Synaptotagmin-1 is the dominant receptor for botulinum neurotoxin B binding in the mouse bladder

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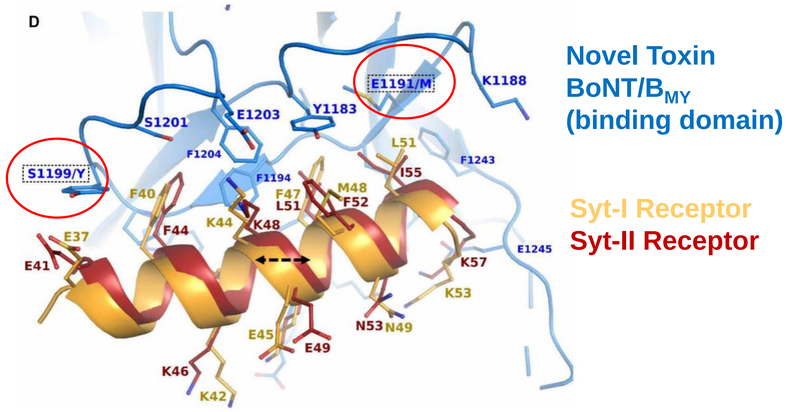
Introduction and Objectives

OnabotulinumtoxinA (Botox, BoNT/A) is FDA approved for overactive and neurogenic bladder patients (OAB, NGB), with clinically effective outcomes. Commercial BoNT/B (Myobloc) is not widely utilized for OAB or NGB as BoNT/B shows a lower activity on human neurons than BoNT/A. This is because binding of BoNT/B to its human receptors, homologous synaptic vesicle membrane proteins, synaptotagmin 1 and 2 (Syt-1/2) is lower than its binding to mouse receptors due to a single residue change in human Syt-2, which has been demonstrated to be the dominant receptor in skeletal muscle. We recently developed an engineered BoNT/B with higher affinity to human Syt than natural BoNT/B, and this engineered toxin showed similar potency as BoNT/A on skeleton muscles, but 10-fold higher activity than BoNT/A on paralyzing bladder tissues. Interestingly, natural BoNT/B also showed ~10-fold higher activity than BoNT/A on bladder tissues. These data suggest that the receptor profile is different in skeleton muscles versus bladder tissues. Here we aim to define the functional receptor for BoNT/B at the bladder in order to establish a mechanism of action and evaluate therapeutic potential of both natural and engineered BoNT/B for treating OAB and NGB.

Novel engineered BoNT/B

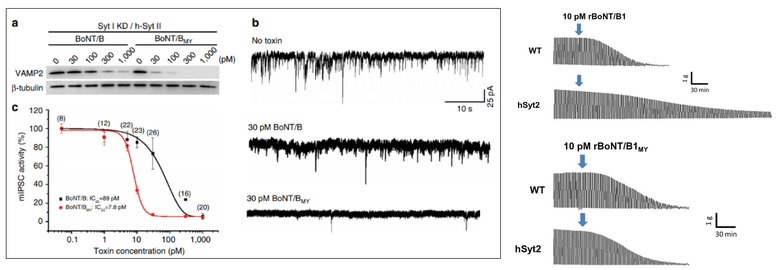


Novel Toxin BoNT/B_{MY} (binding domain)

Syt-I Receptor

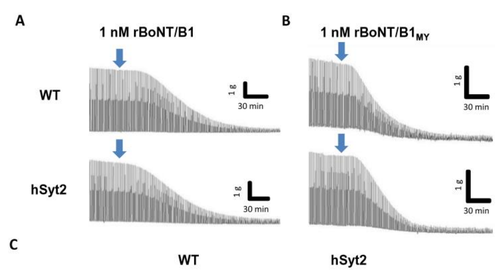
Syt-II Receptor

BoNT/B_{MY} (heavy chain binding domain) superposed with hSyt1 and hSyt2.



BoNT/B_{MY} shows enhanced functional efficacy in neuron cell culture, demonstrated by (a) more VAMP2 cleavage compared to WT BoNT/B, and (b,c) enhanced mIPSC quiescence on whole-cell patch-clamp recordings.

Novel Engineered BoNT/B



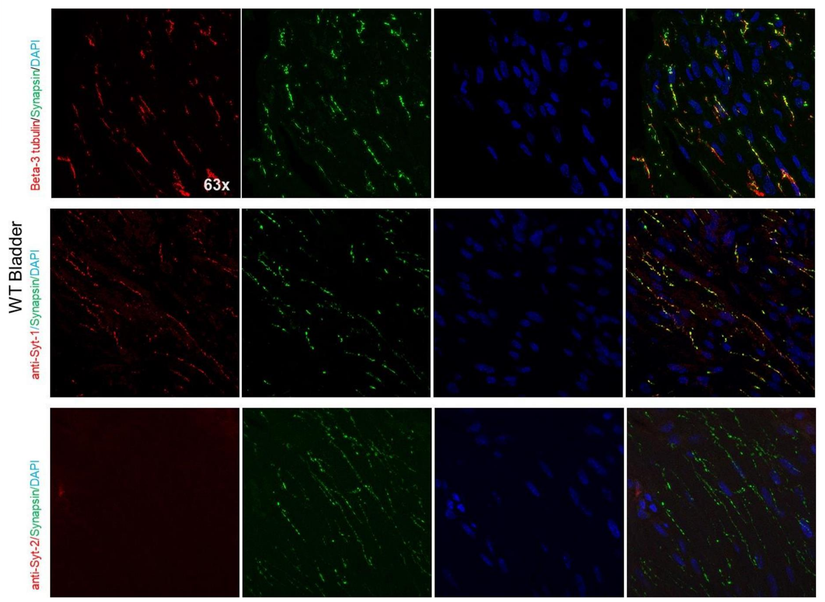
BoNT/B1 and BoNT/B_{MY} induces detrusor paralysis in hSyt2 mice, but without significant difference compared to WT mice.

Methods

To define the function of Syt-1 and Syt-2, we generated two transgenic knock-in (KI) mouse lines, one with Syt-2 containing three point mutations that abolish binding of BoNT/B, the other with Syt-1 containing the same set of mutations. cDNA encoding BoNT/HcB_{MY} was expressed in *E. coli*, and protein concentrates were purified by hydrophobic interaction chromatography.

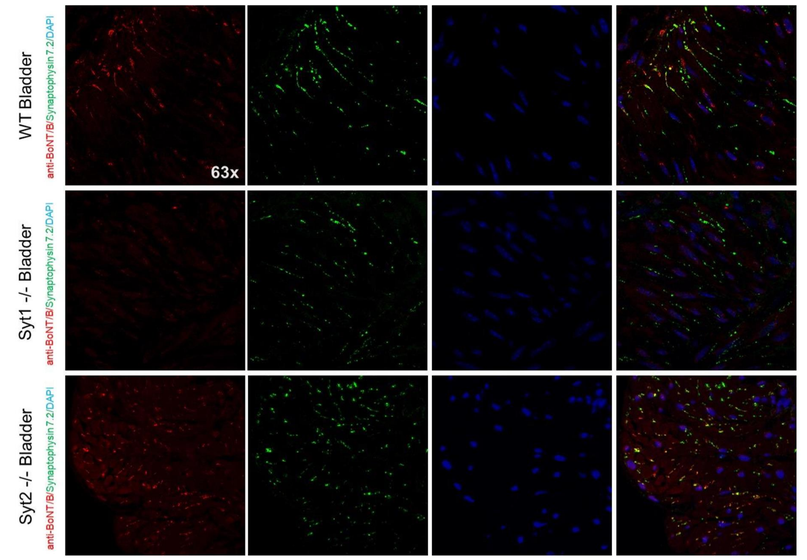
In vitro BoNT/B binding on the wild type & transgenic mouse bladder was examined using immunofluorescence analysis. Primary antibodies were directed against anti-BoNT/B (1:800), β-3 tubulin (1:200), presynaptic neuronal markers (synaptophysin 7.2, 1:1000 and synapsin, 1:500) and anti-Syt-1 or -2 (1:200) with negative controls. Species-specific secondary antibodies were used (1:1000-2000) with DAPI counterstain.

Results



Syt-1 is the dominant receptor on wild type mouse bladders, demonstrated via immunofluorescence analysis. Synapsin and β3 tubulin was labeled as a marker for presynaptic terminals & nerves, with DAPI counterstain.

Results



Bladders were exposed to HcB_{MY} (100nM) followed by immunofluorescence analysis. Bound HcB_{MY} was detected using anti-BoNT/B antibody.
HcB_{MY} shows vigorous binding to wild type and Syt-2 knock in bladders, but not to Syt-1 knock in bladders. Syt-1 knock in thus abolishes binding of HcB_{MY}. Synaptophysin 7.2 was labeled to serve as a presynaptic terminal marker, with DAPI counterstain.

Summary and Conclusions

These results demonstrate that Syt-1, but not Syt-2, is the dominant receptor for BoNT/B in bladder tissues, which is a major difference from skeletal muscle. Further studies testing engineered BoNT/B in these transgenic mice, with functional endpoints, is warranted to characterize the minimally effective dose and potency.

References

Tao, L. *et al.* Engineered botulinum neurotoxin B with improved efficacy for targeting human receptors. *Nat Commun* **8**, 53, doi:10.1038/s41467-017-00064-y (2017).

Elliott, M. *et al.* Engineered botulinum neurotoxin B with improved binding to human receptors has enhanced efficacy in preclinical models. *Sci Adv* **5**, eaau7196, doi:10.1126/sciadv.aau7196 (2019).

Acknowledgements

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