Human and mouse isolated bladder preparations are not equally affected by botulinum neurotoxin serotypes A and B

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Introduction

- **Botulinum Neurotoxin type A (BoNT/A) and BoNT/B products are available for clinical use, with the BoNT/B product requiring higher doses to reach efficacy in skeletal muscle indications.**
- **Few studies have compared the efficacy of both serotypes in smooth muscle preparations in urological indications.**
- **The aim of this study was to investigate and compare the potency of BoNT/A and BoNT/B in isolated bladder strip preparations from human and murine tissues.**

Methods

Human tissue

Human detrusor strip samples were prepared from patients undergoing cystectomy for bladder cancer with no known bladder dysfunction according to their medical chart (n=20). Consent of patients in writing (mentioning data privacy obligations), as well as patient medical history, were obtained.

Murine tissue

Mouse bladder strip samples were prepared from C57Bl6 female mice (n= 30) after isoflurane anaesthesia and decapitation, in compliance with the relevant animal health regulation in place in France (Council Directive No.2010/63/UE of September 22nd 2010 on the protection of animals used for scientific purpose).

Chemicals

Natural Botulinum toxins A and B (150kDa) were purchased from List Biologicals Laboratories, Campbell, CA. The activity of the toxins was confirmed in cell-free assays using BoTest® (Botulinum Neurotoxin Detection Kit, BioSentinel, Madison, WI) and in cell-based assays using rat spinal cord neuronal cultures. All other chemicals were purchased from Sigma-Aldrich (St Louis, MO).

Experimental protocol

Strips were tensed in organ baths and tested for viability and muscarinic receptor responses with KCl and carbachol (CCh), respectively. Strips were then submitted to electrical field stimulation (EFS) using the following stimulation protocols:

- Mouse: trains of 20 pulses (intra-train frequency 10 Hz, single pulse duration 20 µsec) separated by 1 minute intervals.
- Human: trains of 20 Hz, 1 ms pulse duration, 5 s train duration continuously performed by groups of 3 stimulations applied at 1-min interval and followed by a 3-min period of rest.

Once the contractile response was stable, pharmacological agents (tetrodotoxin (a voltage-gated sodium channel blocker), atropine or oxybutinin (both muscarinic receptor antagonists), and/or α-β-methylene-ATP (a P2X receptor desensitizer) or purified botulinum toxins (1, 3, 5 or 10nM) were added into the baths and the signals were recorded for 3 to 4 hours. At the end of the experiment the viability of the tissue throughout the experiment was assessed by adding CCh to the organ bath and comparing CCh responses at the beginning and end of the experiment.

Data analysis

A non-linear regression analysis was performed (four-parameter dose-response logistic curve) to calculate the time to half-amplitude contraction (t50) as compared to control contraction amplitude. This parameter allows to express the potency of a neurotoxin, with the smaller value indicating a higher potency. A two-way ANOVA was performed to assess a potential difference in the effect of the serotypes within each species.

Results

Pharmacological characterisation of electrically evoked contractions in mouse and human bladder strip preparations

Contractions induced by electrical field stimulation were of neurogenic origin in human and murine bladder preparations (Figure 1A and 1C). In murine tissue, approximatively 50% of contractions were blocked by muscarinic receptor antagonism, and 50% by desensitization of the purinergic receptors (Fig 1B). Contractions in the human tissue were exclusively due to muscarinic transmission (Fig. 1D).

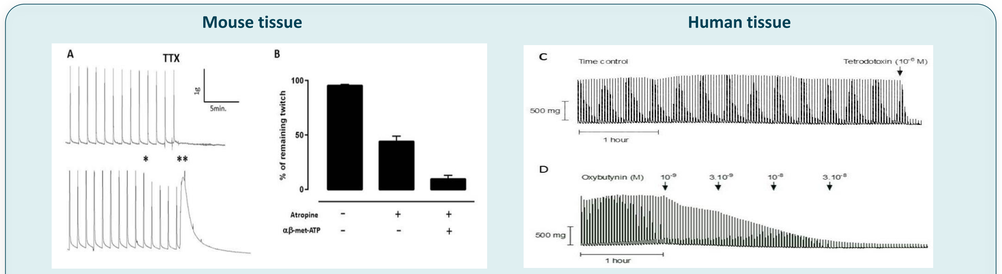


Figure 1. Pharmacological characterisation of EFS-induced contractions in mouse and human detrusor strips. (A) Typical recording illustrating the effect of 1 µM tetrodotoxin (TTX) on EFS-induced contractions of the isolated mouse bladder strips, resulting in complete inhibition of EFS-induced contractions. 1 µM atropine (*) partially inhibited the contractions, while the subsequent addition of 10 µM α–β-met-ATP (* *) completely abolished the remaining signal after a peak in amplitude due to overstimulation of purinergic receptors. (B) Summary data from experiments as shown in A. (C). Recording from a human bladder strip preparation. Recordings were stable for prolonged periods of time. Contractions in human bladder were also of neurogenic origin, as shown by their inhibition by tetrodotoxin. (D) Contractions in human bladder strip preparations were totally abolished by the addition of oxybutinin, a muscarinic receptor antagonist.

Paralysing effect of BoNT serotypes A and B

Human and mouse bladder preparations were sensitive to both serotypes in a concentration-dependent manner. Interestingly, in the mouse bladder preparation, BoNT/B was approximately 3 fold more potent than BoNT/A in inhibiting neurogenically induced contractions. In contrast, BoNT/A and BoNT/B serotypes were approximately equipotent in the human bladder preparations.

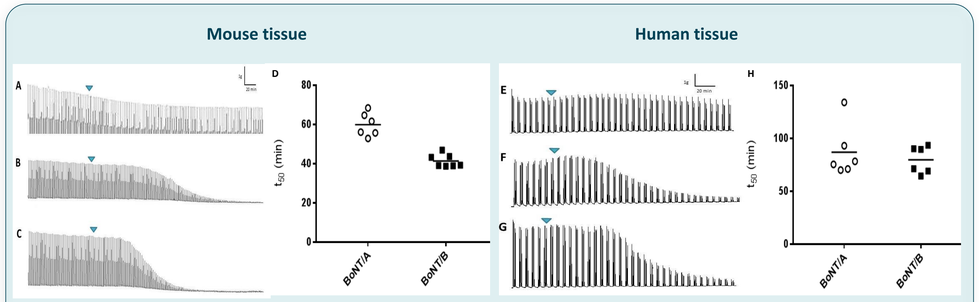
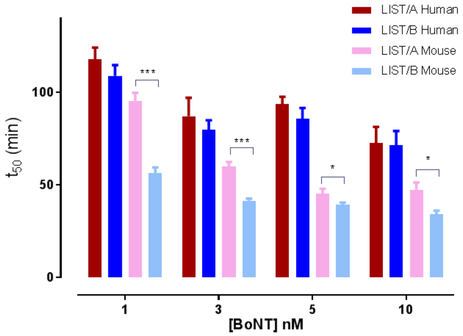


Figure 2. Representative traces of electrically evoked contractions in mouse and human detrusor strips. Left panel. Typical recording of the effect of 3 nM BoNT/A or BoNT/B (B and C, respectively) and control strip of mouse bladder assay (A), showing the stability of the signal over time. Graph D illustrates the summary data for all mouse bladder experiments at 3 nM. Right panel. Traces E, F, and G depict recordings obtained from human bladder strips for control conditions, BoNT/A, and BoNT/B (both at 3 nM), respectively. Graph H shows the summary data for both toxins from all experiments on human bladder strips at 3 nM.

Figure 3. Mouse and human bladder are not equally sensitive to BoNT/A and BoNT/B. While in the human preparation the two serotypes were equipotent at every concentration from 1 to 10 nM, BoNT/B was statistically more potent than BoNT/A in the murine preparation at every concentration tested. 5 to 7 strips were studied per condition. Statistical significance was determined using a 2-way ANOVA followed by the Holm-Sidak multiple comparison test, with alpha = 0.05. (* = p<0.05; *** = p<0.001).



Conclusion

The human bladder strip assay is equally sensitive to BoNT/A and BoNT/B. This finding is at odds with the relative resistance of human skeletal muscle tissue to BoNT/B experienced in the clinic. This may be explained by a preference of BoNT/B for the autonomic nervous system, which innervates the urinary tract, possibly due to differences in synaptotagmin isoform expression between autonomic and somatic nervous system. Supporting this idea is the finding that BoNT/B is more potent than BoNT/A in the mouse bladder preparation, whereas we have previously shown that both toxins are equally potent in the mouse phrenic nerve-hemidiaphragm preparation (Maignel-Ludop et al, in press). Studies exploring the level of expression of the relevant molecular actors of botulinum toxin mechanisms of actions (Syt, SV2, VAMP, SNAP25) in human and mouse tissue may be a way to further explain these apparent differences.

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