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Research paper

Genetic perturbations suggest a role of the resting potential in regulating the expression of the ion channels of the KCNA and HCN families in octopus cells of the ventral cochlear nucleus

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ABSTRACT

Low-voltage-activated K^+ (g_{KL}) and hyperpolarization-activated mixed cation conductances (g_h) mediate currents, I_{KL} and I_h, through channels of the Kv1 (KCNA) and HCN families respectively and give auditory neurons the temporal precision required for signaling information about the onset, fine structure, and time of arrival of sounds. Being partially activated at rest, g_{KL} and g_h contribute to the resting potential and shape responses to even small subthreshold synaptic currents. Resting g_{KL} and g_h also affect the coupling of somatic depolarization with the generation of action potentials. To learn how these important conductances are regulated we have investigated how genetic perturbations affect their expression in octopus cells of the ventral cochlear nucleus (VCN). We report five new findings: First, the magnitude of g_h and g_{KL} varied over more than two-fold between wild type strains of mice. Second, average resting potentials are not different in different strains of mice even in the face of large differences in average g_{KL} and g_h. Third, I_{KL} has two components, one being α-dendrotoxin (α-DTX)-sensitive and partially inactivating and the other being α-DTX-insensitive, tetraethylammonium (TEA)-sensitive, and non-inactivating. Fourth, the loss of Kv1.1 results in diminution of the α-DTX-sensitive I_{KL}, and compensatory increased expression of an α-DTX-insensitive, tetraethylammonium (TEA)-sensitive I_{KL}. Fifth, I_h and I_{KL} are balanced at the resting potential in all wild type and mutant octopus cells even when resting potentials vary in individual cells over nearly 10 mV, indicating that the resting potential influences the expression of g_h and g_{KL}. The independence of resting potentials on g_{KL} and g_h shows that g_{KL} and g_h do not, over days or weeks, determine the resting potential but rather that the resting potential plays a role in regulating the magnitude of either or both g_{KL} and g_h.

1. Introduction

Acoustic information is carried in the timing as well as the rate of firing of brain stem auditory neurons. The interplay between depolarization-activated, low-voltage-activated K^+ conductances (g_{KL}) mediated by ion channels of the Kv1 family and hyperpolarization-activated conductances (g_h) mediated through HCN channels gives auditory neurons the ability to receive and convey precisely timed electrical signals, as has been demonstrated in spiral ganglion cells (Mo and Davis, 1997; Liu et al., 2014; Mo et al., 2002; Kim and Holt, 2013), bushy and octopus cells in the VCN (Oertel, 1983; Manis and Marx, 1991; Rusznak et al., 1996; Cao et al., 2007), neurons in the medial nucleus of the trapezoid body (Brew and Forsythe, 1995; Cuttle et al., 2001), medial superior olive (Scott et al., 2005; Khurana et al., 2011), lateral superior olive (Barnes-Davies et al., 2004), and the ventral nucleus of the lateral lemniscus (Wu, 1999; Berger et al., 2014). In octopus cells g_{KL} and g_h are especially large (Bal and Oertel, 2000, 2001; Ferragamo and Oertel, 2002; Cao and Oertel, 2011; McGinley et al., 2012). These conductances have opposite voltage dependence of activation and at rest the currents flow in opposite directions, I_{KL} flowing outward and I_h flowing inward, so that perturbations of the resting potential in either direction draw the voltage back toward rest.

The presence of g_h and g_{KL} enhances the timing of signaling in neurons in three ways. First, the partial activation of both conductances lowers the resting input resistance and speeds the rise and fall of voltage changes. Second, the voltage sensitivity around

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Abbreviations

DNQX	6,7-dinitroquinoxaline-2,3-dione
DTX	dendrotoxin
g_h	hyperpolarization-activated, mixed cation conductance
g_{KL}	low-voltage-activated potassium conductance
Ih	hyperpolarization-activated, mixed cation current
I_{KL}	low-voltage-activated potassium current
TEA	tetraethylammonium
TTX	tetrodotoxin
VCN	ventral cochlear nucleus

rest and the rapidity of the activation of g_{KL} enables g_{KL} to shorten and sharpen synaptic potentials. Third, the rapidity of the activation of g_{KL} makes the firing of neurons that have this conductance sensitive to the rate at which they are depolarized, and thus makes them effective coincidence detectors. Since the rate of depolarization depends on the temporal scatter of coincident subthreshold inputs, the sensitivity to the rate of depolarization sharpens the window over which octopus cells detect coincident firing in their auditory nerve inputs (Ferragamo and Oertel, 2002; McGinley and Oertel, 2006; Golding and Oertel, 2012).

g_{KL} is mediated through tetrameric, voltage-sensitive K^+ channels of the Kv1 family; four α subunits associate with four β subunits that modulate functional properties. Kv1.1 α subunits are present on the somatic and dendritic membranes of octopus cells as well as at perinodes of auditory nerve axons and octopus cell axons (Oertel et al., 2008; Rusznak et al., 2008; Robbins and Tempel, 2012). Immunohistochemical visualization of Kv1.1 (Oertel et al., 2008; Robbins and Tempel, 2012), Kv1.2 (Rusznak et al., 2008), Kv1.4 (Fonseca et al., 1998; Lujan et al., 2003), and Kv1.6 (Rusznak et al., 2008) subunits suggests that these subunits are expressed in octopus cells. The sensitivity of g_{KL} to DTX K, a blocker that is specific for Kv1.1 containing channels (Robertson et al., 1996), confirms the role of Kv1.1 in g_{KL} (Bal and Oertel, 2001). To be expressed at the membrane, Kv1.1 subunits require coassembly with other subunits, most often with Kv1.2 and Kv1.4 (Hopkins et al., 1994; Manganas and Trimmer, 2000; Ovsepian et al., 2016). The sensitivity of g_{KL} to α-DTX and to tityustoxin, selective for channels containing Kv1.2 subunits (Werkman et al., 1993; Robertson et al., 1996; Owen et al., 1997; Wang et al., 1999a,b; Hopkins, 1998), functionally confirms the role of Kv1.2 subunits in octopus cells (Bal and Oertel, 2001).

g_h is mediated through tetrameric voltage-sensitive channels that are composed of HCN1-4 α subunits (Ludwig et al., 1998; Robinson and Siegelbaum, 2003). HCN1, HCN2 and HCN4 are expressed in the VCN (Moosmang et al., 1999; Koch et al., 2004; Notomi and Shigemoto, 2004) and HCN1 and HCN2 have been shown specifically to be expressed in octopus cells (Koch et al., 2004). Homomeric HCN1 channels have the most rapid kinetics, HCN2 channels are slower, and HCN4 are the slowest; heteromeric channels have intermediate properties (Santoro et al., 1998; Moosmang et al., 1999; Ulens and Tytgat, 2001; Altomare et al., 2003; Whitaker et al., 2007). ZD7288 blocks g_h in many neurons, including in octopus cells (Harris and Constanti, 1995; Khakh and Henderson, 1998; Luthi and McCormick, 1998; Maccaferri and McBain, 1996; Bal and Oertel, 2000). In spiral ganglion cells g_h is formed from HCN1, HCN2, and HCN4 subunits (Kim and Holt, 2013; Liu et al., 2014). Many other auditory neurons also express g_h (Banks et al., 1993; Brew and Forsythe, 1995; Rusznak et al., 1996; Wu, 1999; Cuttle et al., 2001; Dodson et al., 2002; Mo et al., 2002; Svirskis et al., 2004; Cao et al., 2007; Khurana et al., 2011). The HCN channels that mediate I_h are permeable to both Na^+ and K^+ and have a reversal potential of −40 mV so that I_h is inward at the resting potential in octopus and many other types of neurons (Banks et al., 1993; Bal and Oertel, 2000).

HCN1 and Kv1.1 are colocalized in the somatic and dendritic octopus cell membrane (Oertel et al., 2008; Rusznak et al., 2008; Robbins and Tempel, 2012). In an earlier study we examined mice in which HCN1 was eliminated to alter g_h (Cao and Oertel, 2011). We found that in octopus cells of HCN1 null mutants g_{KL} is concomitantly reduced with g_h leading us to conclude that g_h governs g_{KL} (Cao and Oertel, 2011). Here we test the generality of that conclusion by addressing the complementary question, whether removing Kv1.1, a subunit that is present in many g_{KL} channels, alters I_{KL} or I_h or both. The present results support the earlier conclusion that g_h governs the expression of g_{KL} because we find that g_{KL} in Kv1.1 null mutants at the steady state is unchanged, that the loss of Kv1.1 is compensated by other subunits.

The large size of g_h and g_{KL} in octopus cells allows us to resolve and compare the expression of these conductances at the resting potential in different strains of mice. We found that at the resting potential, the magnitude of I_h equals the magnitude of the sum of an α-DTX-sensitive and an α-DTX-insensitive, TEA-sensitive I_{KL} in each of five strains of mutant and wild type mice even when resting potentials varied between cells over nearly 10 mV and g_h varied over a five-fold range. It has been suggested by many investigators that g_h and g_{KL} set the resting potential. That is true in the short term but the present experiments show that in the long term it is the resting potential that regulates the g_{KL} so that I_{KL} balances I_h at rest.

2. Materials and methods

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the School of Medicine and Public Health at the University of Wisconsin-Madison (M005303).

2.1. Mice

Bruce Tempel kindly sent us mice that lack the Kv1.1 subunit (Smart et al., 1998). The null mutant was generated by injecting AB-1 embryonic stem cells that contained a *Kcna1* targeting vector into C57BL/6J blastocysts. Chimeric mice that expressed the null mutation in the germline were then bred to C3HeB/FeJ mice and have been inbred for more than 15 generations. The absence of Kv1.1 leads to seizures and neuromyotonia and to enhanced excitability at neuromuscular junctions at high temperatures (Smart et al., 1998; Zhou et al., 1998). The mouse line was maintained by breeding heterozygote mice. Genotyping was based on tail digests with three primers, a wild-type forward, a mutant forward and a common reverse, as outlined on the Tempel website (http://depts.washington.edu/tempelab/Protocols/KCNA1.html). Alkaline digests were used to obtain DNA from tails. DNA was then combined with nucleotides, primers and Flexi-Go Taq DNA polymerase, amplified over 30 PCR cycles, and run on gels. Measurements in homozygous Kv1.1 null mice were compared with measurements in wild type control mice.

Mice that lack the HCN1 subunit were genetically engineered by Nolan et al. (2003); the mice used for the present experiments were propagated by inbreeding homozygous mutants from a breeding pair purchased from the Jackson Laboratory (ME) (Cao and Oertel, 2011). Experiments were performed using $HCN1^{-/-}$ mice constructed from 129S/SvEv-derived MM13 embryonic stem cells that