Characterization of a hyperpolarization-activated inward current in rat chemosensory petrosal neurons in vitro

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Abstract—Regulation of carotid body chemoafferent discharge in mammals plays an important role in the reflex control of ventilation. A non-selective blocker (cesium) of the inward rectifier is known to inhibit carotid body afferent discharge during hypoxia, but the underlying current in corresponding neurons of the petrosal ganglia has not been characterized. In this study we provide a detailed description of a voltage-dependent, inwardly rectifying, cation non-selective current, $I_h$, that was present in around 78% of cultured rat petrosal neurons. Activation of this current appeared to be the basis of the slowly developing depolarizing sag that was recorded under current clamp during application of hyperpolarizing current pulses. Under voltage clamp, $I_h$ was activated at voltages negative to $-60$ mV and had an estimated reversal potential ($E_h$) of about $33.1 \pm 3.4$ mV ($n = 20$). Raising extracellular $[K^+]_o$ caused a progressive increase in $I_h$ and a positive shift in $E_h$, whereas reducing extracellular $[Na^+]_o$ caused a small reduction in $I_h$ and an opposite shift in $E_h$. Reducing extracellular $[Cl^-]_o$ had no significant effect on $E_h$, though the amplitude of $I_h$ decreased. Tail current analysis revealed that the activation curve for $I_h$ was well fitted by the Boltzmann distribution, with $V_{1/2} = -90.6 \pm 2.2$ mV (mean $\pm$ SEM; $n = 17$) and slope factor $k = 10.8 \pm 0.5$. $I_h$ activated more rapidly at larger hyperpolarizations; elevated $[K^+]_o$ or lowered $[Na^+]_o$ increased the time constant ($\tau$) of $I_h$ activation. The time constant of deactivation of $I_h$ at $-60$ mV was $317.1 \pm 31.9$ ms ($n = 7$). Extracellular cesium (10 mM) almost completely blocked $I_h$, whereas barium suppressed $I_h$ by around 50%, at a similar concentration. These results, combined with the known sensitivity of the hypoxic afferent discharge to extracellular cesium, suggest that $I_h$ likely plays an important physiological role during carotid body chemosensory signaling.

Keywords: Hyperpolarization-activated current; cell culture; sensory neurons; voltage clamp.

1. INTRODUCTION

Since the first description by Katz (1949), anomalous or inward rectification has been identified in a variety of cell types including muscle cells, endothelial cells and neurons (Hille, 1992). The classic anomalous rectifier ($I_{Kir}$) is $K^+$ selective, blocked by barium and cesium, and activates rapidly at potentials negative to the potassium equilibrium potential ($E_K$) (Hagiwara and Takahashi, 1974; Gay and Stanfield, 1977; Constanti and Galvan, 1983). Another type, the hyperpolarization-activated inward current ($I_h$), is cation non-selective (permeable to both $Na^+$ and $K^+$ ions), blocked
by cesium but not or only partially by barium, and activates more slowly upon hyper-
polarization (DiFrancesco, 1981; Crepel and Penit-Soria, 1986; Kamondi and Reiner,
1991; Wollmuth and Hille, 1992). \(I_h\) has also been described as \(I_f\) in pacemaker
cells of the heart (Brown and DiFrancesco, 1980; Irisawa \textit{et al.}, 1993) and as \(I_Q\) in
hippocampal pyramidal cells (Halliwell and Adams, 1982). Although the significance
of \(I_h\) is unclear it is thought to regulate pacemaker activity in spontaneously spiking
cells (Irisawa \textit{et al.}, 1993) or to prevent the over-hyperpolarization of the cell mem-
brane in sensory neurons and thus keep the membrane potential in a range suitable
for the discharge and release of neurotransmitters (Fain and Lisman, 1981; Mayer and
Westbrook, 1983).

Our long-range goal is to understand how petrosal sensory afferents receive and
process information from peripheral chemoreceptors in the mammalian carotid body.
These visceral afferents project to the respiratory control center in the brainstem and
signal changes in arterial \(P_{O_2}\) by regulating spike frequency, apparently in response
to neurotransmitters secreted by \(O_2\) chemoreceptors (Gonzalez \textit{et al.}, 1994). Previous
studies in our laboratory have characterized a variety of voltage-dependent currents in
cultured rat petrosal neurons (Stea and Nurse, 1992). These include both tetrodotoxin
(TTX)-sensitive and TTX-resistant sodium currents, delayed rectifier and calcium-
dependent potassium currents, and L-type calcium currents. In contrast to somatic
sensory neurons (Mayer and Westbrook, 1983; Scoggs \textit{et al.}, 1994), little is known
about \(I_h\) in visceral sensory neurons, though the presence of inward rectification
has been noted in neurons of the nodose and petrosal ganglia in cat (Gallego and
Eyzaguirre, 1978; Gallego, 1983). In the present study we frequently encountered
\(I_h\) during voltage clamp recordings from cultured rat petrosal neurons and provide a
detailed characterization of its properties. In view of a recent report that extracellular
cesium, a non-specific blocker of \(I_h\), inhibits carotid sinus nerve discharges during
hypoxia (Doyle and Donnelly, 1994), it is likely that this current plays an important
role in regulating spike frequency during chemosensory signaling.

2. METHODS

2.1. Cell culture

Dissociated cells from petrosal ganglia were obtained from 2- to 14-day-old rat pups
(Wistar, Charles River, Quebec, Canada) as previously described (Stea and Nurse,
1992). Briefly, excised ganglia were incubated for 1 h at 37°C in an enzymatic so-
lution containing 0.1% collagenase/0.1% trypsin (Gibco, Grand Island, NY, USA).
The enzyme was then replaced by growth medium consisting of F-12 nutrient medium
(Gibco) supplemented with 10% fetal bovine serum (Gibco), 80 U/l insulin (Sigma,
St Louis, MO, USA), 0.6% glucose, 2 mM glutamine and 1% penicillin–streptomycin
(Gibco). The tissues were mechanically dissociated with forceps, triturated to yield
a cell suspension and plated onto a thin layer of Matrigel (Collaborative Research,
Bedford, MA, USA) that was previously applied to the central wells of 35 mm tis-
tue culture dishes. In some experiments petrosal neurons were grown in co-culture.