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IDENTITY, EXPRESSION AND FUNCTIONAL ROLE OF THE SODIUM-ACTIVATED POTASSIUM CURRENT IN VESTIBULAR GANGLION AFFERENT NEURONS

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Abstract—Vestibular afferent neurons (VANs) transmit information from the vestibular end organs to the central nuclei. This information is encoded within the firing pattern of these cells and is heavily influenced by the K^+ conductances expressed by vestibular neurons. In the present study, we describe the presence of a previously unidentified Na^+-activated K^+ conductance (K_{Na}) in these cells. We observed that the blocking of Na^+ channels by tetrodotoxin (TTX) or the substitution of choline for Na^+ in the extracellular solution during voltage clamp pulses resulted in the reduction of a sustained outward current that was dependent on the Na^+ current. Furthermore, increases in the intracellular concentration of Na^+ that were made by blocking the Na^+/K^+ ATPase with ouabain increased the amplitude of the outward current, and reduction of the intracellular Cl^- concentration reduced the TTX-sensitive outward current. The substitution of Li^+ for Na^+ in the extracellular solution significantly reduced the amplitude of the outward current in voltage clamp pulses and decreased the afterhyperpolarization (AHP) of the action potentials in current clamp experiments. These electrophysiological results are consistent with the presence of mRNA transcripts for the K_{Na} subunits Slick and Slack in the vestibular ganglia and in the sensory epithelium, which were detected using reverse-transcription polymerase chain reaction (RT-PCR). These results are also consistent with the immunolabeling of Slick and Slack protein in isolated vestibular neurons, in the vestibular ganglion and in the vestibular sensory epithelium. These results indicate that K_{Na} channels are expressed in VANs and in their terminals. Furthermore, these data indicate that these channels may contribute to the firing pattern of vestibular neurons.

Key words: slack, slick, synapse, inner ear, intrinsic properties, lithium.

INTRODUCTION

Vestibular afferent neurons (VANs) transmit information regarding angular and linear accelerations from the vestibular end organs to the vestibular nuclei during head movements. The discharge properties of VANs at rest and during mechanical stimulation have been examined in several species (Goldberg and Fernández, 1971a,b; Honrubia et al., 1989; Brichta and Goldberg, 2000). In those organisms, VANs have been classified as regular and irregular using the coefficient of variation of their action potential discharge rate at rest. The regularity of the spontaneous discharge and the dynamic responses of VANs vary with the location of their afferent terminals in the sensory epithelium. The morphological characteristics of the synapses between the afferent neurons and hair cells also vary with their location in the epithelium and are likely involved in the setting of the electrical discharge pattern. Morphologically, VANs have been classified into button-shaped, calyx and dimorphic (Fernández et al., 1988). Although the regularity of the afferent neuron discharge was initially attributed to their synaptic input, cumulative evidence indicates that variations in the specific population of K^+ channels that are expressed by these cells play a significant role in the determination of their discharge pattern and consequently in the coding of vestibular information (Limón et al., 2005; Iwasaki et al., 2008; Pérez et al., 2009; Kalluri et al., 2010). Several voltage-dependent K^+ conductances have been described in vestibular ganglion neurons. Briefly, Chabbert et al. (2001) described three voltage-dependent K^+ currents that can be identified by their kinetic properties and by their sensitivity to tetraethylammonium (TEA), to 4-aminopyridine (4-AP) and α-dendrotoxin (α-DTX), and to 4-AP and blood depressing substance (BDS). The presence of TEA- and 4-AP-sensitive K^+ currents was also observed in non-enzymatically altered ganglion neurons (Risner and Holt, 2006) and in recordings from calyx terminals (Dhawan et al., 2010). The hyperpolarization-activated current (I_h) have been described in cultured vestibular ganglion neurons and in the calyx endings of VAN (Almanza et al., 2012; Meredith et al., 2012). Cultured vestibular ganglion neurons differentially display a Ca^{2+}-dependent K^+ current (K_{Ca}), which is composed of BK, IK and SK (big-, intermediate- and small-conductance, respectively). A fourth component that is resistant to classical K_{Ca} channel blockers is also observed in vestibular ganglion neurons (Limón et al., 2005). SK-type K_{Ca} currents were

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Abbreviations: 4-AP, 4-aminopyridine; AHP, afterhyperpolarization; BSA, bovine serum albumin; α-DTX, α-dendrotoxin; FMRP, fragile X mental retardation Protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered solution; RT-PCR, reverse-transcription polymerase chain reaction; TEA, tetraethylammoniun; TTX, tetrodotoxin; VAN, vestibular afferent neurons.

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recently reported in calyx terminals (Meredith et al., 2011). Additionally, the presence of an M-current in large soma-size ganglion neurons and calyx terminals indicates that spike-frequency adaptation in VANs is under the cholinergic control of K^+ currents (Hurley et al., 2006; Pérez et al., 2009, 2010). Interestingly, KCNQ K^+ channels which carry the M-type current, and α-DTX-sensitive Kv1 channels contribute to the low-voltage-activated outward current that participates in conferring irregularity to the stimuli-evoked responses of isolated vestibular ganglion neurons (Kalluri et al., 2010). Furthermore, immunohistochemical studies have demonstrated that KCNQ, $K_V1.1$ and $K_V1.2$ channels are expressed in segregated microdomains of calyx terminals (Lysakowski et al., 2011). These microdomains also express the Na^+ channel subunit $Na_v1.6$, which is sensitive to low concentrations of tetrodotoxin (TTX), and Nav1.5, which is resistant to TTX (Lysakowski et al., 2011). Such subunits contribute, at least in part, to the Na^+ currents that are recorded in calyx terminals (Rennie and Streeter, 2006) and may contribute to those currents that are observed in isolated vestibular neurons (Chabbert et al. 1997; Soto et al., 2002). However, the broad range of the Na^+ current activation that is observed in electrophysiological experiments (Chabbert et al., 1997; Soto et al., 2002; Rennie and Streeter, 2006), as well as the expression of mRNA transcripts for the majority of the TTX-sensitive Na^+ channel subunits (Wooltorton et al., 2007), indicate that the Na^+ current in VANs is due to the activity of a combination of different Na^+ channels, the functional roles of which may not necessarily be temporally limited to the initiation of action potentials (Wooltorton et al., 2007).

The Na^+-dependent K^+ conductance (K_{Na}) is broadly distributed in the brain. K_{Na} channels are tetramers formed by homomers or heteromers of two different subunits encoded by the *Slack* (*Slo2.2*; sequence like a calcium-activated K^+ channel) and *Slick* (*Slo2.1*; sequence like an intermediate conductance K^+ channel) genes (Dryer, 1994; Bhattacharjee et al., 2002). The K_{Na} current (I_{KNa}) is activated by intracellular Na^+ and by intracellular Cl^-, its sensitivity to these ions depending on the subunits forming the channel. In this article, we report that VANs express a K_{Na} that is mediated by at least two different K_{Na} channel subunits, Slack and Slick, which participate in the action potential afterhyperpolarization (AHP) and phase locking of stimuli-evoked action potentials in isolated ganglion neurons.

EXPERIMENTAL PROCEDURES

We used Wistar rats at postnatal days (P) 7–10 for the electrophysiological experiments and for the detection of mRNA transcripts. For the immunohistochemical experiments and to determine the expression pattern of K_{Na} channels during postnatal development, we used P7–P10 and P21–P24 rats. All of the rats were supplied by the ''Claude Bernard'' animal house of the University of Puebla. The protocols that involved animal research were reviewed and approved by the Comité Institucional de Cuidado y uso de Animales de Laboratorio (CICUAL) from the Consejo de Investigación y Estudios de Posgrado de la Vicerrectoria de Investigación y Estudios de Posgrado (VIEP-BUAP). All of the animal care and experimental procedures were performed according to the *Reglamento de la Ley General de Salud en Materia de Investigación para la Salud* of the Secretaría de Salud de México. All efforts were made to minimize animal suffering and to reduce the number of animals used, as outlined in the ''Guide to the Care and Use of Laboratory Animals'', which is issued by the National Academy of Sciences.

Cell culture of vestibular ganglion neurons

To obtain the VAN cultures, we used methods that have been previously described in detail (Soto et al., 2002; Limón et al., 2005). Briefly, the rats were euthanized by decapitation. The brain was then removed, the vestibular nerves were exposed and the vestibular ganglia were isolated. The tissue was first incubated in L-15 culture medium (Gibco, Grand Island, NY, USA) that was supplemented with 1.25 mg/mL collagenase type 1A (Sigma–Aldrich, St. Louis, MO, USA) and 1.25 mg/mL porcine trypsin (USB, Cleveland, OH, USA) for 30 min at 37 °C. This step was followed by a wash with fresh L-15 medium. Following the enzymatic treatment, the tissue was dissociated by mechanical agitation. The dissociated cells were placed in Petri dishes (Nunc, Denmark, 35 mm) in L-15 medium that was modified for CO_2 culture conditions by adding 10% fetal bovine serum (FBS) (Gibco), 10 mM $NaHCO_3$, 10 mM HEPES buffer and 1000 IU/mL penicillin. All of the procedures were performed in a tissue culture room under a laminar flow hood (Nuaire, Plymouth, MN, USA). The dissociated cells were incubated at 37 °C in an atmosphere of 95% air and 5% CO_2 until the electrophysiological recordings (18–24-h cultured cells).

Electrophysiological recordings and data analysis

We used primary cultured VANs to (i) allow for the isolated cells to recover from potential membrane alterations following the dissociation procedure and (ii) to facilitate patching of the cells after the myelin surrounding the soma disappeared during the culturing process. Whole-cell voltage and current clamp experiments were performed at room temperature (23–25 °C). The patch pipettes were pulled from borosilicate glass capillary tubing (WPI, Sarasota, FL, USA) using a Flaming-Brown micropipette puller (80/PC; Sutter Instruments, San Rafael, CA, USA). The pipettes typically had a resistance between 1 and 3.5 MΩ, and the seal resistance exceeded 1 GΩ. We used an Axopatch 200B voltage clamp amplifier (Molecular Devices, Union City, CA, USA). The command signals and data acquisition were generated using an analog–digital converter (Digidata 1200, Molecular Devices) that was controlled by the pClamp 9.0 software (Molecular Devices). In all of the experiments, the cell capacitance and series resistance (80%) were electronically compensated once the whole-cell configuration was obtained. The signals were low-pass filtered at 2 kHz and digitized at 20 kHz for the voltage clamp experiments. The maximum amplitude of the I_{KNa} was estimated by subtracting the *I–V* relationship obtained with the perfusion of a Na^+-free solution, from the control *I–V* relationship. The normalized conductance was approximated by a Boltzmann function:

$$g/g_{max} = 1/(1 + e^{((V_m - V_{1/2})/S)})$$

where g is the conductance, g_{max} is the maximum conductance, V_m is the membrane potential, $V_{1/2}$ is the potential at which half of the maximum current is reached and S is the slope factor.

A subset of cells was recorded in current clamp conditions to examine the effects of ion substitution on their response to the current stimuli. For these experiments, the filters were open to 10 kHz, and square current pulses from −0.5 to 0.5 nA with