (τac.1 and τac.2) at 0 mV determined from the fitted curves were 7.9 and 58.8 ms, respectively (Fig. 4D). The time required for half-maximal activation at 0 mV was 13.8 ± 3.4 ms (n = 10). The threshold potential for the activation of the KV conductance was approximately −80 mV (Fig. 4, C and D).

Fig. 3. K+ selectivity and deactivation of KV current. A: voltage protocol and tail currents under 2 different extracellular K+ concentration ([K+]o) conditions. To block the KIR current, 0.5, 1.5, and 5 mM Ba2+ were added to the bath solution when [K+]o was 3.6, 10, and 36 mM, respectively. Leakage currents were not subtracted. Arrowheads, zero-current level; bars, 2 nA. Tail currents were obtained after activation at 0 mV for 150 ms. B: current-voltage relationship of instantaneous tail currents shown in A. C: reversal potential (Erev) of tail currents plotted against [K+]o (number of observations in parentheses). Slope of straight line obtained after applying linear regression was 52.9 mV/10-fold change in [K+]o. D: voltage protocol and tail currents showing effects of Ba2+ on deactivation. Bars, 2 nA. E: time constant of deactivation (tdeactivation) obtained from single-exponential fittings to tail currents. Continuous lines are best fits to linear regressions: tdeactivation = 39.86 + 0.21E in control condition, and tdeactivation = 10.62 + 0.06E in presence of 2 mM Ba2+.
Boltzmann distribution, and the membrane potential (E_m) at the half-maximal activation and the slope factor were estimated to be −42.6 mV and 17.5 mV/e-fold change in conductance, respectively (Fig. 4D).

The activation curve of the steady-state component recorded using the protocol shown in Fig. 5A was also examined (steady-state data in Fig. 4, C and D). E_m at the half-maximal activation and slope factor estimated by fitting a Boltzmann distribution were −75.0 mV and 6.9 mV/e-fold change in conductance, respectively. These values were apparently different from those of the peak current. Inactivation and recovery from inactivation. The K_V current was inactivated by prolonged depolarization, but inactivation at steady state was incomplete (Fig. 5A): steady-state currents at the end of 10-s voltage pulses normalized to the peak currents were 0.27 ± 0.01 (n = 7) when E_m was set at 0 mV. The time course of inactivation was well fitted by two exponential functions (Fig. 5B). The two time constants (τ_inac.1 and τ_inac.2) at 0 mV determined from the fitted lines were 0.6 and 4.3 s, respectively. Steady-state inactivation was evaluated by a two-pulse protocol and by measuring peak currents at +60 mV after 10-s prepulses (Fig. 6A). Peak currents, normalized to the peak current after a prepulse of −100 mV, showed incomplete steady-state inactivation. The voltage-independent fraction was estimated to be 0.21 from the fitted curve (Fig. 6B).

Recovery from inactivation was studied by varying the interval between two test pulses (Fig. 7A). The time required for complete recovery was dependent on the duration of depolarization. Complete recovery from
inactivation required ~20 s after 0.5-s depolarization and 40 s after 10-s depolarization (Fig. 7B). The above result was taken into account to determine the interval between voltage pulses in other experiments presented in this study.

Drug sensitivity and effect of Ca²⁺ removal from bath solution. Quinine, verapamil, TEA, and Ba²⁺ inhibited both peak and steady-state currents dose dependently (Fig. 8). IC₅₀ values were estimated by fitting the Hill equation (Fig. 8, B and C). IC₅₀ values for verapamil were 4.8 × 10⁻⁵ M (peak) and 2.5 × 10⁻⁴ M (steady state), for quinine were 3.8 × 10⁻⁵ M (peak) and 1.8 × 10⁻⁴ M (steady state), and for TEA were 3.7 × 10⁻² M (peak) and >10⁻¹ M (steady state). The above results indicate that the peak current was more sensitive to blockers than the steady-state current. From the dose-response relationship, the effects of Ba²⁺ at concentrations <10⁻³ M were considered to be relatively small. Although IC₅₀ values for Ba²⁺ were not determined since effects of Ba²⁺ at 0.5 mM cannot be excluded, the IC₅₀ for the peak current is likely to lie between 10⁻³ and 5 × 10⁻³ M (Fig. 8B). The depolarization-activated current was resistant to 4-AP even at a high concentration of 50 mM; the difference between the current magnitude under control conditions and that in the presence of 50 mM 4-AP was not statistically significant (P > 0.05 with Student’s t-test) for both peak and steady-state currents (n = 5).

To exclude the possibility that Ca²⁺ flowed into the cell on depolarization and activated the relevant K⁺ channel, extracellular Ca²⁺ was removed and 1 mM EGTA was added to the bath solution. As shown in Fig. 8A, an increase rather than decrease in the peak current by 49.8 ± 7.9% (n = 9) was observed. The above result and the relatively strong chelation of intracellular Ca²⁺ by 5 mM EGTA make it unlikely that the depolarization-activated K⁺ current was activated by intracellular Ca²⁺. To elucidate the reason for the increase in the peak current under the Ca²⁺-free condi-
Effects of 0.5 mM Ba\(^{2+}\) on the change in maximal conductance could not be explained by the voltage-activated K\(^{+}\) channel (see Fig. 4 in Ref. 37). Removal of extracellular Ca\(^{2+}\) reduced not only the K\(_{IR}\) current but also the K\(_{V}\) current. Removal of ATP from the pipette solution was ineffective for the suppression of the K\(_{IR}\) current.

![Diagram](image)

**Fig. 7.** Recovery of K\(_{V}\) current from inactivation. A: voltage protocol and current recordings with a constant duration of test pulses (t\(_{\text{pulse}}\)) and varied intervals between test pulses (t\(_{\text{interval}}\)). Leakage currents were subtracted. Arrowheads, zero-current level; bars, 2 nA. I\(_0\) and I are peak currents elicited by first and second voltage pulses, respectively. B: normalized peak current (I/I\(_0\)) plotted against t\(_{\text{interval}}\), showing time course of recovery from inactivation (n = 5) for each data point; t\(_{\text{pulse}}\) values were set at 0.5 s (○) and 10 s (▲). Continuous lines are best fits to single-exponential functions: I/I\(_0\) = 1 − 0.31exp(−t\(_{\text{interval}}\)/5.67) when t\(_{\text{pulse}}\) = 0.5 s, and I/I\(_0\) = 1 − 1.25exp(−t\(_{\text{interval}}\)/19.18) when t\(_{\text{pulse}}\) = 10 s.

**DISCUSSION**

Method for separation of the K\(_{V}\) current from the K\(_{IR}\) current. In all patch-clamped intermediate cells, the K\(_{IR}\) current was observed under the experimental conditions described in this study. Thus elimination of the K\(_{IR}\) current was essential for the characterization of the K\(_{V}\) current. It has been reported that K\(_{IR}\) currents are more sensitive to Ba\(^{2+}\) than K\(_{V}\) currents (27). The IC\(_{50}\) of Ba\(^{2+}\) for the K\(_{IR}\) current in the intermediate cell has been shown to be ~10\(^{-5}\) M (37), whereas that for the K\(_{V}\) current is likely to lie between 10\(^{-3}\) and 10\(^{-2}\) M (Fig. 8). Complete suppression of the K\(_{IR}\) current is not likely, since currents at E\(_{m}\) values lower than the E\(_K\) (~93 mV) were essentially null (a in Fig. 2B).

Substantial inhibition of the K\(_{V}\) current by 0.5 mM Ba\(^{2+}\) is not likely, since apparent effects of Ba\(^{2+}\) on this current only appeared at higher concentrations (>1 mM, Fig. 8). Among all the methods tested, the application of 0.5 mM Ba\(^{2+}\) to the bath solution was the only effective method for the selective and complete inhibition of the K\(_{IR}\) current. For example, extracellular Cs\(^{+}\), which is known to inhibit K\(_{IR}\) currents, blocked the inward current but failed to block the outward current, which we regarded as being mediated by the K\(_{IR}\) channel (see Fig. 4 in Ref. 37). Removal of extracellular K\(^+\) reduced not only the K\(_{IR}\) current but also the K\(_{V}\) current. Removal of ATP from the pipette solution was ineffective for the suppression of the K\(_{IR}\) current.

Characteristics of the K\(_{V}\) current in the intermediate cell. Several characteristics of the K\(_{V}\) current in the intermediate cell, such as voltage-dependent activation, speed of activation and inactivation, and independence from the intracellular Ca\(^{2+}\), resemble those of delayed rectifier K\(^+\) currents, whereas the time courses of activation fitted by double-exponential functions were different from those of delayed rectifier K\(^+\) currents that are known to be fitted by sigmoidal functions (28). One of the distinctive characteristics of K\(^+\) conductance is its activation at relatively deep negative E\(_m\) values. Outward K\(^+\) currents activated in a similar E\(_m\) range have been reported (16, 17, 29). Although the majority of cloned voltage-activated K\(^+\) channels are activated at more depolarized E\(_m\) values, a modification
of channel properties may occur in native cells. In relation to the above discussion, it has been reported that cell culture causes a positive shift of activation potential (45). Incomplete inactivation even at the end of a 10-s depolarization is another characteristic of the KV current in the intermediate cell. Similar incomplete inactivation has been reported for KV currents in native cells (17, 23, 29) and those mediated by cloned KV channels (10, 11, 31, 44).

When the steady-state current was analyzed separately, the activation curve was quite different from that of the peak current (Fig. 4D). The above result and the difference in the drug sensitivity between the peak current and the steady-state current (Fig. 8) raise the possibility that the KV current in the intermediate cell is derived from two distinctive channels, although it cannot be excluded that peak and steady-state currents might be derived from different states of one channel.

The ineffectiveness of 4-AP at 50 mM and the relatively high IC_{50} for TEA (37 mM for the peak current) also characterize the KV current in the intermediate cell, since the majority of cloned KV channels are sensitive to 4-AP and/or TEA at lower concentrations.
The above characteristic does not necessarily imply that a large structural difference exists between known K_v channels and the relevant K^+ channel, since even small differences in the molecular structure may have significant effects on certain properties of the channel. For example, it has been reported that K_v channels belonging to the same subfamily but isolated from different animal species differ in certain properties such as drug sensitivity (1).

Possible roles of K_v conductance in the intermediate cell. It is known that K_v currents regulate the duration of action potential in excitable cells (13). In nonexcitable cells, such currents have also been reported (e.g., Refs. 8, 35, 41). With regard to the physiological function of K^+ channels in the intermediate cell, they have been proposed to contribute to the generation of positive EP (32, 42). The K_IR channel reported in the previous study was suggested to contribute to EP (37). Because K_v conductance is activated by depolarization, its contribution to E_m depends on the relative magnitude of other conductances in the intermediate cell, which remain unknown except for K_IR conductance.

To discuss the E_m of the intermediate cell in vivo, K^+ concentration in the intercellular space of the stria vascularis, where the intermediate cell is found, should be considered. Although a general agreement on the above-mentioned K^+ concentration has not been arrived at because of technical difficulties, a low concentration comparable to that found in usual extracellular fluids has been suggested (30). Several lines of research on the Na^+-K^+-ATPase (5, 19, 32) and the Na^+-K^+-Cl^- cotransporter (25, 38, 42) in the basolateral membrane of the marginal cell support the putative low concentration in the intercellular space, because these transporters are thought to take up K^+ and keep K^+ concentration in the intercellular space low (Fig. 1). The Na^+-K^+-ATPase in the intermediate cell (5, 19) may also contribute to taking up K^+ from the intercellular space. Assuming usual intracellular and extracellular K^+ concentrations, E_m would be expected to lie in the range between ~80 and ~95 mV. The above discussion and the activation curve of K_v conductance (Fig. 4D) suggest that a slight depolarization can activate K_v conductance even if the E_m of the intermediate cell in vivo is close to E_K. The activated K_v channel stabilizes the E_m near E_K. Incomplete inactivation of K_v conductance on prolonged depolarization may be favorable for stabilizing E_m in case of prolonged depolarization.

The above speculation was examined by studying E_m in the zero-current clamp mode (Fig. 9). When K_IR conductance was blocked completely by 0.5 mM Ba^{2+}, depolarization by 10.7 mV occurred. Thus K_IR conductance was indispensable for keeping E_m near E_K. However, E_m was still maintained at a relatively deep negative value of ~76.4 mV when K_IR conductance was blocked almost completely. It is very likely that E_m in the presence of 0.5 mM Ba^{2+} was maintained by K_v conductance. Although the slope conductance between ~100 and ~60 mV of the K_v current was ~30% of that of the K_IR current (Fig. 2B), the size of the K_v conductance may be sufficient to maintain the relatively deep negative E_m.

The second possible function could be to provide a pathway for K^+ secretion. K^+ secretion into the intracellular space in the stria vascularis is important as part of the K^+-recycling pathway from the perilymph to the endolymph (20, 32, 33). K^+ secreted into the intercellular space is thought to be taken up by the marginal cell via Na^+-K^+-ATPase and the Na^+-K^+-Cl^- cotransporter, as mentioned above, and finally secreted into the endolymph. As the K_IR channel in the intermediate cell is likely to mediate outward currents at E_m values near E_K (see b and c in Fig. 2B), the K_IR channel is one possible pathway for K^+ secretion. In addition, when the E_m of the intermediate cell in vivo is above the activation threshold of the K_v channel, it would provide an additional route for K^+ secretion into the intracellular space. The incomplete inactivation on prolonged depolarization may favor the open state of this additional route. The above-mentioned supportive role of the K_v channel may be important when the activity of the K_IR channel declines.

Finally, the K_v channel may be involved in cell proliferation reported for the intermediate cell of adult guinea pigs (6) because K_v channels have been proposed to be involved in mitosis of several types of cells (9, 26, 46).

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