519

Su X[1], Nickles A[1], Nelson D[1]
1. *Medtronic, Inc*

EVALUATION OF INTRADETRUSOR INJECTION OF BOTULINUM TOXIN A ON SPINAL NEUROMODULATION IN THE RAT

Hypothesis / aims of study

Botulinum toxin A (BoNT-A) and sacral neuromodulation are both used to treat patients with overactive bladder. However potential interactions between the two therapies have not been explored. Using the rat model of bladder rhythmic contraction (BRC) we have evaluated the bladder inhibitory effects of spinal nerve (SN) stimulation following intradetrusor injection of BoNT-A.

Study design, materials and methods

Female rats were anesthetized with 3% isoflurane. The urinary bladder was exposed via a small suprapubic incision under sterile conditions. BoNT-A (2 units, 0.2 ml) or saline were injected into the detrusor at 12 to 20 sites per rat. The rectus fascia and the skin were closed. Rats then were housed for 2 days, 1 or 2 weeks, or 1 month before neuromodulation study under urethane anesthesia (i.p. 1.2g/kg). Monopolar electrodes were placed under each of the L6 SN bilaterally. A bladder cannula was inserted via the urethra for saline infusion and intravesical pressure recording. The bladder was initially emptied via the bladder catheter and manual compression. Saline infusion (3 ml/hr) induced BRC. Urodynamic parameters including bladder capacity, bladder compliance (bladder capacity divided by threshold minus basal pressure), threshold pressure, amplitude and frequency of BRC were measured. Biphasic pulses (pulse width 0.1 ms) of different intensities at 0.8 fold of motor threshold intensity (0.8 x T_{mot}), T_{mot} or 2 x T_{mot} were applied at a frequency of 10 Hz to stimulate the SN.

Results

BoNT-A treatment alone caused some urodyanmic changes. The bladder capacity in BoNT-A treated rats was first compared with that in saline treated rats. Figure 1A shows raw traces of intravesical pressure recording in rats following either saline or BoNT-A injection (1 month). Time required for inducing the BRC (capacity) in BoNT-A pretreated rat was longer than that in saline pretreated rat. Summarized graph (figure 1B) demonstrates that intradetrusor injection of BoNT-A for 1-2 weeks or 1 month significantly increased bladder capacity (*, p<0.05, unpaired Student's t-test), with a trend towards larger compliance (p=0.13 and p=0.08, respectively, figure 1C) in comparison to saline injection.

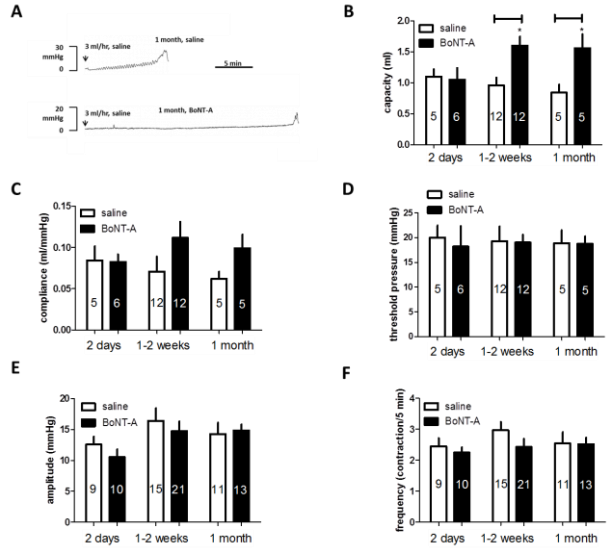

Figure 1. Urodynamic function in rats pretreated with botulinum toxin A (BoNT-A) or saline. **A.** Raw traces of intravesical pressure recordings using intravesical saline infusion 1 month following saline or BoNT-A injections. **B**-**F**. Summary of urodynamic parameters following intradetrusor injection of saline or BoNT-A. The number of animals for each condition tested is indicated in each bar. *p<0.05, Student's t-test.

The values for threshold pressure (figure 1D), amplitude of BRC (figure 1E) and frequency of BRC (figure 1F) in rats pretreated with BoNT-A were not significantly different than the values in rats pretreated with saline (p>0.05, Student's t-test).

Using the BRC we were not able to identify any significant differences in responsiveness to electrical stimulation in animals pretreated with either saline or BoNT-A treatment. Electrical stimulation of the SN attenuated the frequency of bladder contractions, either eliminating bladder contractions or reducing the contraction frequency during electrical stimulation. Figure 2 summarizes the mean responses to different intensities of SN stimulation in saline or BoNT-A treated rats. Student's t test demonstrates that a significant difference of BRC frequency by SN stimulation is produced following either BoNT-A or saline injections (figures 2A, 2C and 2E, * p<0.05, vs time control when stimulation was not applied, - Stim). Two days, 1-2 weeks and 1 month post BoNT-A injection, SN stimulations at T_{mot},10 Hz decreased the frequency of contractions to 70.18 ± 25%, 52.14 ± 13%, and 51.94 ± 12% of controls (vs. 98.77 ± 5% without stimulation), respectively. Using the same stimulation parameters, SN stimulations decreased the frequency of contractions in saline pretreated rats, to 50.32 ± 12%, 55.93 ± 15%, and 45.45 ±

10% of controls (vs. 114.16 ± 8% without stimulation), respectively. Inhibition of the contraction frequency in BoNT-A treated rats was not different from that in saline treated rats ($p>0.05$).
Consistently, SN stimulation at T_{mot} intensity elicited relatively weak inhibition on the amplitude of the bladder contractions. Figures 2B, 2D and 2F summarize effects of SN stimulation on amplitude of BRC.

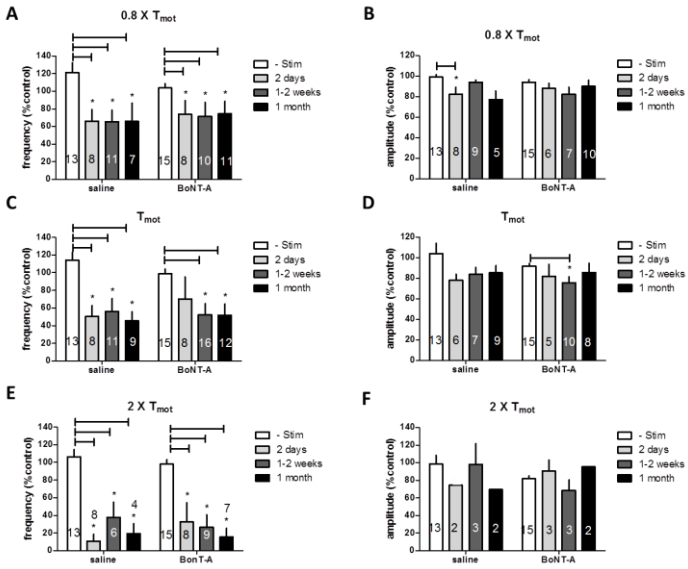

Figure 2. Effects of spinal nerve stimulation on the frequency and amplitude of the bladder rhythmic contraction. The number of animals is indicated either in or above each bar. Responses are represented as a percentage of control (% control), where the baseline response before stimulation is defined as 100%. *p<0.05, Student's t-test.

Interpretation of results

Spinal nerve stimulation attenuated the frequency of BRC in both saline and BoNT-A treated animals. Inhibitory effects of neuromodulation on the BRC is a consequence of an increased bladder capacity (compliance) and is thought to be mediated through the afferent limb of the micturition arc at lower intensities of stimulation and perhaps through the efferent limb with higher intensity stimulation (1). BoNT-A is thought to increase the bladder capacity at least in part through actions at peripheral nerve endings. However BoNT-A does not appear to alter the ability of SN stimulation to inhibit BRC in this model.

Concluding message

Intradetrusor injection of BoNT-A does not change the efficacy of bladder inhibitory response to acute spinal nerve stimulation in the rat. These results support further pre-clinical and clinical studies to evaluate potential interactions or combination therapy with neuromodulation and intra-detrusor BoNT-A therapeutic approaches.

References

1. Su X, Nickles A, Nelson DE. Neuromodulation in a rat model of bladder micturition reflex. American Journal of Physiology-Renal Physiology, 302: F477-F486, 2012.

Disclosures

Funding: Medtronic Inc. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** The Institutional Animal Care and Use Committee of Medtronic and Non-clinical Research Board of Medtronic (Minneapolis, MN).