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CHANGES IN THE SPINAL EXPRESSION OF RECEPTORS INVOLVED IN THE CONTROL OF THE LOWER URINARY TRACT FOLLOWING INTRADETRUSOR BOTULINUM NEUROTOXIN A (BONT/A) INJECTIONS: RESULTS FROM NORMAL RATS

Hypothesis / aims of study

It has been proposed that BoNT/A injected in the overactive human bladder has a complex effect on peripheral neurotransmission, particularly on bladder afferent pathways that are important mediators or inducers of intrinsic or spinal reflexes. Such an effect appears to be exerted via an action on urothelial and suburothelial receptors, neuropeptides and neurotransmitters, as well as via a decrease in bladder afferent nerve firing in response to stretch and detrusor spontaneous contractions.

A possible additional effect at the spinal cord (SC)/dorsal root ganglia (DRG) level has been suggested. In a model of spinal neurogenic bladder overactivity, a central inhibitory effect of BoNT/A was confirmed as significant reductions in the numbers of c-fos immunoreactive cells in the L6/S1 spinal cord segments were found following intravesical application of BoNT/A. Interestingly, earlier studies had already described changes in neurotransmitter (enkephalin - ENK, substance P - SP, Neuropeptide Y – NPY, vasoactive intestinal polypeptide – VIP) and growth factor gene expression at the spinal cord following BoNT/A injection in peripheral skeletal muscles in experimental animal models.

We investigated possible changes in the SC/DRG expression of neuropeptides and receptors associated with lower urinary tract function (Tachykinin 1 [Neurokinin A/Substance P] - Tac1, vanilloid receptor TRPV1, Vesicular Acetylcholine Transporter – VAChT, acetylcholinesterase – AChE, NPY and adrenergic receptors α1A and α1D) in parallel with respective bladder changes following BoNT/A bladder injection in normal rats.

Study design, materials and methods

Twenty-four female Sprague-Dawley rats underwent BoNT/A injections in the bladder wall. Twelve rats received 2U and another 12 received 5U of OnabotulinumtoxinA (Botox®). Another 12 rats received saline injections (saline controls) and 6 received no treatment serving as sham-controls. In accord with previous experiments showing central and peripheral changes at similar time-points after skeletal or bladder muscle BoNT/A injection, rats were sacrificed on days 7 and 14, and tissue was harvested from the bladder, the DRGs and SC at the L6-S1 level, and examined with real-time PCR (RT-PCR) for the genes of interest. Expression levels of transcripts were normalized to GAPDH as endogenous control and changes were expressed as fold change to the endogenous control.

Results

Sensory markers: Tac1 expression significantly increased following treatment with 2U or 5U at 14 days (bladder: p=0.000 and 0.042, DRG: p=0.006 and 0.140, SC: p=0.000 and 0.042, respectively), but it was unchanged at 7 days in the two Botox-treated groups.

No significant changes were seen in TRPV1 bladder expression. TRPV1 expression in the DRGs increased following the 5U injection at 7 days (p=0.000 compared to sham-controls and p=0.001 compared to saline) but returned to controls levels at 14 days. Spinal TRPV1 expression also increased after the 5U injection at 7 days (p=0.005).

Cholinergic markers: VAChT expression changed only with the 2U dose (7 days: bladder p=0.009 and 0.047 compared to controls and saline respectively, SC p=0.009 and 0.028 respectively – 14 days: DRG p=0.003 and 0.015 respectively)

AChE expression showed late central, inconsistent changes (increases in DRG at 14 days: saline vs BOTOX 5U p=0.016 and sham vs BOTOX 5U p=0.009, but decreases in the SC at 14 days: saline vs BOTOX 2U p=0.047 and saline vs BOTOX 5U p=0.009, sham vs BOTOX 5U p=0.028), whereas no significant changes were noted in the bladder for this marker.

Sympathetic markers: NPY expression increased in the DRGs both at 7 and 14 days in animals treated with 5U Botox (p=0.028 compared to saline and p=0.009 compared to sham-controls), as well as in animals injected with 2U Botox (p=0.028 and 0.075 respectively compared to saline). Bladder NPY expression showed a similar trend for increase only at 7 days with the 5U dose (p=0.047 compared to saline-controls and 0.016 compared to sham-controls).

Finally, α1A and α1D receptors showed late bladder changes (at 14 days α1A expression decreased: saline vs BOTOX 2U p=0.047 and saline vs BOTOX 5U p=0.009, while α1D expression increased: saline vs BOTOX 5U p=0.009) and appeared to follow changes in the spinal cord (7 days: saline vs BOTOX 5U p=0.028 for α1A) and the DRG (7 days: sham vs BOTOX 2U p=0.027 and sham vs BOTOX 5U p=0.047 for α1A, sham vs BOTOX 2U p=0.046 and sham vs BOTOX 5U p=0.008 for α1D).

Interpretation of results

Expression of markers of cholinergic, sympathetic and sensory control of the lower urinary tract in the SC/DRGs showed changes following bladder injection of onaBotulinumtoxinA. The explanation for this central effect remains to be elucidated. After skeletal muscle application of radioiodine labeled botulinum toxin, radioactivity could be found in motoneurons, the authors proposing a retrograde transport of the toxin as an explanation for such finding. The viability of such a hypothesis has not been

tested to date. Nevertheless, a recent animal report suggested that both pre- and post-ganglionic nerve fibres associated with intramural ganglia can be affected by bladder BoNT/A.
A neuroplastic effect, possibly centrally mediated, has been also suggested by the partial post-BoNT/A restoration of the impaired levels of urothelial and suburothelial receptors in overactive bladders, and could explain our findings. Additionally, secondary changes in the central nervous system have been proposed following treatment with BoNT/A in pain syndromes.

Concluding message

Our findings from normal rats suggest that bladder injections of Onabotulinumtoxin A may be followed by changes in the expression of sensory, sympathetic and cholinergic markers important in the regulation of bladder function at the DRG/SC level. Parallel bladder changes were noted for tachykinin expression and, to a lesser degree, for the NPY and VAChT expression, whereas adrenergic receptor bladder changes appeared to follow those at the SC and DRGs. Further experiments need to confirm these results in bladder dysfunction states and investigate a neural plasticity as opposed to a direct causative effect of the toxin.

Disclosures

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