Excitatory Cholinergic and Purinergic Signaling in Bladder Are Equally Susceptible to Botulinum Neurotoxin A Consistent with Co-Release of Transmitters from Efferent Fibers

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ABSTRACT

Mediators of neuromuscular transmission in rat bladder strips were dissected pharmacologically to examine their susceptibilities to inhibition by botulinum neurotoxins (BoNTs) and elucidate a basis for the clinical effectiveness of BoNT/A in alleviating smooth muscle spasms associated with overactive bladder. BoNT/A, BoNT/C1, or BoNT/E reduced peak and average force of muscle contractions induced by electric field stimulation (EFS) in dose-dependent manners by acting only on neurogenic, tetrodotoxin-sensitive responses. BoNTs that cleaved vesicle-associated membrane protein proved to be much less effective. Acetylcholine (ACh) and ATP were found to provide virtually all excitatory input, because EFS-evoked contractions were abolished by the muscarinic receptor antagonist, atropine, combined with either a desensitizing agonist of P2X1 and P2X3 or a nonselective ATP receptor antagonist. Both transmitters were released in the innervated muscle layer and, thus, persisted after removal of urothelium. Atropine or a desensitizer of the P2X1 or P2X3 receptors did not alter the rate at which muscle contractions were weakened by BoNT/A. Moreover, although cholinergic and purinergic signaling could be partially delineated by using high-frequency EFS (which intensified a transient, largely atropine-resistant spike in muscle contractions that was reduced after P2X receptor desensitization), they proved equally susceptible to BoNT/A. Thus, equi-potent blockade of ATP co-released with ACh from muscle effecters probably contributes to the effectiveness of BoNT/A in treating bladder overactivity, including nonresponders to anticholinergic drugs. Because purinergic receptors are known mediators of sensory afferent excitation, inhibition of efferent ATP release by BoNT/A could also help to ameliorate acute pain and urgency sensation reported by some recipients.
can be co-released from nerves (Zimmermann, 2008), clinical evidence that BOTOX often attenuates urgency and pain before urodynamic improvement led to a suggestion that inhibition of ATP release from distended urothelium precedes any cholinergic block (Khera et al., 2004). A direct action of BoNT/A on sensory afferents has also been proposed (Apostolidis et al., 2006) because it blocks the release of pain and inflammatory mediators from cultured sensory neurons (Apostolidis et al., 2006; Meng et al., 2009) and brain slices (Meng et al., 2009).

Herein, electrically induced contractions of rat bladder strips were investigated to determine their susceptibility to BoNT serotypes that cleave different proteins essential for synaptic vesicle fusion. Moreover, contributions of cholinergic and purinergic inputs to motor signaling were determined and evidence was obtained that ACh and ATP (or metabolites) are co-released from the same effenter nerve fibers by showing that they are equally susceptible to inhibition by BoNT/A. Possible mechanisms whereby such blockade could contribute to attenuation of sensory and motor signaling in bladder are discussed.

Materials and Methods

**Materials.** PPADS was purchased from Tocris Bioscience (Eching, Germany). BoNTs were obtained from Metabiologics Inc. (Madison, WI) as diachains, except BoNT/E, which was activated with trypsin before use. Specific neurotoxicities of BoNTs after intraperitoneal injection into mice (after nicking, where required) were determined by the manufacturer ($\times 10^6$ median lethal doses per mg): BoNT/A, 2.5; BoNT/B, 0.9; BoNT/C1, 0.2; BoNT/D, 0.6; BoNT/E, 0.6; BoNT/F, 0.2; and BoNT/G, 0.1. Atropine, $\alpha$-methylene-adenosine-5’-triphosphate ($\alpha$MeATP), and buffer salts were supplied by Sigma-Aldrich (Dublin, Ireland).

**Tissue Preparation.** Bladders were excised from adult rats (2–4 months), cut into longitudinal strips, and mounted in 10 ml tissue baths (Myobath; World Precision Instruments, Inc., Stevenson, UK) in Krebs/Ringer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 23.8 mM NaHCO$_3$, 1.2 mM KH$_2$PO$_4$, 2.5 mM CaCl$_2$, and 11.7 mM glucose) and gassed with 5% CO$_2$/95% O$_2$; in some experiments, were determined by the manufacturer (intraperitoneal injection into mice (after nicking, where required) (Madison, WI) as dichains, except BoNT/E, which was activated by Metabiologics Inc., Germany). BoNTs were obtained from Metabiologics Inc.

**Functional Studies.** Because of limitations in the amounts of BoNTs available, a large static bath was used, as done previously by us (Lawrence et al., 2007) and others (Simpson, 1980) to study the action of these proteins on innervated skeletal muscle. This recording format gives a lower efficiency of EFS for nerve (and muscle) because of reduced shunt resistance; hence, the pulse frequencies and widths required to elicit muscle contractions (see figure legends for details) are larger than those reported in studies using smaller baths (e.g., MacKenzie et al., 1982). It is noteworthy that others have successfully used conditions almost identical to those described here (Dveksler et al., 1985). Moreover, to minimize the possibility of direct muscle stimulation, care was taken to ensure that the current amplitude used was no more than 5-fold greater than the threshold for triggering nerve-mediated contractions. The strips were tensioned (0.4 g) and equilibrated (1 h) before EFS, via Ag/AgCl electrodes introduced into the top and bottom of the bath, respectively. Constant current EFS (250 mA) was supplied from a Multistim DE300 stimulator (Digitimer, Welwyn Garden City, U.K.) in square pulses (see figure legends for details); polarity was switched between consecutive pulses. Muscle tension was recorded by using isometric force transducers (FOR525) linked to a LabTrax 4/16 data acquisition system (World Precision Instruments, Inc.). Values for peak changes in muscle tension (i.e., the difference between the maximum force generated during EFS and the basal level immediately before EFS) and contractile area (i.e., the area under the force-time trace) during EFS were measured by using DataTrax 2 software (World Precision Instruments, Inc.).

Results

**BoNT/A Inhibits EFS-Evoked TTX-Sensitive Contractions of Rat Bladder Strips: Other SNAP-25-Cleaving BoNTs Are Equally Effective, Whereas the Vesicle-Associated Membrane Protein-Inactivating Serotypes Proved to Be Less Potent.** With the aim of dissecting neurotransmission into distinct components and delineating their sensitivity to BoNTs, rat bladder strips were stimulated by using a variety of pulse protocols before and upon exposure to these inhibitors of synaptic vesicle fusion. During 1-min excitation with 50-ms pulses at 4 Hz, muscle contractions were most intense during the first seconds and then receded (Fig. 1A, a and b). Raising the pulse frequency to 8 Hz increased contractile force over the whole 1-min stimulation period, particularly at the start. No contractions were observed if strips were stimulated with much narrower pulses, 1.5 ms (not shown), unless the pulse frequency was increased, e.g., to 32 Hz, which induced a transient peak of tension followed by a more slowly declining signal (Fig. 1A, a and b, and see later). A second train produced contractions profiles similar to the preceding signals (Fig. 1Ac) but, in general, of lower tension. Accordingly, contraction force declined steadily during a repetitive series of EFS (Fig. 1B). Elicited responses were caused by nerve stimulation because 1 $\mu$M TTX dramatically reduced the size of contractions (compare trace d with b in Fig. 1A), indicating a requirement for TTX-sensitive Na$^+$ channels that are expressed in neurons but not muscle, as shown previously under similar EFS conditions (Dveksler et al., 1985). Application of 3 nM BoNT/A greatly accelerated the decline in evoked muscle tension, which was virtually abolished within 2 h (Fig. 1Bb). It is noteworthy that subsequent inclusion of 1 $\mu$M TTX exerted minimal effect on the residual response in BoNT/A-treated strips (Fig. 1Cb), unlike control muscle that retained a large TTX-sensitive signal for more than 4 h (Fig. 1B, a and Ca). Thus, in rat bladder BoNT/A selectively inhibits release of excitatory neurotransmitters from a source excited by the opening of TTX-sensitive voltage-activated Na$^+$ channels. It is noteworthy that the rate of neuroparalysis by BoNT/A is concentration-dependent (Fig. 1D). Similar relationships were observed for BoNT/E and BoNT/C1, with the latter being slightly faster (Fig. 1E). BoNT/D, BoNT/F, and BoNT/G were very slow acting, and no dose–response relationship was evident. It is noteworthy that the relationships between concentration and paralysis time for BoNT/E and BoNT/C1 were fit well by a power function (Fig. 1E; Table 1), a property well established for the action of BoNTs at the neuromuscular junction (Simpson, 1980; Lawrence et al., 2007). Although BoNT/A showed more variation, the data could also be fitted with a power function (Table 1). Such concentration dependencies are in accordance with BoNTs being directly responsible for a loss of muscle tension after their application to bladder strips.

**Partial Delineation of Cholinergic and Purinergic Contractile Components.** Shortening of the pulse width induced an initial transient spike in tension preceding a sustained phase; increasing frequency intensified both fea-
Fig. 1. BoNT/A inhibits TTX-sensitive neuromuscular transmission in rat bladder strips: the rate of paralysis is concentration-dependent, as is the blockade by BoNT/C1 or BoNT/E. A, typical traces of muscle tension for two bladder strips (a and b) stimulated for 1 min with 50-ms pulses at 4 or 8 Hz or 1.5-ms pulses at 32 Hz. c and d, responses to second trains, applied 12 min after the first, without (c) or with (d) the addition of 1 μM TTX between trains. B and C, muscle strips were stimulated by EFS (1.5 ms, 32 Hz) in 1-min trains repeated every 5 min while recording tension profiles in the absence (Ba) or presence (Bb) of 3 nM BoNT/A; arrow indicates time of addition. In C profiles are magnified on the time axis; ai and bi were recorded immediately before addition of 3 nM BoNT/A, aii and bii were recorded 2 h later, and aiii and biii were recorded after subsequent addition of 1 μM TTX. D and E, strips were stimulated by 1-min trains of EFS (50 ms, 8 Hz) repeated every 5 min. The contractile area (see Materials and Methods) was normalized as a percentage of the signal immediately before addition of BoNT/A: 0.3 nM (△), 0.5 nM (●), 1 nM (▲), n = 3, 2 nM (▲), n = 3, 3 nM (■), n = 5, or 10 nM (○), n = 4. Control recordings (□) were quantified after the addition of bovine serum albumin (10 mg/ml in bath). E, elapsed time (± S.E.M.; n ≥ 3) for contractile area to be reduced by 50% after the addition of BoNT/A (○), BoNT/C1 (▲), BoNT/D (■), BoNT/E (□), BoNT/F (●), or BoNT/G (○). Strips were obtained from at least two different bladders for each point.

TABLE 1

<table>
<thead>
<tr>
<th>BoNT</th>
<th>A</th>
<th>B</th>
<th>R²</th>
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<tbody>
<tr>
<td>A</td>
<td>97.3 ± 3.8</td>
<td>-0.28 ± 0.04</td>
<td>0.747</td>
</tr>
<tr>
<td>C1</td>
<td>73.5 ± 2.0</td>
<td>-0.27 ± 0.02</td>
<td>0.929</td>
</tr>
<tr>
<td>E</td>
<td>89.2 ± 2.7</td>
<td>-0.24 ± 0.03</td>
<td>0.955</td>
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Power functions fit to the relationship between concentration and time to paralysis of rat bladder strips by various BoNTs. Data sets were fit to the equation $y = A(x^B)$ and $R^2$ calculated by using Prism software (GraphPad Software Inc., San Diego, CA).

Cholinergic and Purinergic Signals Are Blocked by BoNT/A with Equal Efficacy. A proposed involvement of ACh and ATP in the spike and sustained phases was probed using, respectively, atropine to antagonize muscarinic receptors and αβMeATP to activate and desensitize P2X receptors. It is noteworthy that only the sustained phase was reduced significantly by atropine (Fig. 2B); indeed, atropine often had little effect when EFS trains were reduced in duration from 1 min to 10 s, thereby eliciting only the spike (e.g., Fig. 2B). In contrast, αβMeATP selectively removed the spike (Fig. 2C). Sequential application of αβMeATP and then atropine virtually abolished evoked contractions, indicating that ACh plus ATP accounts for virtually all excitatory signaling in rat bladder (Fig. 2D). Whereas atropine reproducibly inhibited the sustained phase more effectively than the initial spike elicited by stimulation with 1.5-ms pulses at 32 Hz (Fig. 2E; p < 0.001, n = 8), upon stimulation with 50-ms pulses at 8 Hz the difference in reduction was not statistically significant (Fig. 2F; n = 8). On the other hand, αβMeATP was reliably more effective in reducing the initial spike than the sustained response after excitation with narrow pulses at high frequency (Fig. 2G; p < 0.05, n = 9) but, again, no significant difference in the reduction of each phase was noted for stimulation with 50-ms pulses at 8 Hz (n = 9). Thus, reducing the width and increasing the frequency of electrical pulses elicits a partial temporal separation of the purinergic and cholinergic components of the excitatory input.

The Innervated Muscle Layer Can Produce Excitatory ATP. Further evidence for excitatory purinergic signals was obtained by sequential application of atropine then a broad-range P2 antagonist, PPADS. Atropine induced a rapid reduction in EFS-evoked muscle tension to a level that was then sustained for several hours (Fig. 3A), but subsequent addition of PPADS after the atropine-resistant responses had stabilized caused a progressive further decrease in muscle tension (Fig. 3B). It is noteworthy that the atropine-sensitive and -resistant signals were preserved in the muscle layer after urothelium removal (Fig. 3C) and the latter were inhibited by PPADS (Fig. 3D). This confirms that both excitatory ATP and ACh can be released from a source within the innervated muscle layer, although it does not exclude urothelium serving as an additional source of these transmitters.

Cholinergic and Purinergic Signals Are Blocked by BoNT/A with Equal Efficacy. The relative susceptibilities...
to BoNT/A of cholinergic and purinergic signals were determined by recording paralysis during sequential 1-min trains of EFS, using various pulse widths and frequencies. B–D, profiles obtained from bladder strips subjected to EFS trains of pulses 1.5 ms wide at 32 Hz. B, trains were alternated between 60 and 10 s in length and atropine was added (1 μM final concentration) at the time indicated. C, EFS was applied for 1 min then αβMeATP was added at the indicated times (bringing the bath concentration up to 100 μM) before applying further trains of EFS. D, αβMeATP addition was followed by two trains of EFS before adding 1 μM atropine and a further EFS train thereafter. E–H, peak heights and contractile areas were measured for sequential responses to EFS (1.5-ms pulses at 32 Hz (E and G) or 50-ms pulses at 8 Hz (F and H)), and the subsequent signals were expressed as a percentage of their antecedents. Where indicated, 1 μM atropine (E and F) or two sequential, additive doses of 50 μM αβMeATP (G and H) were included between the sequential bouts of EFS. For each bar, n = 7; p values were calculated by using Student’s t test. A minimum of four bladders was used per bar. An equal number of control and agonist/antagonist-treated strips were obtained from each bladder.

In summary, EFS causes co-release of ACh and ATP from the same TTX-sensitive source in the rat bladder muscle layer, in the absence or presence of urothelium, and each is inhibited with equal efficacy by BoNT/A. High-frequency stimulation intensifies an initial spike in muscle tension that seems to be caused largely by purinergic signaling, but this remains susceptible to BoNT/A. Unlike the vesicle-associated membrane protein-cleaving toxins, others acting on SNAP-25 (BoNT/C1 and BoNT/E) are also effective inhibitors of excitatory transmission in the bladder and, thus, could potentially be used as therapeutics for OAB.

Discussion

The ability of BoNT/A to inhibit transmitter release has been exploited for treating detrusor overactivity and is well established for its management of skeletal muscle spasms and spasticity (Jankovic et al., 2008). It has proved very effective in reducing voiding frequency and incontinence episodes attributable to lowered bladder pressure during filling and voiding, together with improvements in bladder capacity and compliance (Apostolidis et al., 2006; Schurch and Dykstra, 2009). Patients also report major benefits in reduced urgency sensation. Consequently, much effort has been expended on investigations into the possible effects of BoNT/A on the complex array of cholinergic and additional signaling molecules acting in healthy and diseased bladder (Apostolidis et al., 2006; Chancellor et al., 2008).

Electrically induced atropine-resistant bladder contractions have been known to be blocked by BoNT/A (MacKenzie et al., 1982; Smith et al., 2003), although less effectively than by BoNT/C1, BoNT/C1, and BoNT/E herein, probably because of the lower BoNT concentrations used.
previously (Smith et al., 2003). It is noteworthy that neither natural (Fig. 1) nor recombinant BoNT/D (unpublished observation) proved to be as effective as BoNT/A (BoNT/C1 or BoNT/E), in contrast to a previous report (Smith et al., 2003). The reasons for such a discrepancy are not clear, and direct comparison of the data herein with those in the earlier report is not possible because of differences between the stimulation paradigms used. Purinergic excitation was confirmed herein by using two strategies: 1) P2X desensitization with αβMeATP (Kasakov and Burnstock, 1982) and 2) P2 antagonism with PPADS (North, 2002). Both virtually abolished atropine-insensitive contractions, as expected from an absence of atropine-resistant contrac-
tility in mice lacking P2X1 (Vial and Evans, 2000). Moreover, BoNT/A blocked the cholinergic and purinergic sign-
aling equally, consistent with co-release of ATP and ACh from nerve fibers, possibly the same vesicles [as in other tissues (Volknadl and Zimmermann, 1986; Richardson and Brown, 1987; Zimmermann, 2008)]. Urothelium also releases ATP, which is stimulated by bladder distension (Ferguson et al., 1997), and it has been reported that BoNT/A inhibits urothelial ATP release in a rat spinal cord injury model (Khera et al., 2004). However, it remains unclear how BoNT/A could block release of any signaling molecules from uroepithelial cells because they do not express either SV2 or SNAP-25 [see below and Chancellor et al. (2008) for review]. In fact, immuno-histochemistry has indicated that SNAP-25 is restricted to fibers innervating detrusor muscle (Schulte-Baukloh et al., 2007) and SV2 occurs in neuro-varicosities apposing P2X1 receptor clusters (Hansen et al., 1998). Herein, several pieces of evidence indicate that rat bladder efferents are the source of ATP that is blocked by BoNT/A. Atropine-resistant puri-
nergic signaling was preserved in bladder muscle stripped of urothelium, albeit at a lower level that may reflect some influence of the latter on neuromuscular transmission (see below). Nevertheless, contractile re-
sponses were virtually abolished by TTX that blocks nerve fiber conductance without affecting smooth muscle excit-
bility (Burnstock, 2007) or afferent fiber excitation (Yo-
shimura et al., 2001) and has minimal effect on the urothe-
lial release of ATP (Ferguson et al., 1997; Sadananda et al., 2009). BoNT/A specifically inhibited TTX-sensitive contractions, consistent with the minor TTX-resistant component elicited by direct muscle stimulation (Burn-
stock et al., 1978).

Although neuromuscular transmission in healthy human bladder is >95% atropine-sensitive, the purinergic compo-
nent can increase to 40% in cases of interstitial cystitis (Pa-
lea et al., 1993) or idopathic detrusor instability, possibly because of altered P2X receptor expression (O’Reilly et al., 2002) or reduced expression of ectopic nucleotidases in the detrusor (Harvey et al., 2002). Consequently, abnormally elevated purinergic excitatory signaling may underlie symp-
toms of bladder instability that are resistant to anticholin-
ergics; hence, P2X1 receptors are being considered as a drug target for the relief of detrusor overactivity (Ruggieri, 2006). Most likely, the effectiveness of BoNT/A in the management of OAB, even in nonresponders to antimuscarinics, is caused by its inhibition of the co-release of ATP with ACh.

An intriguing aspect of BoNT/A therapy is the reported alleviation of sensory urgency and pain that seems to be independent of muscle relaxation. A mechanism proposed in a recent review (Chancellor et al., 2009) is that inhibition of efferent ATP release by BoNT/A leads to reduced excitation of sensory afferents. P2X3, enriched on transient receptor

![Fig. 4. BoNT/A inhibits cholinergic and purinergic components of neuromuscular transmission at equivalent rates. Bladder strips were stimulated by 1-min trains of EFS with 50-ms pulses at 8 Hz (A and B) or 1.5-ms pulses at 32 Hz (C and D), separated by 5-min recovery inter-
vals, in the absence (○) or presence of 1 μM atropine (■, A and C) or 100 μM αβMeATP (▲, B and D; added in two steps as in Fig. 2C) for 30 min before adding 10 nM BoNT/A (A) or 3 nM BoNT/A (B–D). Note that inclusion of atropine or αβMeATP reduced contractile area (cf. Fig. 2). Signals recorded after BoNT/A addition (at t = 0 min) were normalized as a percentage of the response immediately before exposure to the toxin. Mean values (± S.E.M.) are plotted for data obtained by using four or more bladder strips from at least two different animals; control and atropine/αβMeATP-treated bladder strips were always obtained from the same rats.](https://jpet.aspetjournals.org/content/1084/4/578)
potential vanilloid type 1- and calcitonin gene related peptide-expressing bladder sensory afferents (Cockayne et al., 2000; Apostolidis et al., 2006), is thought to be important for sensing ATP released from distended urethra and signaling bladder filling (Burnstock, 2007). However, P2X3 expression becomes raised during inflammation (Dang et al., 2008) or OAB (idiopathic detrusor overactivity and, especially, NDO) and, thus, may also be implicated in increased sensory urgency and pain associated with these conditions. Indeed, bladder capacity before reflex voiding is raised and inflammatory pain responses are reduced in the P2X3 knockout mouse (Cockayne et al., 2000). Although a direct action of BoNT/A on afferents to block the release of pain and inflammatory mediators (Lucioni et al., 2008; Meng et al., 2009) could also contribute to its amelioration of sensory symptoms, studies with radio-labeled BoNT/A showed preferential binding to cholinergic (rather than peptidergic or noradrenergic) terminals innervating mouse ileum (Black and Dolly, 1987). Thus, although direct evidence is lacking for afferent excitation via ATP released from bladder afferents (indeed, several other possible mechanisms to alleviate sensory signaling have also been forwarded (Apostolidis et al., 2006; Chancellor et al., 2008, 2009)), blockade of efferent release of nociceptor-activating ATP offers a cogent explanation for relief by BoNT/A of pain/urgency; this would be in accordance with its established selectivity for cholinergic neurons and antinociceptive outcomes in the urology clinic.

It is noteworthy that this model does not exclude a sensory signaling role for ATP released from urethra but, rather, provides an explanation for the apparent non-cholinergic actions of BoNT/A by its inhibition of purine release from a neural source. Indeed, it is possible that relief of sensory symptoms by BoNT/A arises wholly, or in part, from its inhibition of communication from nerve fibers to the urethra. In fact, the latter has been termed a “sensory web” because it integrates chemical and mechanical stimuli into responses that can excite sensory nerve fibers, a concept summarized below but developed and reviewed in detail by others (Apodaca et al., 2007; Khandelwal et al., 2009; Birder, 2010). Both efferent and afferent fibers are localized in close proximity to (and some within) the urethra, and the epithelial cells express a variety of muscarinic and purinergic receptors; thus, both nerve types can transmit chemical signals to the epithelium. In response, uroepithelial cells can themselves release transmitters, including ATP, nitric oxide, prostaglandin growth factors, and (via a nonvesicular mechanism) ACH. Such transmitters can excite neighboring epithelial cells in a paracrine manner, amplify excitatory signaling via autocrine positive feedback mechanisms (e.g., activation of uroepithelial muscarinic receptors can elicit increased urethral release of ATP), and communicate back to nerves, for example, via elevated ATP release to activate P2X receptors on sensory fibers. In addition, both ATP and ACH are released from urethra upon stretch; thus, mechanical stimuli can supplement and amplify chemical signaling. As noted above, injection of BoNT/A into rat bladders modulates the secretion of neurotransmitters from the epithelium (Khera et al., 2004; Smith et al., 2008), but a direct action of secretion from uroepithelial cells is unlikely because of their lack of its substrate, SNAP-25 (Chancellor et al., 2008). Indeed, evoked release of nitric oxide is altered after BoNT/A injection even though this transmitter is not released by vesicular exocytosis (Smith et al., 2008). Another explanation is that inhibition by BoNT/A of ACH and ATP release from nerve fibers blocks excitation by these transmitters of the urethral sensory web. Alternatively (or additionally), a similar outcome may accrue indirectly from a reduction in the frequency and force of detrusor contraction, because this would mitigate mechanical stimulation of the urethra. A third possibility is that BoNT/A blocks the release from affrent fibers of ATP and other transmitters that stimulate the urethral (Apostolidis et al., 2006; Smith et al., 2008). Some investigators have even suggested that BoNT/A might block afferent signaling in the absence of any detectable effect on bladder contractility (Smith et al., 2008); however, because of the observed large variability in contraction force for strips obtained from different subjects, such an interpretation cannot be made conclusively with the small sample group used in that study. Moreover, it is difficult to reconcile this notion with the preferential binding by BoNTs to cholinergic nerves in the peripheral nervous system (Black and Dolly, 1987), as noted earlier. Rather, by inhibiting efferent release of ACH and ATP, BoNT may inhibit both direct activation of nociceptors (via exocytosed ATP) and indirect activation (possibly with amplification) of the sensory fibers through the urethral sensory web.

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