Review - Neuro-urology – Voiding Dysfunction

Proposed Mechanism for the Efficacy of Injected Botulinum Toxin in the Treatment of Human Detrusor Overactivity

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1. Botulinum toxin injections in the overactive human bladder: clinical aspects

Over the past 6 years, the use of Botulinum-A neurotoxin (BoNT/A), the most potent poison known to man, has been pioneered in the treatment of lower urinary tract symptoms (LUTS) such as frequency and urgency incontinence due to intratable neurogenic (NDO) or idiopathic (IDO) overactivity of the detrusor smooth muscle of the
bladder. The treatment was introduced on the theory that BoNT/A would temporarily block the presynaptic release of acetylcholine (ACh) from the parasympathetic innervation and produce a paralysis of the detrusor smooth muscle [1], comparable to its mode of action in skeletal muscle [2]. However, BoNT/A injections have been shown to increase bladder capacity, volume at first reflex detrusor contraction, and bladder compliance [3,4] as well as to induce changes in detrusor function with decreases in detrusor pressures during bladder filling and voiding [3–5]. These urodynamic changes underlie the remarkable symptomatic improvements in frequency and incontinence that patients are reporting [3,5]. Prominent in patients’ clinical responses to BoNT/A is an amelioration in their pathological sensation of urgency [5], a sensation believed to be afferently mediated [6]. These benefits are maintained for a mean of 9–11 mo after treatment [3,7]. Comparable responses have been demonstrated in patients with intractable NDO of various spinal etiologies and in patients with IDO [5,8].

2. Role of the sensory urothelium/suburothelium in the pathophysiology of detrusor overactivity

Novel findings support a role for the suburothelial innervation expressing the capsaicin receptor TRPV1 [9], the purinergic receptor P2X3 [10], and/or the sensory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) [11] in the pathophysiology of human detrusor overactivity (DO). Patients with NDO and, to a lesser degree, those with IDO were found to have increased TRPV1- and P2X3-immunoreactive suburothelial innervation compared to controls [12]. Intravesical instillations of the C-fiber toxin resiniferatoxin in NDO patients resulted in dramatic decreases of TRPV1- and P2X3-immunoreactive fibers in clinical responders only [9,10] and produced significant improvements in LUTS and urodynamic parameters in patients with IDO [13]. Women with IDO were found to have increased density of suburothelial SP- and CGRP-immunoreactive fibers compared to controls [11].

Similarly, urothelial release of neurotransmitters (e.g., ACh, ATP) [14] and neuropeptides including SP [15,16] and the expression of receptors (TRPV1, P2X3) [17,18] strongly imply a role for the urothelium in human bladder mechanosensation. The discovery of a suburothelial nexus of myofibroblasts or interstitial cells [19,20], which are extensively linked by gap junctions [20,21] and may respond to ATP in a mode similar to the activation of ATP-gated P2Y receptors [22], led to the proposal that they may be the substrate for a stretch-receptor organ. This new knowledge and recent clinical and laboratory observations suggest that the mechanism of action of BoNT/A in the overactive bladder may be more complicated than originally believed.

3. Evidence for an effect of BoNT/A on afferent pathways

Despite the long established view that the effect of BoNT/A is selective for ACh-containing nerves inhibiting the presynaptic vesicular ACh release after cleavage of the synaptosomal-associated protein with a molecular weight of 25 KDa (SNAP-25) [2], in vivo and in vitro evidence is now emerging that BoNT/A has additional inhibitory effects on neuropeptides, neurotransmitters, and receptors mediating sensory neurotransmission. In cultured rat dorsal root ganglion (DRG) cells, BoNT/A was shown to induce a delayed, long-lasting inhibition of the release of SP, following the initial cleavage of SNAP-25 [23]. In another study of primary DRG cells, BoNT/A inhibited the protein kinase C (PKC) mediated SNARE-dependent exocytosis of TRPV1 to the plasma membrane [24]. BoNT/A also blocked the release of glutamate in an animal model of inflammatory pain [25], affecting specifically neurogenic inflammation mediated by the release of SP, a neurotransmitter known to be colocalized with glutamate.

4. Evidence for an effect of BoNT/A on bladder afferent pathways

Animal studies first provided evidence for bladder afferent neuromodulation by BoNT/A via the purinergic or TRPV1-mediated pathways. In a rat model of spinal NDO, BoNT/A significantly reduced the abnormal distension-evoked urothelial release of ATP [26]. BoNT/A has also been reported to reduce DO evoked by application of ATP or capsaicin in rat bladders in vivo [27]. In a rat bladder pain model, intravesical BoNT/A administration produced significant improvement in the intervals between bladder contractions in parallel with a reduction of the release of CGRP from the superficial bladder layers [28].

A recent study of biopsies from a mixed population of NDO and IDO patients treated with intradetrusor BoNT/A showed no change in the overall
suburothelial neuronal population both during clinical response [12] and clinical relapse time [29], but it did show progressive decrease and ultimate normalization of P2X₃ and TRPV1 suburothelial nerve immunoreactivity, suggesting that BoNT/A affects sensory receptors’ expression in suburothelial fibers [12]. The fastest change was in P2X₃-immunoreactivity, which correlated with improvements in patients’ sensation of urgency [12]. In support of a possible BoNT/A-induced phenotypic change of bladder afferents, bladder levels of neurotrophic growth factor (NGF) in patients with NDO were reported to be reduced significantly after BoNT/A treatment [30]. Such effect may also explain the absence of significant nerve sprouting in the detrusor after successful BoNT/A treatment [31], unlike that seen in skeletal muscle.

5. Mechanism of BoNT/A’s action in the overactive human bladder

Diffusion of the toxin solution from the detrusor injection sites into the suburothelium has already been demonstrated [32]. Therefore, we propose that BoNT/A injected in the overactive human bladder has a complex inhibitory effect on vesicular release of excitatory neurotransmitters and on the axonal expression of other SNARE-complex-dependent proteins in the urothelium/suburothelium, which are important in mediating those intrinsic or spinal reflexes thought to cause DO.

We propose that the immediate effect of BoNT/A is due to peripheral afferent desensitization and involves inhibition of the vesicular release of ACh, ATP, and SP and of the axonal expression of TRPV1 from the urothelium [14] and suburothelial nerve endings [19,33] (Fig. 1). Furthermore extending the findings that the recently described suburothelial myofibroblasts act as an integrating stretch-receptor organ [19], we propose that BoNT/A induces phenotypic changes on these cells including inhibition of expression of purinergic and proposed SP receptors—assuming a homology of myofibroblasts with the interstitial cells of Cajal [34]—and a reduction in the expression of contractile and gap junction proteins.

The urothelial release of ACh and ATP on bladder filling has been found to increase with aging [14] and in spinal NDO [26], implicating an abnormal release of these neurotransmitters in the pathophysiology of DO. Prevention of SNAP-25 phosphorylation via PKC-mediated pathways could explain the inhibitory effect of BoNT/A on the release of ACh from the urothelium and suburothelial nerve endings, similar to the mechanism proposed for the detrusor [35] based on earlier studies of the effect of BoNT/A on PKC facilitatory pathways of transmitter release [36,37]. By inhibiting urothelial ACh release, BoNT/A may block its proposed excitatory effect on suburothelial afferent and detrusor parasympathetic nerve endings during urine storage, thus affecting the pathophysiology of DO by a mechanism similar to that suggested for oral anticholinergics [38]. Inhibition of the increased urothelial ATP release would reduce its proposed excitatory effect on suburothelial and urothelial P2X₃ receptors and on the P2Y receptors on the myofibroblast network. ATP has been shown to potentiate TRPV1 activity via a PKC-dependent pathway involving metabotropic P2Y receptors [39]. Thus, decreased release of ATP after BoNT/A injection would also minimize potentiation of the TRPV1 receptor.

BoNT/A may also inhibit vesicular SP release from suburothelial nerves. There is evidence that SP shares a common pathway with the muscarinic and purinergic transmitters to activate Ca²⁺-dependent nonselective cation channels (including TRP channels) [40]. SP may also potentiate susceptibility of P2X₃ to ATP via a pathway involving PKC [41]. Inhibition of SP release would reduce activation of TRPV1 and P2X₃ receptors in suburothelial afferents and detrusor muscle, as well as the responsiveness of the myofibroblast network to afferent signaling via decreased activation of proposed NK1 receptors on these cells [34].

Because the axonal expression of TRPV1 depends on interactions with proteins of the SNARE complex [24], destabilization of the complex following cleavage of SNAP-25 by BoNT/A will affect TRPV1 expression in peripheral nerve axons. A similar effect on P2X₃-receptor expression may occur: the presence of P2X₃ in terminal afferent boutons with complex synaptic properties [42] and its colocalization with TRPV1 in the CNS have been shown [43], and there is indirect evidence for colocalization with TRPV1 in bladder suburothelial afferents [9,10].

Were human bladder myofibroblasts demonstrated to express metabotropic purinergic and SP receptors, as has been shown in animals [22], and possess the intermediate filaments alpha smooth muscle actin (α-SMA) and vimentin, as myofibroblasts elsewhere in the body do [44,45], BoNT/A-induced inhibition of P2Y/SP receptor expression and decrease in contractile filaments and gap junctions in these cells [46–49] would result in their reduced activation, contractility, and electrical coupling. Mediation of afferent signaling between the urothelium and the closely apposed nerve
endings would thus be reduced, achieving maximization of the BoNT/A-induced peripheral desensitization.

A secondary effect involves further peripheral inhibition at the synaptic level. Immunofluorescence has demonstrated the long-lasting persistence of BoNT/A-cleaved SNAP-25 (SNAP-25A), which acts as exocytosis inhibitor [2]. SNAP-25A immunofluorescence has been detected up to 40 d after BoNT/A-induced paralysis of mouse motor nerve terminals [2]. Hence, we propose that jamming of the SNARE complex by a persisting presence of SNAP-25A continues for that or an even longer period in humans, further inhibiting the release of SP and the axonal expression of TRPV1- and P2X3-receptor.

Furthermore, the decrease in SP-release would result in attenuation of central sensitization leading to further peripheral desensitization. BoNT/A-induced inhibition of rapid afferent firing has been demonstrated by a reduction of Fos-positive cells in the dorsal horn of formalin-challenged rat models [25]. Increased central c-fos expression has been demonstrated in animal models of NDO [50] and chronic bladder inflammation [51]. A decrease in central SP-receptors has been shown to reduce

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**Fig. 1** - A schematic diagram of identifiable ultrastructural components of the human bladder wall (bl, basal lamina of urothelium; mf, myofibroblast layer; det, detrusor muscle; see [60] for more detailed explanation of ultrastructural components). Superimposed are the known or proposed location of receptors and sites of release of neuropeptides and growth factors involved in bladder mechanosensation. A complex interaction between the release of neurotransmitters and actions on respective receptors located on structural constituents is proposed. All connections identified by arrows are thought to be upregulated in detrusor overactivity and may be reduced with the peripheral afferent desensitization that follows injections of BoNT/A. **Thin solid arrows**, the proposed pathways whereby vesicular ATP release from the urothelium activates urothelial or suburothelial receptors (P2X3-P2Y) or potentiates the response of urothelial/suburothelial receptors (TRPV1) to irritative stimuli; **thin dashed arrows**, proposed pathways whereby vesicular ACh release from the urothelium or suburothelial nerves activates suburothelial or detrusor muscle muscarinic ACh receptors; **thick dashed arrows**, proposed pathways whereby suburothelially released SP acts on NK1 receptors on the myofibroblast cell layer or potentiates the activation of TRPV1-P2X3 receptors in suburothelial afferents (SP is also known to affect the expression of NGF and vice versa); **thick solid arrows**, NGF is thought to affect the expression of urothelial and/or suburothelial TRPV1.
spinal c-fos expression and bladder overactivity [52]. In addition, consequent on the postulated decrease in SP-induced neurogenic inflammation, reduced peripheral production and retrograde uptake of neurotrophic factors to the DRG/spinal cord would result in decreased production of TRPV1/P2X3 in DRGs and their antegrade transport to bladder suburothelial afferents. Because NGF also may activate TRPV1 on small afferent nerves [53], which can in turn promote the vesicular release of SP via an ATP-P2Y mediated pathway [54], a reduction in NGF production, already shown in BoNT/A treated human bladders [30], could lead to further peripheral desensitization. Such proposed cascade mechanisms of neural plasticity might explain the longer-lasting effect of BoNT/A in the overactive bladder compared to its duration of action at other sites.

C-fiber afferents constitute the majority (two-thirds) of bladder afferents. In addition, experimental findings suggest that it is the neuroplasticity of these afferents that is involved in both the reorganization of the micturition reflex in NDO [55,56] and the intrinsic bladder wall reflexes in IDO. However, the role of Aδ-fibers cannot be ignored. Following injections of a BoNT/A endopeptidase conjugate into the intrathecal L4–L5 space of anaesthetized rats, recordings from C-fibers from DRG neurons closest to the zone of the spinal cord injection showed a reduction in activity by 75.8% [57]. The Aδ-fiber-evoked activity was also reduced, although to a lesser extent (42.3%), whereas the Aβ-fiber-evoked responses were unchanged. Following intradetrusor BoNT/A injection patients experience a reduction in sensation of urgency but retain sensation of bladder filling, which could possibly be attributed to a relative sparing of Aδ-fiber activity.

6. BoNT/A and the efferent bladder pathways

Although the emphasis of this proposed mechanism has been that the bladder afferent signaling is affected by BoNT/A, it seems highly likely that its effect on efferent pathways is also considerable. Under high frequencies of electrical field stimulation (EFS) thought to mimic the effect of the parasympathetic nerve overactivity that may occur in patients with DO, detrusor strips removed from rat bladders that had been treated with BoNT/A displayed a significant transient decrease in the neuronal release of ACh compared to strips from sham-treated animals [58]. A subsequent in vitro study using similarly high EFS frequencies concluded a dual inhibition of ACh and ATP release from bladder parasympathetic motor innervation [35].

In clinical studies, the post-BoNT/A decreases in detrusor pressures during both the filling and voiding phases, in conjunction with post-treatment increases in post-void residual urine volumes [3–5] suggest an inhibitory effect of BoNT/A on the motor innervation of the detrusor smooth muscle. A study of the sensory immunohistochemical changes following BoNT/A injection in the human DO bladder found no correlation between the changes in suburothelial P2X3- and TRPV1-immunoreactivity and the changes in maximum filling detrusor pressures or maximum cystometric capacity [12]. A significant correlation with changes in urgency, however, suggested an independent effect [12]. Thus, a synergistic effect of BoNT/A on afferent and efferent pathways is proposed.

Several nonadrenergic noncholinergic (NANC) neurotransmitters, such as the vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), and leucine enkephalin (ENK), have been identified in peripheral efferent bladder pathways, and it has been postulated that these may modulate efferent inputs to the lower urinary tract, but with a mainly inhibitory effect [56]. Evidence of a significant effect of BoNT/A on the release of these transmitters is inconclusive, however, and their putative role in detrusor function remains largely unclear [59]. Further research is needed to determine the effect of BoNT/A on the balance of modulatory transmitter actions on both the afferent and efferent (cholinergic and noncholinergic) pathways of the micturition reflex.

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References


