ErbB’s Palsy? A Molecular Link between Afferent Innervation, Muscle Spindles, and Contractures following Neonatal Brachial Plexus Injury

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Introduction: Neonatal brachial plexus injury (NBPI) occurs in 1-3 per 1,000 live births,^1 leaving permanent paralysis in 20-30% of children.^2 Most permanent injuries involve post-ganglionic ruptures of the C5-6 nerve roots. Following these injuries, secondary shoulder and elbow joint contractures commonly occur due to impaired muscle growth, and no surgical or nonsurgical treatment can reliably and safely restore normal upper extremity function once the contractures have developed. In contrast, children who sustain C5-6 nerve rootlet avulsions do not develop contractures,^3 and regain better motor function following surgical reinnervation than do those with post-ganglionic injuries. Nerve rootlet avulsions are pre-ganglionic in nature and therefore preserve contact between the paralyzed muscles and their afferent neurons in the dorsal root ganglia. Afferent input is essential for normal muscle physiology, particularly proprioception and motor learning, both of which require functioning muscle spindles to sense muscle stretch. The establishment and maintenance of muscle spindles is controlled by myofiber contact with type Ia afferent innervation, and muscle spindles are known to degenerate following post-ganglionic NBPI.^4 We hypothesize that there exists a link between afferent innervation, spindle development, and postnatal muscle growth that is perturbed by post-ganglionic NBPI but preserved by pre-ganglionic NBPI. We hypothesize that this link involves muscle ErbB signaling, which is activated by Neuregulin-1 from afferent neurons and is both required for postnatal spindle maintenance and involved in myogenesis.

Methods: All protocols were approved by the Institutional Animal Care and Use Committee. Post-ganglionic global (C5-T1) NBPIs were created by surgical extraforaminal nerve root excision in order to completely denervate the forelimb. Muscle spindle morphology was analyzed in biceps muscles harvested at 4 weeks post-operatively and processed for Hematoxylin & Eosin staining and imaging under brightfield microscopy at 40x oil magnification. Next, a model of pre-ganglionic C5-7 NBPI was tested in Thy1-YFP mouse (JAX Stock #003709) with fluorescent axons, using confocal imaging after optical clearing in SeeDB to confirm preserved afferent innervation of spindles despite motor end plate denervation. Pre- and post-ganglionic injuries were then created in 5-day-old CD-1 mice under general anesthesia. Four weeks later, total and afferent denervation of the elbow flexors was assessed by musculocutaneous nerve immunohistochemistry. Biceps muscle volume and cross-sectional area were measured by microCT. Elbow flexion contractures were measured by a blinded observer using a validated digital photography technique. Biceps spindle and extrafusal fiber ErbB signaling pathway activity was assessed immunohistochemically. Muscle fiber morphology was assessed histologically using Mason’s trichrome.

Results: Post-operative degeneration of muscle spindles was confirmed by H & E staining of muscle following post-ganglionic global NBPI. We then confirmed the ability to selectively denervate the neuromuscular junction and preserve muscle spindle innervation by pre-ganglionic C5-7 NBPI in Thy1-
YFP mice by confocal scanning of whole biceps muscle. Using this technique of pre-ganglionic NBPI, we found that preservation of afferent innervation prevented contractures despite motor denervation. In the post-ganglionic injury model, ErbB signaling was lost in the degenerated spindles, as evidenced by loss of activated ErbB2 receptor staining and loss of staining for the downstream transcription factor, Egr3. Total ErbB2 receptor expression, however, was upregulated in both the muscle spindle and in extrafusal fibers, suggesting the loss of the ErbB receptor ligand, neuregulin-1. Conversely, following pre-ganglionic injury, activated ErbB2 receptor and Egr3 staining was preserved in the spindles, as was spindle morphology. Total ErbB receptor expression was upregulated in the extrafusal fibers as in the post-ganglionic NBPI, but to a lesser extent. Muscle fiber atrophy and interstitial fibrosis was present in both pre- and post-ganglionic NBPI, suggesting that these histologic findings are independent of afferent innervation and ErbB signaling.

**Discussion:** Pre-ganglionic NBPI preserves afferent innervation, which preserves spindle development, potentially through preservation of ErbB signaling. The role of ErbB signaling and afferent innervation in muscle growth remains unclear, however, as ErbB signaling perturbation was seen in extrafusal fibers despite absence of contractures following pre-ganglionic NBPI. Nonetheless, despite not yet providing a mechanistic link between afferent innervation and the prevention of contractures, we have identified a molecular pathway that can explain the preservation of spindles following pre-ganglionic injury. Pharmacologic manipulation of ErbB signaling in the much more common post-ganglionic NBPI may help mimic the protective effects of maintained afferent innervation seen following pre-ganglionic NBPI.

**Significance:** Understanding the cellular and molecular basis of contracture formation and impaired motor recovery following neonatal denervation will allow novel strategies to prevent contractures while the muscle awaits reinervation and improve muscle functional recovery following reinnervation. Furthermore, understanding the link between innervation and postnatal muscle development will have implications in a wide variety of childhood neuromuscular and musculoskeletal disorders.