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SENSORY NEURON SUBPOPULATION-SPECIFIC DYSREGULATION OF INTRACELLULAR CALCIUM IN A RAT MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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Abstract—The purpose of the present study was to test the prediction that the unique manifestation of chemotherapeutic-induced peripheral neuropathy (CIPN) would be reflected in a specific pattern of changes in the regulation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in subpopulations of cutaneous neurons. To test this prediction, we characterized the pattern of changes in mechanical nociceptive threshold associated with paclitaxel administration (2 mg/kg, iv, every other day for four days), as well as the impact of target of innervation and paclitaxel treatment on the regulation of $[Ca^{2+}]_i$ in subpopulations of putative nociceptive and non-nociceptive neurons. Neurons innervating the glabrous and hairy skin of the hindpaw as well as the thigh were identified with retrograde tracers, and fura-2 was used to assess changes in $[Ca^{2+}]_i$. Paclitaxel was associated with a persistent decrease in mechanical nociceptive threshold in response to stimuli applied to the glabrous skin of the hindpaw, but not the hairy skin of the hindpaw or the thigh. However, in both putative nociceptive and non-nociceptive neurons, resting $[Ca^{2+}]_i$ was significantly lower in neurons innervating the thigh after treatment. The magnitude of the depolarization-evoked Ca^{2+} transient was also lower in putative non-nociceptive thigh neurons. More interestingly, while paclitaxel had no detectable influence on either resting or depolarization-evoked Ca^{2+} transients in putative non-nociceptive neurons, in putative nociceptive neurons there was a subpopulation-specific decrease in the duration of the evoked Ca^{2+} transient that was largely restricted to neurons innervating the glabrous skin. These results suggest that peripheral nerve length alone, does not account for the selective distribution of CIPN symptoms. Rather, they suggest the symptoms of CIPN reflect an interaction between the toxic actions of the therapeutic and unique properties of the neurons deleteriously impacted.

Key words: dorsal root ganglion, neuropathic pain, von Frey test, nociceptor.

INTRODUCTION

The impact of peripheral nerve injury on the regulation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in sensory neurons has received considerable attention because of the importance of neural $[Ca^{2+}]_i$ to transmitter release (Parnas and Parnas, 2010), gene expression (Fields et al., 2005), excitability (Hogan, 2007), and neurotoxicity (Berridge et al., 2000). These are all neural processes that are not only changed by nerve injury (Lekan et al., 1997; Costigan et al., 2002; Yaksh, 2006; Wilson et al., 2012; Ratte et al., 2014) but appear to contribute to the signs and symptoms of peripheral neuropathy (Siau and Bennett, 2006). For example, in models of traumatic nerve injury, which is often associated with paresthesias, dysesthesias, and ongoing pain, there are changes in the regulation of $[Ca^{2+}]_i$ in both putative nociceptive and non-nociceptive neurons in a pattern that appears to depend on whether or not the neurons were injured or were adjacent to those that were injured. That is, the duration of evoked Ca^{2+} transients were shorter in injured, but longer in adjacent putative nociceptive neurons (Fuchs et al., 2007). Similarly there was a decrease in resting $[Ca^{2+}]_i$ in injured putative non-nociceptive neurons with no change in resting $[Ca^{2+}]_i$ in adjacent putative nociceptive neurons (Fuchs et al., 2005). In contrast, in a model of diabetic neuropathy, while there is an increase in the duration of the evoked Ca^{2+} transient in putative nociceptive neurons, there is also an increase in resting $[Ca^{2+}]_i$ (Kostyuk et al., 2001). In both models of peripheral neuropathy, there is evidence to suggest that the mechanisms contributing to the changes in the regulation of $[Ca^{2+}]_i$ contribute to changes in excitability (Tang et al., 2012). While there may be marked differences between peripheral nerve injury models with respect to the pattern of changes in the regulation of $[Ca^{2+}]_i$, there are only relatively subtle differences in the pain behavior associated with each model, highlighting the fact that the nervous system is able to achieve the same phenotype via multiple mechanisms.

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Abbreviations: ANOVA, analysis of variance; CIPN, chemotherapeutic-induced peripheral neuropathy; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbo-cyanine perchlorate; DMSO, dimethylsulfoxide; DRG, dorsal root ganglion; HEPES, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); NCX, sodium/calcium exchanger.

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One potential explanation for the differences between models with respect to the pattern of changes in the regulation of $[Ca^{2+}]_i$, is that each model impacts a different subpopulation of sensory neurons and as a result different subpopulations of neurons contribute to the observed pain behavior. For example, spontaneous activity in muscle afferents appears to play a particularly important role in pain behavior associated with traumatic nerve injury (Michaelis et al., 2000), while neurons innervating more superficial targets appear to play a more prominent role on diabetic neuropathy (Johnson et al., 2008). Furthermore, there are differences between subpopulations of sensory neurons with respect to the regulation of $[Ca^{2+}]_i$ (Lu et al., 2006). Consequently, the specific pattern of changes in the regulation of $[Ca^{2+}]_i$ may be a reflection of an interaction between the unique properties of specific subpopulations of sensory neurons and the relative impact of the nerve injury.

We reasoned that a chemotherapy model of peripheral neuropathy might enable us to begin to test this suggestion. Chemotherapeutic-induced peripheral neuropathy (CIPN) is associated with a unique distribution, in what is referred to as a ''stocking-glove'' pattern, with signs and symptoms of neuropathy largely restricted to the hands and feet. More interestingly, CIPN also presents with what appears to be a differential impact on subpopulations of sensory neurons. Numbness and tingling appear to be associated with a loss of intra-epidermal nerve fiber density thought to reflect a selective dye-back of low-threshold fibers, while the pain and hypersensitivity is thought to reflect the sensitization of nociceptive afferents (Tanner et al., 1998; Dougherty et al., 2004). Thus, we predicted, that this unique manifestation of CIPN would be reflected in a specific pattern of changes in the regulation of $[Ca^{2+}]_i$ in subpopulations of cutaneous neurons. To test this prediction, we employed a combination of behavioral analysis and fura-2 based microfluorimetry to study the impact of paclitaxel-induced CIPN on different subpopulations of sensory neurons. An electronic von Frey device was used to assess the pattern of changes in mechanical sensitivity in the hindlimb. Retrograde tracers were used to identify subpopulations of cutaneous neurons innervating the glabrous and hairy skin of the hindpaw and skin of the thigh. Acutely dissociated dorsal root ganglion (DRG) neurons were used to assess the impact of paclitaxel-induced CIPN on the regulation of $[Ca^{2+}]_i$ in subpopulations of neurons defined by target of innervation. Our results support the suggestion that the unique manifestation of CIPN is reflected in a specific pattern of changes in the regulation of $[Ca^{2+}]_i$ in subpopulations of cutaneous neurons and argue against a prevailing hypothesis that nerve length can account for the manifestation of CIPN.

EXPERIMENTAL PROCEDURES

Animals

Adult (250–320 g) male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were used for all experiments. Rats were housed two per cage in a temperature and humidity controlled, Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited animal housing facility on a 12-h:12-h light:dark schedule with food and water available *ad libitum*. All procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and performed in accordance with National Institutes of Health guidelines for the use of laboratory animals in research.

Tissue labeling

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbo-cyanine perchlorate (DiI) was injected intradermally at three different locations, one per animal, so as to label subpopulations of cutaneous afferents identified based on the target of innervation. These sites included the glabrous skin of the hind paw, the hairy skin on the dorsal side of the hind paw, and the hairy skin of the upper inner thigh. The hair covering the thigh was removed with an electrical shaver before retrograde labeling with DiI. DiI was injected with a 30-g needle under isoflurane (Abbott Laboratories, North Chicago, IL, USA) anesthesia at 3–5 sites per target for a total volume of 10 μL in the dorsal and ventral hindpaw and 20 μL in the thigh.

Paclitaxel treatment

One week following the DiI injection, rats were anesthetized with isoflurane and injected into the tail vein with 2 mg/kg paclitaxel or its vehicle (1:1:23, cremophor EL:ethanol:0.9% saline). The tail vein injection was repeated three more times every other day for a total of four injections.

Behavioral assessment of mechanical hypersensitivity

All behavioral data were collected in the Rodent Behavior Analysis Core of the University of Pittsburgh Schools of Health Sciences. Rats were habituated to the testing procedure, equipment, and the experimenter for two to three days before the collection of baseline data. An electronic von Frey (IITC Plantar Test Analgesia Meter 2390; IITC Life Sciences Inc., Woodland Hills, CA, USA) fitted with a rigid tip (1.0-mm tip diameter) was used to assess changes in mechanical threshold. For assessment of changes in mechanical threshold in the glabrous skin, rats were placed in acrylic clear boxes on an aluminum mesh, which were separated by opaque dividers and, the tip was applied to the center of the middle of the hind paw from below with steady vertical pressure until the paw was lifted off the mesh floor. For assessment of mechanical threshold on the dorsal side of the hindpaw, rats were gently restrained in cotton socks cut so that their hind legs and tail protruded from the back. This enabled application of the mechanical probe in a manner comparable to that used for the glabrous skin of the hindpaw, perpendicular to the plane of the skin. Mechanical threshold was assessed in a similar manner at mid-thigh following removal of the hair