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).
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