Mechanism of action of sacral nerve stimulation using a transdermal amplitude-modulated signal in a spinal cord injury rodent model

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Cite as: *Can Urol Assoc J* 2012;6(4):227-30. http://dx.doi.org/10.5489/cuaj.11249

Abstract

Introduction: Sacral neuromodulation (SNM) is an effective treatment modality for several urological problems, including neurogenic bladder. However, the invasiveness of this technique makes it unsuitable for many patients. We present a novel transdermal amplitude-modulated signal (TAMS) that may provide a non-invasive alternative to implantable SNM to treat neurogenic detrusor overactivity (NDO).

Methods: In this study, we investigated the mechanism of action of non-invasive SNM using TAMS on our established spinal cord injury (SCI) animal model. We demonstrated that spinally transected rats develop urinary bladder hyper-reflexia after 3 weeks of SCI, indicated by the presence of uninhibited contractions, increased resting pressure, increased threshold pressure and increased maximum voiding pressure.

Results: Short-term neurostimulation affected urodynamics parameters by significantly reducing the threshold pressure ($p = 0.02$). Spinal transection also increased calcitonin gene-related protein (CGRP) concentration in the L6 dorsal root ganglia; whereas, neurostimulation significantly reduced CGRP concentration in L6 ($p = 0.03$).

Conclusion: TAMS caused a reduction in NDO by inhibiting C-fibre activity.

Introduction

Sacral neuromodulation (SNM) is a relatively new concept for the treatment of various lower urinary tract dysfunctions, yet it has gained wide acceptance especially in managing patients with conditions refractory to conventional methods. The U.S. Food and Drug Administration has approved SNM for three indications: urge incontinence, urge frequency and nonobstructive urinary retention.[1] The mechanism of action of SNM is still unknown. It has been suggested that SNM inhibits bladder afferent activities through its action on somatic afferent pathways and thereby blocks abnormal sensory input to the spinal cord and brain.[2] SNM has become more appealing because of simpler, minimally invasive implant techniques and shorter hospital stay.

We present a novel, transdermal amplitude-modulated signal (TAMS) that enables non-invasive stimulation of sacral nerves in a spinal cord injury (SCI) rodent model. The signal uses an amplitude-modulated waveform with a high frequency carrier, modulated by a low frequency envelope. The carrier waveform is designed to be of sufficient frequency to overcome skin and tissue impedance. The pulse envelope contains selective frequency, pulse width, amplitude and waveform shape designed to stimulate specific nerves. The efficacy of TAMS has already been demonstrated in a feline overactive bladder (OAB) model.[3,4] We examined TAMS as a treatment modality for neurogenic detrusor overactivity (NDO) caused by SCI. We also assessed the role of C-fibre inhibition by SNM as a possible mechanism of action.

Methods

In the present study, 28 female Sprague Dawley rats (250 g) were stratified into three groups (4 to 6 rats per group): (1) normal controls (C); (2) spinally transected at T10 (S); and (3) spinally transected and electrically stimulated bilaterally at S1 with TAMS for 2 hours before sacrifice (N). All protocols for the experiments were approved by the University Health Network, University of Toronto, Animal Care Committee, in accordance with the policies established in the Guide to Care and Use of Experimental Animals prepared by the Canadian Council on Animal Care.

Spinal cord transection

Spinal cord transection was performed.[5] Under general anesthesia using a combination of xylazine (5 mg/kg) and ketamine (50 mg/kg), rats underwent a midline incision

through the skin overlying the upper part of the thoracic spinal column and the eighth intervertebral space was identified using anatomical landmarks. After performing a limited laminectomy to the eighth thoracic vertebrae to expose the spinal cord, we used a sharp micro-scissor to perform a complete transection of the spinal cord under direct visual control and then aided by an operating stereomicroscope (Spencer, American Optical Company, NY). To ensure a complete transection of the spinal cord, we passed the tip of a 16-G needle several times around the inner surface of the exposed vertebra.

The care of spinalized rats and neurostimulation

The rats' body temperature was monitored and maintained during and after the procedure using a heating pad controlled by an electronic rectal body core thermometer. Supplementary subcutaneous lactated Ringer's solution (20 mL/kg body weight) was administered during transection and in the first 2 days afterward, and perioperative antibiotics in the form of Clavamox (amoxicillin/clavulanic acid) were added to water for 5 days postoperatively. The rats were kept in low-height cages for easy access to food and water in a 24 to 25°C warm room. The rats' bladders were evacuated by manual expression following the spinal cord transection 3 times per day. Electrodes (Noraxon Dual Electrodes, Scottsdale, AZ), with a diameter of 1 cm and an inter-electrode distance of 2 cm, were attached to anesthetised rats' bare skin around the area of S1 dorsally. Each rat in group N was electrically stimulated for 2 hours prior to sacrifice. A high frequency carrier waveform (210 kHz) amplitude-modulated by low frequency (10 Hz), monophasic rectangular pulses (1 ms pulse width), was utilized for stimulation (Fig. 1). The carrier waveform has minimal impedance for the flow of electric current through the skin.[6] Output from a custom-designed signal generator was used to stimulate the rats, and amplitude was adjusted to 80% of the value that produced tail movements.

Three weeks after spinal cord transection in groups S and N, filling cystometrogram (CMG) was conducted in 11 animals through a silicone tube implanted into the bladder one day earlier. The tube was tunnelled subcutaneously to emerge from the skin at the back of the neck. After expressing the bladder to empty it, the bladder was filled at 0.2 mL/min using an infusion pump. Several voiding cycles were recorded in each study, without expressing the bladder between cycles. The intravesicular pressure was recorded using a Grass polygraph and a pressure transducer. The endpoint of any micturition cycle was either spontaneous micturition in normal rats or leakage in spinally transected rats. The variables evaluated in normal animals were the resting bladder pressure, the threshold pressure and the maximum voiding pressure. In groups S and N, detrusor contractions

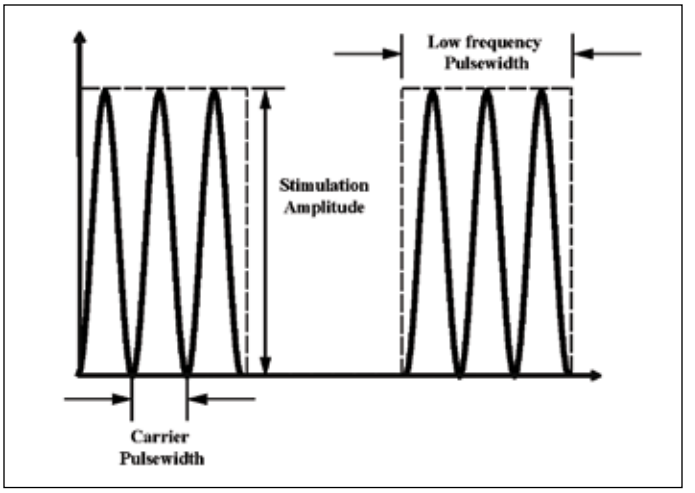

Fig. 1. Transdermal amplitude-modulated signal waveform: Dotted line (rectangular) represents the low frequency modulating waveform while the solid line (sine wave) represents the high frequency carrier waveform.

of ≥15 cm H2O were considered uninhibited. The degree of detrusor overactivity was evaluated in groups S and N as the number of uninhibited contraction until leakage and the volume that induced the first of these contractions.

Dorsal root ganglia-CGRP quantification

In separate groups of rats (n = 16), CGRP was extracted from the dorsal root ganglia (DRG) of the L5 and L6 roots and quantified by radioimmunoassay (RIA). L5 and L6 DRGs were dissected using ultrafine forceps within 30 min after sacrifice. They were frozen in liquid nitrogen and stored at -80°C until peptide extraction. Peptides were extracted from DRGs with an acid buffer containing 2M of acetic acid, 11.4 mM HCl, 1 mM disodium EDTA, 1 mM dithiothreitol, and 4% protease inhibitor cocktail (Sigma) to prevent degradation. Tissues were boiled in 500 μL extraction buffer for 15 minutes, sonicated on ice for 20 seconds and centrifuged at 3900 g in 4°C for 15 minutes. The supernatant was collected in a fresh Eppendorf tube and total protein content was determined with a bicinchoninic acid (BCA) assay (Pierce) for standardization. The remaining supernatant was stored at -80°C. Prior to the assay, samples were lyophilized for 24 hours. A commercial RIA kit (Phoenix Pharmaceuticals, Burlingame, CA) was used, according to the manufacturer's instructions, to quantify CGRP in samples. Univariate ANOVA was used to analyse all data and the statistical significance was set at $p < 0.05$.

Results

Complete transection of the spinal cord at T8-9 segments resulted in total flaccid paralysis of the lower limbs accompanied by bladder areflexia. We also observed that the rats

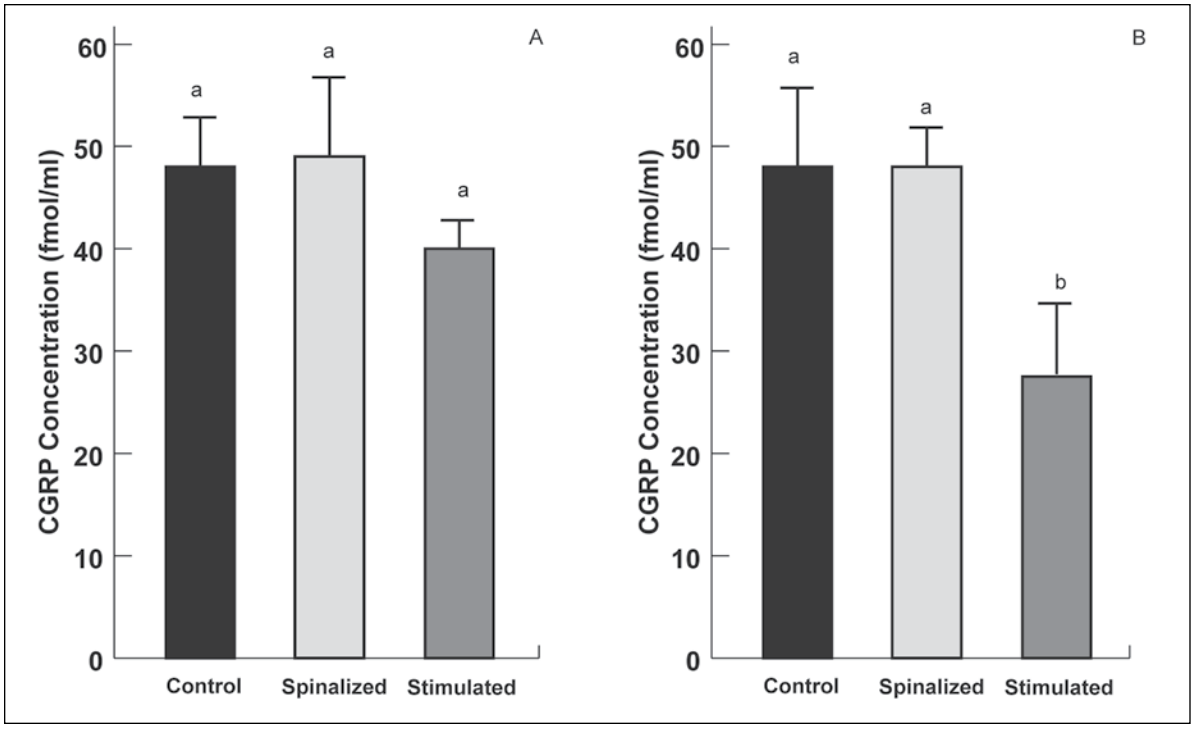

Fig. 2. Calcitonin gene related protein (CGRP) content ± SD in L5 and L6 DRG of Sprague Dawley rats. A. CGRP content in L5 DRG. B. CGRP content in L6 DRG. Means followed by the same letter within each ganglion are not significantly different ($p < 0.05$) according to Benforonni posthoc analysis.

started to regain bladder contractility at about 10 days after the surgery as evidenced by smaller evacuated volumes of urine during regular daily bladder squeezing. Furthermore, a small improvement in locomotor function was noted by day 14 in most rats, as they were able to move their hips and knee joints. We, however, did not appreciate any weight bearing ability in rats during the 3-week study period. Mortality rate ranged from 0% to 20% among study groups. The main reasons for death were shock following spinal cord transection and acute renal failure triggered by dehydration and urinary tract infection.

Spinally transected rats developed urinary bladder hyperreflexia after 3 weeks, indicated by the presence of uninhibited contractions, increased resting pressure, increased threshold pressure and increased maximum voiding pressure. Short-term neurostimulation affected urodynamics by significantly reducing the threshold pressure ($p = 0.02$), but not the other parameters (Table 1).

Spinal transection increased CGRP concentration in the L6 DRG compared with the control, while neurostimulation significantly reduced CGRP concentration in L6 ($p = 0.03$) (Fig. 2).

Discussion

SCI remains a significant cause for morbidity and mortality in North America.[7] As medical protocols have significantly improved, patients are expected to live longer, therefore quality of life has become very important for SCI patients.[8] SNM has recently gained wide acceptance as a treatment modality for bladder overactivity, including those caused by neurologic causes such as SCI.[9] Even if its mechanism of action is still unknown, SNM inhibits the emergence of new reflex circuits mediated by C-fibre afferents, as recent evidence indicates.[2] Normally, C-fibres are quiescent and unresponsive to bladder distension but, as a result of several neurologic and inflammatory disorders, these fibres evolve to respond to bladder distension and hence activate void-

Table 1. Mean bladder pressure ± SD parameters of Sprague Dawley rats

Group	Resting pressure (cmH2O)	Threshold pressure (cmH2O)	Maximum voiding pressure (cmH2O)	No. uninhibited contractions
Control (n=4)	3.8±0.6*a*	14.6±2.4*a*	26.2±2.1*a*	0*a*
Spinalized (n=4)	9.2±1.2*b*↑	43.8±6.5*b*↑	56.5±3.3*b*↑	5.8±2.6*b*↑
Neurostimulated (n=4)	8.6±0.7*b*↑	30.5±2.9*c*↑ ($p = 0.02$)	54.3±6.2*b*↑	4.6±3.1*b*↑

Means in a column followed by the same letter are not significantly different ($p < 0.05$).

ing reflexes. SNM is thought to block C-fibre activities and hence inhibit abnormal voiding reflexes.[2]

Most experimental studies of SCI have centered on using the rat as an experimental model. Rats have several advantages over larger animals because they provide inexpensive and reliable methods to characterize complex clinical problems. For instance, in our laboratory, we characterized the lower urinary tract dysfunction following spinal cord transection at T8 in rats.[10] We found that the signs of uncoordinated ineffective voiding after spinal cord transection in the rat resemble the clinical situation observed in patients following SCI. The measurement of intravesical pressure by a catheter inserted through the dome of the bladder is standard. Several disadvantages are associated with this method, including direct manipulation and potential irritation of the bladder wall and also a possible limitation of bladder movement during filling. Some studies have demonstrated that the bladder becomes irritated 1 to 3 days after implantation of the catheter.[11] However, during the first day after catheter implantation, this irritative change in bladder activity is not evident.[12,13] In this study, we inserted the catheter only a few hours before the CMG assessment to minimize the possibility of bladder irritation.

CGRP, a neuropeptide, is present exclusively in the neuronal bodies of the C-fibre afferents and also known to be inhibited by the neurotoxin capsaicin.[14] Hence, CGRP content of neuronal bodies, including the DRG, can reflect the activity of the C-fibre afferents. Furthermore, several studies have shown that the arbours of small-diameter primary C-fibre afferents can enlarge greatly in rats and mice after SCI potentially leading to increased reflex excitation of preganglionic neurons, via interneuronal pathways.[15] These afferent neurons are CGRP-immunoreactive, and are believed to mediate the spinal reflex pathways to the sympathetic preganglionic neurons, and hence play a significant role in the pathogenesis of NDO.[11,15]

Conclusion

In this study, we demonstrated that non-invasive sacral nerve stimulation using TAMS reduced CGRP content at L6 DRG which may therefore explain the modulatory effect on the C- fibre afferents supplying the urinary bladder. This CGRP reduction could be due to reduced expression caused by SNM. Even if acute stimulation did not show a significant change in all urodynamic parameters, the CGRP reduction at L6 after acute SNM, in addition to the results of previous studies conducted on invasive SNM, may suggest that chronic stimulation is required to produce significant changes in all CMG parameters.

Acknowledgements: This study was supported by Ethicon Endo-Surgery, Inc., a Johnson & Johnson company. Financial assistance (Postdoctoral Fellowship in the Area of Incontinence) was provided to Dr. Elkelini through a partnership program of the Canadian Foundation for Research on Incontinence and the Canadian Institutes of Health Research.

Competing interests: None declared.

This paper has been peer-reviewed.

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