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Regular Article

Regulation of neuronal and glial galectin-1 expression by peripheral and central axotomy of rat primary afferent neurons

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Abstract

Galectin-1 (Gal1) is an endogenously-expressed protein important for the embryonic development of the full complement of primary sensory neurons and their synaptic connections in the spinal cord. Gal1 also promotes axonal regeneration following peripheral nerve injury, but the regulation of Gal1 by axotomy in primary afferent neurons has not yet been examined. Here, we show by immunohistochemistry and in situ hybridization that Gal1 expression is differentially regulated by peripheral nerve injury and by dorsal rhizotomy. Following peripheral nerve injury, the proportion of Gal1-positive DRG neurons was increased. An increase in the proportion of large-diameter DRG neurons immunopositive for Gal1 was paralleled by an increase in the depth of immunoreactivity in the dorsal horn, where Gal1-positive terminals are normally restricted to laminae I and II. Dorsal rhizotomy did not affect the proportions of neurons containing Gal1 mRNA or protein, but did deplete the ipsilateral dorsal horn of Gal1 immunoreactivity, indicating that it is transported centrally by dorsal root axons. Dorsal rhizotomy also resulted in an increase in Gal1 mRNA the nerve peripheral to the PNS–CNS interface (likely within Schwann cells and/or macrophages), and to a lesser extent within deafferented spinal cord regions undergoing Wallerian degeneration. This latter increase was notable in the dorsal columns and along the prior trajectories of myelinated afferents into the deeper dorsal horn. These results show that neuronal and glial expressions of Gal1 are tightly correlated with regenerative success. Thus, the differential expression pattern of Gal1 following peripheral axotomy and dorsal rhizotomy suggests that endogenous Gal1 may be a factor important to the regenerative response of injured axons.

Keywords: DRG; Rhizotomy; Axotomy; L-14.5; RL-14.5; IB4; CGRP; NF200

Introduction

Galectins are highly-conserved proteins that are distinguished by homologous carbohydrate recognition domains and specificity for galactosidase-containing oligosaccharides (Cooper and Barondes, 1999; Gray et al., 2004; Hernandez and Baum, 2002). These proteins, which may be found in the extracellular matrix and on cell membranes, have the ability to promote cell–cell or cell–substrate interactions. Galectin-1 (Gal1) was the first galectin to be identified and is widely expressed throughout the body: it is found in most organs, and in a variety of cells including nerve, placental, and muscle cells (Perillo et al., 1998; Poirier et al., 1992; Regan et al., 1986; Wasano et al., 1990). In the developing nervous system, Gal1 expression is initiated in DRG neurons, spinal motoneurons, cranial motoneurons, and olfactory neurons after the last cell division and remains elevated until their axonal targets are reached (Hynes et al., 1990; Regan et al., 1986; St John and Key, 1999). Gal1 is required for the typical termination

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pattern of axons in olfactory and somatic primary afferent systems (McGraw et al., 2005; Puche et al., 1996), indicating that Gal1 is necessary for proper targeting of subpopulations of axons during development.

Like many developmentally-important gene products, Gal1 influences the success of axonal regeneration in the adult. Horie et al. (1999) were the first to suggest that Gal1 has a role in the initiation of axonal regeneration following peripheral nerve injury, and subsequent studies have attempted to further elucidate the underlying mechanisms (Fukaya et al., 2003; Horie and Kadoya, 2000; Horie et al., 2004). For the most part, previous studies have focused on the effects of the application of exogenous Gal1 and function-blocking antibodies; however, the regulation of endogenous Gal1 by axotomy has received little attention. Recently, it has been found that Gal1 mRNA is increased in facial motoneurons following peripheral axotomy (Akazawa et al., 2004; McGraw et al., 2004a). We also found that the upregulation of Gal1 mRNA in neuronal somata of the rubrospinal tract was positively correlated with its regenerative potential (McGraw et al., 2004b).

The apparent developmental importance of Gal1 in sensory systems suggests that it may also be involved in primary afferent regeneration in the adult. Manipulation of the dorsal root ganglion (DRG) neuron, which has one peripheral and one central projection, provides a unique opportunity to study gene and protein regulation following peripheral and central axotomy. While axons projecting peripherally from sensory neurons have the ability to regenerate and reconnect with peripheral targets following injury, those that project centrally cannot regenerate past the peripheral nervous system–central nervous system (PNS–CNS) interface (dorsal root entry zone, DREZ) to reconnect with central targets in the spinal dorsal horn or brainstem. The selective induction of a regenerative program in DRG neurons by peripheral (but not central) axotomy is characterized by changes in gene expression, including upregulation of growth-associated proteins, neuropeptides, and cytoskeletal proteins (Donnerer, 2003; Fenrich and Gordon, 2004; Hokfelt et al., 1994). Other proteins that are upregulated following axotomy have important roles in neuropathic pain, including the neurotrophin nerve growth factor (Ramer et al., 1998; Ro et al., 1999) and specific sodium channel subtypes (Chung and Chung, 2004; Hains et al., 2003).

DRG neurons can be divided into modality-specific subgroups based on soma (and axon) size and by specific protein markers. Approximately 70% of sensory neurons are small diameter (Snider and McMahon, 1998). Small-diameter neurons can be sub-classified as peptidergic, which express the neuropeptide calcitonin gene-related peptide (CGRP); or non-peptidergic, which bind the lectin *Bandeiraea simplicifolia* (IB4) and express the glial cell-line derived neurotrophic factor signaling receptor c-Ret (Bradbury et al., 1998; Chen et al., 1995; Molliver et al., 1997). Larger DRG neurons are identifiable by their expression of the heavy neurofilament NF200 (Lawson et al., 1984). Although most DRG neurons are Gal1-immunoreactive, small-diameter sensory neurons that express c-Ret mRNA have been shown to have the highest Gal1-immunoreactivity in the adult (Regan et al., 1986; Sato and Perl, 1991; Sango et al., 2004).

Here, we examine the distribution of Gal1 expression in peptidergic small-diameter (CGRP-expressing), non-peptidergic small-diameter (IB4-binding), and large-diameter (NF200-expressing) neurons. We subsequently characterize the regulation of Gal1 protein and mRNA expression in these neurons and their associated glial environments after peripheral axotomy and after dorsal rhizotomy.

Materials and methods

Surgery

A total of 30 adult male Wistar rats (University of British Columbia Animal Care Facility, weight 200–250 g) were used for this study. All surgery was performed in accordance with the Canadian Council for Animal Care and approved by the University of British Columbia Animal Care Committee. Rats were anaesthetized with an intraperitoneal injection of ketamine hydrochloride (72 mg/kg; Bimeda-MTC, Cambridge, ON) and xylazine hydrochloride (9 mg/kg; Bayer Inc, Etobicoke, ON), and all surgery was carried out under sterile conditions. To reduce post-operative pain and lessen blood flow to muscle during surgery, 0.4 mL of 2% lidocaine with epinephrine (Vétoquinol, Quebec, QC) was injected into the exposed superficial musculature around the spinal column. Dorsal rhizotomy or a peripheral axotomy of primary afferents was performed unilaterally at the 6th cervical level (C6) to the 1st thoracic level (T1). Dorsal rhizotomy was performed as described in (Ramer et al., 2001b). Briefly, small pieces of vertebrae from C6-T1 were removed, exposing the dorsal roots. The roots were transected midway between the DRG and DREZ. For unilateral peripheral axotomy, nerves exiting the spinal column at C6-T1 were transected and a 5-mm section of nerve was removed, ensuring that all the neurons within a particular DRG were axotomized.

Perfusion/cryosectioning

At either 7 or 14 days after injury (at least 4 animals per group and 2 spinal segments per animal), rats were injected with a lethal dose of chloral hydrate. Upon loss of nociceptive reflexes, animals were perfused intracardially with PBS followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brain and spinal cord were removed and the tissue post-fixed for 24 h in 4% paraformaldehyde at 4°C. Tissue was cryoprotected in a 22% sucrose solution in 0.1 M PB. After cyroprotection, tissue was rapidly frozen in dry ice-cooled 2-methylbutane