Experimental Neurology 223 (2010) 143–152

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Rolipram-induced elevation of cAMP or chondroitinase ABC breakdown of inhibitory proteoglycans in the extracellular matrix promotes peripheral nerve regeneration

E. Udina [a,b], A. Ladak [c], M. Furey [a], T. Brushart [d], N. Tyreman [a], T. Gordon [a,*]

[a] Division of Physical Medicine and Rehabilitation, Faculty of Medicine and Dentistry, Center for Neurosciences, University of Alberta, Edmonton, Alberta, Canada T6G 2S2
[b] Institut Neurosciences, Department Cell Biology, Physiology and Immunology and Centro Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Universitat Autonoma de Barcelona, Barcelona 08913, Spain
[c] Division of Plastic Surgery, University of Alberta, Edmonton, Alberta, Canada T6G 2S2
[d] Departments of Orthopedics and Neurology, John Hopkins School of Medicine, Baltimore, MD 21287, USA

ARTICLE INFO

Article history:
Received 3 April 2009
Revised 25 August 2009
Accepted 26 August 2009
Available online 4 September 2009

Keywords:
cAMP
Chondroitinase ABC
Axon regeneration
Axon outgrowth
Staggered regeneration
Motoneurons
DRG neurons

ABSTRACT

The inhibitory growth environment of myelin and extracellular matrix proteoglycans in the central nervous system may be overcome by elevating neuronal cAMP or degrading inhibitory proteoglycans with chondroitinase ABC (ChABC). In this study, we asked whether similar mechanisms operate in peripheral nerve regeneration where effective Wallerian degeneration removes myelin and extracellular proteoglycans slowly. We repaired transected common peroneal (CP) nerve in rats and either elevated cAMP in the axotomized neurons by subcutaneous rolipram, a specific inhibitor of phosphodiesterase IV, and/or promoted degradation of proteoglycans in the distal nerve stump by local ChABC administration. Rolipram treatment significantly increased the number of motoneurons that regenerated axons across the repair site at 1 and 2 weeks, and increased the number of sensory neurons that regenerated axons across the repair site at 2 weeks. Local application of ChABC had a similar effect to rolipram treatment in promoting motor axon regeneration, the effect being no greater when rolipram and ChABC were administered simultaneously. We conclude that blocking inhibitors of axon regeneration by elevating cAMP or degrading proteoglycans in the distal nerve stump promotes peripheral axon regeneration after surgical repair of a transected nerve. It is likely that elevated cAMP is sufficient to encourage axon outgrowth despite the inhibitory growth environment such that simultaneous enzymatic proteoglycan degradation does not promote more axon regeneration than either elevated cAMP or proteoglycan degradation alone.

Crown Copyright © 2009 Published by Elsevier Inc. All right reserved.

Introduction

Axons in the peripheral (PNS) but not the central nervous system (CNS) regenerate after injury (Fu and Gordon, 1995a,b, 1997). Nevertheless there is only a short window of opportunity for effective PNS regeneration to occur, whereby regenerative success of injured nerves progressively decreases after prolonged axotomy and Schwann cell denervation (Fu and Gordon, 1995a,b; Gordon et al., 2003; Sulaiman and Gordon, 2000). Axon outgrowth from the proximal stump of transected and surgically repaired nerves is a slow process (Brushart et al., 2002) and this lengthy period when regenerating axons wander in the suture site of surgically repaired peripheral nerves (Cajal, 1928; Witzel et al., 2005) accounts, at least in part, for the delays of several weeks for all regenerating axons to cross a repair site (Brushart et al., 2002). Chondroitin sulfate proteoglycans (CSPGs) of the extracellular matrix and myelin-associated inhibitors, both potent inhibitors of neuronal regeneration in the CNS (Mueller, 1999; Sandvig et al., 2004; Tang, 2003), are also present in the peripheral nerve. These molecules are up-regulated after nerve injury, show neurite-inhibitory activity (Braunewell et al., 1995; Shen et al., 1998; Zuo et al., 1998), and may play a role in delayed axon outgrowth following peripheral nerve injury. The prolonged time course of weeks for effective removal of myelin debris by macrophages and Schwann cells (Avellino et al., 1995; Fansa and Keilhoff, 2003; George and Griffin, 1994; Stoll et al., 1989) and for degradation of glycoproteins of the extracellular matrix (Hughes et al., 2002) may account for the staggered outgrowth of axons from the proximal stump of an injured nerve (Al-Majed et al., 2000; Gordon et al., 2003).

Injured nerves in the CNS may be stimulated to regenerate their axons despite the inhibitory environment by interfering with signaling pathways that are activated by inhibitory myelin associated molecules (Cai et al., 1999; Dergham et al., 2002; Lehmann et al., 1999; Neumann and Woolf, 1999; Neumann et al., 2002; Qiu et al., 2002). The inhibition can be overcome by increasing neuronal cAMP levels *in vivo* (Cai et al., 1999; Dergham et al., 2002; Lehmann et al., 1999; Neumann and Woolf, 1999; Neumann et al., 2002; Qiu et al., 2002) and *in vitro* (Cai et al., 1999) and by selectively cleaving

* Corresponding author. Faculty of Medicine, Division of Physical Medicine and Rehabilitation/Centre for Neuroscience, 525 Heritage Medical Research Centre, University of Alberta, Edmonton, AB, Canada T6G 2S2. Fax: +1 780 492 1617.
E-mail address: tessa.gordon@ualberta.ca (T. Gordon).

0014-4886/$ – see front matter. Crown Copyright © 2009 Published by Elsevier Inc. All right reserved.
doi:10.1016/j.expneurol.2009.08.026

glycosaminoglycan (GAG) side chains from the protein core of proteoglycans with chondroitinase ABC (ChABC) (Fawcett and Asher, 1999). However, applying these pharmacological approaches to PNS regeneration has generated conflicting data. Some have reported that cAMP does promote axon outgrowth (Gershenbaum and Roisen, 1980; Kilmer and Carlsen, 1987; Pichichero et al., 1973) while others have failed to demonstrate increased axon outgrowth (Black and Lasek, 1979; Han et al., 2004; McQuarrie et al., 1977). The use of ChABC in the PNS has been shown to accelerate axon outgrowth into distal nerve stumps and acellular nerve grafts in rats (Krekoski et al., 2001; Zuo et al., 2002). Analyses of the distance of regenerating axons of *thy-1 TFP-H* transgenic mice demonstrated longer axon profiles in nerve grafts from wild-type littermates with ChABC, which specifically degrades CSPGs but not other proteoglycans such as heparin sulfate proteoglycans (Groves et al., 2005).

Using retrograde labeling techniques, we aim to further investigate whether elevation of cAMP or removal of GAG side chains from inhibitory proteoglycans in axotomized motor and sensory neurons in the peripheral nerve promotes PNS regeneration. We also question whether a combinational strategy of rolipram and ChABC that has proven to be effective in CNS regeneration (Fouad et al., 2005; Houle et al., 2006; Tropea et al., 2003) is also effective in the PNS.

Material and methods

All experiments were performed on adult female Sprague–Dawley rats (200–220 g) and approved by local authorities (Health Sciences Laboratory Animal Services, University of Alberta) according to the Canadian Council for Animal Care guidelines.

Surgery: nerve repair and delivery of rolipram and/or chondroitinase ABC (ChABC)

All surgeries were performed under Ketamine (Vetalar, Bioniche, Bellville, Ontario) and Xylazine (Rompun, Bayer, Toronto, Ontario) intraperitoneal anesthesia at doses of 0.6 and 0.4 mg/kg, respectively. Eye lubricant (Duratears Naturale, Alcon, Ontario) was used to prevent corneal damage during surgery.

Using aseptic technique, the right CP nerve was exposed distal to the sciatic notch and transected 10 mm proximal to its entrance into the flexor muscle group including the tibialis anterior muscle (Fig. 1A). Silicone silastic guides (Helix Medical, Inc., Carpinteria, CA) of 0.76 mm interior diameter and 3 mm length were implanted, and the proximal and distal CP nerve stumps were approximated within the nerve guide using 9-0 Ethicon nylon suture (Ethicon, Inc. ,Somerville, NJ) as described in Furey et al. (2007). An Alzet mini-osmotic pump (Durect Corporation, Cupertino, CA) was then implanted subcutaneously on the back of the rat to release its content of either rolipram or saline systemically. The Alzet pump (model 2ML2) was filled with the appropriate solution for 24 h and incubated at 37 °C prior to implantation. The pump delivered either rolipram (Nikulina et al., 2004) diluted in 1:1 saline/dimethyl sulfoxide (DMSO) (rolipram group, $n = 26$) or vehicle solution (1:1 saline/DMSO; control group, $n = 22$), both delivered at a rate of 0.4 μmol/kg/h continuously over 7, 14 or 21 days. The surgical wound was sutured closed and the rats were monitored and kept warm until they recovered from the anesthetic.

Chondroitinase ABC application

In two groups of rats, the CP nerve was exposed and transected under surgical anesthesia 10 mm proximal to the entrance of the nerve into the flexor muscle group of the hindlimb. In the first group of animals, 8 mm of the cut end of the distal stump was dipped into a vaseline well containing 20 U/ml protease free ChABC (Seikagaku, Japan) in 1% protease free bovine serum albumin for 1 h. The nerve ends were then repaired using a silicone silastic guide. An Alzet mini-osmotic pump was then placed on the back of the animal and a vehicle solution of 1:1 saline/DMSO was delivered systemically over 2 weeks at a delivery rate of 5 μl/ml (ChABC group $n = 9$). In the second group of rats, the ChABC was again applied to the distal nerve stump as described, and an Alzet pump implanted on the back of the rat was filled with rolipram solution for systemic delivery over a 2-week period at the same rate as for the vehicle solution group (Roli + ChABC group, $n = 8$). The skin was closed after surgery and the rats were warmed and monitored during recovery from the anesthetic.

Surgical application of retrograde dyes

A second sterile surgery was carried out under surgical anesthesia at 7–21 days following the CP nerve section and repair, and for the systemic delivery of saline/DMSO vehicle or rolipram, and/or local application of ChABC to the CP nerve suture site. Fluororuby (Dextran, tetramethylrodamine, Invitrogen, Molecular Probes, Eugene, OR) was either injected 3 mm distal to the site of the nerve repair or applied directly to the proximal stump of the cut CP nerve 10 mm distal to the repair site (Figs. 1B, C). For the microinjection, the CP nerve was crushed just distal to the cuff (3 mm distal to the suture site) and the dye was injected via a micropipette that was attached to a picospritzer (Intercel Picospritzer III) (Brushart et al., 2002). For the application of the dye to the proximal stump of the CP nerve cut 10 mm distal to the suture site, the stump was dipped into a 5% fluororuby solution contained within a vaseline well for 1 h. Following the dye exposure, excess dye was carefully rinsed off before suturing the skin and allowing the rats to wake. In control experiments, we injected the dye 3 mm distal to the suture site immediately after nerve repair rather than after 7, 14, and 21 days. This was done to ensure that the injected dye was confined to the injection site within the distal nerve stump and did not penetrate to the axons in the proximal nerve stump.

Tissue removal and analysis of the backlabeled neurons

Rats were deeply anesthetized 5–6 days after backlabeling of the neurons and transcardially perfused with 200 ml saline followed by 500 ml of 4% paraformaldehyde at pH 7.4. After perfusion, the spinal cord was dissected and spinal cord segments T11 to L2 (containing the CP motoneuron pool) and L4 and L5 dorsal root ganglia (DRGs) (containing most of the cell bodies of the CP sensory nerves) were harvested and post-fixed with 30% sucrose in 4% paraformaldehyde solution overnight. The tissue was then frozen in liquid nitrogen after been embedded in OCT Tissue-Tek Liquid (Sakura, Japan).

Frozen tissues were sectioned in a cryostat (Jung 3000). Longitudinal spinal cord sections were cut at 50 μm thickness and DRG cross-sections were cut at 25 μm thickness. The fluorescent bodies of the labeled motor and sensory neurons of the CP nerve were visualized and counted at 40× magnification under fluorescent microscopy at barrier filters of 580 nm (Fig. 2). The number of neurons counted was corrected according to the thickness of the sections and the diameter of the neuron cell bodies (30 and 15 μm for motor and DRG neurons, respectively) by the method of Abercrombie and Johnson (1946). The correction factor in our samples was 0.635 for motoneuron counts in the spinal cord sections and 0.574 for the DRG sensory neurons.

Morphological analysis of nerve sections

In a third set of rats, surgery was performed to administer rolipram ($n = 9$) or vehicle solution ($n = 9$) immediately after CP nerve transection and repair via an Alzet miniosmotic pump for periods of 4 or 14 days. The surgeries were the same as described for the previous sets of rats. A segment of the CP nerve 3 mm distal to the suture site was removed at 4- and 14-day time points. The nerve pieces were fixed with gluteraldehyde (3% in 0.1 M phosphate buffer),

Download English Version:

https://daneshyari.com/en/article/6019369

Download Persian Version:

https://daneshyari.com/article/6019369

Daneshyari.com