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Regulation of excitability in tonic firing substantia gelatinosa neurons of the spinal cord by small-conductance Ca^{2+}-activated K^+ channels

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ABSTRACT

The excitability of substantia gelatinosa (SG) neurons in the spinal dorsal horn determines the processing of nociceptive information from the periphery to the central nervous system. Small conductance Ca^{2+}-activated K^+ (SK) channels on neurons supply strong negative feedback control on neuronal excitability by affecting afterhyperpolarization (AHP). However, the role of SK channels in regulating tonic-firing SG neuron excitability remains elusive. In the present study, whole-cell recordings were conducted in SG neurons from acute spinal cord slices of adult rats. The SK channel opener 1-ethyl-2-benzimidazolinone (1-EBIO) attenuated spike discharges and increased AHP amplitudes; this effect was mimicked by a high Ca^{2+} external solution. Systemic administration of 1-EBIO attenuated the thermal-induced nociception behavior. Conversely, the inhibition of SK channels with apamin, a specific SK channel inhibitor, increased neuronal excitability and decreased the AHP amplitudes; this effect was mimicked by a Ca^{2+}-free external solution. Apamin increased excitatory synaptic transmission by increasing the amplitudes of evoked excitatory postsynaptic potentials (eEPSPs). This facilitation depended on N-methyl-D-aspartate (NMDA) receptors, extracellular Mg^{2+} and intracellular Ca^{2+}. Voltage-gated Ca^{2+} channels (VGCCs) were also involved in the apamin-induced effects. Strikingly, 1-EBIO action on decreasing excitability persisted in the presence of apamin, indicating that 1-EBIO manipulates SK channels via a pathway rather than via apamin-sensitive SK channels. The data reveal a previously uncharacterized mechanism for manipulating SG neuronal excitability by Ca^{2+} conductances via both apamin-sensitive and apamin-insensitive pathways. Because SG neurons in the dorsal horn are involved in regulating nociception, manipulating neuronal excitability via SK channels indicates a potential therapeutic target.

1. Introduction

Nociceptive information from the periphery is transmitted to the spinal cord dorsal horn and is subsequently projected to the thalamus and cortex (Fields et al., 1991; Todd, 2010; Willis and Coggeshall, 1991). Within the spinal cord dorsal horn gray matter, lamina II (substantia gelatinosa; SG) is of particular importance in nociception. Constituted mainly of interneurons, the SG receives direct synaptic input from fine nociceptive primary afferent fibers (Todd, 2010; Wu et al., 2010; Yang et al., 2003) and modulates nociception by multiple neurotransmitters (Basbaum et al., 2009; Mason, 1999). Since the introduction of the "gate theory of pain," extensive scientific effort has been invested in studying the SG neurons that are responsible for these presynaptic effects (Mendell, 2014; Wu et al., 2010). Composed of multiple excitatory and inhibitory interneurons (Heinke et al., 2004; Kim et al., 2012; Punnakkal et al., 2014), SG neurons are thought to play important roles in processing and modulating pain information from the peripheral to the central nervous system (CNS). Therefore, changes in

Abbreviations: 1-EBIO, 1-ethyl-2-benzimidazolinone; aCSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AP-5, D-2-amino-5-phosphonopentanoic acid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CGP52432, 3-[[[(3,4-dichlorophenyl)methyl]amino]propyl] (diethoxy-methyl) phosphinic acid; CNS, central nervous system; DIC, differential interference contract; DRG, dorsal root ganglion; EPSCs, excitatory postsynaptic currents; GABA, γ-aminobutyric acid; IPSCs, inhibitory postsynaptic currents; NMDA, N-methyl-D-aspartate; SG, substantia gelatinosa; SK channels, small conductance Ca^{2+}-activated K^+ channels; TTX, tetrodotoxin; VGCCs, voltage-gated calcium channels.

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the excitability of the SG neurons could be a factor contributing to the modulation and modification of nociceptive information from the periphery to the CNS.

A principal determinant of neuronal excitability is the after-hyperpolarization (AHP) that follows individual action potentials or action potential bursts (Bean, 2007; Bond et al., 2004, 2005). The AHP is mediated by K^+ channels that are opened in response to elevated intracellular Ca^{2+} and supplies a strong negative feedback control over neuronal excitability (Bahia et al., 2005; Safronov, 1999). Three different kinetic components of AHP are recorded in different types of neurons: large conductance voltage- and Ca^{2+}-dependent K^+ (BK) channels underlie fast AHP, while small conductance voltage-independent Ca^{2+}-dependent K^+ (SK) channels contribute to medium AHP (Pedarzani et al., 2005; Sah, 1996; Stocker, 2004). The underlying mechanisms for the slow phase of AHP remain elusive, KCNQ2 and KCNQ3 (Tzingounis and Nicoll, 2008) or KCa3.1 (King et al., 2015) may underlie the slow AHP currents. SK channels are widely expressed in the central and peripheral nervous system, including putative nociceptors of the dorsal root ganglion (DRG) and superficial laminae of the spinal dorsal horn (Bahia et al., 2005; Gymnopoulos et al., 2014; Tsantoulas and McMahon, 2014), while intermediate conductance Ca^{2+}-activated K^+ (IK) channels are much more limited in the nervous system, but are important in many non-neuronal tissues (Pagadala et al., 2013; Pedarzani et al., 2005; but see Tsantoulas and McMahon, 2014). Although SK channels are widely expressed in both the central and peripheral nervous system (Kohler et al., 1996) and control sensory input into the spinal cord (Bahia et al., 2005; Pagadala et al., 2013), little is known about the modulation mechanisms of SK channels in spinal dorsal horn SG neurons.

Several lines of evidence suggest that SG neurons show considerable heterogeneity in firing patterns in response to depolarizing current injections. At least four different firing patterns have been introduced: tonic, delayed, irregular and single spike (Hantman et al., 2004; Lu et al., 2008; Punnakkal et al., 2014). Tonic-firing neurons comprise the majority of GABA- and glycine-containing inhibitory interneurons (Punnakkal et al., 2014). In the present experiments, the analysis was centered on tonic-firing neurons to study the role of SK channels in postsynaptic membrane potentials and the AHP following the action potentials (Heinke et al., 2004; Kim et al., 2008; Kohler et al., 1996), where Ca^{2+}-dependent conductance does not contribute to adapting firing (Melnick et al., 2004a, 2004b). Here, I report the role of SK channels in the modulation excitability of SG tonic-firing neurons, focusing on the spike modulation and the roles of different sources of intracellular Ca^{2+}, which underlies the activation of SK channels.

2. Materials and Methods

2.1. Acute spinal cord slices and electrophysiology

All of the procedures used in the present study were approved by The Animal Use Committees of Jiangsu University and the University of Maryland School of Medicine. The methods for the preparation and recording of transverse spinal cord slices have been detailed previously (Yang et al., 2001). In brief, male Sprague–Dawley rats (5–9 weeks old) were anesthetized with intraperitoneal nembutal sodium (50 mg kg^{-1} bodyweight). Animals were kept in a warm environment, and a laminectomy was carried out. The lumbosacral cord trunk was rapidly removed and immersed in chilled (~0 °C) artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 124, KCl 3.6, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 11 (mOsm ~300, pH 7.2 when carbogenated with 95% O_2/5% CO_2 gas mixture). Three transverse slices (300–400 μm thick) were excised from one rat spinal cord trunk with a Vibratome slicer (Vibratome 1000, St. Louis, MO) and were recovered in warm oxygenated aCSF at ~36 °C for 40 min before recording.

One slice was transferred to a custom-made submersion-type recording chamber (volume ~1 ml) and continuously perfused with aCSF (8–10 ml min^{-1}) at room temperature (22–24 °C). For nominally calcium-free (0 mM Ca^{2+}) external solution, Ca^{2+} was omitted without replacement for calcium ions and the solution was quickly switched to the perfusion system for 5 min. After perfusion, the "normal" aCSF was reinstated. Slices were anchored to the glass bottom of the recording chamber and placed on the stage of an upright microscope equipped with a differential interference contast (DIC) system (Nikon E600FN or Olympus BX51WI). The SG was distinguished using a low-power objective (X4) as a translucent band in the dorsal part of the gray matter under transillumination, and a high-power DIC objective (X40) was used to identify individual neurons within the SG. Whole-cell current- or voltage-clamp recordings were performed with a glass pipette having a resistance of 6–8 MΩ with an internal solution composed of (in mM): K-gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 0.2, HEPES 5 and Mg-ATP 5 (pH 7.2 modulated by KOH, mOsm 290). In a set of experiments, a Cs^+-based internal solution (in mM: Cs-gluconate 117, NaCl, 2.8, $MgCl_2$ 5, HEPES 20, Na-ATP 2, Na_2-GTP 0.3 and EGTA 0.6) was used to block postsynaptic K^+ conductances. In another set of experiments, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA; 5 mM) was included to buffer postsynaptic intracellular Ca^{2+} (Fakler and Adelman, 2008). Conventional whole-cell configurations were first established in voltage-clamp mode and then switched to current-clamp in I-clamp mode. Presynaptic focal stimulation was carried out with rectangular pulses (0.1 ms) using a concentric bipolar electrode (MicroProbe Inc., Gaithersburg, MD) pre-located 80–200 μm from the recorded neurons. Current-clamp signals were amplified by Axoclamp 1A (Axon Instruments) while voltage-clamp recordings were carried out by Axopatch 200B (Molecular Devices, Union City, CA). Signals were digitized at 50 kHz with Digidata 1200A (current-clamp recordings) or Digidata 1322 (voltage-clamp recordings) (both from Molecular Devices) and stored on a personal computer using Clampex9 or Clampex10 software (Molecular Devices). Action potential independent miniature excitatory postsynaptic currents (mEPSCs) were recorded in the presence of tetrodotoxin (TTX; 300 nM) at a holding potential of −70 mV (Yang et al., 2001).

2.2. Action potentials and excitatory postsynaptic potentials (EPSPs) induction

To assess the role of SK channels on SG neuron excitability, we first compared the action potential firing rates across conditions. Action potentials were initiated by either injecting a constant depolarization current or by their responsiveness to a 1-s step current injection. The amplitude of the AHP was measured from the resting membrane potential to the peak of the AHPs (Ibáñez-Sandoval et al., 2010). Presynaptic stimulation-induced glutamatergic evoked EPSPs (eEPSPs) were pharmacologically isolated by bath application of antagonists for glycinergic (strychnine 1 μM), $GABA_A$ receptor-mediated (picrotoxin 100 μM) and $GABA_B$ receptor-mediated (CGP52432 1 μM) components.

2.3. Behavior tests

The methods for the behavioral tests have been introduced elsewhere (Zhang et al., 2008). In brief, 7 male rats (7 weeks old) were used in a paw withdrawal latency (PWL) test before and after the systemic administration (subcutaneously to the neck skin) of 1-ethyl-2-benzimidazolinone [1-EBIO; 10 mg/ml in a Tween-80