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Protein tyrosine phosphatase receptor type O regulates development and function of the sensory nervous system

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ABSTRACT

The roles of protein tyrosine phosphatases (PTPs) in differentiation and axon targeting by dorsal root ganglion (DRG) neurons are essentially unknown. The type III transmembrane PTP, PTPRO, is expressed in DRG neurons, and is implicated in the guidance of motor and retinal axons. We examined the role of PTPRO in DRG development and function using PTPRO$^{-/-}$ mice. The number of peptidergic nociceptive neurons in the DRG of PTPRO$^{-/-}$ mice was significantly decreased, while the total number of sensory neurons appeared unchanged. In addition, spinal pathfinding by both peptidergic and proprioceptive neurons was abnormal in PTPRO$^{-/-}$ mice. Lastly, PTPRO$^{-/-}$ mice performed abnormally on tests of thermal pain and sensorimotor coordination, suggesting that both nociception and proprioception were perturbed. Our data indicate that PTPRO is required for peptidergic differentiation and process outgrowth of sensory neurons, as well as mature sensory function, and provide the first evidence that RPTPs regulate DRG development.

Introduction

The development of the sensory nervous system requires the differentiation of sensory neuron subtypes, coupled with the guidance of their axons to appropriate targets in the spinal cord. Both subtype differentiation and axon guidance are regulated by a family of receptor tyrosine kinases, the tropomyosin-related kinase (Trk) receptors, among others (Huang and Reichardt, 2003; Masuda et al., 2008; Togashi et al., 2006). For example, differentiation and axon targeting of nociceptive neurons requires signaling through TrkA, and differentiation and targeting of proprioceptive neurons requires signaling through TrkC (Ben-Zvi et al., 2008; Genc et al., 2004; Marmigere and Ernfors, 2007). Although signaling through tyrosine phosphorylation requires the coordinated activity of tyrosine kinases together with protein tyrosine phosphatases (PTPs), essentially nothing is known about the roles of PTPs in sensory development.

Among vertebrate PTPs, the transmembrane receptor PTPs (RPTPs) are most clearly implicated in the regulation of axon guidance. RPTPs with cell adhesion molecule-like extracellular domains (type II and type III RPTPs) have been shown to be important for the guidance of a variety of axons in the central nervous system (Johnson and Van Vactor, 2003). Although two type II RPTPs, PTP-σ and LAR, are known to modulate Trk signaling (Faux et al., 2007; Yang et al., 2006), and to regulate the speed and efficiency of peripheral nerve regeneration (Van der Zee et al., 2003; Xie et al., 2001), their roles in sensory development have not been examined. The roles of type III RPTPs in sensory development and regeneration are unknown.

The type III RPTP known as protein tyrosine phosphatase receptor type O (PTPRO) is clearly implicated in the developmental guidance of axons. PTPRO is selectively expressed on developing neurons, their axons, and their growth cones (Beltran et al., 2003; Bodden and Bixby, 1996; Ledig et al., 1999). In vitro, the PTPRO extracellular domain, consisting of eight fibronectin type III repeats, inhibits adhesion and neurite outgrowth, causes growth cone collapse, and repels the growth cones of retinal axons (Stepanek et al., 2001). Further, loss of function experiments using RNAi and/or dominant-negative constructs show that PTPRO mediates guidance of motor axons and optic nerve axons in vivo (Shintani et al., 2006; Stepanek et al., 2005). PTPRO is strongly expressed on developing TrkC-positive and TrkA-positive sensory neurons (Beltran et al., 2003), but its functions in

Abbreviations: PTP, protein tyrosine phosphatase; RPTP, receptor PTP; CGRP, calcitonin gene-related peptide; PV, parvalbumin; Trk, tropomyosin-related kinase; PTPRO, protein tyrosine phosphatase receptor type O.

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sensory differentiation and axon guidance have not been studied. To begin to address the role of PTPRO during development of mammalian sensory neurons, we examined these neurons in PTPRO$^{-/-}$ mice at the time of birth (P0). Sensory development was clearly altered by loss of PTPRO. PTPRO$^{-/-}$ mice had fewer nociceptive peptidergic neurons, while the total number of sensory neurons was unchanged. This difference persisted into the adult. Importantly, pathfinding in neonates was altered both in these nociceptive neurons and in proprioceptive neurons. Finally, adult PTPRO$^{-/-}$ mice performed abnormally on tests of thermal pain and of sensorimotor function. These results indicate that PTPRO is required for sensory neuronal differentiation and process outgrowth, as well as mature sensory function, including the perception of thermal pain.

Results

Decreased number of nociceptive neurons in PTPRO-deficient mice

All populations of DRG sensory neurons, including peptidergic nociceptors, arise from neural crest stem cells in response to Wnt/β-catenin signaling (Lee et al., 2004). Migratory neural crest cells from the dorsal neural tube then coalesce to form the developing DRG. Later in DRG development, the neuronal determination gene neurogenin1 (Ngn1) is required for the formation of TrkA-expressing cells (Bertrand et al., 2002), and Ngn2 is required for the generation of TrkC-expressing cells (Ma et al., 1999). TrkA, TrkB, and TrkC are receptors for the neurotrophins nerve growth factor (NGF), brain derived growth factor (BDNF), and neurotrophin-4/5 (NT-4/5), as well as neurotrophin-3 (NT-3), respectively (Bibel and Barde, 2000; Huang and Reichardt, 2001; Huang and Reichardt, 2003). Cells expressing TrkA include the populations that will develop into peptidergic and non-peptidergic nociceptive neurons (Woolf and Ma, 2007). The majority of TrkB expressing cells develop into mechanoreceptive neurons, while most of the cells expressing TrkC become proprioceptive neurons.

Nociceptive neurons are small in diameter, and express TrkA at P0; in mature animals roughly 50% of these neurons express the neuropeptides calcitonin gene-related peptide (CGRP) and/or substance P (Averill et al., 1995). In contrast, proprioceptive cells are large in diameter, comprise about 20% of the DRG neuronal population at birth, and express TrkC and parvalbumin (PV) (Ernfors et al., 1994). PTPRO is expressed on most TrkC-positive DRG neurons and some TrkA-positive DRG neurons at embryonic day 16 (Beltran et al., 2003), and PTPRO is still expressed in DRG neurons at P0 (unpublished). To determine the effects of PTPRO loss of function on these neuronal populations, we examined lumbar DRG from wild-type and PTPRO-deficient mice at P0, at which time neurogenesis and developmental cell death are complete in wild-type animals (Silos-Santiago et al., 1995). This period coincides with peak PTPRO expression in the brain (Beltran et al., 2003).

The total number of neurons in the L4 DRG was the same in wild-type and PTPRO$^{-/-}$ mice (Figs. 1G–I) suggesting that PTPRO is not necessary for neuronal determination in the DRG or for neuronal

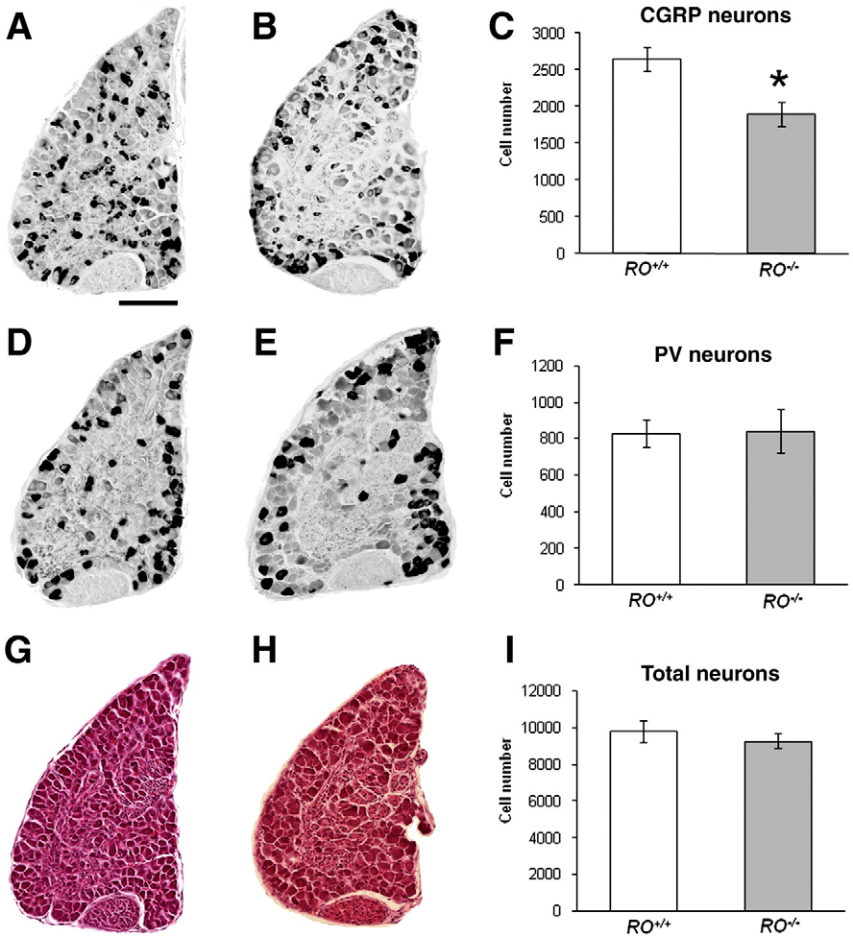

Fig. 1. Decreased number of nociceptive neurons in PTPRO$^{-/-}$ mice. (A–C) P0 L4 DRG sections stained for CGRP to label nociceptive neurons. (A) Wild-type ($n=$ 3 animals; 6 DRG). (B) PTPRO$^{-/-}$ ($n=$ 5 animals; 10 DRG). (C) PTPRO$^{-/-}$ DRG had fewer nociceptive neurons. (D–F) P0 DRG sections stained for parvalbumin to label proprioceptive neurons. (D) Wild-type ($n=$ 5 animals; 10 DRG). (E) PTPRO$^{-/-}$ ($n=$ 5 animals; 10 DRG). (G–I) All neurons were identified in HE stained sections. (G) Wild-type ($n=$ 4 animals; 8 DRG). (H) PTPRO$^{-/-}$ ($n=$ 4 animals; 8 DRG). (F and I) Neither proprioceptive neuron counts nor total neuron counts were altered in the PTPRO$^{-/-}$ mice. Two-tailed Student's t tests were used for this and all statistical tests to test for differences between wt and PTPRO$^{-/-}$. *, $p<$ 0.003. Scale bar, 100 μm.