Neuroscience 179 (2011) 244–255

SUBTYPE-SPECIFIC REDUCTION OF VOLTAGE-GATED CALCIUM CURRENT IN MEDIUM-SIZED DORSAL ROOT GANGLION NEURONS AFTER PAINFUL PERIPHERAL NERVE INJURY

J. B. McCALLUM,[a] H.-E. WU,[a] Q. TANG,[a] W.-M. KWOK[a,b] AND Q. H. HOGAN[a,c]*

[a]*Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI, USA*

[b]*Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, USA*

[c]*Department of Anesthesiology, Zablocki VA Medical Center, Milwaukee, WI, USA*

Abstract—Sensory neurons express a variety of voltage-gated Ca^{2+} channel subtypes, but reports differ on their proportionate representation, and the effects of painful nerve injury on each subtype are not established. We compared levels of high-voltage activated currents in medium-sized (30–40 μm) dorsal root ganglion neurons dissociated from control animals and those subjected to spinal nerve ligation, using sequential application of semiselective channel blockers (nisoldipine for L-type, SNX-111 or ω-conotoxin GVIA for N-type, agatoxin IVA or ω-conotoxin MVIIC for P/Q-type, and SNX-482 for a component of R-type) during either square wave depolarizations or action potential waveform voltage commands. Using sequential administration of multiple blockers, proportions of total Ca^{2+} current attributable to different subtypes and the effect of injury depended on the sequence of blocker administration and type of depolarization command. Overall, however, N-type and L-type currents comprised the dominant components of I_{Ca} in sensory neurons under control conditions, and these subtypes showed the greatest loss of current following injury (L-type 26–71% loss, N-type 0–51% loss). Further exploration of N-type current identified by its sensitivity to ω-conotoxin GVIA applied alone showed that injury reduced the peak N-type current during step depolarization by 68% and decreased the total charge entry during action potential waveform stimulation by 44%. Isolation of N-type current by blockade of all other subtypes demonstrated a 50% loss with injury, and also revealed an injury-related rightward shift in the activation curve. Non-stationary noise analyses of N-type current in injured neurons revealed unitary channel current and number of channels that were not different from control, which indicates that injury-induced loss of current is due to a decrease in channel open probability. Our findings suggest that diminished Ca^{2+} influx through N-type and L-type channels may contribute to sensory neuron dysfunction and pain after nerve injury. Published by Elsevier Ltd on behalf of IBRO.

Key words: Ca^{2+} currents, nerve injury, neuropathic pain, neurotoxins, dorsal root ganglion, sensory neuron.

Progress in devising successful treatments for neuropathic pain has been slow, in part due to incomplete understanding of the molecular basis of neuronal dysfunction in these conditions. Investigations in our laboratory and others have shown that peripheral nerve injury leading to neuropathic pain in animal models is accompanied by loss of voltage-gated Ca^{2+} influx (I_{Ca}) through the sensory neuron plasmalemma, including both low-voltage activated (LVA) currents, as well has high-voltage activated (HVA) currents (Baccei and Kocsis, 2000; Hogan et al., 2000; Abdulla and Smith, 2001; McCallum et al., 2003). Reduced I_{Ca} after injury is associated with diminished resting cytoplasmic Ca^{2+} levels (Fuchs et al., 2005), decreased transient elevations in cytoplasmic Ca^{2+} levels during neuronal activation (Fuchs et al., 2007a), and heightened neuronal excitability (Lirk et al., 2008). Because of the role of cytoplasmic Ca^{2+} in regulating diverse functions such as membrane currents, gene expression, neurotransmitter release, and cell death, understanding the I_{Ca} deficit following injury in greater detail may contribute to clarifying the molecular mechanisms of neuropathic pain.

Sensory neurons contain a rich variety of Ca^{2+} current subtypes identified according to the genetic structure of the pore-forming α_1 subunit, as well as pharmacological and functional properties. Among HVA currents, L-type I_{Ca} typically requires a strong depolarization for activation, shows minimal inactivation, and is blocked by dihydropyridine antagonists. N-type and P/Q-type I_{Ca}, which also require strong depolarization for activation, are relatively unaffected by dihydropyridines but are blocked by specific polypeptide toxins from snail and spider venoms. A residual R-type current is insensitive to blockers of these other channels and is probably made up of several components in dorsal root ganglion (DRG) neurons (Wilson et al., 2000). The LVA T-type calcium current is activated by weak depolarizations, inactivates rapidly during sustained depolarization, and is resistant to both the dihydropyridines and the peptide toxins used to define the N-, P/Q-, and R-type HVA I_{Ca}.

*Correspondence to: Q. H. Hogan, Department of Anesthesiology, Medical College of Wisconsin, Room M4280, 8701 Watertown Plank Road Milwaukee, WI 53226, USA. Tel: +1-414-456-5727; fax: +1-414-456-6507.
E-mail address: qhogan@mcw.edu (Q. H. Hogan).

Abbreviations: AgaIVA, agatoxin IVA; AP, action potential; DRG, dorsal root ganglion; EGTA, ethylene glycol-bis(2-amino-ethylether)-N,N,N',N'-tetra-acetic acid; GDNF, glial-derived neurotrophic factor; GVIA, ω-conotoxin GVIA; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HVA, high-voltage activated; I_{Ca}, Ca^{2+} current; LVA, low-voltage activated; L5, fifth lumbar vertebral level; MVIIC, ω-conotoxin MVIIC; SNL, spinal nerve ligation; TEA, tetraethylammonium chloride; TTX, tetrodotoxin.

0306-4522/11 $ - see front matter. Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2011.01.049

To some extent, the functional roles for these current subtypes are distinct. T-type current prolongs the Ca^{2+} entry associated with action potentials due to its slow deactivation (Scroggs and Fox, 1992a), and may influence patterns of repetitive firing (Nelson et al., 2005). L-type current is a dominant path for Ca^{2+} that triggers genetic events associated with neuronal activity (Dolmetsch et al., 2001). While various HVA currents may interact synergistically at certain CNS synapses to produce neurotransmitter release, the central terminals of DRG neurons demonstrate distinct roles for various I_{Ca} channel subtypes. N-type channels are the principal source for Ca^{2+} that triggers excitatory synaptic transmission in the dorsal horn of the spinal cord, with a lesser contribution by P/Q-type currents (Bao et al., 1998; Heinke et al., 2004; Rycroft et al., 2007). However, P/Q-type currents predominate at inhibitory synapses (Takahashi and Momiyama, 1993; Heinke et al., 2004). Subcellular colocalization may functionally link I_{Ca} subtypes with specific Ca^{2+}-dependent processes, including opening distinct subtypes of Ca^{2+}-activated K^+ currents that regulate neuronal excitability (Marrion and Tavalin, 1998; Cordoba-Rodriguez et al., 1999).

Loss of I_{Ca} is a consistent and robust consequence of various models of primary sensory neuron injury in rats (Baccei and Kocsis, 2000; Hogan et al., 2000; Abdulla and Smith, 2001; McCallum et al., 2006). However, apart from the injury-induced depression of T-type current in medium-sized neurons (Andre et al., 2003; McCallum et al., 2003) and increased T-type current in small neurons (Jagodic et al., 2008), the specific subtypes of I_{Ca} affected by injury have not been defined. In order to identify the distribution of injury-induced I_{Ca} loss among specific HVA subtypes in a clinically relevant model of painful peripheral nerve injury, we examined currents in axotomized neurons of the fifth lumbar (L5) DRG from rats after L5 spinal nerve ligation and section (SNL), compared to currents in neurons from control rats. A corresponding loss of I_{Ca} is not found in adjacent L4 neurons after SNL (McCallum et al., 2006), so the present study examines only the directly injured L5 neurons. We focussed our study on medium-sized neurons (30–40 μm diameter) since 74% of neurons with these diameters have a slow conduction velocity and long action potential (AP) duration typical of lightly myelinated A∂ neurons (intracellular recordings from intact DRGs, data not shown), which undergo the greatest I_{Ca} loss after injury (McCallum et al., 2006). N- and L-type channels conduct the bulk of inward Ca^{2+} flux in most DRG neurons (Regan et al., 1991; Cardenas et al., 1995; Rusin and Moises, 1995; Wu and Pan, 2004), so we hypothesized that these would be the most affected by injury. Finally, we examined N-type current in greater detail as indirect measures have highlighted its role in plasticity of dorsal horn sensory transmission after injury (Matthews and Dickenson, 2001), and because N-type blockade has shown efficacy as analgesics in animal and human subjects (Staats et al., 2004).

EXPERIMENTAL PROCEDURES

Animal preparation

Adult male Sprague-Dawley rats (Taconic, Hudson, NY, USA) weighing 125–150 g were studied under approval of the Medical College of Wisconsin Animal Care and Use Committee. Rats in the neuropathic group were subjected to SNL modified from the original description (Kim and Chung, 1992), specifically ligation and section of the right L5 and L6 spinal nerves approximately 5 mm distal to the DRG, while control rats received skin incision and closure only. Following recovery from isoflurane anesthesia, rats were returned to individual cages under climate and light controlled conditions. Sensory function was evaluated as previously described (Hogan et al., 2004). Briefly, a pin was applied to the plantar skin with pressure adequate to indent but not puncture it. In response to this noxious stimulus, rats either briefly withdraw their paw as a reflexive flinch or display a sustained (>2 s), complex lifting, shaking, and grooming of the paw. This later response is rarely seen in control animals but increases in frequency following nerve injury, and is selectively associated with conditioned place avoidance (Wu et al., 2010). These behavior types were tabulated by a blinded observer while rats rested on a 0.25 inch wire grid in clear plastic enclosures. Five needle applications were made in turn to the plantar skin of each hindpaw at 2 s intervals and repeated 3 min later, allowing calculation of the frequency of the complex hyperalgesia-type response averaged over three separate tests between 10 and 17 days postoperatively.

Neuronal dissociation

After a postsurgical interval of 23.5±2.0 days for control rats (n=47) and 19.2±0.7 days for SNL animals (n=56), L5 ganglia were removed following decapitation under isoflurane anesthesia and placed into a 35 mm petri dish containing Ca^{2+}/Mg^{2+}-free, iced Hank's balanced salt solution (Gibco, Carlsbad, CA, USA) for mincing with iris scissors. Cleaned ganglia were incubated in 0.05% blendzyme 2 (Roche, Penzberg, Germany) in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 with glutaMAX (Invitrogen, Carlsbad, CA, USA) for 30 min, washed, vortexed, and resuspended in 0.0625% trypsin (Sigma-Aldrich Corp., St. Louis, MO, USA) and 0.0125% Dnase (Sigma-Aldrich) for another 30 min. Dissociated neurons were placed in Trypsin inhibitor (Sigma, Type II), centrifuged, lightly triturated, and resuspended in adult neural basal media (1X) (Invitrogen) containing 2% (v:v) B27 supplement (50×) (Life Technologies, Carlsbad, CA, USA), 0.5 mM glutamine, 0.02 mg/ml gentamicin, and 100 ng/ml nerve growth factor 7S (Alomone Labs Ltd., Jerusalem, Israel) for plating onto poly-L-lysine (70–150 kDa) coated 12 mm coverslips (Spiegelglas #2, Carolina Biological Supply, Burlington, NC, USA) and placed in a 95:5 O_2:CO_2 incubator for 2 h before study. All neurons were studied 6–8 h after dissociation. Medium-sized (30–40 μm diameter) neurons from the L5 DRG after SNL and from uninjured control animals were compared, as previous studies have shown that I_{Ca} loss and increased excitability occurs predominantly in axotomized neuronal group after SNL (Sapunar et al., 2005; McCallum et al., 2006).

Voltage and current recording

Voltage and currents were recorded in the whole cell configuration of the patch-clamp technique. Patch pipettes, ranging from 2 to 5 MΩ resistance, were formed from borosilicate glass (Garner Glass Co., Claremont, CA, USA) and fire polished. Coating with Sylgard (Dow Corning Corp., Midland, MI, USA) was used in noise analysis experiments. Currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz through a 4-pole Bessel filter, and digitized at 10 kHz with a Digidata 1320 A/D interface and pClamp 9 software (Molecular