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ELECTROPHYSIOLOGICAL, PHARMACOLOGICAL AND MOLECULAR PROFILE OF THE TRANSIENT OUTWARD RECTIFYING CONDUCTANCE IN RAT SYMPATHETIC PREGANGLIONIC NEURONS *IN VITRO*

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Abstract—Transient outward rectifying conductances or A-like conductances in sympathetic preganglionic neurons (SPN) are prolonged, lasting for hundreds of milliseconds to seconds and are thought to play a key role in the regulation of SPN firing frequency. Here, a multidisciplinary electrophysiological, pharmacological and molecular single-cell rt-PCR approach was used to investigate the kinetics, pharmacological profile and putative K^+ channel subunits underlying the transient outward rectifying conductance expressed in SPN. SPN expressed a 4-aminopyridine (4-AP) sensitive transient outward rectification with significantly longer decay kinetics than reported for many other central neurons. The conductance and corresponding current in voltage-clamp conditions was also sensitive to the Kv4.2 and Kv4.3 blocker phrixotoxin-2 (1–10 μM) and the blocker of rapidly inactivating Kv channels, pandinotoxin-Kα (50 nM). The conductance and corresponding current was only weakly sensitive to the Kv1 channel blocker tityustoxin-Kα and insensitive to dendrotoxin I (200 nM) and the Kv3.4 channel blocker BDS-II (1 μM). Single-cell RT-PCR revealed mRNA expression for the α-subunits Kv4.1 and Kv4.3 in the majority and Kv1.5 in less than half of SPN. mRNA for accessory β-subunits was detected for Kvβ2 in all SPN with differential expression of mRNA for KChIP1, Kvβ1 and Kvβ3 and the peptidase homologue DPP6. These data together suggest that the transient outwardly rectifying conductance in SPN is mediated by members of the Kv4 subfamily (Kv4.1 and Kv4.3) in association with the β-subunit Kvβ2. Differential expression of the accessory β subunits, which may act to modulate channel density and kinetics in SPN, may underlie the prolonged and variable time-course of this conductance in these neurons.

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Abbreviations: aCSF, artificial cerebrospinal fluid; BDS-II, Blood depressing substance II; BSA, bovine serum albumin; DPP6, dipeptidyl aminopeptidase-like protein; DTX-I, Dendrotoxin-I; IML, intermediolateral cell column; I_{TR}, transient outward rectifying potassium conductance; KChIP, Kv channel interacting proteins; Kv, voltage gated potassium channel; NCS, neuronal calcium sensor; PaTx-2, phrixotoxin-2; PiTx-Kα, pandinotoxin-Kα; scRT-PCR, single-cell reverse transcriptase polymerase chain reaction; SPN, sympathetic preganglionic neuron; TiTx-Kα, tityustoxin-Kα; 4-AP, 4-aminopyridine.

Key words: A-current, KChIP, DPP6, rtPCR, patch clamp, spinal cord.

Outward currents through potassium (K^+) channels are the primary means by which excitable cells oppose membrane excitability. Several distinct types of K^+-mediated conductances have been identified in sympathetic preganglionic neurons (SPN; Polosa et al., 1988; Pickering et al., 1991; Sah and McLachlan, 1995; Miyazaki et al., 1996; Wilson et al., 2002). One such conductance, the transient outward rectifying conductance or current (I_{TR}), is a hallmark of SPN and is used as an identifiable, characteristic electrophysiological feature of these neurons (Yoshimura et al., 1987; Pickering et al., 1991; Inokuchi et al., 1993). This conductance has historically been referred to as the A-like conductance in SPN, based upon its similarity to A-type K^+ currents and conductances described in other neurons (see Jerng et al., 2004a for review). The unusual feature of the conductance in SPN is its variable and often prolonged time-course, lasting for hundreds of milliseconds to seconds.

Transient outward currents are voltage-dependent K^+ currents that possess rapidly activating and inactivating kinetics. In many neurons, A-type channels are unavailable at resting membrane potentials due to pronounced steady-state inactivation and only become available in these cells during afterhyperpolarization following action potential discharge, when the membrane potential becomes sufficiently negative to remove inactivation. A-type channels activate during the decay of the afterhyperpolarization and tend to delay depolarization. In this manner, they are believed to participate in the regulation of firing frequency (Connor and Stevens, 1971; Miyazaki et al., 1996) and in some neurons, sustain spike repolarization virtually alone (Storm, 1987).

At the molecular level, voltage-gated K^+ channels (Kv) mediate these rapidly inactivating outward currents. Kv channels are composed of two classes of subunits; the α-subunit, which forms the channel pore and auxiliary (β) subunits. The Kv α-subunit is a typical six transmembrane spanning protein, with unusually large intracellular N- and C-termini loops. These confer control of the activity-dependent regulation of channel closure (channel inactivation) and are the site of protein–protein interactions (interaction with cytoplasmic β-subunits) within the channel. At least 18 genes for Kv-channels are expressed in the CNS (Jan and Jan, 1997). They are grouped into four conserved subfam-

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ilies; Kv1.1–1.8 (Shaker), Kv2.1, 2.2 (Shab), Kv3.1–3.4 (Shaw) and Kv4.1–4.3 (Shal) (see Gutman et al., 2005). Four mammalian β-subunits, (Kvβ1–4) have also been identified (Scott et al., 1994; Rettig et al., 1994; Heinemann et al., 1995; Jan and Jan, 1997). Expression studies have shown that six α-subunits from three Kv subfamilies mediate rapidly inactivating outward currents: Kv1.4, Kv3.3, Kv3.4, Kv4.1, Kv4.2 and Kv4.3 (Song et al., 1998; Jerng et al., 2004a,b). Moreover, Kv1 subfamily channels that alone yield delayed rectifier currents, inactivate rapidly in the presence of specific β-subunits (Rettig et al., 1994; Heinemann et al., 1995) for example, Kv1.5 and Kvβ3 (Leicher et al., 1998).

Although pore-forming α-subunits form rapidly inactivating outward currents in heterologous cells, these differ considerably from native currents (An et al., 2000). However, four Kv channel interacting proteins (KChIP 1–4) have been identified, which when expressed with Kv4 subunits reconstitute several features of native channels (An et al., 2000; Morohashi et al., 2002). All four KChIP proteins are differentially expressed in the CNS (Xiong et al., 2004) with KChIP1, KChIP3 (An et al., 2000) and KChIP4 (Rhodes et al., 2004) being highly expressed in neuronal tissues. Other modulators of Kv channel kinetics include the neuronal calcium sensor-1 (NCS-1, frequenin; Nakamura et al., 2001a), expressed throughout the gray matter of the spinal cord (Averill et al., 2004), dipeptidyl aminopeptidase-like protein 6 (DPP6) (DPPX, BSPL; Kin et al., 2001; Nadal et al., 2003) and DPP10 (DPPY, Jerng et al., 2004b; Zagha et al., 2005). As such, differential expression of these subunits may be a contributing factor prolonging the time-course of the transient conductance observed in SPN.

Thus, in the present study, using a multidisciplinary whole-cell electrophysiological, pharmacological and molecular approach, we have characterized further the electrophysiological and pharmacological profiles of I_{TR} in SPN. Furthermore, using single-cell RT-PCR we have begun to identify putative molecular constituents of the ion channels forming the substrates underlying this conductance and accessory subunits that may contribute to the prolonged time-course observed in SPN.

EXPERIMENTAL PROCEDURES

Slice preparation

Electrophysiological recordings were made from transverse thoracolumbar spinal cord slices as described previously (Pickering et al., 1991; Wilson et al., 2002). Protocols were in accordance with the UK. Animals (Scientific Procedures) Act (1986) and the Canadian Council for Animal Care guidelines, and approved by the Ottawa Hospital Research Institute Animal Care and Use Committee. Care was taken to minimize the number of animals used and to reduce their suffering. Briefly, Wistar Kyoto rats, aged 6–14 days (either sex), were terminally anaesthetized using 4% enflurane in O_2, cervically dislocated, decapitated, the spinal cord removed and thoracic sections cut into 300–400 μm thick slices using a Leica VT1000 S tissue slicer. Slices were maintained in artificial cerebrospinal fluid (aCSF) at room temperature for 1 h after slicing before experimentation was performed. For recording, individual slices were held between two grids in a custom built chamber continuously perfused with aCSF at a rate of 4–10 ml min^{-1}, illuminated from below and viewed under a dissection microscope. The aCSF was of the following composition (mM); NaCl, 127; KCl, 1.9; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgCl_2$, 1.3; $NaHCO_3$, 26; D-glucose, 10; equilibrated with 95% O_2-5% CO_2.

Recordings

Whole cell recordings were performed at room temperature (17–21 °C) from neurons in the intermediolateral cell column (IML) with an Axopatch 1 D amplifier (MDS Analytical Technologies, Sunnyvale, CA, USA), using the blind version of the patch clamp technique (Pickering et al., 1991). Patch pipettes were pulled from thin-walled borosilicate glass and had resistances of between 3 and 8 MΩ when filled with intracellular solution of the following composition (mM): Kgluconate, 130; KCl, 10; $MgCl_2$, 2; $CaCl_2$, 1; EGTA-Na, 1; HEPES, 10; Na_2ATP, 2 and Lucifer Yellow, 2 (or Biocytin, 5); pH adjusted to 7.4 with KOH, osmolarity adjusted to 310 $mosmol^{-1}$ with sucrose. In voltage clamp recordings, tetrodotoxin (TTX; 500 nM) and carbenoxolone (100 μM) were added to the extracellular solution to block voltage-dependent sodium channels and gap-junctions, respectively.

Series resistance compensation of approximately 70–80% was applied for whole-cell voltage-clamp experiments. Access resistance ranged between 5 and 25 MΩ. Neuronal input resistance was measured by injecting small rectangular-wave hyperpolarizing current pulses (10–100 pA) and measuring the amplitude of resulting electrotonic potentials. Recordings were monitored on an oscilloscope (Gould 1602, Gould Instrument Systems) and displayed on a chart recorder (Gould, Easygraf TA240) along with being stored on digital audio tapes (Biologic, DTR-1205) for subsequent analysis off-line. In addition, data were filtered at 2–5 kHz, (1 kHz for voltage clamp data), digitized at 2–10 kHz (Digidata 1322, MDS Analytical Technologies) and stored on PC running pCLAMP 9 data acquisition software. Analysis of electrophysiological data was carried out using Clampfit 9 software (MDS Analytical Technologies).

Cell identification

SPN were identified by their characteristic electrophysiological properties, a long duration action potential (5–10 ms) with a shoulder on the repolarization phase, a large amplitude (18–30 mV) and prolonged action potential afterhyperpolarization, and the expression of inwardly rectifying and transient outwardly rectifying conductances (Pickering et al., 1991; Wilson et al., 2002). To further confirm recordings from SPN, the neuronal morphology was also routinely determined retrospectively with Lucifer Yellow (dipotassium salt, 1 mg ml^{-1}) or Biocytin (5 mM) in the patch pipette solution. Methods for visualizing filled SPN have been reported in detail previously, (see Pickering et al., 1991 for Lucifer Yellow and Spanswick et al., 1998 for Biocytin).

Cell harvest and single cell RT-PCR

For RT-PCR experiments, thoracic spinal cord sections were cut into 200–300 μm thick slices using a Leica VT1000 S tissue slicer. Whole cell recordings were performed at room temperature (17–21 °C) from neurons in the IML with an Axopatch 1 D amplifier (MDS Analytical Technologies, Sunnyvale, CA, USA), using the visualized version of the patch clamp technique. A Zeiss Axioskop FS2 microscope (Carl Zeiss Ltd., Welwyn Garden City, UK) fitted with a 63× water-immersion objective lens together with gradient contrast optics (Luigs and Neumann, Ratingen, Germany) was used to view slices. Light in the infrared range (>740 nm) was used in conjunction with a contrast-enhancing Newvicon camera (Hamamatsu, Hamamatsu City, Japan) to resolve individual neurons within slices (Stuart et al., 1993). Recording solutions were as above.