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TITLE: Use of Optical Mapping to Evaluate Mechanisms and New Therapies for Bladder Dysfunction
Due to Spinal Cord Injury

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14. ABSTRACT There are ~300,000 individuals in the United States with spinal cord injury (SCI), where ~22% are veterans [1,2]. While their quality of life is significantly affected by lower urinary tract symptoms (LUTS), most treatments are palliative or ineffective. We focused on the therapeutic benefits of β_3 adrenoceptor agonists, botulinum neurotoxin type A (BTX-A) intradetrusor injections and their combination. BTX-A inhibits neurotransmitter release from nerve terminals. This has therapeutic effects on bladder dysfunction by inhibiting parasympathetic nerves to decrease reflex contractions, and afferent nerves, to reduce sensory symptoms. However, by also inhibiting sympathetic nerves, BTX-A decreases norepinephrine release and stimulation of detrusor β_3 adrenoceptors thereby decreasing relaxation and bladder compliance. Accordingly, we assessed the effects of BTX-A and β_3 adrenoceptor agonists in combination. In control mice, β_3 agonists had little effect as β_3 adrenoceptors are not normally expressed in mice. In SCI mice, β_3 agonists were beneficial by abolishing intrinsic bladder contractions and enhancing bladder compliance suggesting that β_3 adrenoceptors are upregulated in pathology. In BTX-A treated SCI mice, β_3 agonists significantly improved bladder compliance compromised by the toxin. Thus, β_3 adrenoceptor agonists in combination with BTX-A are beneficial in improving bladder function in SCI patients.					
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INTRODUCTIONS

There are an estimated 250,000 to 400,000 individuals in the United States with spinal cord injury/disease, where 22% are military veterans [1, 2]. From this subset of individuals, approximately 40% would have received the injury during active service. While the quality of life for these individuals is considerably affected by lower urinary tract dysfunction, most treatments are only palliative and so there is a significant need for further research to develop improved treatments for these patients. Accordingly, our studies are designed to further our understanding of lower urinary tract complications associated with acute and chronic spinal cord injury, with the overriding goal being to improve the quality of life for these patients through improved treatment methods.

Spinal cord injury can have significant consequences on lower urinary tract function. In upper lesions (above thoracic level, T12), the sacral micturition center and sacral reflex arc remain intact and continue to function, but are disconnected from supraspinal control centers. This can result in detrusor-sphincter-dyssynergia (DSD) where patients are unable to void efficiently due to discoordination of the contraction of the bladder and relaxation of the urethral sphincters. This can cause urinary retention, hypertrophy and damage to the bladder and kidneys. However, there is partial recovery of bladder voiding function after spinal cord injury (SCI) which has been attributed to remodeling of the neural connections within the spinal cord [3].

β_3 -adrenoceptors have shown promise as therapeutic targets for treating bladder overactivity. They are highly expressed throughout the human bladder and their activation relaxes detrusor smooth muscle [4, 5]. While these receptors are thought to be the main target of β_3 agonist therapy for bladder overactivity [6, 7, 8], the effects of their stimulation on bladder sensory function have yet to be fully elucidated.

BTX-A has therapeutic effects on bladder dysfunction by inhibiting acetylcholine release from parasympathetic nerves. This decreases reflex contractions and inhibits neuropeptide release from afferent nerves which reduces sensory symptoms. However, BTX-A inhibition of sympathetic nerves may decrease norepinephrine release and stimulation of detrusor β_3 adrenoceptors in humans to adversely affect bladder compliance.

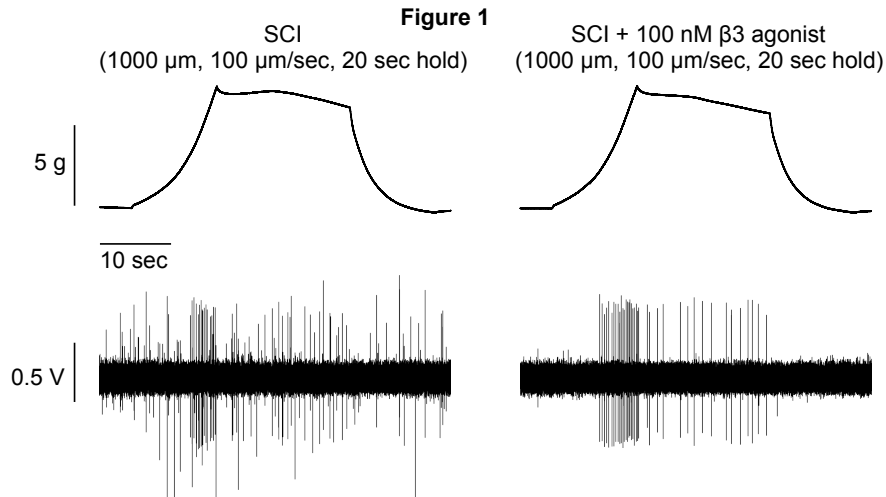
BODY

In these studies, we evaluated the effect of a β_3 adrenoceptor agonist (BRL-37344) on urinary bladder function and afferent nerve activity in control and spinal cord injured (SCI, T8-T9) mice. We have also assessed the effect of BTX-A and β_3 adrenoceptor agonist combination therapy.

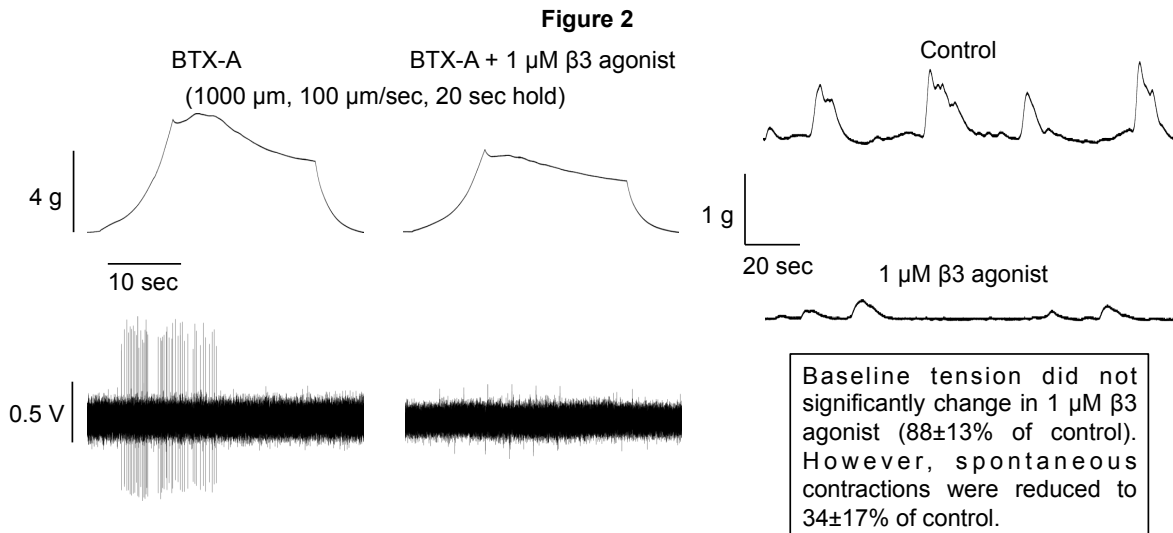
Adult female C57Bl/10 mice were used for *in vivo* decerebrate cystometry and *in vitro* bladder-pelvic spinal nerve (L6-S1) recordings. For nerve recordings, bladders were connected to a tension transducer and spinal nerves were passed into adjacent oil recording chambers. The bladders were stretched *via* a computer-controlled stepper motor to evoke mechanosensitive firing. The β_3 agonist (10nM – 1 μ M) was added to the perfusate. For cystometry, the β_3 agonist or the β_3 antagonist (L-748,337) were given IP at 0.5 mg/kg. All the studies were done in the presence of 100nM propranolol to block β_1 and β_2 adrenoceptors.

BTX-A was injected (2 units) *in vivo* into mouse bladder walls. After 48 hours, the animals were used for cystometry or their bladders were excised for nerve recordings. When injected IP, 1 unit of BTX-A is, by definition, the LD₅₀ in mice. However, when injected into the bladder wall, 2 units are not lethal but decrease nerve mediated contractions by 70%.

In control mice, addition of β_3 agonist did not alter stretch-evoked afferent activity (not shown). However, SCI mice exhibit large amplitude spontaneous detrusor contractions and afferent firing not seen in controls. The spontaneous activity was eliminated by β_3 agonist while stretch-evoked firing was not affected (**Figure 1**).

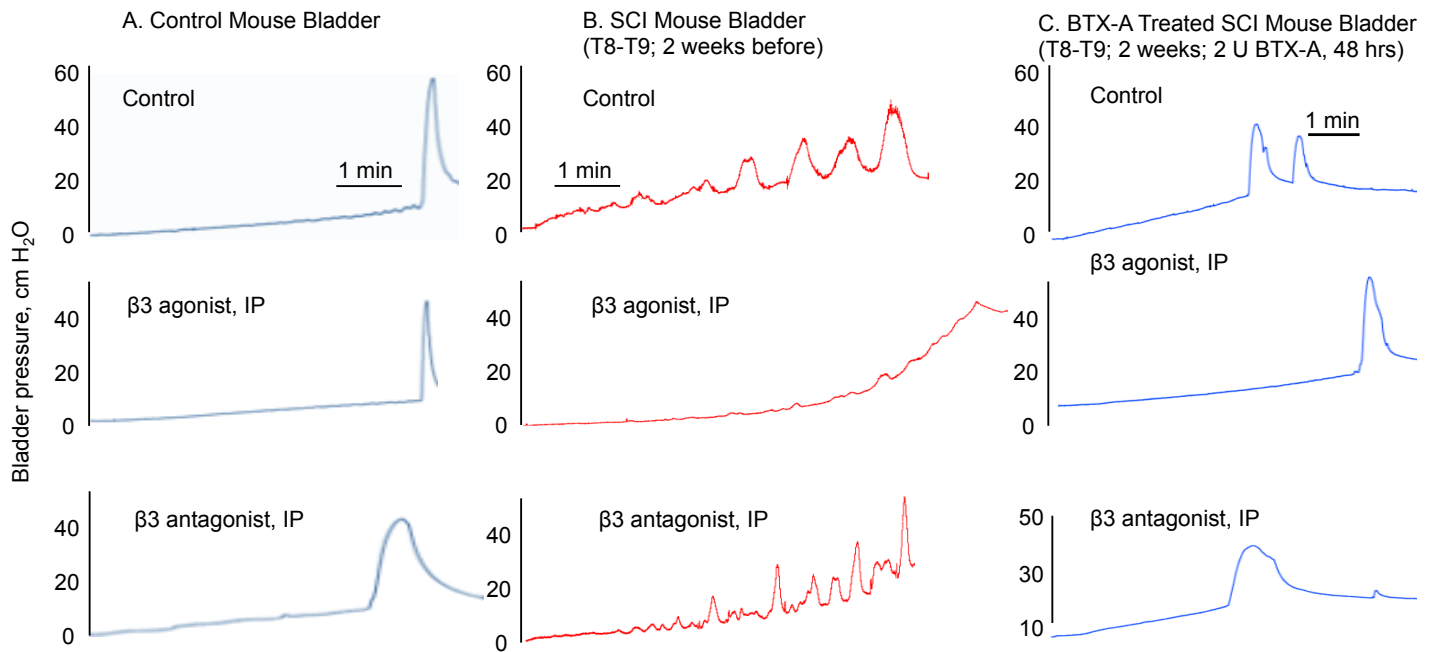


The efficacy of β_3 agonist was enhanced in SCI rodents treated with BTX-A (**Figure 2**).



In cystometric studies of the bladders of untreated control mice, the β_3 agonist was without effect (**Figure 3A**). However, in SCI mice, β_3 adrenoceptor agonist was beneficial by abolishing intrinsic bladder contractions and increasing compliance (**Figure 3B**). In BTX-A treated SCI mice, β_3 agonist dramatically improved bladder compliance which was decreased by the toxin (**Figure 3C**).

Figure 3



The results in figure 3 suggest that β_3 adrenoceptors do not have a significant role in normal mouse bladders and that bladder relaxation in these animals may be mediated by the β_2 or β_1 receptor subtype. Following SCI, however, our data suggest that β_3 receptors are upregulated. While the β_3 subtype predominates over β_1 and β_2 in human bladders, it may also be upregulated in pathology thereby increasing the efficacy of β_3 agonists. Moreover, in SCI patients treated with BTX-A, combination with a β_3 adrenoceptor agonist may be beneficial by improving bladder function.

KEY RESEARCH ACCOMPLISHMENT

- β 3 adrenoceptor agonists inhibit nociceptive but not stretch-sensitive afferent nerves. This should decrease painful nociception without adversely affecting stretch-evoked micturition.
- BTX-A inhibits nociceptive and stretch-sensitive afferent nerves. While this will decrease painful sensation, given that BTX-A also inhibits parasympathic nerves, this may inhibit bladder contraction requiring catheterization.
- β 3 adrenoceptor agonists inhibit spontaneous bladder contractions which may also decrease nociception.
- β 3 adrenoceptors are normally absent in mouse bladders but upregulated following SCI. While these receptors are present in human bladders, they may also be upregulated in pathology making β 3 adrenoceptor agonists more efficacious.
- β 3 adrenoceptor agonists increase bladder compliance while BTX-A decreases it. Accordingly, in cases where BTX-A is used, combination with a β 3 adrenoceptor agonist may be therapeutically beneficial.

REPORTABLE OUTCOMES

Presentations at the International Consultation on Incontinence – Research Society (ICI-RS) meeting:

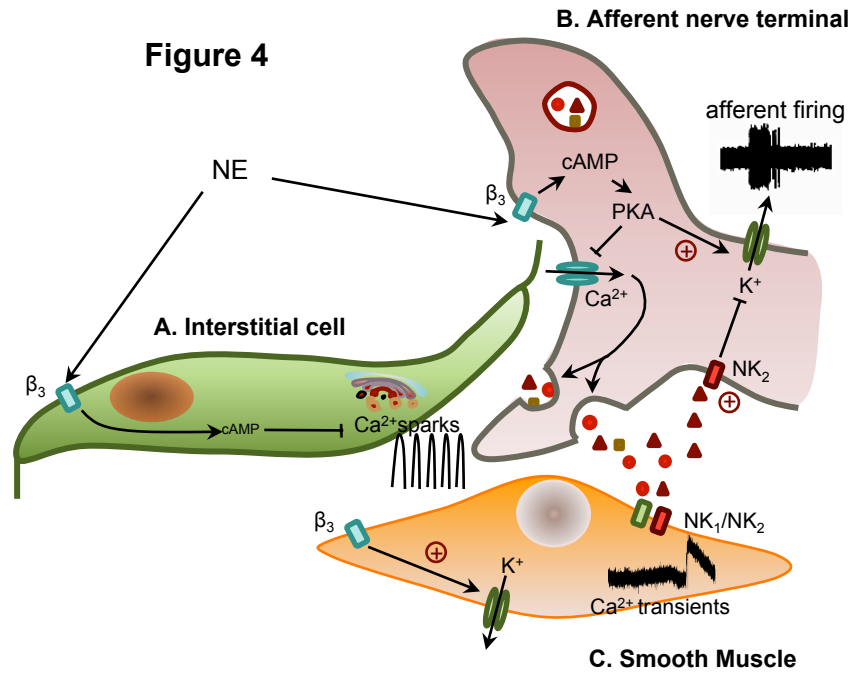
1. Kanai AJ, Ikeda Y, Hanna-Mitchel A. Do we understand any more about LUT interstitial cells?
2. Zabbarova IV, Gajewski J. Does our limited knowledge of the mechanisms of neuromodulation limit its benefits for patients

Manuscripts:

1. Y. Ikeda, I.V. Zabbarova, L.A. Birder, W.C. de Groat, C.J. McCarthy, A.T. Hanna-Mitchell, A.J. Kanai. Botulinum neurotoxin serotype A suppresses neurotransmitter release from afferent as well as efferent nerves in the urinary bladder. Eur. Urol. Mar 23, (2012).

CONCLUSION

Our results demonstrate that β_3 adrenoceptor agonists increase bladder compliance while BTX-A decreases it. Accordingly, in cases where BTX-A is used, combination with a β_3 agonist may be therapeutically beneficial. The putative sites and mechanisms of action of these receptors in the LUT are shown in figure 4 below.



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APPENDICIES

<http://www.ncbi.nlm.nih.gov/pubmed/22480459>