Increased Expression of the Neuroregenerative Peptide Galanin in the Major Pelvic Ganglion Following Cavernous Nerve Injury

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ABSTRACT

Introduction. Erectile dysfunction (ED) remains a frequent complication of radical prostatectomy due to injury to the cavernous nerves (CNs). A recent microarray showed the neuropeptide galanin to be one of the most strikingly upregulated genes in the rat major pelvic ganglion (MPG) after bilateral CN crush injury (BCNI).
Aim. The aim of this study is to evaluate the temporal regulation of galanin in the MPG after BCNI and its relationship to functional nerve regeneration.
Methods. Changes in galanin, galanin receptor (galR), and c-JUN mRNA expression were assessed in Sprague-Dawley rats after sham operation (n = 10) and at 48 hours (n = 10), 7 (n = 10), 14 (n = 5), 21 (n = 5), 30 (n = 5), and 60 (n = 5) days after BCNI using quantitative PCR. Erectile function was assessed by measuring intracavernous pressure (ICP) divided by mean arterial pressure (MAP) during CN electrostimulation. Immunohistochemistry was performed on the MPG in sham-operated animals and 5 days after BCNI.
Main Outcome Measures. ICP/MAP upon CN stimulation; galanin, galR1, -2, -3, and c-JUN mRNA expression at various time points after BCNI; and nNOS, galanin, and galR distribution in the MPG of sham-operated rats and after BCNI.
Results. After BCNI, ICP/MAP values quickly deteriorate, while after 60 days, spontaneous restoration of erectile responses to CN stimulation is observed, reflecting CN regeneration. Galanin mRNA in the MPG is up to 186-fold upregulated compared with sham-operated rats at 48 hours and 7 days after BCNI and gradually declines with increasing time from injury, whereas galanin receptor expressions decrease and c-JUN gradually increases. Galanin expression shows a strong inverse correlation with erectile responses to CN stimulation with time from injury. Injured MPGs show a colocalization between galanin- and nNOS-positive neuronal cell population in the MPG.
Conclusions. Galanin is upregulated in the MPG in the early phase after CN injury after which it gradually decreases and is present in nNOS-positive neurons of the ganglion. We hypothesize that galanin upregulation is an important factor in the endogenous neuroregenerative response to CN injury. **Weyne E, Albersen M, Hannan JL, Castiglione F, Hedlund P, Verbist G, De Ridder D, Bivalacqua TJ, and Van der Aa F. Increased expression of the neuroregenerative peptide galanin in the major pelvic ganglion following cavernous nerve injury. J Sex Med 2014;11:1685–1693.**

Key Words. Autonomic Nerve Regeneration; c-JUN; Galanin Receptor; Major Pelvic Ganglion; Neurogenic Erectile Dysfunction; Neuroregeneration; Neuronal Nitric Oxide Synthase; Parasympathetic; Radical Prostatectomy; Cavernous Nerve Injury

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Introduction

Patients undergoing radical prostatectomy with good baseline erectile function have high expectations concerning the preservation of erectile function following the procedure [1]. However, erectile dysfunction (ED) remains a frequent complication of radical prostatectomy due to injury to the cavernous nerves (CNs), which innervate the erectile tissue of the corpora cavernosa.

While various clinical studies have aimed at preserving the integrity of corpus cavernosum structure and function by the administration of phosphodiesterase 5-inhibitors, the functional outcome of these studies has been disappointing [2,3]. Therefore, researchers are interested in increasing CN regeneration in order to reduce the time the erectile tissue lacks innervation [4–7]. However, these attempts have been made with little background on the actual changes that occur in the major pelvic ganglion (MPG) and CNs after nerve injury. In order to develop appropriate therapies, it is helpful to discern the molecular pathways that are altered in the MPG and CN following neuropraxia and axonotmesis. In that perspective, a recent microarray was performed in order to identify important changes in mRNA expression in the rat MPG after CN injury. The neuropeptide galanin was one of the most strikingly upregulated genes. The three currently known G-protein coupled galanin-receptors (galR1,-2,-3) were not significantly altered as a result of injury [8]. While the microarray focused on two time points (48 hours and 14 days), we have added additional time points to investigate the temporal changes.

Galanin, a 29 amino acid peptide (named after the N-terminal glycine and C-terminal alanine), is a predominantly inhibitory neuropeptide which was first identified in 1983 in porcine intestinal extracts [9,10]. It has been shown to be a cotransmitter in erectile biology in cats, acting in synergy with NO-cGMP signaling [11]. The three receptors for the galanin family peptides are expressed in the central nerve system (CNS) and in sensory neurons in the dorsal root ganglia [12,13]. Little is known, however, on the expression and regulation of galanin and its receptors in the parasympathetic nervous system. Autonomic neurons commonly respond to axonal injury with increased expression of various neuropeptides. This increased peptide expression is believed to play a role in enhancing neuronal survival and nerve regeneration after injury [14]. As such, upregulation of galanin and other neuropeptides has been suggested to confer neuroregenerative and neuroprotective mechanisms [15]. Girard et al. recently confirmed the presence of galanin and its receptors in the MPG and elegantly showed that galanin was upregulated in the MPG after unilateral CN transection and MPG explantation [16]. However, these models do not reflect the neuropraxia which occurs during radical prostatectomy and are not suitable to investigate the link between galanin and in vivo neuroregeneration. In this study, we aim to evaluate temporal expression of galanin in the MPG after bilateral CN crush injury (BCNI) and its relationship to functional nerve regeneration. We hypothesized that galanin mRNA is significantly upregulated by the infliction of CN crush injury.

Materials and Methods

Experimental Animals

All animal studies were approved by the IACUC and the Ethical Committee for Animal Research of Johns Hopkins School of Medicine and University of Leuven. Adult male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 150–250 g (7–9 weeks of age) were used (n = 66). Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine/xylazine (100 + 10 mg/kg). The prostate was exposed via a midline abdominal incision. The CN and MPG were identified posterolateral to the prostate. In order to study the pathophysiology of CN injury on molecular signaling in the MPG, the following groups of experimental rats were utilized: (i) sham operation with exposure of bilateral CNs and no manipulation of the nerves in age-matched control rats; and (ii) exposure of bilateral CNs and associated nerve crush injury. Crush injury was induced as previously described by applying Dumont #5 forceps (Fine Science Tools, Foster City, CA, USA) to the nerve 2–3 mm distal to the MPG. The forceps were held to closure three times for 15 seconds each, causing a moderate crush injury, reflecting clinical neuropraxia or axonotmesis [8,17].

In Vivo Erectile Function Measurement

Rats were anesthetized with an intraperitoneal injection of ketamine/xylazine (100 + 10 mg/kg) and placed on a thermoregulated surgical table, and a standard in vivo experimental protocol was conducted (n = 6–8/group) with the needle for pressure recording placed at the site of the crus