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Disruption of Fast Axonal Transport in the Rat Induces Behavioral Changes Consistent With Neuropathic Pain

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Abstract: Studies of peripheral nerve inflammation (neuritis) suggest that some symptoms of neuropathic pain can be generated from inflamed but otherwise uninjured axons. We have previously inferred a role for inflammation-induced axonal transport disruption in the underlying mechanisms. In the present study, we have investigated the development of sensory hypersensitivities following vinblastine-induced axonal transport disruption. Similar to neuritis, locally applied .1 mM vinblastine caused the rapid development of mechanical hypersensitivity within the first week postsurgery. The same animals did not develop heat hypersensitivity. Because aberrant firing from primary sensory neurons is considered necessary to drive spinal mechanisms that lead to hypersensitivities, the levels of ongoing activity and axonal mechanical sensitivity were examined. Recordings from A- and C-fiber neurons did not reveal differences in the levels of ongoing activity between vinblastine-treated (<5.8%) and saline-treated control animals (<4.6%). However, 28% of C-fiber axons were mechanically sensitive at the vinblastine treatment site. Using kinesin immunohistochemistry, we confirmed a reduction of anterograde axonal transport in vinblastine-treated and neuritis animals. In summary, this study has revealed an alternative pain model, which may be relevant to conditions that are not accompanied by frank nerve injury.

***Perspective:** In this study, we expand our previous reports and demonstrate that focal reduced axonal transport causes distal mechanical hypersensitivity considered consistent with neuropathic pain but in the absence of nerve injury. These findings may inform pain conditions that have a neural inflammatory component.*

***Key words:** Axonal transport disruption, neuritis, pain hypersensitivity, neuropathic pain, vinblastine.*

Over the last 25 years, the use of animal models such as the chronic constriction injury and spinal nerve ligation models[4,35] has advanced our understanding of the mechanisms of neuropathic pain. However, these models are associated with substantial nerve injury, whereas many of the patients with symptoms of neuropathic pain do not have signs of an overt nerve injury on routine clinical examination. Examples of such conditions include complex regional pain syndrome, repetitive strain injuries (eg, nonspecific arm pain), and lower back pain. Studies using a model of localized nerve inflammation (neuritis) suggest that symptoms in these patients may be generated from inflamed but otherwise uninjured axons.[5,8,22,25] For example, neuritis produces pain behaviors (ie, mechanical and heat hypersensitivities) in the absence of Wallerian degeneration or demyelination.[25] In this model, intact C-fiber axons develop ongoing activity, which refers to activity that develops in the absence of applied stimulus, and mechanical sensitivity at the inflamed site.[5,8,22,24] Such altered neuronal physiology is associated with the symptoms of spontaneous pain (ie, ongoing activity) and movement-evoked radiating pain (ie, axonal mechanical sensitivity) that are reported by patients with radiating pain.[9,29]

In injured neurons, axonal mechanical sensitivity and ongoing activity can develop at regenerating axonal sprouts.[13,27,41,48,50] However, in intact (uninjured) axons

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in the neuritis model, as well as in some nerve injury models,[23] axonal continuity is maintained and the sensitivities may arise through an alternative mechanism. We have previously suggested a mechanism of inflammation-induced axonal transport disruption,[20] following the reports that inflammation can disrupt axonal transport.[1,3] Because ion channel components are transported from the dorsal root ganglia to the periphery by fast axonal transport,[37] we have hypothesized that the disruption of axonal transport by localized nerve inflammation may cause an increased density of mechanically sensitive ion channels, forming a "hot spot" of excitability.

Experimentally, axonal transport along a peripheral nerve can be disrupted by the local application of antimitotic agents such as vinblastine or colchicine. At low doses, these agents can effectively block axonal transport without causing axonal damage or inflammation.[20,26,32-34,36,59] We have previously shown that the local disruption of axonal transport along the sciatic nerve causes axonal mechanical sensitivity in otherwise normal C-fiber neurons.[20] Conversely, the application of vinblastine proximal to the site of inflammation/injury can reduce the development of axonal mechanical sensitivity during neuritis,[20] ongoing activity from a neuroma,[18] and heat hypersensitivity in the chronic constriction injury model.[58] These results further infer a role for axonal transport in pathologic pain mechanisms.

The vinblastine model provides a novel means to reveal the effects of axonal transport on pain physiology. The main aim of the present study was to determine whether axonal transport disruption induced by the local application of vinblastine could cause pain behaviors associated with nerve injury or inflammation. Because axonal transport disruption results in the development of axonal mechanical sensitivity,[20] we also designed experiments to determine whether reduced axonal transport can cause neurons with A- and C-fiber axons to develop ongoing activity. As well as a role in spontaneous pain, such aberrant peripheral activity into the spinal cord is reputed to drive spinal mechanisms that lead to mechanical hypersensitivity.[11,28,38,49,54] Finally, this study examined axonal transport disruption in the neuritis model and following vinblastine treatment. Our results show that vinblastine-induced axonal transport blockade leads to mechanical hypersensitivity, but in the absence of ongoing activity. We also demonstrate that neuritis and vinblastine disrupt anterograde axonal transport. These findings further emphasize an important role for axonal transport disruption in clinical pain mechanisms.

Methods

Animals and Surgery

Experiments were carried out in strict accordance with the UK Animals (Scientific Procedures) Act (1986) and the Animal Care and Use Committee of the University of New England. A total of 58 adult male Sprague Dawley rats (175–300 g; Harlan, Bicester, Oxon, United Kingdom, and Charles River Laboratories, Wilmington, MA) were used in this study.

Vinblastine and Neuritis Surgery (for Behavioral and Electrophysiological Studies)

The sciatic nerves of adult male Sprague Dawley rats were treated with vinblastine (n = 14) as previously described.[20] Animals were anesthetized and maintained on isoflurane (1.75%) in oxygen. The left sciatic nerve was exposed in the mid-thigh by blunt dissection and a 7 to 8 mm length was carefully freed from its surrounding connective tissue. A strip of Parafilm (6 × 20 mm; Pechiney Plastic Packaging, Menasha, WI) was positioned under the nerve to prevent leakage of the agent onto the surrounding tissue. A length (5 × 5 × 10 mm) of sterile Gelfoam (Spongostan; Ferrosan, Denmark) saturated in .1 mM vinblastine (diluted in sterile .9% saline) was wrapped around the nerve. After 15 minutes, the Gelfoam and Parafilm were removed and the nerve was rinsed copiously with sterile saline. The location of the treatment site was carefully noted. The muscle and skin were closed using 4/0 monofilament sutures (Vicryl; Ethicon, West Lothian, United Kingdom) and the animals were allowed to recover. In 11 "sham" animals, the same surgical procedure was followed, except that the nerve was exposed to .9% saline alone.

In an additional group of adult male Sprague Dawley rats (n = 6), a localized sciatic nerve neuritis was induced using previously described methods.[21,45] In these animals, a 7- to 8-mm segment of nerve was dissected in the proximal thigh. A strip of sterile Gelfoam (2 × 2 × 10 mm) saturated in complete Freund's adjuvant (approximately 150 μL; diluted 50% in sterile saline) was loosely wrapped around the nerve. The muscle and skin were closed using 4/0 monofilament sutures and the animals were allowed to recover.

Nerve Ligation Surgery (for Assessment of Anterograde Axonal Transport)

Anterograde axonal transport was assessed by partially ligating the sciatic nerve distal to the neuritis or vinblastine treatment site and assessing the accumulation of kinesin proximal to the suture (see Fig 1). Kinesin is the primary "motor" of fast anterograde axonal transport[15,42,43] and is critical to the mechanism that transports mechanically sensitive ion channel components toward the periphery.[37]

Twenty-four adult male Sprague Dawley rats underwent a partial nerve ligation. Animals were anesthetized and maintained on isoflurane (2.0–2.25%) in oxygen. The left sciatic nerve was exposed in the mid-thigh by blunt dissection and carefully freed from its surrounding connective tissue. Approximately half of the nerve was tightly ligated using 7/0 monofilament suture. In 1 group of 8 rats (vinblastine-treated nerve ligation group), a small cup made of Parafilm was positioned to surround the nerve, and a small pledget of cotton soaked with .1 mM vinblastine in sterile saline was placed around approximately 5 mm of the nerve, proximal to and including the ligation site. After 15