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EFFECT OF BOTULINUM NEUROTOXIN-A ON BLADDER CONTRACTILE RESPONSES TO ACTIVATION OF EFFERENT NERVE TERMINALS, SMOOTH MUSCLE AND AFFERENT NERVE TERMINALS IN RAT BLADDER

Hypothesis / aims of study
Botulinum neurotoxin-A (BoNT/A) is one of the novel therapeutic agents for urinary dysfunction including overactive bladder (OAB). The inhibitory mechanism of BoNT/A is considered mainly to be the inhibition of acetylcholine exocytosis from cholinergic efferent nerve terminals; however, the inhibitory effect of BoNT/A on afferent nerve terminals has also been reported. In addition, we recently reported that BoNT/A directly inhibits voltage-gated Ca^{2+} channels (VGCC) in rat and human detrusor smooth muscle using patch-clamp techniques (ICS 2010). In the present study, we therefore compared the effects of BoNT/A on rat bladder smooth muscle contractions induced by activation of efferent nerve terminals, afferent nerve terminals and VGCC on to clarify which mechanism(s) are most important for the inhibitory effects of BoNT/A.

Study design, materials and methods
Bladder strips were obtained from female SD rats and the urothelium was removed. These strips were incubated for 3 h at 37°C in Krebs solution with different concentrations of BoNT/A (0.3-100nM) and tension development was measured in an organ bath. To examine the effect of BoNT/A on neurotransmitter release from efferent nerve terminals, changes in electrical field stimulation (EFS: 20V; 0.5-64 Hz)-induced contractions were evaluated. To examine the effect of BoNT/A on neurotransmitter release from afferent nerve terminals, changes in capsaicin (1 μ M)-induced contractions were evaluated. To examine the effects of BoNT/A on VGCC of smooth muscle, changes in the contraction elicited by 70mM KCl solution, which induce smooth muscle membrane depolarization and activate nifedipine-sensitive VGCC, were evaluated. In addition, the contractile responses to carbachol (CCh), a cholinergic receptor agonist, were also evaluated. All data were compared with those obtained from vehicle-incubated control muscle strips.

Results
The contractile responses induced by EFS and 70mM KCl were dose-dependently inhibited by the incubation with BoNT/A (Fig. 1, 2). The significant inhibitory effect of BoNT/A on EFS-induced contractions was observed at a concentration of 10nM or higher, and the maximal inhibition at 100nM was 70% compared to control strips (Fig. 1). KCl-induced contractions were significantly inhibited by the incubation with BoNT/A of 3nM or higher, and the maximal inhibition at 100nM was 30% compared to control strips (Fig. 2). However, the contractile responses to CCh were not altered by the incubation with BoNT/A even at the highest concentration of 100nM. Capsaicin-induced contractions were not altered by the incubation with 100nM BoTN/A for 3h. However, the overnight incubation with 100nM BoNT/A significantly inhibited the capsaicin-induced contraction by 30% compared to control strips (Fig. 3).

Interpretation of results
BoNT/A have an inhibitory effect on neurotransmitter release from efferent and afferent nerve terminals as well as activation of VGCC. The order of inhibitory potency seems to be efferent nerve terminals > VGCC > afferent nerve terminals. Although BoNT/A has greater inhibitory effects on nerve-mediated contractions than those on depolarization-induced contractions of rat detrusor smooth muscle, BoNT/A-induced inhibition of these two different contractile responses occur at the similar level of BoNT/A concentrations. In contrast, a high tissue concentration of BoNT/A may be necessary to suppress neurotransmitter release from afferent nerve terminals because a longer incubation with BoNT/A was needed to inhibit capsaicin-induced contractions.

Concluding message
The clinical efficacy of BoNT/A in the treatment of OAB may include the inhibition of not only acetylcholine exocytosis from efferent nerve terminals, but also VGCC on detrusor smooth muscles. In addition, when compared with these two mechanisms, suppression of neurotransmitter release from afferent nerve terminals seems to contribute at a lesser degree to the BoNT/A-mediated inhibition of bladder activity.

Fig. 1

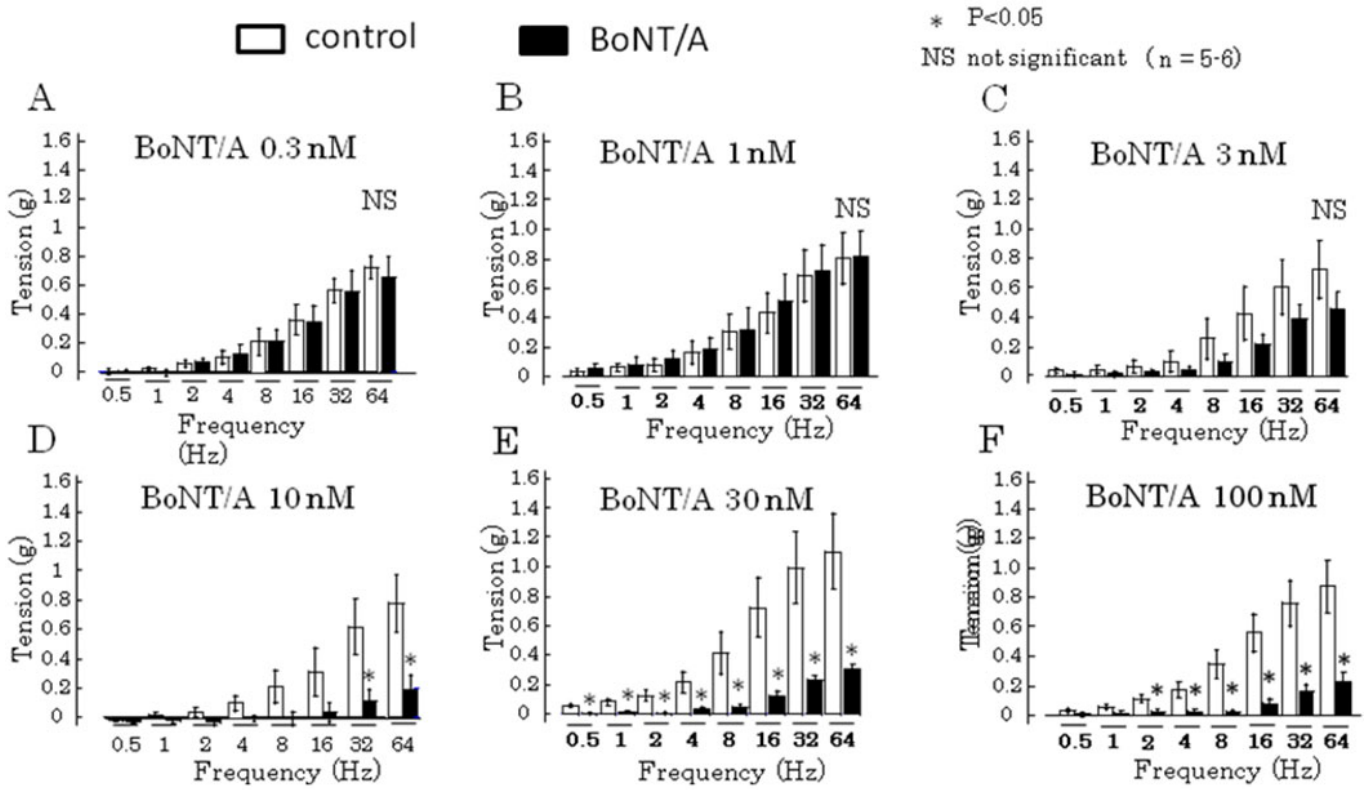

control
BoNT/A
* P<0.05
NS not significant (n = 5-6)
A
BoNT/A 0.3 nM
NS
B
BoNT/A 1 nM
NS
C
BoNT/A 3 nM
NS
D
BoNT/A 10 nM
E
BoNT/A 30 nM
F
BoNT/A 100 nM
Tension (g)
Frequency (Hz)
0.5 1 2 4 8 16 32 64

Fig. 2

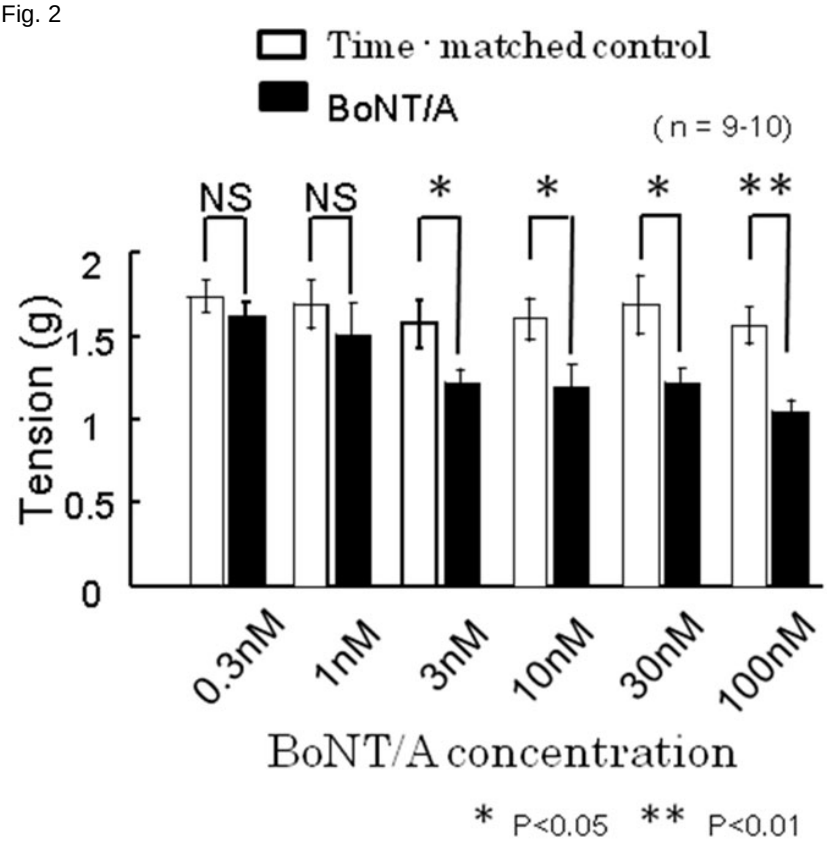
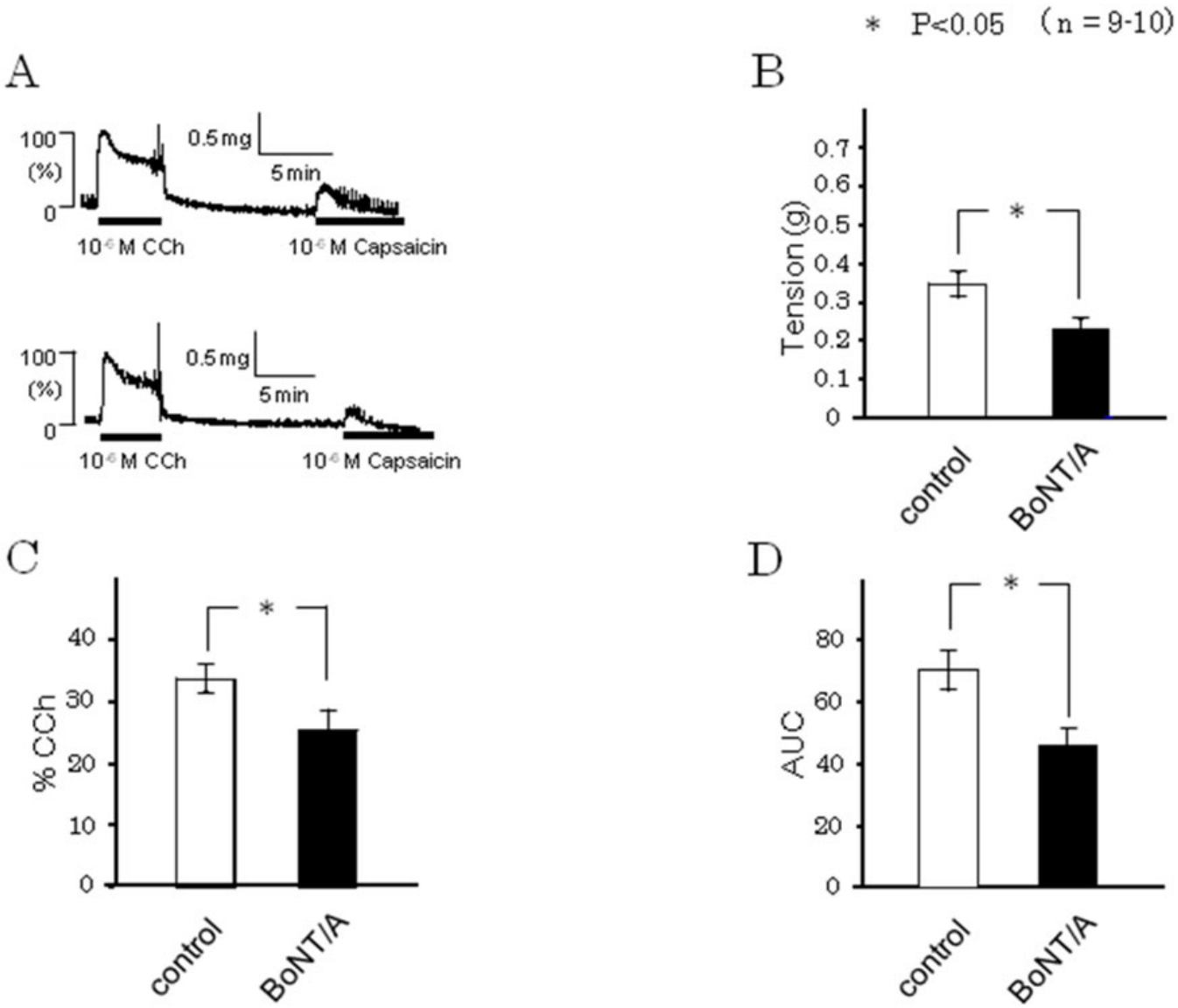

Time · matched control
BoNT/A
(n = 9-10)
NS NS * * * **
Tension (g)
0 0.5 1 1.5 2
0.3nM 1nM 3nM 10nM 30nM 100nM
BoNT/A concentration
* P<0.05 ** P<0.01

Fig. 3



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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 Committees at the University of Pittsburgh**