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Research report

Regulation of TRPV2 by axotomy in sympathetic, but not sensory neurons

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Abstract

Neuropathic pain results from traumatic or disease-related insults to the nervous system. Mechanisms that have been postulated to underlie peripheral neuropathy commonly implicate afferent neurons that have been damaged but still project centrally to the spinal cord, and/or intact neurons that interact with degenerating distal portions of the injured neurons. One pain state that is observed following peripheral nerve injury in the rat is thermal hyperalgesia. The noxious heat-gated ion channel TRPV1 may be responsible for this increased sensitivity, as it is up-regulated in L4 dorsal root ganglion (DRG) neurons following L5 spinal nerve lesion (SpNL). The TRPV1 homologue TRPV2 (or VRL-1) is another member of the TRPV subfamily of TRP ion channels. TRPV2 is a nonselective cation channel activated by high noxious temperatures (>52 °C) and is present in a subset of medium- to large-diameter DRG neurons. To establish whether TRPV2 is endogenous to the spinal cord, we examined its expression in the dorsal horn following rhizotomy. We found no significant decrease in TRPV2 immunoreactivity, suggesting that TRPV2 is endogenous to the spinal cord. In order to determine whether TRPV2, like TRPV1, is regulated by peripheral axotomy, we performed L5 SpNL and characterized TRPV2 distribution in the DRG, spinal cord, brainstem, and sympathetic ganglia. Our results show that peripheral axotomy did not regulate TRPV2 in the DRG, spinal cord, or brainstem; however, TRPV2 was up-regulated in sympathetic postganglionic neurons following injury, suggesting a potential role for TRPV2 in sympathetically mediated neuropathic pain.

Theme: Sensory systems
Topic: Somatic and visceral afferents

Keywords: Neuropathy; Nociception; TRP channel; Dorsal root ganglion; Sympathetic ganglion; Dorsal horn

1. Introduction

Neuropathic pain can result from trauma or disease of the nervous system. Several types of peripheral nerve injury paradigms are used to model this pathology in the rat. One established model of neuropathic pain is lesion to the fifth lumbar spinal nerve (L5 SpNL), first described by Kim and Chung [16]. This model allows for the study of distinct populations of injured and uninjured dorsal root ganglion (DRG) neurons and induces measurable pain states that are associated with neuropathy in humans: allodynia, hyperalgesia, and spontaneous pain.

One protein that has been implicated in thermal hyperalgesia following peripheral axotomy is the heat-activated cation channel TRPV1 (vanilloid subclass of the transient receptor potential family of ion channels, originally named VR1). TRPV1 mRNA and protein are up-regulated in neurons of the L4 DRG following L5 SpNL [9,12]. The up-regulation of TRPV1 in the DRG adjacent to injury (containing undamaged afferents) is one mechanism that could underlie increased sensitivity to noxious thermal stimuli (thermal hyperalgesia) that results from L5 SpNL. There are seven temperature-sensitive ion channels that have been identified, and their thresholds range from noxious cold to noxious hot temperatures. Four of these are members of the TRPV subfamily of TRP cation channels [3,25]. TRPV1 was the first TRPV channel to be characterized [5]. TRPV1 is sensitive to moderately noxious temperature (>43 °C), vanilloid compounds (such

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as capsaicin), and proton fluxes involved in the response to peripheral inflammation. In the DRG, TRPV1 is localized to small-diameter C-type polymodal neurons that are associated with the transmission of nociceptive information [5–7]. DRG neurons from TRPV1 knockout mice have decreased responsiveness to moderate noxious heat [7] providing further evidence that TRPV1 is involved in thermal sensation.

TRPV2 (originally VRL-1), a TRPV1 homologue, is unresponsive to vanilloid compounds, protons, or moderately noxious thermal stimuli; instead, it is activated by high-intensity noxious heat, with a threshold of activation of approximately 52 °C [1,6]. TRPV2 immunoreactivity (-IR) has been found in the DRG [1,6], cranial ganglia [13–15], and the spinal cord [18]. In the spinal cord, sympathetic preganglionic cells in the intermediolateral horn are among cells that are labeled. In the DRG, it has been demonstrated that TRPV2 is present mainly in medium- to large-diameter Aδ- or Aβ-type primary afferent neurons that are not TRPV1-positive [6,20]. Because TRPV1 and TRPV2 activate at different thermal thresholds and are present and functional in distinct subpopulations of DRG neurons, it is inferred that both of these channels contribute to nociceptive processing over a range of thermal stimuli. Given the homology of these channels and their similar functions in DRG neurons, it is possible that TRPV2 contributes to neuropathic pain in a manner similar to that proposed for TRPV1.

In this study, we test whether TRPV2 protein expression is regulated by peripheral axotomy. First, we examine the pattern of distribution of TRPV2 in the rat spinal cord following rhizotomy to determine whether TRPV2 is endogenous to the spinal cord. Next, we characterize TRPV2-IR in the spinal cord, brainstem, and injured and uninjured DRG neurons that project to the sciatic nerve 14 days after L5 SpNL. Finally, we characterize TRPV2-IR in the lumbar sympathetic ganglia following sciatic nerve injury. We show that TRPV2 is endogenous to the spinal cord, and that TRPV2 is up-regulated after peripheral nerve injury in sympathetic postganglionic neuron cell bodies.

2. Materials and methods

2.1. Surgical and tracing procedures

All experiments conformed to guidelines of the University of British Columbia and the Canadian Council on Animal Care. Adult male Wistar rats (200–300 g) were deeply anaesthetized with ketamine–xylazine (70 and 10 mg/kg, respectively). In three animals, a unilateral laminectomy was performed to expose the cervical (C-)6 to thoracic (T-)1 spinal cord and dorsal roots. After cutting the overlying dura mater, a quadruple rhizotomy (dorsal root transection) was performed. To remove ascending and descending input, three animals received unilateral sextuple rhizotomy (C4-T1) in combination with double unilateral hemisection of the spinal cord, two segments apart (caudal to C5 and C7). Another group ($n=12$) received unilateral axotomy of the spinal nerve, 2–5 mm distal to the DRG [16]. The transverse process of L6 was removed to expose the L5 spinal nerve, which was transected, and a 3-mm piece of nerve was removed to ensure that regeneration did not occur. A fourth group ($n=4$) received a unilateral sciatic nerve lesion (ScNL). The common sciatic nerve was exposed by blunt dissection of the upper thigh, and a small piece of nerve was removed. All animals recovered and allowed to survive to 14 days. Rats with sciatic nerve injury, and an additional group of intact rats ($n=4$), were anaesthetized with ketamine–xylazine 3 days prior to sacrifice, and 2 μl of 5% fluorogold (Fluorochrome) was injected into the left sciatic nerve using a micropipette. Although injected fluorogold is taken up by all axons of the nerve, its purpose was to identify sympathetic postganglionic neurons that project to the sciatic by examining cell bodies in the sympathetic ganglia of the trunk.

2.2. Immunohistochemistry

Animals were killed with an overdose of chloral hydrate (1400 mg/kg) and were transcardially perfused with PBS followed by 4% paraformaldehyde in 0.1 M phosphate buffer. In rhizotomized animals, spinal cord and DRGs were removed from C6 to T1. From animals that received L5 SpNL, we removed L4 and L5 DRGs, the corresponding segments of spinal cord, and the brainstem. Finally, sympathetic ganglia from the trunk of intact animals and those that had received ScNL were removed. Tissue was postfixed in 4% paraformaldehyde overnight and was then immersed in 24% sucrose for at least 1 day. Tissue was cut into transverse sections (10 μm for ganglia, 20 μm for spinal cord and brainstem) and, after blocking in 10% normal donkey serum (NDS), immunohistochemistry was performed for two neuronal markers of interest: neuropeptide tyrosine (NPY; 1:2000, host rabbit; Bachem-Peninsula Labs, San Carlos, CA) and TRPV2 (1:200, host rabbit; Abcam, Cambridge, UK). The TRPV2 antibody is specific for the C-terminus of TRPV2, and its properties have been demonstrated previously [6,18]. For TRPV2, we added biotin anti-rabbit secondary antibody (1:200, host donkey; Jackson ImmunoResearch, West Grove, PA) to amplify the signal and fluorescein isothiocyanate (FITC)-conjugated extr-avidin (1:500, 1.5 h; Jackson ImmunoResearch) for fluorescent microscopy. When double-staining for TRPV2 and NPY (both raised in rabbit), another blocking step with 10% NDS was included before incubating with anti-NPY. Secondary antibodies (1:200, host donkey; Jackson ImmunoResearch) were conjugated to aminomethylcoumarin (AMCA), Cy3, or FITC. Tissue was incubated with primary antibodies overnight, and with secondaries for 2 h. All antibodies were diluted with PBS, 0.2% Triton X-100, and 0.1% sodium azide.

2.3. Image analysis

Images were captured using an Axioplan 2 microscope (Zeiss, Jena, Germany), QImaging (Burnaby, BC) digital camera, and Northern Eclipse 6.0 (Empix Imaging, Mississauga, ON) software. Images were analyzed using SigmaScan Pro 5 software (SPSS, Leesburg, VA).

For image analysis, treatments were coded to prevent investigator bias. All images for a given marker were captured under the same exposure settings. In the dorsal horn of the spinal cord, each image of NPY and TRPV2 was thresholded. The densities of both markers were then measured along three 25-μm-wide strips extending 225 μm down from the uppermost border of the grey matter. Strips were placed either directly beneath Lissauer's tract or more laterally. Three sections, a minimum of 100 μm apart, were captured from each of three rats. Density measurements were averaged across sections at each depth in each rat and mean ± S.E.M. densities were plotted against depth. Student's *t*-tests were used to compare individual points on each graph, and data were considered significantly different if $p < 0.05$.

For DRG and sympathetic ganglia, cellular profiles were outlined to create an artificial overlay, which was then used to measure the average intensity of immunoreactivity and Feret diameter of each profile for both the NPY- and TRPV2-stained DRG images, and for the fluorogold-labeled TRPV2-IR sympathetic ganglia images. The threshold for immunoreactivity for each of the captured images was determined by averaging the intensity of three cells (two negative for the antigen and one slightly positive) in order to account for small variations in background signal on a section-by-section basis. We considered cells that had high levels of NPY and TRPV2 to be positive for analysis (there was a subpopulation of cells that was less immunoreactive for these markers). It should be noted that this profile-based analysis gives an overestimation of both large- and small-diameter neurons. The recursive translation method [27] was applied in order to correct for these effects. This method calculates the size distribution of a given cell population (in this case DRG and sympathetic neurons) using the diameters of their profiles in section. Kolmogorov–Smirnov tests were used to determine whether the distributions of neuronal size-frequency differed between populations ($p < 0.05$).

3. Results

3.1. TRPV2 immunoreactivity in the DRG and rhizotomized spinal cord

First, we investigated TRPV2-IR in the uninjured DRG and cervical dorsal horn. A subset of medium- to large-diameter DRG neurons was highly immunoreactive for TRPV2 (Fig. 1a). Like Caterina et al. [6], we found that there were two main patterns of TRPV2-IR in neuronal cell bodies (Fig. 1a′). Some somata had TRPV2 present only in the membrane, while others had intense cytoplasmic staining with TRPV2; only the latter were considered TRPV2-positive for image analysis. TRPV2-IR was also

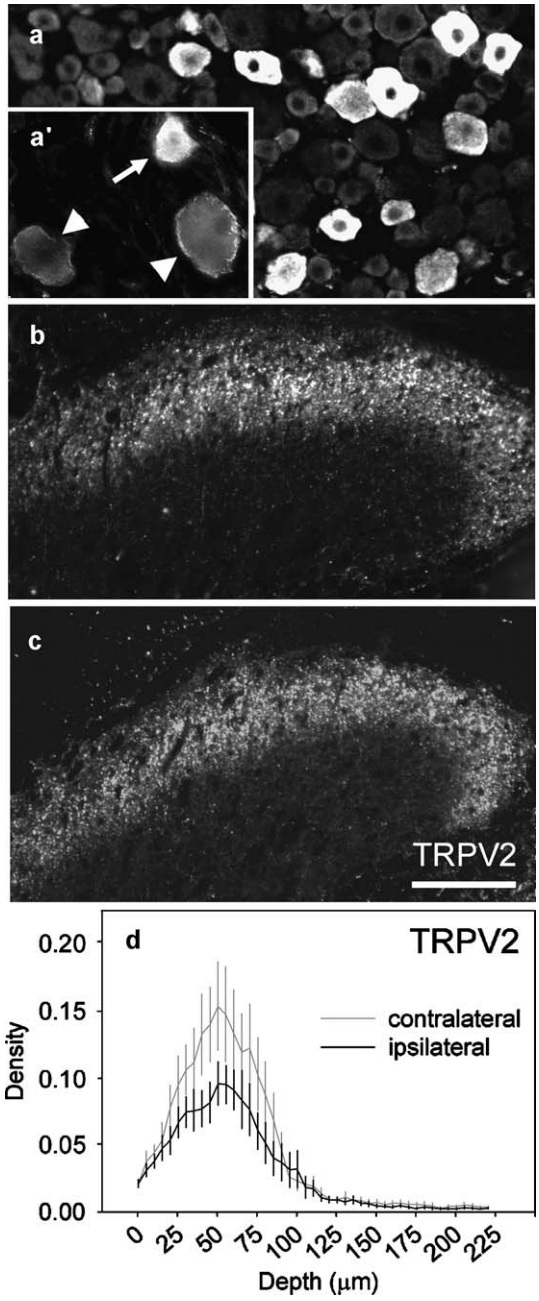

Fig. 1. TRPV2 immunoreactivity in the rat DRG and spinal cord. TRPV2 is present in a subset of DRG neurons (a). Two patterns of TRPV2 staining were observed: TRPV2-IR confined to the membrane (a′ , arrowheads) and high levels of TRPV2 throughout the neuronal cell body (TRPV2-positive for analysis; a′ , arrow). In the spinal cord, TRPV2 was observed in the superficial laminae of the dorsal horn contralateral to injury (b). Following quadruple dorsal rhizotomy, a similar amount of TRPV2 staining was present in the superficial dorsal horn ipsilateral to injury (c) to that of the contralateral side. Quantification confirmed that TRPV2-IR did not change significantly on the ipsilateral side following rhizotomy (d). Scale bar: 100 μm (a, b, c); 50 μm (a′).

present in the superficial laminae of the spinal cord, with some reactivity in lamina I and strong reactivity throughout lamina II (Fig. 1b). To determine whether the pattern of TRPV2-IR in the spinal cord is endogenous to the cord or is derived from centrally transported TRPV2 in primary afferents, we performed a quadruple dorsal rhizotomy. There was no obvious change in TRPV2-IR in the dorsal horn following rhizotomy (Fig. 1c), and image analysis did not show a significant reduction in the amount of TRPV2 in the dorsal horn ipsilaterally (Fig. 1d). Because multisegment deafferentation did not eliminate TRPV2 staining, TRPV2 is likely to be expressed by interneurons of the spinal cord and/or in descending supraspinal neurons. The absence of TRPV2-IR cell bodies in the dorsal horn would argue for the latter, although dorsal spinal hemisections did not appreciably reduce TRPV2-IR in the dorsal grey matter (data not shown).

3.2. TRPV2 and NPY immunoreactivity in the DRG, spinal cord, and brainstem following peripheral axotomy

TRPV2 and NPY were examined immunohistochemically in the DRG 14 days after L5 SpNL. NPY was used as a marker for a subpopulation of large-diameter DRG neuron cell bodies. We compared TRPV2- and NPY-IR in the DRG associated with injury [ipsilateral L5; L5 (i)] with DRGs contralateral [L5 (c)] and adjacent [L4 (i)] to injury (Fig. 2). In the contralateral DRG (Fig. 2a), many of the NPY-positive neuronal cell bodies also expressed TRPV2 ($77 \pm 10\%$; Fig. 2d), confirming that both proteins are present in a medium- to large-diameter subpopulation of DRG neurons [6,8]. Examination of the DRG adjacent to injury (Fig. 2b) revealed that there was no significant change in NPY-IR or TRPV2-IR in neuronal cell bodies following injury. In the injured DRG (Fig. 2c), there was a significant increase in NPY-IR following injury; however, there was no significant change in TRPV2-IR in these DRGs (Fig. 2d). In the L5 (i) DRG, we established the percentage of cells that were immunoreactive for both proteins of interest. It was found that the majority ($67 \pm 8\%$) of TRPV2-positive cells expressed NPY. This was not significantly different from the proportion of TRPV2-positive cells which expressed NPY in adjacent ($64 \pm 7\%$) or contralateral ($65 \pm 10\%$) DRGs. The similarity in NPY expression in TRPV2-positive neurons between ganglia suggests that NPY up-regulation occurs in a distinct subpopulation of large TRPV2-negative DRG neurons.

We also determined the distribution of DRG neuron cell body diameters of all cells, NPY-positive cells, TRPV2-positive cells, and cells immunoreactive for both proteins after axotomy (Fig. 2e–h). The distribution of all cells was not different among the three DRGs studied (Fig. 2e). In agreement with qualitative observations, the proportion of NPY-IR cells was significantly increased following SpNL, and the increase was restricted mainly to medium-to-large cell bodies (Fig. 2f). TRPV2-positive neurons were present in the same proportions and the same size-frequency distribution following SpNL (Fig. 2g).

NPY-IR and mRNA have been shown increase ipsilaterally in both the deep dorsal horn of the rat spinal cord and the gracile nucleus of the rat brainstem after various injuries that induce neuropathic pain [24,28,29,32], but changes in NPY-IR have not yet been described following L5 SpNL. An increased density of NPY-positive axons and terminals

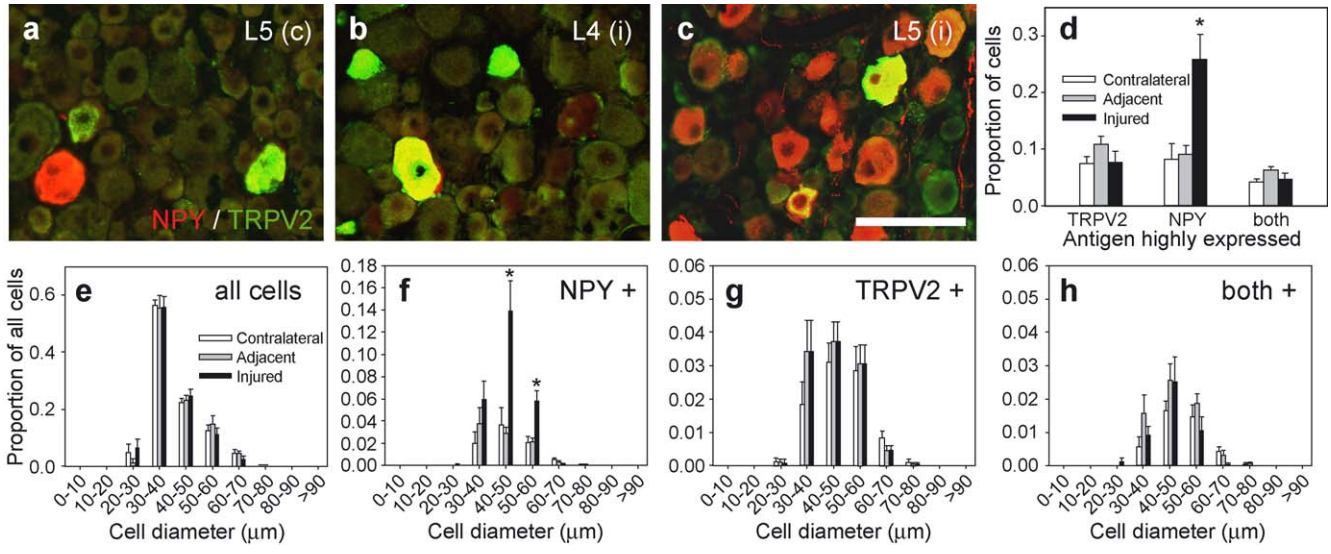

Fig. 2. Colocalization of NPY and TRPV2 in the rat DRG following L5 SpNL. Photomicrographs show NPY and TRPV2-IR in DRGs contralateral [a; L5 (c)] and adjacent [b; L4 (i)] to injury, as well as in the DRG associated with the injury [c; L5 (i)]. Image analysis revealed that TRPV2 and NPY often colocalized in a subset of DRG neurons and that NPY-IR, but not TRPV2-IR, was significantly increased in DRG cell bodies 14 days after L5 SpNL (d; * indicates $p < 0.01$ vs. contralateral). Approximately 7–8% of DRG neurons were highly immunoreactive with TRPV2 (d). A study on cell size distribution was also performed (e–h). Both NPY (f) and TRPV2 (g) were found in a subset of medium- to large-diameter DRG neurons, and they colocalized in this range of cell sizes (h). Scale bar: 100 μm.

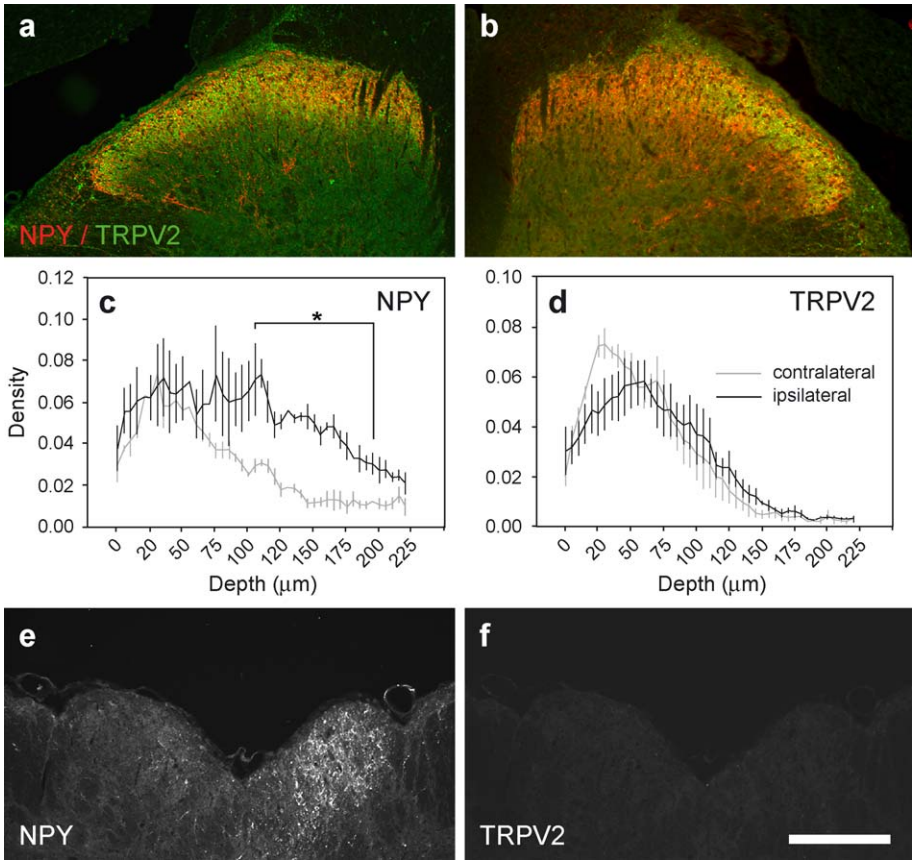

Fig. 3. Immunoreactivity of NPY and TRPV2 in the rat lumbar spinal cord (a–d) and brainstem (e, f) 14 days postaxotomy. NPY and TRPV2 showed much colocalization in the uninjured superficial dorsal horn contralateral to L5 SpNL (a). Ipsilateral to injury, there was still colocalization noted in superficial laminae of the dorsal horn (b), although there was more NPY staining in deeper areas of the grey matter. Quantification confirmed that NPY-IR was increased in deeper laminae of the ipsilateral dorsal horn (c; * indicates $p < 0.05$ vs. contralateral), whereas TRPV2-IR did not change significantly (d) compared to the contralateral side. NPY- and TRPV2-IR were examined in the gracile nuclei of the brainstem (e and f). NPY-IR was greatly increased on the ipsilateral side following L5 SpNL (e). There was very little TRPV2-IR present in gracile nuclei contralateral or ipsilateral to injury (f). Scale bar: 200 μm.

was present in the ipsilateral dorsal horn and gracile nucleus 14 days following L5 SpNL (Fig. 3). In the dorsal horn, increased NPY-IR was observed in deeper laminae following injury (Fig. 3a–c), whereas TRPV2-IR was not significantly changed (Fig. 3a, b, and d). Likewise, in the gracile nucleus of the brainstem, axotomy-induced NPY up-regulation was not accompanied by a change in TRPV2-IR (Fig. 3e and f). Given that NPY density increases following injury in areas of the DRG, the dorsal horn, and the gracile nucleus, and that injury does not induce significant changes in TRPV2-IR in these regions, we infer that axotomy increases NPY production in a subpopulation of DRG neurons that do not express TRPV2.

3.3. TRPV2 immunoreactivity is increased in sympathetic postganglionic neurons in response to peripheral axotomy

After observing high TRPV2-IR in axons of the grey ramus communicans at the entry point into the spinal nerve (Fig. 4a), we proceeded to characterize TRPV2-IR in sympathetic neurons of the lumbar chain. TRPV2-IR was examined in fluorogold-labeled sympathetic neurons following sciatic nerve lesion. We compared TRPV2-IR in sympathetic postganglionic neurons from uninjured and injured animals (Fig. 4). Examination of sympathetic ganglia of animals without sciatic nerve injury revealed that TRPV2 was present within a population of fluorogold-labeled uninjured sympathetic neurons that project to the sciatic nerve (Fig. 4b). Following sciatic nerve injury, an increase in TRPV2-IR was noted in the fluorogold-labeled injured sympathetic neurons (Fig. 4c). Image analysis indicated that there was a significant increase in TRPV2-IR in axotomized sympathetic neurons (Fig. 4d). We observed no TRPV2-IR in sympathetic baskets surrounding DRG neuron cell bodies following axotomy.

4. Discussion

This study demonstrated the localization of TRPV2 in various nervous tissues. We observed TRPV2-IR in laminae I and II of the spinal cord, which are areas involved in nociceptive processing. In the DRG, a subpopulation of medium- to large-diameter neuronal cell bodies was highly

immunoreactive for TRPV2, and these cells were often found to be NPY-positive. TRPV2-IR was present at lower levels in many DRG cell bodies. Taken together, we suggest that TRPV2 is likely to be expressed mainly in subpopulations of medium- to large-diameter, myelinated Aδ and Aβ afferents, which terminate in the superficial dorsal horn. Although these large-diameter sensory neurons are not normally associated with nociception, there is evidence that a proportion of these fibers are activated by noxious stimuli [17]. In the uninjured sympathetic ganglion, TRPV2 was present in many neuronal cell bodies, and high TRPV2-IR was observed in 20 ± 1% of the neurons. Axotomy of the peripheral branch of sympathetic neurons caused an increased proportion of TRPV2-positive cells (31 ± 3%).

Dorsal rhizotomy did not induce a significant change in TRPV2-IR in the dorsal horn of the spinal cord, although we did note a trend of decreased immunoreactivity on the ipsilateral side in accordance with previous findings [18]. The decrease that was observed by Lewinter et al. [18] was found to be significant but not large (26% in laminae I and II), which corresponds well with our findings. This suggests that there is some central transport of TRPV2 and thus some afferent-derived TRPV2 in the dorsal horn. However, because strong TRPV2-IR persists after multiple dorsal rhizotomy, there must be sources of TRPV2 endogenous to the central nervous system (also proposed by Lewinter et al. [18]). To determine whether supraspinal sources or neurons from other spinal segments were contributing to the presence of TRPV2 in the spinal cord, we performed a sextuple rhizotomy in combination with two unilateral hemisections, separated by two segments. This procedure produced two segments with only descending input, two segments with no input from ascending nor descending tracts, and two segments with input only from ascending tracts. Again, we found that there was no significant change in TRPV2-IR in the ipsilateral dorsal horn after this injury (data not shown), which implies that TRPV2 is endogenous to the spinal cord and may be expressed by interneurons there, in addition to being transported centrally by primary afferents.

We used NPY as a marker for medium- to large-diameter DRG neuron cell bodies. Approximately 7 ± 1% of DRG neurons were highly immunoreactive for TRPV2, which is in accordance with previous studies (7.3% [10]), and like Ma [20], Greffrath et al. [10], and Lewinter et al. [18], we found that TRPV2 was largely localized in cells of larger diameter. The presence of TRPV2 in most NPY-positive uninjured DRG neurons implies that these neurons are involved in nociception. Because the TRPV2 homologue

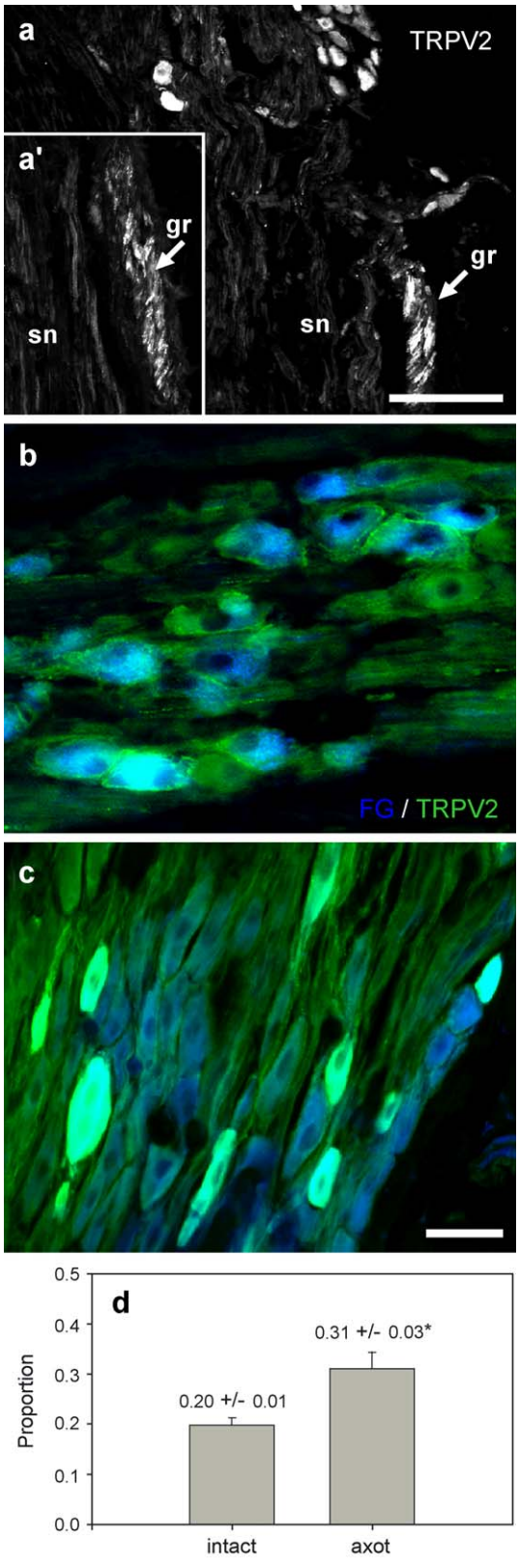

Fig. 4. TRPV2 immunoreactivity in postganglionic sympathetic neurons. TRPV2-IR was observed in the grey ramus (gr) where sympathetic axons enter the spinal nerve (a; sn). To study the effect of axotomy on TRPV2 expression in sympathetic neurons, fluorogold was injected into intact (b) and axotomized (c) sciatic nerves. TRPV2 colocalized with uninjured sympathetic neurons that project to the sciatic nerve (i.e., labeled with fluorogold; (b). Following a sciatic nerve lesion, more TRPV2-IR were noted in the fluorogold-labeled sympathetic neurons (c). Quantification indicated that a greater proportion of fluorogold-labeled axotomized sympathetic neurons show TRPV2-IR compared to the fluorogold-labeled uninjured sympathetic neurons (d). Scale bar: 200 μm (a); 150 μm (a′); 200 μm (b and c).

TRPV1 is up-regulated in sensory neurons of the L4 DRG following L5 SpNL [9,12] and is implicated in hyperalgesic neuropathic pain [7], we were interested in studying the regulation of TRPV2 after L5 SpNL. We were also interested in the colocalization of TRPV2 with NPY following axotomy, because NPY is known to be up-regulated in the same size of neurons after peripheral nerve injury [19,22,28]. We found that there was no significant change in TRPV2-IR following this injury in both the DRG associated with injury (L5) and the DRG adjacent to injury (L4), relative to the contralateral side. The cell size distributions did not change, nor did the proportion of all cells expressing TRPV2. In contrast, NPY-IR was greatly increased in the L5 DRG ipsilateral to injury, as has been shown before in various models of neuropathic pain [19,22,28]. Because the amount of cells that expressed both TRPV2 and NPY was the same in injured and contralateral L5 DRGs, the population of cells that showed increased NPY-IR were cells that did not express TRPV2. Given that TRPV2-IR is not changed after L5 SpNL, we conclude that TRPV2 does not have a role in neuropathic pain through up-regulation in DRG neurons that undergo peripheral axotomy nor in neurons in adjacent DRGs that interact with degenerating peripheral branches of the injured afferents.

A similar pattern of regulation of NPY and TRPV2 was found to occur in the lumbar spinal cord and brainstem after L5 SpNL. TRPV2-IR was not significantly changed in the superficial dorsal horn, although there was a trend of decreased IR in laminae I and II of the dorsal horn, which are areas of nociceptive input termination. NPY-IR was significantly increased in deeper laminae (III and IV) of the dorsal horn ipsilateral to injury, as has been described previously [21,24,28–30]. Because up-regulation of NPY following peripheral axotomy is also known to occur in the ipsilateral gracile nucleus of the brainstem [19,31], we were interested in whether this target of spinal projections up-regulated TRPV2 after injury. Again, we found that TRPV2-IR did not change after injury; in fact, it was not detectable in either gracile nucleus, despite being present in the spinal trigeminal tract in the same sections. This is in contrast with previous observations [18], which suggested that TRPV2-IR was present in the gracile nucleus and was abolished by unilateral hemisection. We suggest that the disparity in results arises in part from the use of different immunohistochemical techniques; Lewinter et al. [18] primarily used diaminobenzidine immunostaining, while we used fluorescent microscopy. The former can be troublesome because the intensity of staining is heavily dependent on the duration of the horseradish peroxidase (HRP) reaction, as well as the efficacy of the initial quenching of endogenous peroxidases; lengthy HRP reactions and insufficient peroxidase quenching can occasionally lead to false positive results. It is noteworthy that despite the reported TRPV2-IR in the dorsal column nuclei, there was none observed in the ascending dorsal column axons (which terminate in the dorsal column nuclei) either in the Lewinter's study [18] or our own. Axonal TRPV2 staining was evident in the periphery in both studies, indicating that the disparate results are not underpinned by an inability to visualize axons.

While examining DRGs, we noticed that the grey ramus communicans, just distal to the DRG, was strongly immunoreactive for TRPV2. Despite the presence of TRPV2 in the grey ramus, no TRPV2-IR was observed in sympathetic baskets (which appear following spinal nerve lesion [23]) surrounding DRG neuron cell bodies. TRPV2 immunohistochemistry of the lumbar sympathetic chain confirmed that many postganglionic sympathetic neurons contained at least some TRPV2, and a subpopulation was highly immunoreactive for TRPV2. Interestingly, we found that approximately 20% of lumbar sympathetic neurons that project to the sciatic nerve are TRPV2-positive, which is a much higher proportion than has been reported in sympathetic neurons of the superior cervical ganglion, where only 2% of neurons show TRPV2-IR [14] (the authors of that study used the same antibody that was used in this work). Such a discrepancy in proportions of neuronal subsets along the neuraxis is not unprecedented; other ion channels (such as the ATP receptor P2X3) are expressed in different proportions in sensory ganglia at the cranial and caudal levels [26], and it is reasonable to surmise that different proportions of neuronal phenotypes reflect different peripheral targets. After injury to the peripheral projections of sympathetic neurons by sciatic nerve transection, we found that there was a 50% increase in the proportion of sympathetic neurons that were immunoreactive for TRPV2. This increase in TRPV2-IR in sympathetic neurons is a potential mechanism by which TRPV2 could contribute to neuropathic pain. TRPV2-IR has also been reported in parasympathetic pterygopalatine and submandibular ganglia, where approximately two-thirds of neurons are TRPV2-positive [14].

Why would an ion channel that is activated by high temperatures be present in sympathetic neurons? There are two possible roles of TRPV2 in these cells. First, TRPV2 might have other functions that have not yet been identified. TRPV2 mRNA has been detected in a variety of nonexcitable tissues (e.g., lung, spleen, skeletal muscle) [2,6], which implies that TRPV2 may not be involved in sensation of noxious thermal stimuli alone. Another possible role of TRPV2 in sympathetic neurons is more intriguing. It is conceivable that in addition to their normal efferent function, sympathetic neurons may play an afferent role. This type of dual role has been demonstrated in sensory neurons, which are normally associated with the detection of mechanical, thermal, chemical, or proprioceptive inputs. Efferent functions of DRG neurons have been described, although there is some controversy over whether individual neurons have dual functions or whether there are distinct groups of efferent and afferent DRG neurons (see Ref. [11]). In particular, stimulation of DRG neurons can result in the release of peptide transmitters from their peripheral terminals, which causes neurogenic inflammation [4]. This state is characterized by local vasodilation and plasma extrava-

sation and is similar to conditions induced by autonomic efferent neurons. In an analogous fashion, it is possible that sympathetic efferents have an afferent function. The presence of TRPV2 in these neurons provides support for this notion, because TRPV2 is assumed to retain its sensitivity to noxious heat. This means that the subpopulation of TRPV2-expressing postganglionic sympathetic neurons could potentially be excited by high temperature in an afferent role.

In summary, peripheral axotomy causes an increase in TRPV2-IR in sympathetic neurons but does not regulate TRPV2 in DRG neurons or in the dorsal horn of the spinal cord. We conclude that TRPV2 may have a role in sympathetically mediated neuropathic pain, and that the presence of TRPV2 in sympathetic neurons may be indicative of a potential afferent function.

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