Sonic Hedgehog Is Neuroprotective in the Cavernous Nerve with Crush Injury

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ABSTRACT

Introduction. The cavernous nerve (CN) is commonly injured during prostatectomy, resulting in erectile dysfunction (ED). Although peripheral nerves have a limited ability to regenerate, a return of function typically does not occur due to irreversible down stream morphological changes in the penis that result from CN injury. We have shown in previous studies that sonic hedgehog (SHH) is critical for CN regeneration and improves erectile function after crush injury.

Aims. Examine a new direction, to determine if SHH is neuroprotective to the pelvic ganglia (PG)/CN after crush injury. A secondary focus is to examine if SHH signaling decreases with age in the PG/CN.

Methods. Sprague–Dawley rats underwent bilateral CN crush and SHH and glial fibrillary acidic protein were quantified by western analysis of the PG/CN (N = 6 rats at each time point) at 1, 2, 4, 7, and 14 days, and the apoptotic index was measured in the penis. SHH was quantified by western in the PG/CN with blockade of anterograde transport (N = 4 rats) in comparison to mouse IgG (N = 4 rats). If SHH is neuroprotective was examined at 4 (N = 14 rats) and 7 days (N = 16 rats) of treatment after CN crush. SHH protein was quantified in aging (P200-300, N = 5 rats) PG/CN in comparison to normal adult (P115-120, N = 3 rats) PG/CN.

Main Outcome Measures. SHH pathway was examined in PG via immunohistochemistry, *in situ*, western, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

Results. SHH is neuroprotective in the PG/CN with injury. SHH localization in the PG/CN suggests SHH interaction in neuronal/glial signaling. SHH protein is significantly decreased in the PG/CN after crush injury and in the aged PG/CN. Signals from the PG are required to maintain SHH in the CN.

Conclusions. There is a window of opportunity immediately after nerve insult in which manipulation of SHH signaling in the nerve microenvironment can affect long-term regeneration outcome. **Angeloni N, Bond CW, Harrington D, Stupp S, and Podlasek CA. Sonic hedgehog is neuroprotective in the cavernous nerve with crush injury. J Sex Med 2013;10:1240–1250.**

Key Words. Sonic Hedgehog; Cavernous Nerve; Neuroprotective; Regeneration; Transport; Aging; Neuroprotective

Introduction

The cavernous nerve (CN) is a peripheral nerve that provides innervation to the penis. The CN commonly undergoes resection, crush, or tension injuries during prostatectomy, which results in erectile dysfunction (ED). Neuropathy of the CN also frequently occurs in diabetic patients as well as in aging patients, which results in ED. Our previous studies show that the secreted protein sonic hedgehog (SHH) is essential to maintain and regenerate the CN because: SHH is abundant in Schwann cells of the CN, SHH is

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necessary for maintenance of CN morphology, SHH inhibition causes demyelination and axonal degeneration of CN fibers, and SHH protein treatment promotes CN regeneration [1,2]. In this study, we propose to examine a new direction of research to determine if SHH is neuroprotective to the pelvic ganglia (PG)/CN in the first weeks after crush injury. A secondary hypothesis is that SHH signaling is decreased in the PG/CN with age, thus proposing a potential mechanism of how aging related ED may develop. A neuroprotective role for SHH is supported by observations in the literature that delivery of SHH to the facial nerve after axotomy promoted motor neuron survival for 3–5 days [3,4], *Shh* mRNA was elevated and SHH protein had prominent localization within the regenerating axons 24 hours after sciatic nerve crush [5,6], injured sciatic nerves had enhanced recovery in the presence of SHH protein [7], and *Shh* expression was significantly lower in mice with impaired Wallerian degeneration [8]. If SHH is neuroprotective in addition to promoting CN regeneration, as shown in past studies [2], is unknown, but a better understanding of SHH signaling in the PG/CN is critical to manipulate the nerve microenvironment to induce regeneration more quickly.

CN injury has a significant impact on quality of life of ED patients and their partners. ED affects 61% of men between the ages of 40–69, 77% of men over 70 [9] and has been shown to be an early warning sign for cardiovascular disease [10]. ED occurs in 16–82% of patients treated by prostatectomy [11], which results from injury to the CN. Tissues innervated by the damaged nerve have deteriorating function, morphological remodeling including induction of smooth muscle apoptosis [12] and fibrosis [13], which affect the responsiveness of penile smooth muscle. A recent study showed that only 36% of prostatectomy patients recover erectile function without intervention [14] and PDE5 inhibitors improve erectile function in only ~31% of prostatectomy patients [15]. Thus new therapies that address both the down stream morphological changes in the penis and the underlying cause of the dysfunction, injury to the CN, are needed. As is the case with other peripheral nerves, efforts to regenerate the CN have so far had limited success in animal models, and these treatments have not yet translated into clinical therapies. Since our previous studies show that SHH treatment is effective in promoting CN regeneration [2], a better understanding of SHH signaling in the PG/CN is critical for development of new treatments. In this study we examine if SHH is neuroprotective to the PG/CN in the first weeks after injury and if SHH signaling is decreased in the aged PG/CN, suggesting a potential mechanism of how aging related ED may develop.

Materials and Methods

Animals

Male Sprague–Dawley rats postnatal day 115–120 (P115–P120) and retired male Sprague–Dawley breeder rats (P200–P300) were obtained from Charles River (Wilmington, MA, USA).

Ethics Statement

All animals were cared for in accordance with institutional IACUC approval and the National Research Council publication *Guide for Care and Use of Laboratory Animals*.

In Vivo SHH Protein Delivery by Peptide Amphiphile (PA)

PAs were synthesized at the Northwestern Institute for BioNanotechnology in Medicine Chemistry Core Facility as described previously [16]. The PA used in this study had the structure (C_{16})-$V_2A_2E_2$-(NH_2). PA was prepared [2] by adding 20 mM $CaCl_2$ to a glass slide. To form the linear PA, 8 μL of 100 mM PA plus either 2.27 μg SHH or BSA (control) proteins were pipetted onto the slide.

CN Crush Time Course

PG/CN were exposed in rats. Microforceps (size 0.02 × 0.06 mm) were used to crush the CN bilaterally for 30 seconds [17–19]. Sham surgery was performed by exposing, but not crushing, the CN. The reproducibility of the crush injury was previously verified [2]. Rats were sacrificed 1, 2, 4, 7, and 14 days after surgery. Six PG/CN (from six rats) for each time point were divided into three groups and were homogenized for western analysis. Pooling of tissue was required for homogenization because of the small size of the PG/CN. Samples were run in duplicate and the results were averaged for each time point. Penis tissue was fixed in 4% paraformaldehyde and embedded in paraffin for TUNEL.

Interruption of Anterograde Transport in Vivo by Anti-Kinesin Treatment of the PG

Affi-Gel beads (100–200 mesh, Bio-Rad, Hercules, CA, USA) were equilibrated with mouse anti-kinesin (0.9 mg/mL, Sigma, St. Louis, MO, USA,