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Paclitaxel-induced increase in NCX activity in subpopulations of nociceptive afferents: A protective mechanism against chemotherapy-induced peripheral neuropathy?

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ABSTRACT

We recently demonstrated, in a rat model of chemotherapy-induced peripheral neuropathy (CIPN), that there is a significant decrease in the duration of the depolarization-evoked Ca^{2+} transient in isolated somata of putative nociceptive afferents innervating the glabrous skin of the hindpaw, but no change in transient magnitude or the resting concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Because the Na^+-Ca^{2+} exchanger (NCX) only contributes to the regulation of the duration of the evoked Ca^{2+} transient, in putative nociceptive dorsal root ganglion (DRG) neurons, we hypothesized that an increase in NCX activity underlies the CIPN-induced change in this subpopulation of neurons. Acutely dissociated retrogradely labeled sensory neurons from naïve, vehicle-, and paclitaxel-treated rats were studied with fura-2 based Ca^{2+} imaging. There was no difference in the relative level of NCX activity between glabrous neurons from paclitaxel-treated or control rats. However, in contrast to the relatively large and long lasting Ca^{2+} transients needed to evoke NCX activity in neurons from naïve rats, there was evidence of resting NCX activity in glabrous neurons from both vehicle- and paclitaxel-treated rats. More interestingly, there was a paclitaxel-induced increase in NCX activity in putative nociceptive neurons innervating the thigh, neurons in which there is no evidence of a change in the depolarization-induced Ca^{2+} transient, or a body site in which there was a change in nociceptive threshold. Furthermore, while the majority of NCX activity in glabrous neurons is sensitive to the NCX3-preferring blocker KB-R7943, the increase in NCX activity in thigh neurons was resistant to KB-R7943 but sensitive to the NCX1-preferring blocker SEA0400. These results suggest that a mechanism(s) other than NCX underlies the paclitaxel-induced decrease in the duration of the evoked Ca^{2+} transient in putative nociceptive glabrous skin neurons. However, the compensatory response to paclitaxel observed may also explain why only subpopulations of sensory neurons are impacted by paclitaxel, raising the intriguing possibility that CIPN is due to the failure of injured neurons to appropriately compensate for the deleterious consequences of this compound.

1. Introduction

Chemotherapeutic-induced peripheral neuropathy (CIPN) is a painful condition mainly restricted to the hands and feet [1]. We have recently shown that a rat model of CIPN is associated with a significant decrease in the duration of the depolarization-evoked Ca^{2+} transients in isolated cell bodies of putative nociceptive afferents, but no changes in the magnitude of the transient, or in resting levels of Ca^{2+} [2]. Moreover, the degree of this change in duration was significantly larger in neurons innervating the glabrous skin of the hindpaw, than those targeting the hindpaw hairy skin or the inner thigh where no change in transient duration was detected. Interestingly, paclitaxel-induced mechanical hypersensitivity was only detected in the glabrous skin [2]. These data indicate the presence of a subpopulation-specific dysregulation of Ca^{2+} caused by paclitaxel treatment in this model.

The purpose of the present study was to test the hypothesis that increased activity of the Na^+-Ca^{2+} exchanger (NCX) is the underlying mechanism for the paclitaxel-induced changes in the evoked Ca^{2+} duration. This hypothesis was based on several observations: 1) NCX is a major Ca^{2+} extrusion mechanism, but with a low

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affinity for Ca^{2+}, it is only activated with relatively high $[Ca^{2+}]_i$ such as during depolarization-induced Ca^{2+} transients [3,4]. In the sensory neuron cell soma, NCX plays a major role in the regulation of the duration of the evoked Ca^{2+} with no influence on transient magnitude [5,6]. Consequently, NCX has the biophysical properties in DRG neurons to account for the selective paclitaxel-induced changes in the evoked Ca^{2+} transient. 2) Among sensory neurons, NCX is active only in putative nociceptive neurons [5,6], the same subpopulation in which we observed the paclitaxel-induced decrease in the evoked Ca^{2+} transient duration [2]. 3) There is evidence that a change in NCX activity is associated with inflammatory hypersensitivity, albeit, a decrease in NCX activity [5]. Thus, given the often opposing cellular response to inflammation and nerve injury [7], it is possible that paclitaxel-induced neuropathy is associated with an increase in NCX activity.

To test this hypothesis, retrograde tracer-labeled, small-diameter, IB4+, capsaicin responsive DRG neurons from naïve, vehicle-treated, and paclitaxel-treated rats were studied with ratiometric Ca^{2+} imaging in combination with a variety of pharmacological manipulations. Our results suggest that the paclitaxel-induced decrease in the duration of the evoked Ca^{2+} transient is not due to an increase in NCX activity. However, both vehicle and paclitaxel treatments were associated with NCX sensitization. A compensatory Ca^{2+} regulatory mechanism was also present in afferents innervating target areas where there was no detectable evidence of a chemotherapy-induced change in mechanical sensitivity. Furthermore, paclitaxel treatment affects NCX subtypes differentially based on target of innervation.

2. Experimental procedures

2.1. Animals

Adult (250–320 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) 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.

2.2. Tissue labeling

1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbo-cyanine perchlorate (DiI) was injected intradermally at three different sites, one location per animal, so as to label subpopulations of 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. DiI was injected with a 30 g needle under isoflurane (Abbott Laboratories, North Chicago, IL) 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.

2.3. Paclitaxel treatment

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

2.4. Sensory neuron isolation

Rats were deeply anesthetized with an intraperitoneal injection (1 ml/kg) of an anesthetic cocktail containing ketamine (55 mg/kg), xylazine (5.5 mg/kg) and acepromazine (1.1 mg/kg). L4 and L5 DRG were removed bilaterally, enzymatically treated, and mechanically dissociated. DRG neurons were plated on laminin (Invitrogen, Grand Island NY, 1 mg/ml) and poly-L-ornithine (Sigma-Aldrich, St Louis MO, 1 mg/ml) coated glass cover slips as previously described [6]. All subsequent experiments were performed within 8 h of tissue harvest. Only neurons containing the retrograde label DiI were included for further analysis.

2.5. Ca^{2+} imaging

Neurons were first incubated with 2.5 μM Ca^{2+} indicator fura-2 AM ester (TEF Laboratories, Austin TX) with 0.01% Pluronic F-127 (TEF Laboratories) for 20 min at room temperature. Neurons were then incubated with FITC-conjugated IB4 (5 μg/ml) for 10 min at room temperature. Following labeling, neurons were placed in a recording chamber and continuously superfused with a HEPES-buffered bath solution (HBS) consisting of (in mM): 130 NaCl, 3 KCl, 2·5$CaCl_2$, 0.6 $MgCl_2$, 10HEPES, 10 glucose, pH 7.4, osmolality 325 mOsm. Fluorescence data were acquired on a PC running Metafluor software (Molecular Devices, Sunnyvale, CA) via an EMCCD camera (Photometrics, Tucson, AZ; model QuantEM 512SC). The ratio (R) of fluorescence emission (510 nm) in response to 340/380 nm excitation (controlled by a DG-4 (Sutter Instrument, Novato, CA)) was acquired at 1 Hz during application of KCl or capsaicin, which were applied through a computer-controlled, piezo-driven perfusion system (switching time <20 ms; Warner Instruments, Hamden, CT, USA, Fast-Step Model SF-77B). The concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) was determined from fura-2 ratio according to the equation $[Ca^{2+}]_i$ (nM) = K_d (S_{f2}/S_{b2}) (($R-R_{min}$)/($R_{max}-R$)) following *in situ* calibration as described previously [8], where K_d is the dissociation constant for fura-2 for Ca^{2+} at room temperature (224 nM); S_{f2}/S_{b2} is the fluorescence ratio of the emission intensity excited with the 380 nm wavelength in the absence of Ca^{2+} to that in the presence of saturating Ca^{2+}; R_{min} and R_{max} are the minimal and maximal fluorescence ratios, respectively. Sf2/Sb2, R_{min} and R_{max} were determined empirically with calibration experiments as described previously [9], run periodically throughout the data collection period.

2.6. Chemicals

The retrograde tracer, DiI, was dissolved at 170 mg/ml in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, MO, USA) and diluted 1:10 in 0.9% sterile saline. Paclitaxel (Sigma-Aldrich), was dissolved at 25 mg/ml in 1:1 Cremophor EL (Sigma-Aldrich): ethanol and freshly diluted 1:12.5 in 0.9% sterile saline prior to injections. FITC-conjugated Isolectin B4 (IB4, Sigma-Aldrich) was dissolved in dH_20 as a stock solution of 1 mg/ml, and then diluted to a final concentration of 10 μg/ml in HBS the day of use. Fura-2 acetoxymethyl (AM) ester was dissolved in DMSO as a 2.5 mM stock solution and diluted to a final concentration of 2.5 μM in HBS. Pluronic F-127 was dissolved in DMSO as a 20% stock solution and diluted to 0.01% in HBS. Capsaicin (Sigma-Aldrich) was dissolved in ethanol as a 10 mM stock solution and diluted to 500 nM in HBS. LiCl (Sigma-Aldrich) was used to replace NaCl in HBS. KB-R7943 mesylate (Tocris, Bristol, UK) was dissolved in DMSO as a 100 mM stock solution and diluted to 100 nM in HBS. SEA0400 (ChemScene, Monmouth Junction, NJ, USA) was dissolved in DMSO as a 100 mM stock solution and diluted to 1 μM in HBS.