INVESTIGATIVE UROLOGY

Effect of botulinum toxin A on the autonomic nervous system of the rat lower urinary tract

Smith CP. Franks ME, McNeil BK, Ghosh R, De Groat WC, Chancellor MB,
Somogyi GT
From the Departments of Urology and Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania
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Purpose: The magnitude and duration of the effects of botulinum toxin A on acetylcholine (ACh) and norepinephrine release from the bladder and urethra of rats were measured using a radiochemical method.

Materials and Methods: Saline (sham treatment) or botulinum toxin A was injected into the bladder (50 µl.) or urethra (30 µl.) in separate groups of animals. The release of ^{3}H-norepinephrine or ^{14}C-choline was measured at 2 time points after injection (5 or 30 days).

Results: The fractional release of ACh in botulinum toxin A treated animals was significantly inhibited at higher frequencies of electrical field stimulation (20 Hz.) but not at lower frequencies (2 Hz.) 5 days after

injection. However, ACh release recovered to sham injected values 30 days after toxin injection. No significant differences in the fractional release of norepinephrine from sham injected or botulinum toxin A bladders were observed. In contrast, norepinephrine release from the urethra was inhibited by botulinum toxin A for at least 30 days after injection. Similar to its effect on transmitter release in the bladder, botulinum toxin A inhibited norepinephrine release in the urethra at high (20 Hz.) but not at low (4 Hz.) electrical stimulation frequencies.

Conclusions: These data indicate that the clinical effects of botulinum toxin A on the lower urinary tract may vary depending on the site of injection and level of nerve activity.

Editorial Comment

Since its introduction into clinical use in the 1980's, botulinum toxin A (BTX-A) has been successfully used to treat various conditions including blepharospasm, strabismus, focal dystonias, muscle spasms and spasticity, axillary hyperhidrosis, and achalasia. Urological applications of BTX-A have been primarily associated with cases of detrusor external sphincter dyssynergia (DESD), as a viable option for patients that are not capable of performing clean intermittent catheterization (1).

In addition to classic neuropathic DESD, the urological indications for use of BTX-A have been expanded to include patients with a variety of bladder outlet obstructions. BTX-A was successfully used to treat voiding dysfunction in multiple sclerosis patients with DESD, patients with pelvic floor spasticity, and even in an acontractile multiple sclerosis patient who wished to void by Valsalva (2). Recently, it was reported a case of functional urethral obstruction and detrusor acontractility following pubovaginal sling surgery that was successfully treated by BTX-A urethral sphincter injection (3).

The authors are pioneers in the field, and the present work represents the expansion and in deep presentation of a previous abstract, on which the same group of researchers demonstrated that the clinical success of BTX-A is supported by laboratory research (4). The work demonstrated marked decreases in the release of labeled norepinephrine and acetylcholine in BoNT/A (laboratory grade botulinum toxin) injected rat urethral sphincters (4). While the therapeutic effect of inhibiting acetylcholine release is obvious, blockage of norepinephrine release may provide clinical benefit by inhibiting sympathetic transmission and smooth muscle dyssynergia (1).

In the present work, saline or BTX-A was injected into the bladder or urethra in separate groups of female Sprague-Dawley rats. The release of ^{3}H-norepinephrine or ^{14}C-choline was measured at 2 different times after injection (5 or 30 days). The results indicate that BTX-A injected into the bladder and urethra at different times before the experiment could depress the release of neurotransmitters in a frequency and time dependent manner. In the bladder ACh release was depressed 5 days after treatment but it recovered at 30 days. On the other hand, BTX-A depression of norepinephrine release in the urethra was delayed in onset but lasted at least 30 days, indicating that the mechanism of action or diffusion of BTX-A through the tissue is different in the bladder and urethra.

References

1. Smith CP, Somogy GT, Chancellor MB: Botulinum toxin treatment of urethral and bladder dysfunction. Int Braz J Urol. 2002, 28: 545-52.
2. Phelan MW, Franks M, Somogyi GT, Yokoyama T, Fraser MO, Lavelle JP, Yoshimura N, Chancellor MB: Botulinum toxin urethral sphincter injection to restore bladder emptying in men and women with voiding dysfunction. J Urol. 2001, 165: 1107-10.
3. Smith CP, O'Leary M, Erickson J, Somogyi GT, Chancellor MB: Botulinum toxin urethral sphincter injection resolves urinary retention after pubovaginal sling operation. Int Urogynecol J Pelvic Floor Dysfunct. 2002, 13: 185-6.

4. McNeil BK, Smith CP, Franks ME, Ghosh R, de Groat WC, Chancellor MB, Somogyi GT: Effect of botulinum toxin A on urethral neurotransmitter release: Implications on somatic/autonomic nerve transmission. J Urol. 2001, 165: 277 (Abst).

Dr. Francisco J.B. Sampaio
Chairman, Urogenital Research Unit
State University of Rio de Janeiro
Rio de Janeiro, Brazil

Nitric oxide synthase in the external urethral sphincter of the sheep: immunohistochemical and functional study

González-Soriano J, Martín-Palacios S, Rodríguez-Veiga E, Triguero D, Costa G, Garcia-Pascual A
From the Departments of Anatomy and Physiology, Veterinary School, Complutense University, Madrid, Spain

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Purpose: We studied the distribution of neuronal nitric oxide synthase (nNOS) and the effects of nitric oxide (NO) modulating drugs on contractile function of the external urethral sphincter of lambs. Gender differences were evaluated.

Materials and Methods: Longitudinal and transverse sections of the external urethral sphincter from 10 female and 10 male lambs were studied using reduced nicotinamide adenine dinucleotide phosphate-diaphorase histochemistry and nNOS immunocytochemistry. Isometric contractile responses to electrical field stimulation were recorded from external urethral sphincter preparations from 47 female and 45 male lambs and the effects of NO modulating drugs were evaluated.

Results: We detected nNOS in the sarcolemma of some but not all striated fibers, where nNOS seems to be concentrated at the neuromuscular junction. In addition, nNOS was present in nerve fibers and intramural ganglia. The density of innervation decreased toward the distal part of the external urethral sphincter and was higher in male preparations. No significant functional effects of the NOS inhibitor N^G-nitro-L-arginine (10 mM.) or the NO donors diethylamine and spermine NONOate (Sigma Chemical Co., St. Louis, Missouri) (5 mM. each) on external urethral sphincter isometric contractility were found in either gender.

Conclusions: Despite the evidence for nNOS at the sarcolemma and nerve fibers of the external urethral sphincter the physiological relevance of these immunohistochemical findings remains to be determined.

Editorial Comment

The authors used sheep for studying because from an anatomical standpoint, these animals present a very clear distinction between the smooth and striated components of the external urethral sphincter. In this elegant study, histological and immunohistochemical techniques were combined with analysis on the effects of nitric oxide modulating drugs on external urethral sphincter contractile function in males and females.

Under light microscopy, it was identified the external urethral sphincter in the mid and caudal third of the urethra, both in males and females. No important morphological differences were identified between genders.

Staining for nNOS immunoreactivity demonstrated that the sheep external urethral sphincter is densely innervated by nNOS containing nerve fibers, which also occurred in the intramural ganglia, nerve trunks and

even in the sarcolemma of striated fibers. These observations provide the morphological basis for a functional role of endogenous production of NO in the external urethral sphincter, as a neurotransmitter or as a neuromodulator.

Nevertheless, even with these morphological evidences for the presence of nNOS in the external urethral sphincter, the authors did not observe any effect on in vitro contractility of the external urethral sphincter after NOS inhibition by N^G-nitro-L-arginine. Therefore, the physiological relevance of these findings remains to be determined.

Dr. Francisco J.B. Sampaio
Chairman, Urogenital Research Unit
State University of Rio de Janeiro
Rio de Janeiro, Brazil