Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Neurology 200 (2006) 19–25
Experimental Neurology
www.elsevier.com/locate/yexnr

Membrane-bound CSPG mediates growth cone outgrowth and substrate specificity by Schwann cell contact with the DRG neuron cell body and not via growth cone contact

Cristina Castro, Damien P. Kuffler *

Institute of Neurobiology, Medical Sciences Campus, University of Puerto Rico, 201 Boulevard, del Valle, San Juan 00901, Puerto Rico

Received 25 October 2005; revised 18 January 2006; accepted 1 February 2006
Available online 10 March 2006

Abstract

The central nervous system and peripheral nervous system (CNS/PNS) contain factors that inhibit axon regeneration, including myelin-associated glycoprotein (MAG), the Nogo protein, and chondroitin sulfate proteoglycan (CSPG). They also contain factors that promote axon regeneration, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Axon regeneration into and within the CNS fails because the balance of factor favors inhibiting regeneration, while in the PNS, the balance of factor favors promoting regeneration. The balance of influences in the CNS can be shifted toward promoting axon regeneration by eliminating the regeneration-inhibiting factors, overwhelming them with regeneration-promoting factors, or making axon growth cones non-receptive to regeneration-inhibiting factors. The present in vitro experiments, using adult rat dorsal root ganglion (DRG) neurons, were designed to determine whether the regeneration-inhibiting influences of Schwann cell CSPG are mediated via Schwann cell membrane contact with the DRG neuron cell body or their growth cones. The average longest neurite of neurons in cell body contact with Schwann cells was 7.4-fold shorter than those of neurons without Schwann cell–neuron cell body contact (naked neurons), and the neurites showed substrate specificity, growing only on the Schwann cell membranes and not extending onto the laminin substrate. The neurites of naked neurons showed no substrate specificity and extended over the laminin substrate, as well as onto and off the Schwann cells. After digesting the Schwann cell CSPG with the enzyme C-ABC, neurons in cell body contact with Schwann cells extended neurites the same length as those of naked neurons, and their neurites showed no substrate selectivity. Further, the neurites of naked neurons were not longer than those of naked neurons not exposed to C-ABC. These data indicate that the extent of neurite outgrowth from adult rat DRG neurons and substrate specificity of their growth cone is mediated via contact between the Schwann cell membrane-bound CSPG and the DRG neuron cell body and not with their growth cones. Further, there was no apparent influence of diffusible or substrate-bound CSPG on neurite outgrowth. These results show that eliminating the CSPG of Schwann cells in contact with the cell body of DRG neurons eliminates the sensitivity of their growth cones to the CSPG-induced outgrowth inhibition. This may in turn allow the axons of these neurons to regenerate through the dorsal roots and into the spinal cord.
© 2006 Published by Elsevier Inc.

Keywords: Inhibiting regeneration; DRG; CSPG; Beta-xyloside; Schwann cells; C-ABC; MAG; Nogo; Nogo-A

Introduction

Axon regeneration within the adult CNS requires a cellular environment that is permissive to and promoting of neurite outgrowth. Although reactive astrocytes express many growth factors, including BDNF and NGF, axon regeneration into and within the CNS fails because of the ubiquitous presence of potent neurite outgrowth inhibiting factors, such as myelin-associated glycoprotein (MAG) (Domeniconi and Filbin, 2005; Mingorance et al., 2005), Nogo-A, associated with oligodendrocytes and DRG Schwann cells (Buss et al., 2005; Caroni and Schwab, 1988; Chen et al., 2000; Fouad et al., 2005), and chondroitin sulfate proteoglycan (CSPG), associated with activated astrocytes (Canning et al., 1996). Although the same axon regeneration-inhibiting factors are present in the PNS, regeneration is successful because and the balance of regeneration-promoting factors vs. regeneration-inhibiting favors promoting axon regeneration.

MAG promotes neurite outgrowth in the embryonic and neonatal CNS but inhibits neurite outgrowth in adults (McKerracher et al., 1994; Mukhopadhyay et al., 1994; Shen

* Corresponding author. Fax: +1 787 721 1235.
E-mail address: dkuffler@hotmail.com (D.P. Kuffler).

0014-4886/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.expneurol.2006.02.001

et al., 1998; Tang et al., 2001). In the PNS, within the first 2 weeks after birth, sectioned nerves change from regeneration promoting to increasingly inhibitory, which in part results from the increased synthesis and presence of MAG (Torigoe and Lundborg, 1998). MAG is also present in adult PNS myelin where it inhibits axon regeneration (Bahr and Przyrembel, 1995; Shen et al., 1998). If following nerve injury myelin is completely removed from the PNS and CNS, the regeneration-inhibiting role of MAG is eliminated, and axons can regenerate in the CNS and more rapidly in the PNS (Savio and Schwab, 1990).

Nogo-A inhibits axon regeneration into and within the spinal cord, as well as through peripheral nerves (Fouad et al., 2005; Hunt et al., 2003; Kim et al., 2004; Pot et al., 2002; Raineteau et al., 2001; Seymour et al., 2005; Simonen et al., 2003). This inhibitor of regeneration is also present on the surface of fibroblasts, DRG neurons, myoblasts, myelin, oligodendrocytes, and presynaptic terminals of the neuromuscular junction (Chen et al., 2000; Dodd et al., 2005). Nogo-A is recognized by the monoclonal antibody IN-1, which neutralizes its regeneration-inhibiting action, which allows axons to regenerate into and through the CNS, and more extensively through peripheral nerves (Buss et al., 2005; Chen et al., 2000; Pot et al., 2002; Schnell and Schwab, 1990).

Inhibition of axon growth by myelin and CSPG is in part controlled by Rho GTPase, which mediates growth cone collapse (Mizuno et al., 2004). Endogenous neuronal levels of cAMP levels determine the neuronal responsiveness to myelin-associated neurite growth inhibitors by regulating Rho GTPase activities (Oertle et al., 2003). Thus, elevating the cAMP concentration in neurons blocks Nogo-A and MAG induced inhibition of neurite outgrowth by suppressing GTPase RhoA activities (ras homology protein) or of its downstream target Rho-associated coiled kinase (ROCK) (Borisoff et al., 2003; Brabeck et al., 2004; Domeniconi and Filbin, 2005; Oertle et al., 2003; Schwab et al., 2004). Increasing cAMP levels and inhibiting Rho is one mechanism by which inhibition of regeneration following a nerve lesion can be overwhelmed and axons regeneration into the mammalian spinal cord can be induced.

Reactive astrocytes are negative modulators of axon growth following adult CNS injury, due to their production of neurite outgrowth inhibitory molecules. Although immature astrocytes are a supportive substrate for axon outgrowth in vivo (Bahr and Przyrembel, 1995, Silver et al., 1993) and in vitro (Ard et al., 1991, Bahr and Przyrembel, 1995), reactive astrocytes are a non-permissive substrate for axon regeneration (Bahr and Przyrembel, 1995; Canning et al., 1996; Gilbert et al., 2005; Le Roux and Reh, 1996; McKeon et al., 1991, 1995). Further, reactive astrocytes inhibit Schwann cell proliferation and myelin formation (Guenard et al., 1994). Thus, reactive astrocytes present following trauma contribute to the creation of a regeneration-inhibiting environment.

CSPG is also a potent inhibitor of axon regeneration within the PNS. Following a peripheral nerve lesion, Schwann cells show a 7-fold increase in their concentration of CSPG, which restricts neurite elongation (Zuo et al., 1998a,b). The inhibition of growth cone elongation following contact with CSPG results from an induced increase in the concentration of intracellular calcium that modifies the cytoskeleton and growth cone migration (Snow et al., 1994). When CSPG is eliminated by in vivo administration of the CSPG synthesis inhibitor beta-D-xyloside (Zuo et al., 1998a,b), or the CSPG is digested by injecting the injury site with chondroitinase ABC (C-ABC) (Zuo et al., 2002), neurons extend longer neurites (Zuo et al., 1998a,b). Similarly, digesting CSPG at the DREZ allows axons to regenerate into and within the CNS (Brabeck et al., 2004; Bradbury et al., 2002; Grimpe and Silver, 2004; Morgenstern et al., 2002; Steinmetz et al., 2005; Yick et al., 2000; Zuo et al., 1998a,b).

Materials and methods

Isolating and plating neurons

Adult rat DRG neurons were isolated according to methods previously described (Cruz and Kuffler, 2005). Briefly, thoracic ganglia were removed and cleaned of their connective tissue capsule, placed in a siliconized glass dish (Sigmacoat, Sigma), in DMEM (Sigma) and F-12 tissue culture medium (Sigma), 50/50 by volume adjusted to pH 7.2, cut into small pieces and incubated in collagenase P (3 mg/ml) (Boehringer–Mannheim), neutral dispase type II (8 mg/ml) (Boehringer–Mannheim), and DNase (0.3 mg/ml) (Boehringer–Mannheim) and placed in an O_2/CO_2 incubator for 1 h. The pieces of ganglia were gently triturated through a siliconized Pasteur pipette with a tip just larger than the pieces of ganglia. Following complete dissociation, using a siliconized micropipette attached to a mouth sucker, individual neurons were plated into a cover slip in a 35-mm culture dish that had been treated for 1 h with poly-L-lysine (1 mg/ml) followed by 1 h with laminin (5 mg/ml) (Sigma Chemical) in DMEM + F-12 medium (50/50 by volume) adjusted to pH 7.6. Viable neurons adhered to the substrate immediately. After 5 min, heat inactivated fetal bovine serum (Sigma Chemical) was added to the medium to a final concentration of 10%. Addition of serum before plating the neurons prevented them from adhering to the substrate. The dishes were placed in an O_2/CO_2 incubator at 37°C, in a saturated water environment. The cultures were maintained without any neurotrophic or other factors. The cultures were analyzed after 2 days in vitro.

Morphometric analysis

Images of the neurons were captured using a Zeiss Axiovert microscope with an ORCA digital camera (Hamamatsu) or a 3-chip color camera (Hamamatsu) and were displayed on a monitor. The length of the longest neurite of each neuron was measured. This provides reliable comparative information on the relative lengths of neurites of neurons under different conditions and when exposed to different factors (Dobretsov et al., 1994). Neurite lengths were measured using a Metamorph Imaging Analysis System (Universal Imaging Corp., PA). The length of a neurite on a monitor was traced using a cursor

Download English Version:

https://daneshyari.com/en/article/3057384

Download Persian Version:

https://daneshyari.com/article/3057384

Daneshyari.com