ACUTE APPLICATION OF BOTULINUM TOXIN TYPE-A INHIBITS MUSCARINIC BUT NOT PURINERGIC RECEPTOR INDUCED CONTRACTILE ACTIVITY IN MOUSE BLADDERS

Hypothesis / aims of study
Botulinum toxin type A (BTX-A) has been examined widely through clinical trials as a therapeutic agent to ameliorate bladder overactivity. However, its mechanism of action has not been fully elucidated. In this study we examined the effect of BTX-A on nerve-evoked and agonist induced contractions of a bladder sheet to determine the onset-of-action and the effect on muscarinic receptors.

Study design, materials and methods
Female C57bl/10 mice (6-7 weeks) were sacrificed humanely and the bladder excised. The bladder was cut open from outlet to dome along the dorsal aspect to form a sheet. The outlet was pinned to a fixed platform (with the mucosal surface facing up) in a recording chamber and the dome was tied with 7-0 suture to a bar connected to a tension transducer. The recording chamber was filled with 10 ml of modified Tyrodes solution (95% O2 and 5% CO2, pH 7.35) and maintained at 37°C. Bladder sheets were stretched to 1g of resting tension and allowed to equilibrate for at least 30 min. The preparation was electrically field-stimulated (EFS; 3 s train, 1 ms pulses, 10V, 2-40 Hz) before and once every hour (up to eight hours) following the addition of BTX-A (50 U) to the bath. The effect of arecadine (10 µM) was determined pre- and post-BTX-A (8 hrs), while α-β-methylene ATP (10 µM) and high KCl (80 mM) were tested post-BTX-A. The tested drugs were added from 10 mM stocks to the perfusion solution to give working concentrations.

Results
The contractile response of the bladder sheet was initially reduced, specifically at higher frequencies (16-40Hz), 4 hours following the addition of BTX-A to the bath. Over the subsequent 4 hrs, contractions at all frequencies were progressively reduced (80-100% at 8 hrs). Remaining contractions were sensitive to 1µM tetrodotoxin.

Interpretation of results
These results suggest that the onset-of-action of BTX-A on nerve-evoked contractions in the in vitro bladder sheet preparation is approximately 4 hours and the effect increases thereafter. This significant reduction (80-100%) in the magnitude of EFS contractions indicates that the release of both Ach and ATP are blocked by BTX-A. The inhibition of arecadine but not α-β-methylene ATP or KCl suggests that BTX-A has an inhibitory effect on muscarinic receptors in the bladder.

Concluding message
This is the first study to determine the on-set-action of BTX-A in mouse bladders using an in vitro whole sheet preparation. Moreover, in addition to the established presynaptic effects of the toxin, we have demonstrated that BTX-A also inhibits muscarinic but not purinergic post synaptic receptor-induced contractile activity in mouse bladders.

Specify source of funding or grant
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Is this a clinical trial?
No
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<th>What were the subjects in the study?</th>
<th>ANIMAL</th>
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<td>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</td>
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