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Control of somatic membrane potential in nociceptive neurons and its implications for peripheral nociceptive transmission

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ABSTRACT

Peripheral sensory ganglia contain somata of afferent fibres conveying somatosensory inputs to the central nervous system. Growing evidence suggests that the somatic/perisomatic region of sensory neurons can influence peripheral sensory transmission. Control of resting membrane potential (E_{rest}) is an important mechanism regulating excitability, but surprisingly little is known about how E_{rest} is regulated in sensory neuron somata or how changes in somatic/perisomatic E_{rest} affect peripheral sensory transmission. We first evaluated the influence of several major ion channels on E_{rest} in cultured small-diameter, mostly capsaicin-sensitive (presumed nociceptive) dorsal root ganglion (DRG) neurons. The strongest and most prevalent effect on E_{rest} was achieved by modulating M channels, K2P and 4-aminopiridine-sensitive K_V channels, while hyperpolarization-activated cyclic nucleotide-gated, voltage-gated Na^+, and T-type Ca^{2+} channels to a lesser extent also contributed to E_{rest}. Second, we investigated how varying somatic/perisomatic membrane potential, by manipulating ion channels of sensory neurons within the DRG, affected peripheral nociceptive transmission in vivo. Acute focal application of M or K_{ATP} channel enhancers or a hyperpolarization-activated cyclic nucleotide-gated channel blocker to L5 DRG in vivo significantly alleviated pain induced by hind paw injection of bradykinin. Finally, we show with computational modelling how somatic/perisomatic hyperpolarization, in concert with the low-pass filtering properties of the t-junction within the DRG, can interfere with action potential propagation. Our study deciphers a complement of ion channels that sets the somatic E_{rest} of nociceptive neurons and provides strong evidence for a robust filtering role of the somatic and perisomatic compartments of peripheral nociceptive neuron.

1. Introduction

In contrast to the majority of central nervous system neurons, peripheral somatosensory neurons normally generate action potentials (APs) at peripheral nerve endings, not at the axon hillock [2,3]. While somatic APs and electrogenesis are not required for AP propagation from the periphery to the spinal cord [4], sensory neuron somata are electrically excitable [3,12,111,122], and ectopic somatic activity [3,12,77,111,122], along with ectopic peripheral fibre activity [22,23,125], is thought to contribute to many chronic pain conditions. Moreover, measurements [28,33,110,113] and simulations [79] suggest that the axonal bifurcation (t-junction) within dorsal root ganglia (DRG) influences the transmission of spikes on their way to the spinal cord. Hitherto unexplained recent clinical studies have established that direct electrical stimulation (“neuromodulation”) of the DRG provides efficacious pain relief in neuropathic pain patients [20,95]. Taken together, these findings suggest that sensory ganglia may play a much stronger role in peripheral nociceptive transmission than is generally accepted. Moreover, sensory ganglia may represent a novel target for pain therapeutics [95]. Yet, surprisingly little has

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been done so far to directly test how electrophysiological properties of somatic/perisomatic compartment of sensory neuron affect peripheral somatosensory transmission.

The aims of this study were 1) to identify major ion channels influencing the resting membrane potential (E_{rest}) of nociceptive DRG neurons and 2) to investigate if (and how) manipulation with the activity of these channels within the somatic/perisomatic compartments of DRG would affect peripheral nociceptive transmission. In the first part we focused on the ion channels that are known to be expressed in nociceptive DRG neurons and would be expected to be active at, and possibly contribute to, the E_{rest} of these neurons. These channels included 4-aminopiridine (4-AP)-sensitive voltage-gated K^+ (K_V) channels [26], slow-activating M channels (Kv7, KCNQ) [26,58,71,74,92,93,100,101], 2-pore K^+ "leak" channels (K2P) [1,80,115], sodium-activated K^+ channels (Slo2.x, K_{Na}) [32,91,116]; hyperpolarization-activated cyclic nucleotide-gated channels (HCN) [13,30,31,128], low voltage-activated T-type Ca^{2+} channels ($Ca_V3.x$) [50,90,107,114], and voltage-gated Na^+ channels (VGNC) [6,60]. These analyses identified M channels, 4-AP-sensitive K_V and K2P "leak" channels as those having most significant influence over the E_{rest}. We then investigated how the hyperpolarization of somatic/perisomatic E_{rest} in the DRG achieved by modulating somatic/perisomatic ion channel activities can influence nociceptive transmission in vivo. Finally, a computational model of a nociceptive DRG neuron was used to better understand how the relationship between morphology, membrane potential, and ion channels active at E_{rest} influence nociceptive signal propagation. Our study identifies major ion channels that set somatic E_{rest} of nociceptive neurons and provides firm evidence for a much stronger role of sensory ganglia in the peripheral nociceptive transmission than is generally thought.

2. Materials and methods

2.1. Neuronal cultures and slice preparation

DRG neurons were cultured as described previously ([74,76]; see [59] for step-by-step protocol). Briefly, adult male Sprague-Dawley rats (180-200 g) were humanely euthanized by cervical dislocation under the isoflurane anaesthesia. DRG from all spinal levels were removed and treated at 37°C in Hank's Balanced Salt Solution supplemented with collagenase (1 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) and dispase (10 mg/mL; Invitrogen, Life Technologies, Grand Island, NY, USA) for ~30 minutes. Ganglia were then gently triturated, washed twice, and resuspended in 600 μL culturing media (approx. 500,000 cells per isolation); this suspension was then plated as dense cultures onto glass coverslips coated with poly-D-lysine and laminin. Neurons were cultured for 2 to 5 days. No nerve growth factor was added to the culture to avoid inflammatory insult; we found that densely plated cultures survive well without trophic factors added. It is of note that our dissociation protocol provides cultures that are enriched with small-diameter, high-threshold (presumed nociceptor) neurons because large-diameter, low-threshold mechanoreceptors mainly die during trituration due to the mechanical overstimulation, unless specifically protected [36,59].

For sharp electrode recording, DRG slices were prepared from 12-day-old Wistar rats as described earlier [101], with slight modifications. Briefly, DRG were embedded in agar and sliced (300 μm) in ice-cold extracellular solution using a vibrating blade microtome (VT100S; Leica Microsystems, Buffalo Grove, IL, USA). Slices were then stored at room temperature for the remainder of the day in carbogenated (95% O_2-5% CO_2) extracellular solution containing (in mM): 115 NaCl, 25 $NaHCO_3$, 11 D-Glucose, 5.6 KCl, 2 $MgCl_2$, 1 NaH_2PO_4, and 2.2 $CaCl_2$ (pH 7.4).

2.2. Electrophysiology

Whole-cell and perforated patch recordings in current clamp configuration were performed at room temperature (unless indicated otherwise). Patch pipettes (resistance 2–4 MΩ) were fabricated from borosilicate glass capillaries using a DMZ-universal horizontal puller (Zeitz, Martinsried, Germany) or a Sutter P-97 puller (Sutter, Novato, CA, USA). Currents were amplified and recorded using an EPC-10 patch amplifier and Patchmaster 2.2 software (HEKA Electronik, Lambrecht, Germany) or an Axon patch 700B amplifier and pCLAMP 10.0 software (Axon Instruments, Union City, CA, USA), and were sampled at a frequency of 5 kHz. Liquid junction potentials were calculated with the algorithm developed by P.H. Burry [7] using pCLAMP software and subtracted post acquisition. Continuous current-clamp recording with no current injection was used for E_m monitoring. Linear ramps of currents from 0 to 1 nA (1-second duration) were injected for measuring rheobase and other AP parameters. The extracellular solution contained (in mM): 160 NaCl, 2.5 KCl, 5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 8 glucose, pH 7.4. The intracellular solution for perforated patch experiments [70] contained (in mM): 150 KCl, 5 $MgCl_2$, 10 HEPES, pH 7.4. (with 0.2-0.4 mg/mL amphotericin B, Sigma). The intracellular solution for whole-cell recordings from cultured DRG neurons contained (in mM): 150 KCl, 5 $MgCl_2$, 10 HEPES, 4 adenosine triphosphate (ATP; magnesium salt), pH 7.4. For whole-cell recordings from DRG slices, extracellular solution contained (in mM): 115 NaCl, 25 $NaHCO_3$, 5.6 KCl, 1 NaH_2PO_4, 1 $MgCl_2$, 2.2 $CaCl_2$, 11 glucose, pH 7.4, and intracellular solution contained (in mM) 130 KCl, 5 $MgCl_2$, 4.63 $CaCl_2$, 5 EGTA, 5 HEPES, 3 ATP (dipotassium salt), pH 7.4. Whole-cell current clamp recordings were performed as previously described [101].

Sharp electrode recordings were performed from DRG slices held in a submerged-type chamber and perfused with carbogenated extracellular solution (4-5 mL/min) at room temperature. Electrodes were pulled using a DMZ-universal horizontal puller to resistances of 70-120 MΩ when filled with a solution containing 1 M K-acetate (plus 1 mM KCl; pH 7.2 adjusted with acetic acid). Some recordings were performed with electrodes filled with 1 M KCl (plus 10 mM HEPES titrated to 7.2 with potassium hydroxide). Recordings were made using an SEC-05L amplifier (npi electrotonic, Tamm, Germany) and digitized (10 kHz) with a PC-based system (Digidata 1200 and Clampex 9.3, Molecular Devices, Sunnyvale, CA, USA) and analysed off-line (Clampfit 10.1). To measure the rheobase and to analyse AP properties, a family of 600-ms current injections (between −0.35 and +1 nA with 0.05 nA increment) was used. Because liquid junction potential should be <1 mV [89], no correction was applied.

To identify neurons as nociceptive, capsaicin (1 μM) has been applied at the end of the recording in all recording paradigms. Due to the small number of capsaicin-insensitive neurons and due to the fact that it was not always possible to apply capsaicin (eg, due to the premature loss of the recording), data from capsaicin-sensitive and capsaicin-insensitive neurons were not analysed separately.

2.3. Experiments with recombinant channels

In experiments testing specificity of K_{Na}-modulating drugs, plasmids encoding human Kv7.2 and Kv7.3 (GenBank accession no. NM000218 and AF091247) were transfected into Chinese hamster ovary (CHO) cells using Lipofectamine 2000 (Invitrogen). In experiments testing effect of ST101 on Cav3.2, the plasmid encoding human Cav3.2 (GenBank accession no. AF051946; kind gift from Prof. Chris Peers, University of Leeds, UK) was transfected into human embryonic kidney (HEK293) cells and whole-cell recordings were performed. The recordings were made using an Axon 700B