Voltage-clamp errors cause anomalous interaction between independent ion channels

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In voltage-clamp, uncompensated series resistance results in steady-state voltage errors that scale with the amplitude of the elicited current and are often correctable offline. However, while investigating mechanoelectric transduction currents at hair cells’ resting potential, voltage-gated calcium channels and calcium-activated potassium channels (BK) were activated in voltage-clamp by displacing the sensory hair bundle. This resulted from steady-state voltage errors (< 1.5 mV) induced by series resistance changing the holding potential. Thus, uncompensated series resistance, interacting with an elicited current, resulted in a voltage error that could induce the erroneous activation of other currents. This error is not correctable offline. Recognizing this type of error is critical when investigating multiple voltage-dependent conductances with steep voltage dependence. NeuroReport 16:943–947 © 2005 Lippincott Williams & Wilkins.

Key words: Auditory hair cells; BK potassium channels; Calcium channels; Mechanoelectric transduction; Patch clamp; Series resistance

INTRODUCTION

Series resistance (SR) errors, associated with the whole-cell voltage-clamp technique have both steady-state and kinetic components [1]. Whereas the kinetic component sets the speed of the voltage-clamp circuit and is equivalent to the product of the SR and membrane capacitance, steady-state errors change the voltage at which a cell is being clamped, equivalent to the product of the SR and the elicited current. Typically, with small currents and low resistances, steady-state errors are not significant and the resulting small changes in voltage can be compensated offline. However, such compensation may not be possible under conditions in which multiple conductances are involved: activation of one conductance alters the voltage at which the cell is clamped and thus changes the activation properties of a second conductance. The significance of this problem is revealed when investigating the interaction between mechanically gated currents and voltage-sensitive conductances in auditory hair cells.

Hair cells are mechanoreceptors whose receptor potential is regulated by the sum of several conductances. In addition to current through mechanoelectric transducer (MET) channels [2,3], conductances in the basolateral cell membrane, including L-type calcium channels and BK-type calcium-activated potassium channels, create the hair cell receptor potential [4]. Voltage-clamp investigation of MET channel properties near the hair cell’s resting potential revealed that steady-state SR errors led to contamination of the MET currents through activation of the basolateral channels. The data presented here provide a clear example of the seriousness of this artifact, where a mechanically gated current activates a voltage-dependent current by changing the holding potential.

METHODS

Hair cells were recorded in the intact auditory papilla of turtles (Pseudemys scripta elegans), using established methods [5]. Procedures followed National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee. Turtles (carapace length 75–125 mm) were rapidly decapitated and the head sectioned along the midline with a razor blade. Inner ear organs were removed and the papilla separated and mounted in a recording chamber using single strands of dental floss.

Whole-cell patch recordings, with potassium as the major cation, were used to assess the characteristics of BK and MET conductances at resting potential. Electrodes were filled with (in mM): KCl (125), MgCl₂ (4), Na₂ATP (5), creatine phosphate (5), D-glucose (8), ethylene glycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) (5) and N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES) (10), pH 7.2 for recordings. Either an Axopatch 200b or an EPC-8 amplifier was used for the recordings. Electrode resistances ranged from 1 to 3 MΩ. The extracellular solution contained (in mM): NaCl (125), KCl (4), MgCl₂ (2.2), CaCl₂ (2.8), Na HEPES (10), Na pyruvate (2), ascorbate (2), creatine (2) and D-glucose (6), pH 7.6 with NaOH and osmolality 275 mmol/kg. The bath contained Apamin (130 nM) to block SK potassium channels.

Hair bundles were placed using a rigid glass fiber, fire-polished to a 0.5–1 μm diameter and fixed to a piezoelectric bimorph [3,5]. The fiber tip was placed on the hair bundle just above the row of the shortest stereocilia. Whereas positive deflections toward the kinocilium opened MET channels, negative deflections away from the kinocilium closed them. The fiber’s movement was driven with voltage...
steps from Signal Software using a CED D/A interface (Cambridge Electronic Design, Cambridge, UK) and filtered at 1–5 kHz. All positive and negative displacements were greater than those needed to saturate the MET current [6]. BK activation curves were generated from tail currents following a series of 20-ms voltage steps (mean of 4 sweeps/step) from a holding potential of ca. −60 mV. Test solutions were either bath applied or pressure applied with a picospritzer (General Valve). All data are presented as mean ± standard deviation.

RESULTS

A comparison of MET currents at a holding potential of −83 mV (where no other conductances are activated) with the MET currents near the hair cells’ resting potential (−54 ± 5 mV) revealed smaller (981 ± 285 vs. 508 ± 137 pA, n=32) and slower (both activation and adaptation) currents in the more depolarized cells. Surprisingly, near the cell’s resting potential, larger deflections of the hair bundle activated an outward conductance that superimposed upon the MET current (Fig. 1a and b), appearing as a decrease in the MET current over time. This was never observed at the more hyperpolarized potentials. The outward current following the deflection of the hair bundle (tail current) was used to characterize this conductance, as it was not contaminated by the MET current. The average amplitude of this current was 133 ± 44 pA (n=32). This current had a steep voltage dependence (2.0 ± 0.5 mV, Fig. 1d) and a half activation of −52 ± 0.5 mV (n=11), measured by fitting the

![Fig. 1. (a and b) Mechanical deflection of the hair bundle elicited saturating mechanoelectric transducer (MET) currents when holding at −83 mV but subsequently activated a second current (indicated by arrows) at −47 mV. Activation of this second current was steeply voltage-dependent. (c) Examples at different holding potentials. (d) shows the normalized voltage-conductance plot (Ek used for reversal potential = −135 mV) fit with a Boltzmann equation (see methods). Whereas the black symbols and curve are for mechanical stimulation (Vh=−52 ± 0.5 mV; slope = 2.0 ± 0.3 mV), the gray curve represents conductance at different voltage steps (Vh=−53 ± 0.8 mV; slope = 1.6 ± 0.9 mV n=4; symbols not shown). Lower inset: 30 ms duration ± 1 ms steps were given during a 60 ms depolarizing step to a potential (−50 mV) on the steep part of the activation curve, demonstrating BK sensitivity similar to the mechanically elicited outward tail currents. Scale bar: 100 pA, 10 ms. Upper inset: expanded x-axis with error bars. (e) and (f) Activation of this second current was fast. (f) varies the duration of the mechanical stimulus from 0.3 ms to 12 ms demonstrating that the peak outward tail occurs with a stimulus of ~3 ms. (f) is a summary plot of relative tail current amplitude against stimulus duration. An exponential fit gave a time constant of 0.6 ± 0.3 ms (n=8). Inset: expanded x-axis. Horizontal bars show time of mechanical displacements of the bundle with step size and direction indicated above (positive toward kinocilium).
data with a Boltzmann function:

$$g/g_{\text{max}} = 1/(1 + e^{(V_{1/2} - V)/K})$$

where $V_{1/2}$ is the half-activating voltage and $K$ is the limiting slope (voltage dependence). These activation properties are remarkably similar to those of the basolateral BK conductance, which had a half activation of $-53 \pm 0.8 \text{mV}$ and a slope factor of $1.6 \pm 0.9 \text{mV}$ ($n=4$; Fig. 1d). BK currents, comparable in amplitude to the outward tail, are elicited by $\pm 1 \text{mV}$ steps from a potential on the steep part of the BK activation curve (Fig. 1d, inset). The current activated faster than MET current activation (Fig. 1e). Stimulus durations as short as 3 ms could elicit a peak response (time constant to peak of $0.6 \pm 0.3 \text{ms}$; $n=8$) (Fig. 1f). Because the cell was in voltage-clamp during these recordings two possibilities arose as to the mechanism of activation: direct mechanical activation or calcium-induced activation.

The first question addressed was about what channel is responsible for this additional conductance and how this channel is activated given that the cell is in voltage-clamp mode. The similarity in activation properties implicated BK potassium conductance. Data exist suggesting that BK channels can be mechanosensitive [7,8]; to test whether activation of this current was due to either direct mechanical coupling or MET channel activation, the MET current was blocked with tubocurarine (Fig. 2a, 200 $\mu\text{M}$) [9,10]. Blocking the MET current abolished BK current activation, suggesting that activation was not due to a mechanical coupling, but was indirect and driven by MET current activation.

Given that the cells were in voltage-clamp, it was surmised that perhaps Ca$^{2+}$, through the MET channels, was activating a BK current that, in order to account for the rapid kinetics, needed to be in the stereocilia or in close proximity to the MET channels. Bath application of potassium (125 mM) caused the current to reverse polarity and become inward (Fig. 2c, $n=10$) [11,12], while bath application of tetraethyl ammonium (TEA, 3 mM) blocked the current (Fig. 2b, $n=11$), supporting the conclusion that a potassium channel distinct from the MET channel was being activated. Intracellular Cs$^+$ also blocked the current (Fig. 2d, $n=3$), further demonstrating that this was a potassium conductance. However, local application of the high K$^+$ solution ($n=10$) or TEA ($n=3$) to the sensory hair bundle and apical cell surface had no effect on this conductance, indicating that the channels were not located on the apical surface or stereocilia.

To further test whether the channel being activated was Ca$^{2+}$-dependent, the internal Ca$^{2+}$ buffer was changed from 5 mM EGTA to 1 mM BAPTA, resulting in a depolarizing shift of the activation curve, from $-52 \pm 0.5 \text{mV}$ to $-45 \pm 1 \text{mV}$, ($n=8$ for BAPTA cells) consistent with a Ca$^{2+}$-dependent conductance [13,14]. Because both Ca$^{2+}$ diffusion down the stereocilia [15] and Ca$^{2+}$ release from stores would be too slow for the measured BK activation kinetics, the source of Ca$^{2+}$ at this point was unclear.

Might voltage-dependent calcium channels activate despite being in voltage-clamp? This question was answered by simultaneously stimulating the bundle mechanically and bath applying Nimodipine, which blocks L-type Ca$^{2+}$ channels in these hair cells [16], while leaving MET channels unaffected [10]. While no effect was found on the MET current, the potassium conductance was blocked, suggesting that its activation followed the activation of an L-type Ca$^{2+}$ channel (Fig. 2e, $n=8$). Nimodipine had no effect when locally applied to the hair bundle.

How then does a voltage-dependent conductance become activated while the cell is in voltage-clamp? SR errors have both temporal and steady-state consequences. Voltage-clamp speed is equal to the product of cell capacitance and uncompensated SR. Average hair cell capacitance of $12 \pm 3 \text{pF}$ ($n=32$) and uncompensated SR of $4 \pm 2 \text{M} \Omega$ ($n=32$) gives a clamp speed of 48 $\mu\text{s}$. Thus, clamp speed is unlikely to be responsible for the activation of the BK or Ca$^{2+}$ current, because the conductance is activated on a much shorter time scale.
mode implicates steady-state SR errors as the underlying fact that these channels are activated while in voltage-clamp sium current. These channels are likely the basolateral activation from this source of Ca\(^{2+}\). Further support of a [11], the increased BK current argues against a direct Ca\(^{2+}\) to the apical hair bundle, the MET current amplitude cell further, activating more BK current. By applying low increasing the MET current amplitude would depolarize the be expected to result in larger SR error, it is predicted that the current is activated [13]. As larger MET currents would it activates over \(< 2\) mV, and at rest, a significant portion of the current is activated [13]. As larger MET currents would be expected to result in larger SR error, it is predicted that increasing the MET current amplitude would depolarize the cell further, activating more BK current. By applying low Ca\(^{2+}\) to the apical hair bundle, the MET current amplitude increased as a result of removal of a Ca\(^{2+}\) block of the channel [11]. Performing this experiment near the hair cell’s resting potential increased the BK current amplitude (Fig. 3a) because of the increased MET current, supporting the conclusion that the MET current amplitude determined the level of BK channel activation, likely due to a steady-state SR error. Additionally, because Ca\(^{2+}\) entry through the MET channels was reduced during low Ca\(^{2+}\) application [11], the increased BK current argues against a direct activation from this source of Ca\(^{2+}\). Further support of a SR artifact is the relationship between the voltage error associated with uncompensated SR and the magnitude of the BK current activated during mechanical stimulation (Fig. 3b). The solid line in Fig. 3b was plotted using currents predicted by the BK activation curve (Fig. 1d gray) for each SR voltage error.

**DISCUSSION**

Data presented demonstrate that mechanical stimulation of the sensory hair bundle near the cell’s resting potential leads to rapid activation of an L-type Ca\(^{2+}\) current and subsequent activation of the BK calcium-activated potassium current. These channels are likely the basolateral channels and are not localized near the MET channels. The fact that these channels are activated while in voltage-clamp mode implicates steady-state SR errors as the underlying mechanism. The consequence of this error is that the MET currents cannot be analyzed independently at the cell’s resting potential because of contamination by other conductances. Pharmacological antagonists would be required to block the BK or calcium current (Fig. 2) to investigate the MET properties. This solution, however, limits the ability to investigate the physiological response properties of the cell. The implications of this problem are far reaching in that many biophysical investigations of excitable cells rely on voltage-clamp protocols for isolating multiple types of voltage-gated conductances without considering how small SR errors might lead to interaction between conductances. For example, cells often have multiple potassium conductances with different activation kinetics. Voltage errors due to uncompensated SR interacting with the conductance that activates more hyperpolarized will contaminate the properties of the second conductance in a manner that cannot be corrected offline. The example presented here for the hair cell is straightforward in that current through a mechanically gated channel is instigating the error and any voltage changes must be artifacts, as no change occurs in the command voltage. In contrast, in most excitable cells where multiple voltage steps are employed, leading to different voltage errors, the effects on additional conductances will be much more complex. The hair cell example used here illustrates just how SR errors can prevent accurate measures of simultaneously activating conductances. When recording at resting potential to examine the functional interactions between the mechanical and basolateral conductances, the BK conductance exacerbates SR artifacts because of the steepness of its activation curve.

**CONCLUSION**

The effect of steady-state SR errors, determined by the SR and the current amplitude, on holding potential can typically be corrected offline, if only a single conductance is involved. Present work demonstrates that when multiple conductances are involved, such errors result in erroneous activation properties being recorded that are uncorrectable offline.

**REFERENCES**


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