

## THE EFFECTS OF BOTULINUM NEUROTOXIN-A ON L-TYPE AND T-TYPE VOLTAGE-GATED CA<sup>2+</sup> CURRENT IN DETRUSOR SMOOTH MUSCLES

### Hypothesis / aims of study

Botulinum neurotoxin-A (BoNT/A) is one of novel therapeutic agents for urinary dysfunction including overactive bladder. The inhibitory mechanism of BoNT/A in the bladder is considered mainly as that it cleaves synaptosome-associated protein of 25,000 daltons (SNAP-25), resulting in failure of acetylcholine exocytosis from efferent nerve terminals. However, it is not known whether BoNT/A has any interaction with voltage-gated Ca<sup>2+</sup> channels (VGCC) in detrusor smooth muscle. We therefore investigated the effects of BoNT/A on L-type and T-type VGCC in rat and human detrusor.

### Study design, materials and methods

Bladder smooth muscle strips were obtained from female rats and humans. Muscle strip tension was measured in an organ bath in the presence of 500nM tetrodotoxin, and we analyzed the effect of BoNT/A on spontaneous and high K<sup>+</sup> induced contractions. For whole-cell patch clamp recordings, single myocytes were obtained by enzymatic digestion, and we analyzed the effect of BoNT/A on VGCC, which was evaluated as barium current. In immunohistological study, we analyzed the effect of BoNT/A on the binding affinity of anti-SNAP-25 antibody, which targets C-terminus of SNAP-25.

### Results

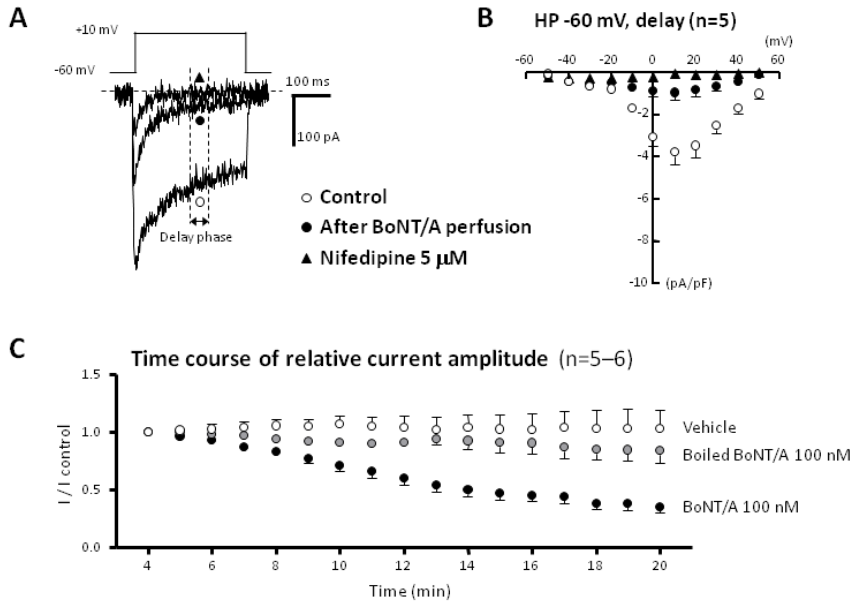
In tension measurements, spontaneous myogenic contractions of rat detrusor were observed in 8 of 8 muscle strips in the vehicle pretreated control group and 5 of 8 strips in the 10µg/ml BoNT/A pretreated group. The frequency and the peak amplitude of spontaneous contractions as well as the peak amplitude of rat detrusor contractions induced by high K<sup>+</sup> solutions (25, 40 and 70mM) were significantly reduced by pretreatment of BoNT/A. The effects of intracellular application of BoNT/A on VGCC were then investigated in single detrusor cells using patch-clamp methods. We monitored the time course of amplitude of L-type inward currents evoked by depolarization to +10mV from the holding potential of -60mV. After 16 min intracellular perfusion of BoNT/A-light chain (LC) 100 nM, the amplitude of L-type current was significantly reduced in both rat (Figure 1) and human (Figure 2) detrusor cells (relative value to control, 0.57 ± 0.048, n=6 in rat; 0.63 ± 0.039, n=5 in human). Similar result was demonstrated by intracellular perfusion of a peptide, C-terminus 9 amino-acids of SNAP-25 (SNAP-25C). Next we monitored the time course of peak amplitude of T-type inward currents evoked by depolarization to -40mV from the holding potential of -90mV. After 16 min intracellular perfusion of BoNT/A-LC 100 nM, the peak amplitude of T-type current was significantly increased (relative value to control, 1.6 ± 0.082, n=6), and similar result was demonstrated by intracellular perfusion of SNAP-25C. After intracellular perfusion of BoNT/A-LC or SNAP-25C, time to peak of T-type current was prolonged and the V<sub>h</sub> value of steady-state inactivation curve was changed to negative potential. In immunohistological analysis, Cav1.2 which is the subunit that forms L-type channels, and SNAP-25 were coexpressed on the plasma membrane in a single rat detrusor cell. The intensity of SNAP-25 staining was weakened by pretreatment of BoNT/A.

### Interpretation of results

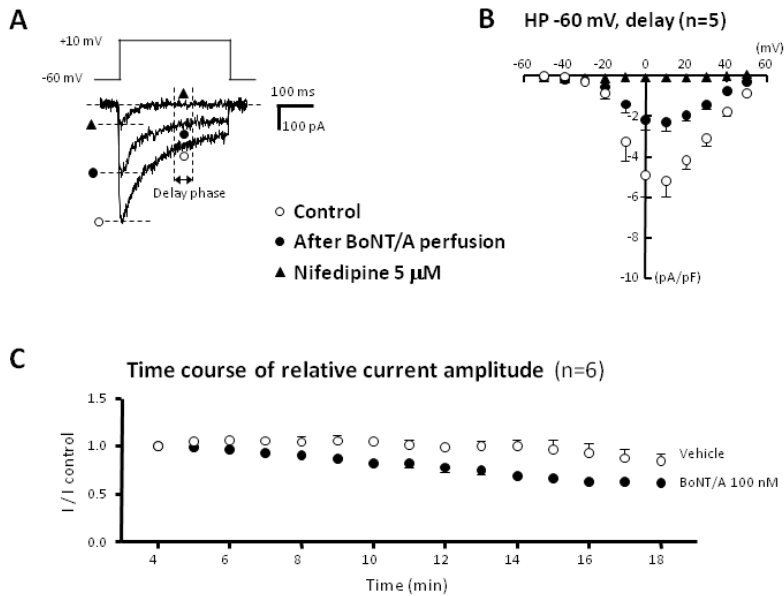
These results suggest that BoNT/A inhibits L-type VGCC in detrusor smooth muscle to reduce detrusor muscle contractility, and this effect seems to be mediated by cleaved SNAP-25 C-terminus. And further, BoNT/A changes the ion channel kinetics of T-type VGCC and it can result in less excitability in detrusor cell.

### Concluding message

BoNT/A inhibits myogenic contractions and L-type VGCC in detrusor smooth muscle. This mechanism may contribute to therapeutic effect of the BoNT/A to suppress detrusor overactivity in OAB patients.



**Figure 1**



**Figure 2**

Specify source of funding or grant	none
Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Institutional Animal Care and Use Committee at the University of Pittsburgh

