Hyperpolarization-activated (I_h) current in mouse vestibular primary neurons

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The presence of a hyperpolarization-activated inward current (I_h) was investigated in mouse vestibular primary neurons using the whole-cell patch-clamp technique. In current-clamp configuration, injection of hyperpolarizing currents induced variations of membrane voltage with prominent time-dependent rectification increasing with current amplitudes. This effect was abolished by 2 mM Cs^+ or 100 μM ZD7288. In voltage-clamp configuration, hyperpolarization pulses from −60 mV to −140 mV triggered a slow activating and non inactivating inward current that was sensitive to the two blockers, but insensitive to 5 mM Ba^{2+}. Changing Na^+ and K^+ concentrations demonstrated that I_h current is carried by both these monovalent cations. This is the first demonstration of a I_h current in vestibular primary neurons. *NeuroReport* 12:2701–2704 © 2001 Lippincott Williams & Wilkins.

Key words: Current- and voltage-clamp; Cs^+; I_h; Vestibular primary neurons; ZD7288

INTRODUCTION

A hyperpolarization-activated inward current (I_h), carried by K^+ and Na^+ ions, has been described in a number of neuronal and non-neuronal cells [1]. This current is blocked by extracellular Cs^+ [2] and ZD7288 [3] but is insensitive to Ba^{2+}. I_h, once activated, induces a slow depolarization of the cell. In neurons, I_h is known to be involved in setting the resting membrane potential, and in providing pacemaker depolarization inducing rhythmic activity [1].

Discrepancy in the discharge properties of vestibular primary afferents has previously been attributed to a difference in the composition of membrane ionic currents in these neurons [4]. In this context, we recently described a large variability in the expression of outward depolarization-activated K^+ currents in vestibular primary neurons (VPN) [5]. This observation could explain in part the difference in discharge properties of these neurons. However, the properties of I_h make it a putative candidate in controlling discharge properties in vestibular afferents. We therefore investigated whether I_h was present in VPN acutely isolated from mice using the current- and voltage-clamp configurations of the whole-cell patch-clamp technique. We report and characterize for the first time a hyperpolarization-activated inward current that resembles I_h described in other neuronal preparations [1].

MATERIALS AND METHODS

Whole-cell recordings of hyperpolarization-activated currents were performed in VPN using an isolation procedure described previously [5]. The standard extracellular solution contained (in mM): NaCl 135, KCl 5, HEPES 10, glucose 10, $MgCl_2$ 1. For the Na^+-free solution, NaCl was replaced by equimolar choline-Cl. Patch pipettes (2 and 3 MΩ) were filled with the following intracellular solution (in mM): KCl 135, EGTA 10, HEPES 25, MgATP 3, NaGTP 1, glucose 10. The pH of the solutions were adjusted to 7.35 and the osmolarity to 300 mOsm/l. Data were recorded with an Axopatch 200B and analyzed with Pclamp software (Axon Instrument, Forster City, CA). Series resistance in the range 5–9 MΩ were 80% compensated after cancellation of the capacitive transients. Data presented are corrected online for junction potentials (−7 mV). In current-clamp mode, action potentials (AP) were elicited with brief (1.5 ms) depolarizing pulses from the resting membrane potential.

Activation curves were best fitted with a single Boltzmann function of the form $I/Imax = 1/(1+\exp((V-V1/2)/k))$. Time constants (τ) were best fitted with a single exponential function of the form $A.\exp(-t/\tau)$. Pooled data are given as mean ± s.d. Statistical significances were examined using ANOVA. ZD7288 was obtained from Tocris, and all other chemicals from Sigma.

RESULTS

Recordings were made from neurons with a mean input resistance of 223.5 ± 100.6 MΩ and a mean membrane capacitance of 16.0 ± 6.5 pF ($n = 93$). The mean resting membrane potential of the neurons studied in current-clamp experiments was −63.1 ± 4.7 mV ($n = 23$). We tested the effect on membrane voltage of hyperpolarizing current injections. In control solution, injection of currents in 50 pA increments up to 300 pA induced variations of membrane voltage, with prominent time-dependent rectification that

increased with the amplitude of the hyperpolarizing currents (Fig. 1aa,ca). The time-dependent rectification was emphasized by plotting the amplitude of the voltage variations taken at the peak and the end of the current injections (Fig. 1ab,cb). In the presence of 2 mM Cs^+ (Fig. 1b) or 100 μM ZD7288 (Fig. 1d), the time-dependent rectification of the membrane voltage was abolished. The resting membrane potential of the recorded neurons was not significantly affected by these blockers ($p > 0.5$, Cs^+ $n = 6$, ZD7288 $n = 9$).

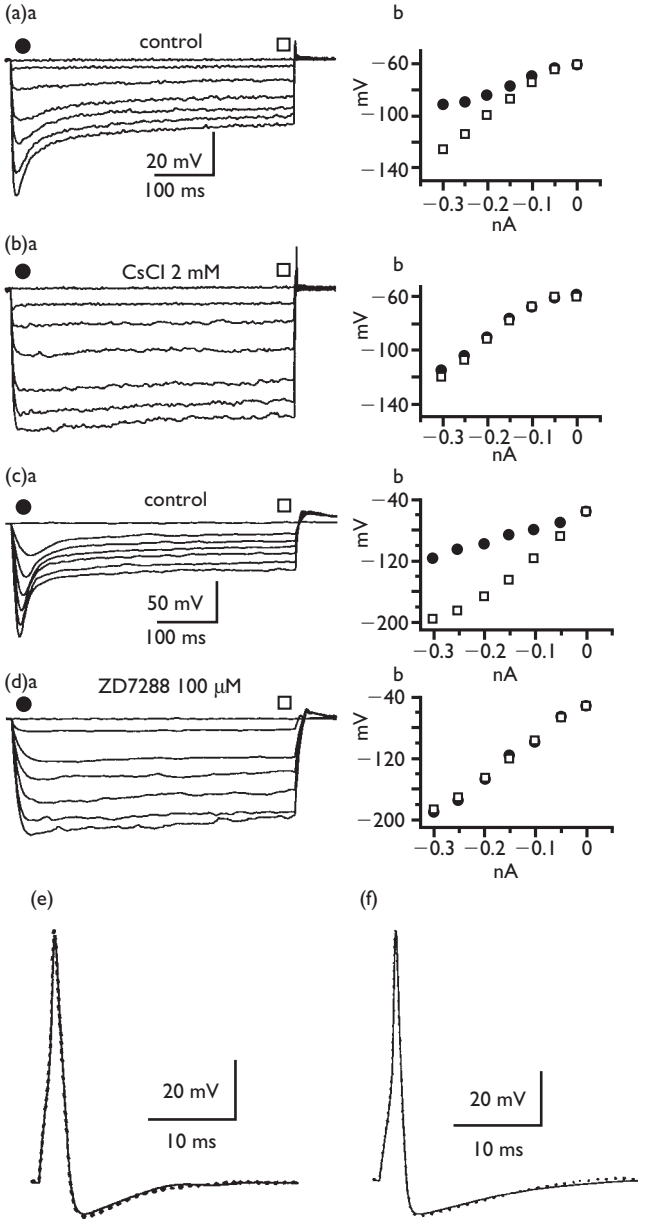

Fig. 1. Effects of current injections on membrane voltage. (**aa**–**da**) Variations of membrane voltage elicited by the injection of hyperpolarizing currents (−50 to −300 pA, −50 pA increments). (aa,ca) Control solution; (ba) 2 mM Cs^+; (da) 100 μM ZD7288. (**ab**–**db**) Plot of voltage amplitudes as a function of the hyperpolarizing current (squares, peak voltage; circles, 10 ms before the end of the current injection). (ab,cb) control solution; (bb) 2 mM Cs^+; (db) 100 μM ZD7288. (**e**,**f**) Solid traces, action potential elicited by 1.5 ms 400 pA depolarizing pulses. Dot traces: effects of Cs^+ (e) and ZD7288 (f). Note the absence of change in the depolarization, repolarization phases and the trajectory of the AHP.

To test whether the hyperpolarization-activated current contributes to the after hyperpolarization (AHP) trajectory, 2 mM Cs^+ ($n = 10$) or 100 μM ZD7288 ($n = 5$) were applied on neurons in which a single action potential was elicited by short depolarizing pulses (Fig. 1e,f). No change was observed either in the depolarization or repolarization phase of the action potential. The amplitude and the trajectory of the AHP following the AP were also unaffected by the application of the two blockers.

We then characterized the hyperpolarization-activated inward current using the voltage-clamp configuration from a holding potential of −60 mV. In control solution, hyperpolarising voltage steps to −70 and −80 mV evoked an instantaneous inward current, whereas hyperpolarizations below −90 mV activated a slow activating inward current (Fig. 2aa–ca). Applications of 2 mM Cs^+ (Fig. 2ab) or 100 μM ZD7288 (Fig. 2bb) prevented the activation of the slow activating component, without affecting the instantaneous one. Applications of 5 mM $BaCl_2$ (Fig. 2cb) abolished the instantaneous component, leaving the slow activating component unaffected. Inward currents were isolated by subtracting traces evoked in the presence of the blockers from those elicited in control external solution (Fig. 2ac–cc).

Voltage-dependent steady-state activation of the inward current was studied by plotting the mean relative amplitudes of the subtracted tail currents against the test voltage pulses (Fig. 2d). Instantaneous tail currents were taken at −110 mV, a voltage level at which we assume that the inward current sensitive to 2 mM Cs^+ is the only voltage-activated current activated, and were normalized to those obtained after the most hyperpolarized pulse (−140 mV). The half activation potential ($V_{ac(1/2)}$) was 109.9 ± -1.9 mV, and $k = 11.1 \pm 2.1$ mV ($n = 8$). Relative activation of the inward current sensitive to ZD7288 was plotted on the same figure. In a set of six neurons $V_{ac(1/2)}$ was -112.5 ± 0.8 mV, and k was 8.1 ± 0.7 mV. These $V_{ac(1/2)}$ values obtained using pharmacological isolations were compared to those obtained in control solution, following the procedure reported previously [6]. The slowly activating component of the inward current could the isolated from the instantaneous one by fitting current traces with single exponential function. Its voltage-dependent steady-state activation was studied by plotting normalized amplitude as a function of the test pulses. This procedure gave $V_{ac(1/2)}$ of -110.6 ± 0.9 mV, and k of 10.0 ± 0.9 mV ($n = 11$; Fig. 2d). The $V_{ac(1/2)}$ values isolated following the three different procedures did not differ significantly ($p > 0.5$). The time constant for activation (τ_{ac}) of the inward current sensitive to 2 mM Cs^+ plotted as a function of the test potential (Fig. 2e) showed a strong voltage dependence with a slope of 29.1 ± 2.4 ms/mV ($n = 8$). τ_{ac} of the inward current sensitive to ZD7288 and those obtained by the fitting method were 30.4 ± 2.9 ms/mV ($n = 6$) and 30.5 ± 3.2 ms/mV ($n = 11$) respectively. Values for τ_{ac} obtained using the three different procedures did not differ significantly ($p > 0.5$).

No correlation between the capacitance of the recorded

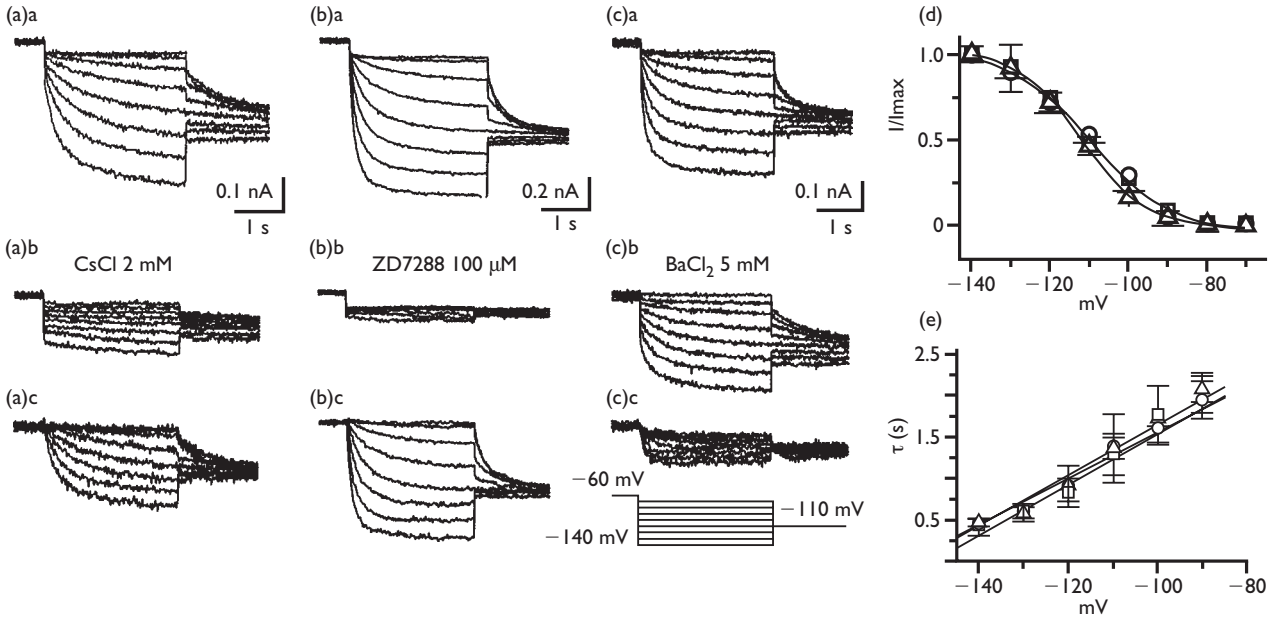

Fig. 2. Characterization of the hyperpolarization-activated inward current. (**a**–**c**) current traces evoked by application of hyperpolarizing voltage pulses between −70 and −140 mV from HP −60 mV. (**aa**–**ca**) Control solution, (**ab**–**cb**) application of 2 mM Cs^+, 100 μM ZD7288, and 5 mM Ba^{2+} respectively. (**ac**–**cc**) subtracted traces. (**d**) Plot of mean relative amplitude of the instantaneous tail currents as a function of the test voltage pulses. (**e**) Plot of the time constant for activation of the slowly activating inward current as a function of the test potential. In panels (d) and (e) cirlcles indicate Cs^+-sensitive current ($n = 8$), squares indicate ZD7288-sensitive current ($n = 6$), triangles indicate current isolated in control solution using the fitting method ($n = 11$), the solid lines represent the Boltzman and the best linear fits respectively.

neurons and the amplitude of the largest current was noticed ($n = 45$), indicating that the hyperpolarization-activated current is not expressed differentially between neurons.

The ionic nature of this current was investigated using the method described recently [7]. Reversal potential was determined in control solution, and in solutions in which the concentrations of Na^+ and K^+ had been changed. The slowly activating inward current was measured at different levels by the application of 2 s voltage pulses around the half activation potential. Voltage pulses between −90 and −140 mV were then applied, and the current-voltage relationship of the instantaneous current was estimated by linear fits. The reversal potential was determined from the intersection of the linear fits. The intersection of the linear fits gave a reversal potential of -49.4 ± 2.3 mV ($n = 5$) in control external solution (Fig. 3b), -62.0 ± 1.6 mV ($n = 4$) in Na^+-free solution (Fig. 3c), and -11.2 ± 2.6 mV ($n = 4$) in 20 mM K^+ solution (Fig. 3d). These results indicated that in VPN, the hyperpolarization-activated inward current is carried by the monovalent cations, Na^+ and K^+.

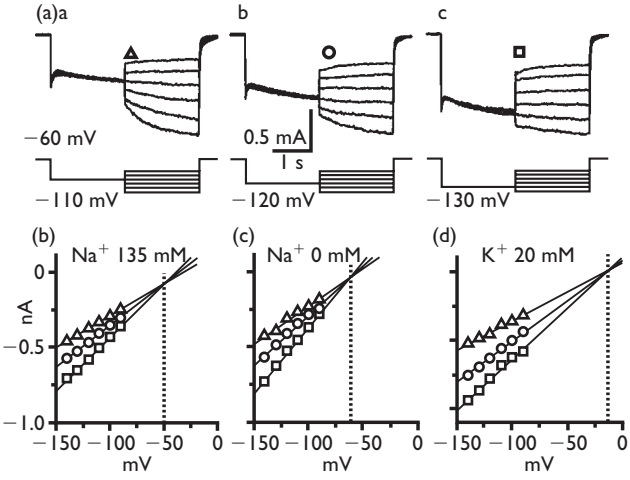

Fig. 3. Ionic nature, of the hyperpolarization-activated inward current. (**a**) Current traces induced in control solution, by applications of 2 s conditioning voltage pre-pulses at −110, −120 and −130 mV from HP −60 mV, followed by voltage test-pulses between −90 and −140 mV in 10 mV increments. (**b**–**d**) Plots of instantaneous current measured immediately after the prepulses at −110 (triangles), −120 (circles), −130 mV (squares), as a function of test voltages. In (b–d) solid lines represent the extrapolated linear fits to the data points. Vertical dotted lines show the voltage at the intersection of the three linear fits. Control solution (b), Na^+-free solution (c), K^+-rich solution (d).

DISCUSSION

The slowly activating hyperpolarization-activated inward current found in VPN shares most of the properties that define I_h in other neuronal preparations, namely a slow and voltage-dependent activation, absence of time-dependent inactivation, sensitivity to Cs^+ and ZD7288, insensitivity to Ba^{2+} and a mixed cations composition of the current [1]. In that matter, it differs from the other hyperpolarization-activated inward currents (I_{KIR}) which are carried by K^+, and are sensitive to Ba^{2+} [8]. The I_h found in VPN differs from that described in other neuronal preparations in several specific characteristics. First, it activates at a membrane potential which is around 20 mV more hyperpolarized than the actual resting membrane

potential measured in these cells. Moreover, application of Cs^+ and ZD7288 did not affect the resting potential. These observations indicate that, unlike other neuronal preparations [7], I_h found in VPN is not involved in setting resting membrane potential. Secondly, full activation of I_h in VPN requires several seconds, whereas it activates within 1 s in a number of other neurons [1]. This property gives some insights into the identification of the subunits that underlie the I_h channel. Of the four members of the HCN gene family that have been cloned, only two (HCN2, HCN4) have slow rates of activation [9]. Lastly, with a half-activation voltage around −110 mV, the activation range of I_h in VPN is relatively hyperpolarized compared to most other neurons in which it has been measured [7]. However, $V_{1/2}$ values between −100 and −120 mV have been reported in guinea pig spiral ganglion neurons [10], and mouse cerebellar basket cells [11]. In VPN, although we took specific care in estimating $V_{1/2}$, we cannot exclude the possibility that our recording conditions may modify the gating properties of the I_h channel. Assuming that such hyperpolarized activation threshold and $V_{1/2}$ values reflect the *in vivo* situation, it is unlikely that I_h is activated during the AHP since, in our recording conditions, AHP never exceeded −80 mV (unpublished observations). This suggestion is confirmed by the absence of any effect of the I_h blockers on the AHP.

Additional roles for I_h in extrinsic or metabolic regulations have been proposed in other neuronal models [1] because I_h is modulating by neurotransmitters and/or extrinsic stimuli via the intracellular cAMP and cGMP [12]. Such possibilities need to be investigated in VPN. A role for I_h in protecting against excessive hyperpolarizations could also be considered.

CONCLUSION

The present study is the first report of a hyperpolarization-activated current in vestibular primary neurons. This current displays most of the pharmacological and kinetic properties reported for I_h in other neuronal preparations. In VPN, however, I_h displays a low threshold of activation, and pharmacological studies indicate that it is not involved in setting resting membrane potential, nor in shaping AHP. Its presence in all investigated neurons, without variation in its expression, suggests that it could be involved in extrinsic functions or metabolic regulations that remain to be investigated.

REFERENCES

1. Pape HC. *Annu Rev Physiol* **58**, 299–327 (1996).
2. Halliwell JV and Adams PR. *Brain Res* **250**, 71–92 (1982).
3. BoSmith RE, Briggs I and Sturgess NC. *Br J Pharmacol* **110**, 343–349 (1993).
4. Smith CE and Goldberg JM. *Biol Cybem* **54**, 41–51 (1986).
5. Chabbert C, Chambard JM, Sans A *et al*. *J Neurophysiol* **85**, 1017–1026 (2001).
6. Perkins KL and Wong RK. *J Neurophysiol* **73**, 911–915 (1995).
7. Bal R and Oertel D. *J Neurophysiol* **84**, 806–817 (2000).
8. Kubo Y, Reuveny E, Slesinger PA *et al*. *Nature* **364**, 802–806 (1993).
9. Santoro B and Tibbs CR. *Ann NY Acad Sci* **868**, 741–764 (1999).
10. Chen C. *Hear Res* **110**, 179–90 (1997).
11. Southan AP, Morris NP, Stephens GJ *et al*. *J Physiol* **526**, 91–97 (2000).
12. Ingram SL and Williams JT. *Neuron* **13**, 179–186 (1994).

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