510

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DISTRIBUTION OF CLEAVED SNAP25 IN THE BONT/A-TREATED GUINEA PIG URINARY BLADDER

Hypothesis / aims of study

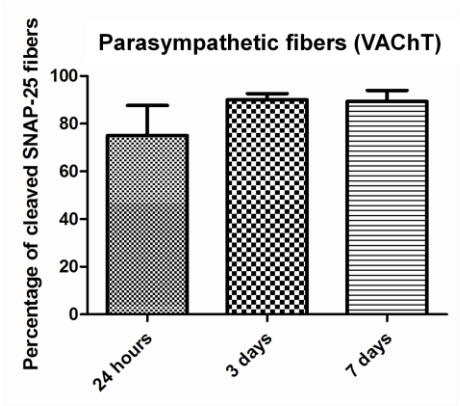
Botulinum toxin type A (BoNT/A) injection in the detrusor is an option to the treatment of refractory detrusor overactivity. BoNT/A acts by cleaving the SNARE complex protein SNAP-25 and blocking neurotransmitter release. In the present study, the expression of cleaved SNAP-25 was analyzed in the guinea-pig urinary bladder after BoNT/A administration and the time course of its appearance determined. In addition, the neurochemistry of the positive cleaved SNAP-25 structures was studied.

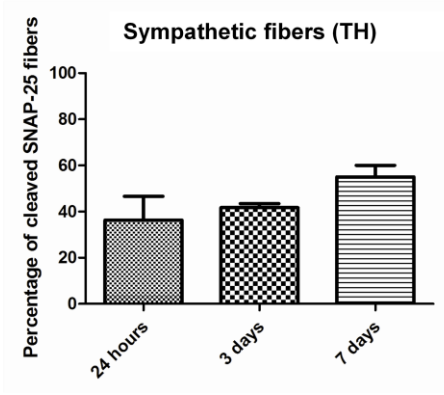
Study design, materials and methods

10 U of BoNT/A or its vehicle were injected in Guinea-pig bladders. 24 hours, 3 and 7 days after BoNT/A administration the bladders were collected and sections were processed for immunohistochemistry against intact and cleaved SNAP-25. Co-localization with the pan neuronal marker β 3-tubulin was performed. The neurochemistry of BoNT/A affected fibers was studied by double-labeling using antibodies against cleaved SNAP-25 and vesicular acetylcholine transporter (VAChT), tyrosine hydroxilase (TH) or calcitonin-gene related peptide (CGRP) to label parasympathetic, sympathetic and sensory fibers respectively.

Results

SNAP-25 co-localize totally with the pan neuronal marker β 3-tubulin. The protein was distributed throughout the mucosa and muscular layer of the bladder wall but was not detectable in the urothelium. Cleaved SNAP-25-immunoreactivity (IR) was found 24 hours after BoNT/A administration. The percentage of parasympathetic fibers containing cleaved SNAP-25 was 85%, significantly higher than sympathetic or sensory fibers, 50% and 45% respectively. No differences in the amount of cleaved SNAP-25 were found at different time points studied (figure 1).





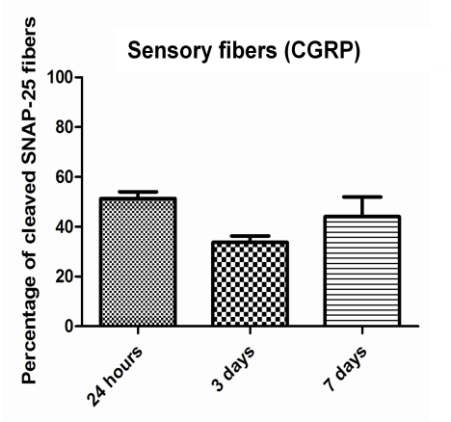


Figure 1: Expression of cleaved SNAP-25 in parasympathetic, sympathetic and sensory fibers 24 hours, 3 and 7 days after botulinum toxin type A injection in the Guinea-pig bladder

Interpretation of results

SNAP-25 was found in the Guinea-pig bladder exclusively in nerve fibers as suggested by a 1:1 co-localization with the pan neuronal marker β 3-tubulin. Cleaved SNAP-25 is predominantly expressed in parasympathetic fibers but is also detectable in sympathetic and sensory fibers. Twenty-four hours after BoNT/A injection, cleaved SNAP-25 is already detectable and its percentage of expression is maintained at least during one week.

Concluding message

In the normal Guinea-pig bladder, BoNT/A acts swiftly, impairing predominantly almost all parasympathetic nerve fibers. However, the expression of cleaved SNAP-25 roughly detected in half of sympathetic and sensory fibers may also contribute to the net effect of BoNT/A in the bladder. The absence of cleaved SNAP-25 from the urothelium seems to exclude urothelial cells as a relevant target for BoNT/A action.

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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	**Ethics Committee of Faculty of Medicine**