EX VIVO COMPARISON OF THREE BONT SEROTYPES IN THE AUTONOMIC NERVOUS SYSTEM AND THE NEUROMUSCULAR JUNCTION

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BACKGROUND

Botulinum neurotoxins (BoNTs) are widely used as therapeutic agents and in aesthetic indications. Despite the richness of serotypes and sub-serotypes within the BoNT family, only A1 and B1 are currently used in clinical practice. BoNT serotypes A1 and B1 are known to be longer acting and F1 is known to be faster acting[1]. In contrast to the skeletal neuromuscular junction (NMJ), the autonomic nervous system (ANS), while targeted clinically, is less explored in BoNT research. One model of ANS hyperactivity is the isolated bladder strip preparation in which electrical field stimulation of the parasympathetic nerves induces hyperactivity of the detrusor smooth muscle. Moreover, BoNT/A1 is the major serotype used in clinical practice, while BoNT/B1 is used to a lesser extent given the lower affinity of BoNT/B for its protein receptor, Synaptotagmin 2 (Syt2), in humans due to a mutation in the binding domain[(1)] which is not present in rodents.

OBJECTIVES

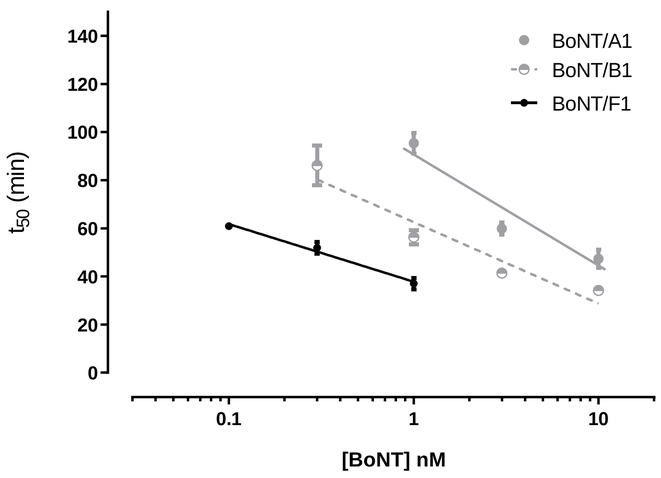
The aim of this study was to compare the paresis induced by 3 serotypes of BoNT in *ex vivo* models of the skeletal NMJ and autonomic hyperactivity, to assess inter-species and inter-tissue variability in the sensitivity to BoNTs. The different receptors/SNARE targets of the selected serotypes are presented in the table below.

Serotype	Protein Receptor	Intracellular Target (SNARE)
A	SV2	SNAP25
B	Syt2	VAMP
F	SV2	VAMP

METHODS

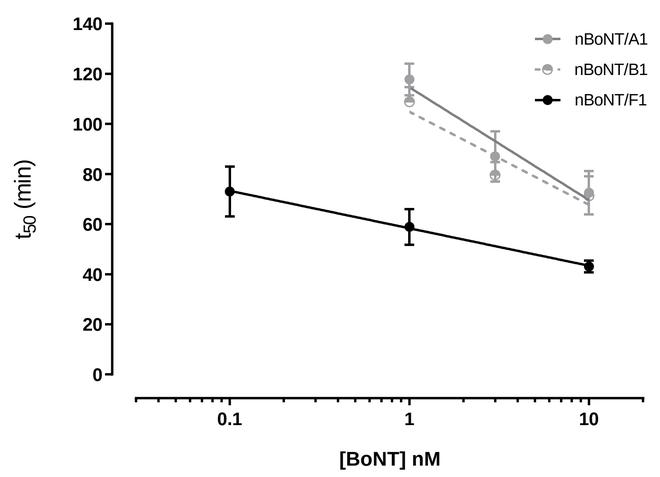
Detrusor strips (*n*=3-7 per concentration), prepared from C57Bl6 mouse, WKY rat, and human bladders were tensed in tissue baths and subjected to species-specific electrical field stimulation to generate neurogenic contractions, as previously described[(2)]. Purified, natural BoNT/A, /B, or /F was added from 0.1 nM to 10 nM in independent baths and signals recorded for 4 hours. Potency was expressed as time to reach half-paralysis (t_{50}). For technical reasons rat bladder strips (with urothelium) were used with a single concentration of BoNT (3 nM). Potency of BoNTs at the NMJ was assessed in the mouse Phrenic Nerve Hemi-Diaphragm (mPNHD) assay, measuring muscle contractility in the presence of 1pM to 1nM neurotoxin using the method previously described[(2)]. Relative potency for each toxin was estimated using a linear regression[(3)].

A) Effect of BoNT/A1, /B1 and /F1 in the mouse detrusor strip assay



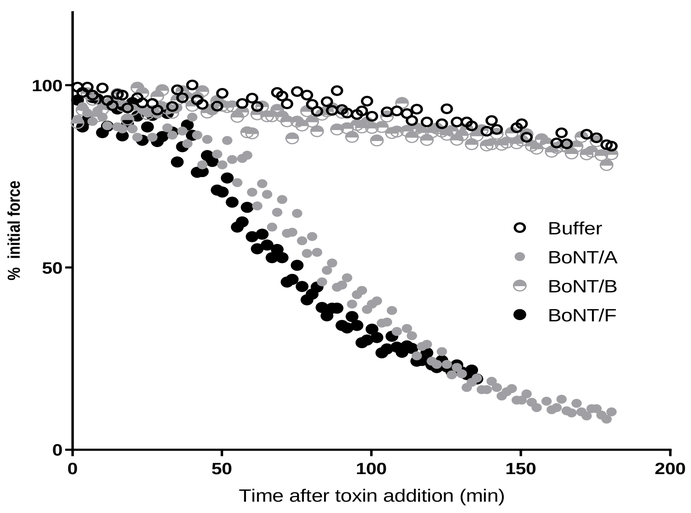
	Estimated [BoNT] resulting in t_{50} of 70 mins (nM)	Potency relative to BoNT/A1
BoNT/A1	2.438	1.0
BoNT/B1	0.206	11.8
BoNT/F1	0.045	54.0

B) Effect of BoNT/A1, /B1 and /F1 in the human detrusor strip assay



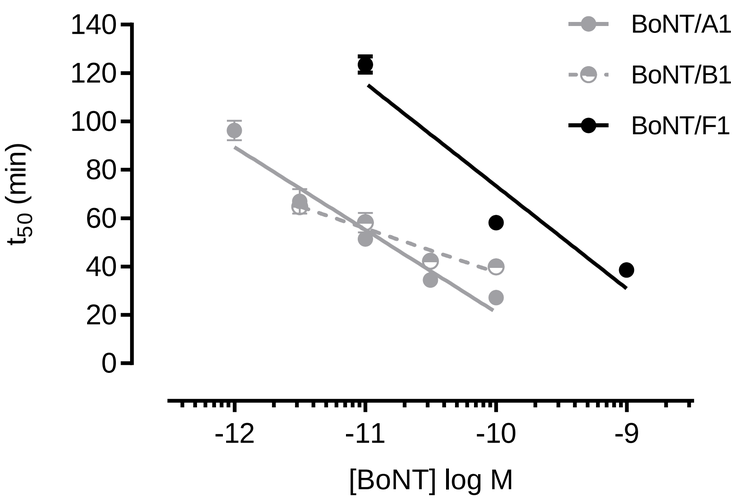
	Estimated [BoNT] resulting in t_{50} of 70 mins (nM)	Potency relative to BoNT/A1
BoNT/A1	12.130	1.0
BoNT/B1	10.218	1.2
BoNT/F1	0.308	39.4

C) Paresis induced by 3 nM BoNT/A1, /B1 and /F1 in the rat bladder strip assay



	Estimated t_{50} (min)
BoNT/A1	71.6 ± 7.5 (*n*=5)
BoNT/B1	N/A (*n*=3)
BoNT/F1	85.4 ± 10.2 (*n*=4)

D) Effect of BoNT/A1, /B1 and /F1 in the mouse PNHD assay



	Estimated [BoNT] resulting in t_{50} of 70 mins (pM)	Potency relative to BoNT/A1
BoNT/A1	3.66	1.0
BoNT/B1	1.22	3.0
BoNT/F1	121.5	0.03

RESULTS

BoNT/F was the most potent toxin in each preparation of detrusor strips (A, B, C), while it was the least potent in the mouse PNHD assay (D). In the mouse bladder, BoNT/B was more potent than BoNT/A at inhibiting neurogenically-induced contractions (A). In contrast, BoNT/A and /B were approximately equipotent in the human bladder preparation (B). Interestingly, rat bladder was resistant to BoNT/B (C), in contrast to BoNT/A and BoNT/F, which were equipotent at this concentration. Nevertheless, Vesicle-Associated Membrane Protein (VAMP)2, which is cleaved by BoNT/B, and VAMP1, which is not cleaved by BoNT/B due to a point mutation in the scissile bond (which does not affect humans or mice[(4)]), were both detected in rat bladder by immunohistochemistry (data not shown).

References

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(2) Maignel-Ludop J, *et al*. Pharma Res Per. 5(1): e00289 (2017)

(3) Weisemann J, *et al.* Toxins (Basel). 7(12):5035-54 (2015)

(4) Schiavo, G. *et al*. Nature. 359, 832-5 (1992).

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(6) Merz, B. *et al*. Clin Auton Res. 13 : 337–338 (2003)

CONCLUSIONS

We have shown human bladder to be equally sensitive to BoNT/A and /B. This finding is at odds with the observed low sensitivity of human skeletal muscle to BoNT/B[(5)] and lower affinity for the human form of its receptor, Synaptotagmin (Syt). A preference of BoNT/B for the ANS over skeletal muscle has been noted previously[(6)] and is supported by the finding that BoNT/B is more potent than BoNT/A in mouse bladder strips. Syt1 and VAMP1 appear to effect parasympathetic-driven contractions of the detrusor, with VAMP2 seems likely to be involved in another aspect of bladder innervation.

BoNT/F showed a very different activity in the mPNHD assay (low) compared to the bladder assay in human and mouse preparations (high). The reason for this discrepancy (which may be due to different receptor and/or SNARE expression in the different species and/or tissue fibre types) will require further investigation.

Together, these data support an interest in using BoNT serotypes other than BoNT/A to affect autonomic disorders.

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