SCIENTIFIC ARTICLE

Afferent Innervation, Muscle Spindles, and Contractures Following Neonatal Brachial Plexus Injury in a Mouse Model

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Purpose We used an established mouse model of elbow flexion contracture after neonatal brachial plexus injury (NBPI) to test the hypothesis that preservation of afferent innervation protects against contractures and is associated with preservation of muscle spindles and ErbB signaling.

Methods A model of preganglionic C5 through C7 NBPI was first tested in mice with fluorescent axons using confocal imaging to confirm preserved afferent innervation of spindles despite motor end plate denervation. Preganglionic and postganglionic injuries were then created in wild-type mice. Four weeks later, we assessed total and afferent denervation of the elbow flexors by musculocutaneous nerve immunohistochemistry. Biceps muscle volume and cross-sectional area were measured by micro computed tomography. An observer who was blinded to the study protocol measured elbow flexion contractures. Biceps spindle and muscle fiber morphology and ErbB signaling pathway activity were assessed histologically and immunohistochemically.

Results Preganglionic and postganglionic injuries caused similar total denervation and biceps muscle atrophy. However, after preganglionic injuries, afferent innervation was partially preserved and elbow flexion contractures were significantly less severe. Spindles degenerated after postganglionic injury but were preserved after preganglionic injury. ErbB signaling was inactivated in denervated spindles after postganglionic injury but ErbB signaling activity was preserved in spindles after preganglionic injury with retained afferent innervation. Preganglionic and postganglionic injuries were associated with upregulation of ErbB signaling in extrafusal muscle fibers.

Conclusions Contractures after NBPI are associated with muscle spindle degeneration and loss of spindle ErbB signaling activity. Preservation of afferent innervation maintained spindle development and ErbB signaling activity, and protected against contractures.

Clinical relevance Pharmacologic modulation of ErbB signaling, which is being investigated as a therapy for congestive heart failure, may be able to recapitulate the protective effects of afferent innervation in spindle development and contracture prevention. Muscle spindle preservation may also have implications in proprioception and motor learning, both of which are impaired in NBPI. *(J Hand Surg Am. 2015;40(10):2007–2016. Copyright © 2015 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Afferent innervation, brachial plexus, contracture, ErbB signaling, muscle spindle.

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Neonatal brachial plexus injury (NBPI), which occurs in 1.5 per 1,000 live births, is the most common nerve injury in childhood.[1] In the 20% to 30% of children who do not have complete neurological recovery,[2] secondary contractures occur, especially shoulder internal rotation and elbow flexion contractures.[3] These contractures markedly impair function and quality of life[4–6] and are the most common reason for surgery after NBPI.

We have previously shown that impaired growth of neonatally denervated muscle contributes to contracture pathogenesis after NBPI.[7] Furthermore, we have shown that early reinnervation prevents contractures in a dose-dependent manner, with an apparent threshold effect in which muscles innervated with more than 40% to 50% of the normal number of axons do not develop contractures.[8] Understanding the relative contributions of motor and/or sensory denervation to muscle growth and contractures will narrow the search for targets for intervention to prevent contracture formation after NBPI.

Patients with rare C5 to C6 preganglionic nerve root avulsion injuries do not develop contractures at the shoulder and elbow,[9] whereas the more common C5 to C6 postganglionic nerve root ruptures lead to contractures. Although both injuries cause an identical distribution of motor denervation, preganglionic avulsion injuries leave the afferent neuron cell bodies in the dorsal root ganglion connected to the muscle, preserving afferent innervation of the muscle (Fig. 1). It is therefore possible that this retained afferent innervation preserves muscle growth and protects against contractures. Muscle afferent neurons themselves are known to secrete growth factors[10,11] that may be important for the longitudinal growth of muscle. Sensory innervation also allows the muscle to sense passive stretch, which is an important signal for longitudinal muscle growth.[12] Such sensory function depends on muscle spindles, which are stretch sensory organs located within the belly of skeletal muscles.[13] Neonatal afferent denervation has been shown to impair postnatal muscle spindle development,[14] potentially implicating the muscle spindle as a link between denervation and impaired muscle growth after NBPI.

This neonatal dependence of spindle development on afferent innervation involves the growth factor neuregulin (NRG)-1 and the ErbB family of tyrosine kinase receptors. Neuregulin-1 is synthesized by dorsal root ganglion cells and activates ErbB receptors in muscle by stimulating differentiation of intrafusal spindle fibers.[15,16] Furthermore, the role of this pathway in muscle development extends beyond spindle formation, because ErbB signaling is also involved in myogenesis and myoblastic differentiation[17–19] and muscle regeneration after injury.[16] The NRG-1/ErbB pathway is a particularly compelling target for investigation because recombinant human NRG-1 is currently in clinical trials for treatment of congestive heart failure, because cardiomyocyte function similarly depends on ErbB activation.[20] Demonstrating the relevance of this pathway to contracture pathophysiology in NBPI could stimulate further investigation into the possibility of medically preserving muscle growth and development while awaiting spontaneous or surgical motor reinnervation. The current study thus used an established mouse model of NBPI to test the hypothesis that preservation of afferent innervation prevents contractures after NBPI associated with preservation of muscle spindle development and ErbB signaling.

MATERIALS AND METHODS

Our institutional animal care and use committee approved all animal procedures and analyses.

Confirmation of preganglionic NBPI model

A previously reported model of surgical extraforaminal NBPI[7] was modified to create preganglionic injuries of the C5 to C7 nerve roots (Fig. 1). To demonstrate that preganglionic injury could cause complete motor denervation but preserve afferent innervation of muscle spindles, we first performed the surgery in mice that constitutively express yellow fluorescent protein (YFP) in all axons (Thy1-YFP, JAX strain 003709). Under isoflurane general anesthesia, 7-day-old mouse pups underwent unilateral surgical exposure of the left brachial plexus. The C5 to C7 nerve roots were traced to the foramina and the corresponding lamina were transected and reflected medially. The dura mater was incised longitudinally to expose the dorsal and ventral rootlets, which were transected as they exited the spinal cord. The dural rent was sealed with fibrin glue (Tisseel; Baxter Healthcare Corporation, Westlake Village, CA). We assessed the relative afferent denervation of muscle spindles and efferent denervation of motor end plates 3 weeks after NBPI. Biceps muscles were harvested immediately after death, fixed in 4% (w/v) paraformaldehyde for 2 hours, and optically cleared using the SeeDB protocol.[21] Whole biceps muscles were imaged using a Nikon Eclipse Ti inverted microscope on a Nikon A1R confocal with the multiline argon laser at 514 nm (Nikon Instruments Inc, Melville, NY). Overview z-stacked tile scans were performed using the Plan Apo ×4 objective and selected features were scanned at higher magnification using the Plan Apo VC ×20 DIC N2 objective. We analyzed