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Galanin Administration Partially Restores Erectile Function After Cavernous Nerve Injury and Mediates Endogenous Nitrergic Nerve Outgrowth In Vitro

Emmanuel Weyne, MD,[1] Johanna L. Hannan, PhD,[2,3] Thomas Gevaert, MD, PhD,[4] Mohammad Ayodhia Soebadi, MD,[5,6] Hotaka Matsui, MD,[2,7] Fabio Castiglione, MD,[1,8] Koenraad van Renterghem, MD, PhD,[9] Dirk De Ridder, MD, PhD,[1] Frank Van der Aa, MD, PhD,[1] Trinity J. Bivalacqua, MD, PhD,[2] and Maarten Albersen, MD, PhD[1]

ABSTRACT

Background: Previously, we found that the neuropeptide galanin was strongly upregulated soon after bilateral cavernous nerve injury (BCNI) and that galanin and its receptors were expressed in nitrergic erectile innervation. Galanin has been observed to exert neuroregenerative effects in dorsal root ganglion neurons, but evidence for these effects in the major pelvic ganglion (MPG) after BCNI is lacking.

Aim: To evaluate the neurotropic effects of galanin receptor agonists and antagonists in vitro in nitrergic neurons and MPG and in vivo in rats after BCNI.

Methods: Male Sprague-Dawley rats underwent BCNI and sham surgery. Organ culture and single-cell neuron culture of the MPG were performed. Osmotic pump treatment with the galanin agonist in vivo and measurement of erectile response to electrostimulation after BCNI, immunohistochemical localization of galanin and receptors in the human neurovascular bundle, and myographic analysis of rat corpus cavernosum smooth muscle relaxation to galanin receptor agonists were investigated.

Outcomes: Neurite outgrowth in vitro and erectile response to electrostimulation after BCNI in vivo, immunohistochemical localization of galanin and receptors, and penile muscle relaxation in vitro.

Results: Galanin showed neurotrophic action in vitro and inhibition of endogenous galanin significantly impaired neurite outgrowth in nitrergic but not in sympathetic MPG neurons. In vivo administration of a selective galanin receptor-2 agonist, M1145, resulted in partial recovery of erectile function (EF) after BCNI. Galanin did not act as a direct vasodilator on corpus cavernosum muscle strips.

Clinical Translation: Endogenous neurotrophins such as galanin could be used as a strategy to improve EF for patients after BCNI from radical prostatectomy.

Strengths and Limitations: We evaluated the effect of galanin on nerve regeneration and EF recovery in vivo and in vitro. Limitations include the lack of washout period for the in vivo experiment and absence of differences in the expression of neuronal markers between treatment groups.

Conclusions: We identified galanin as a potential endogenous mechanism for nerve regeneration after BCNI, which could play a physiologic role in EF recovery after radical prostatectomy. In vivo treatment with exogenous galanin was beneficial in enhancing EF recovery after BCNI, but further research is necessary to understand the underlying mechanisms. **Weyne E, Hannan JL, Gevaert T, et al. Galanin Administration Partially Restores Erectile Function After Cavernous Nerve Injury and Mediates Endogenous Nitrergic Nerve Outgrowth In Vitro. J Sex Med 2018;15:480–491.**

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[1]Department of Urology, KU Leuven and University Hospitals, Leuven, Belgium;
[2]Brady Urological Institute, Johns Hopkins, Baltimore, MD, USA;
[3]Department of Pathology, KU Leuven and University Hospitals, Leuven, Belgium;
[4]Department of Physiology, East Carolina University, Greenville, NC, USA;
[5]Laboratory of Experimental Urology, Department of Development and Regeneration, KU Leuven, Leuven, Belgium;
[6]Department of Urology, Airlangga University School of Medicine, Dr Soetomo General Hospital, Surabaya, Indonesia;
[7]Department of Urology, University of Tokyo, Tokyo, Japan;
[8]University College of London, London, UK;
[9]Department of Urology, Jessa Hospital, Hasselt, Belgium

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INTRODUCTION

Erectile dysfunction (ED) remains a common sequela of radical prostatectomy (RP). Currently, younger and healthier patients are diagnosed with localized prostate cancer and have high expectations of regaining erectile function (EF) after surgery.[1] Despite the refinement of nerve-sparing techniques and technical advancement with the introduction of robotic surgery, ED ranges from 14% to 69% after RP.[2] ED occurs as a direct consequence of damage by traction, compression, and use of electrocautery in the neighborhood of cavernous nerves (CNs) located on the dorsolateral aspect of the prostate. This nerve injury will initiate a neurodegenerative cascade called Wallerian degeneration, resulting in temporary denervation of the corpus cavernosum. This denervation eventually leads to fibrosis and loss of cavernosal smooth muscle mass, leaving the patient with a severe form of ED that is difficult to treat.[3]

Currently no causal therapies exist to restore EF after RP. Current therapies, such as phosphodiesterase type 5 inhibitors, attempt to restore relaxation of the end organ, and penile rehabilitation with these agents have not shown conclusive results toward EF preservation and recovery.[4] The autonomic nervous system has a limited capacity to regenerate after nerve injury, which is mediated by the endogenous release of neurotropic factors stimulating the survival and outgrowth of neuronal cells.[5] It is of utmost importance to better understand the mechanism and neurotrophic factors that are involved in CN regeneration to find new treatments that can truly maximize EF recovery after RP. The exogenous administration of neurotrophic and growth factors in preclinical rat models has resulted in increased EF recovery after CN injury (CNI).[1]

In a previous study, we found that expression of the neurotrophin galanin was 180-fold upregulated in the major pelvic ganglion (MPG) of rats in the early phase after CNI. Furthermore, galanin and its 3 known receptors were located in neuronal nitric oxide synthase (nNOS)-positive neurons of the MPG, providing erectile innervation to the penis.[6] Galanin has 3 known G-protein−coupled receptors that are abundantly expressed in the central nervous system and dorsal root ganglion (DRG) of sensory neurons.[7,8] Galanin was found to act as a neurotrophic factor in peripheral nerves because galanin knockout mice showed increased long-term sensory and motor deficits after nerve injury that could be salvaged by the addition of galanin.[9] Neuroregenerative action of galanin is mediated by galanin receptor-2 (GalR2), which stimulates small GTPases to increase growth cone dynamics and neurite outgrowth in regenerating nerves.[10] The role of galanin and its neurotrophic potential in autonomic nerves such as the CN has not been investigated.

In this study, we investigated the neurotropic potential of galanin in CNI. In the 1st step, a whole mount culture of the MPG was used to evaluate the neurotrophic action of galanin in vitro. A dissociated nerve culture was used to investigate the in vitro differential effect on growth of sympathetic vs parasympathetic nerves. In a 2nd step, we evaluated whether systemic galanin administration through an osmotic pump could improve EF recovery after CNI in vivo. As a 3rd step, the translational potential of galanin and its receptors was evaluated by performing immunohistochemical analysis on human neurovascular bundle specimens obtained after RP. Attached as supplementary data, we investigated whether galanin has a direct relaxation effect on human penile muscle strips in vitro.

METHODS

Animals and Experimental Design

Male Sprague-Dawley rats (Strain RjHan:SD, Janvier, Le Genest-Saint-Isle, France) 10 to 12 weeks old were used in this study. All experiments were approved by the animal ethics committee of the Ku Leuven (P169-2013) and Johns Hopkins (RA13M60). Rats were housed in standard laboratory conditions with a 12-hour light-dark cycle with access to food pellets and tap water ad libitum. Rats underwent sham or bilateral CNI (BCNI) surgery as described previously.[6] Briefly, animals were anesthetized using 2% isoflurane, the prostate was exposed through a midline laparotomy, and the MPG and CN were identified bilaterally. In the BCNI groups, the 2 CNs were injured by crushing with forceps 3 times for 15 seconds each time 2 to 3 mm distal to the MPG.[5] Adequate crush was confirmed by an observable nerve decoloration, with the neurolemma remaining intact. In sham-operated animals, the CN was identified and the abdomen was closed. All BCNI surgeries were performed by the same surgeon.

Pharmacologic Compounds

For the different experiments, different galanin receptor agonists and antagonists were used (all from Tocris, IoTechne, Lille, France). M40 (non-selective) and M871 (selective for GalR2) served as antagonists. Rat galanin (non-selective) and M1145 (selective for GalR2) served as agonists. These compounds were dissolved and aliquoted in distilled water according to the manufacturer's instructions.

Whole Mount Culture of MPG

After sham or BCNI surgeries, MPGs were carefully dissected out at 48 hours after injury to be cultured in Matrigel (n = 4−5/group). Whole MPGs were excised and embedded on reduced growth factor Matrigel in serum-free medium (RPMI-1640 with 1% penicillin and streptomycin; Gibco, Grand Island, NY, USA) as described previously.[5] MPGs were covered with medium 1 mL with or without the addition of pharmacologic agents, which was changed every 24 hours and maintained at 37°C in a humidified atmosphere with 5% CO_2. Sham MPGs were treated with culture