Insight into New Potential Targets for the Treatment of Overactive Bladder and Detrusor Overactivity

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Key Words
Bladder dysfunction · Overactive bladder · Detrusor overactivity · Therapy · Urothelium

Abstract
Although overactive bladder (OAB) and detrusor overactivity (DO) are not synonyms, they share therapeutic options and partially underlying physiopathological mechanisms. The aim of this overview is to give insight into new potential targets for the treatment of OAB and DO. A narrative review was done in order to reach this goal. Ageing, pelvic floor disorders, hypersensitivity disorders, morphologic bladder changes, neurological diseases, local inflammations, infections, tumors and bladder outlet obstruction may alter the normal voluntary control of micturition, leading to OAB and DO. The main aim of pharmacotherapy is to restore normal control of micturition, inhibiting the emerging pathological involuntary reflex mechanism. Therapeutic targets can be found at the levels of the urothelium, detrusor muscles, autonomic and afferent pathways, spinal cord and brain. Increased expression and/or sensitivity of urothelial-sensory molecules that lead to afferent sensitization have been documented as a possible pathogenesis of OAB. Targeting afferent pathways and/or bladder smooth muscles by modulating activity of ligand receptors and ion channels could be effective to suppress OAB.

Introduction
Overactive bladder (OAB) is a symptoms syndrome characterized by 'urgency, with or without urge urinary incontinence, usually with frequency and nocturia ... if there is no proven infection or other obvious pathology' [1]. This combination of symptoms is suggestive of urodynamically demonstrable detrusor overactivity (DO), defined as 'an urodynamic observation characterized by involuntary detrusor contractions during the filling phase' [1]. OAB is not a synonym of DO and vice versa. Actually, DO is only detected in about half of patients with OAB by conventional technique and, on the other hand, up to 50% of patients having DO did not complain of relevant clinical symptoms [2, 3]. The only currently available tool to link them is urodynamics. Nevertheless, they share therapeutic options and, partially, a virtual common underlying physiopathological mechanism [2, 3].
Ageing, pelvic floor disorders, hypersensitivity disorders, morphologic bladder changes, neurological diseases, local inflammations, infections, tumors and bladder outlet obstruction may alter the normal voluntary control of micturition, leading to OAB and DO [4]. Currently, antimuscarinics represent the first-line treatment in cases of OAB [5–7]. When antimuscarinic therapy fails, other drugs for facilitation of urine storage can be used. Their pharmacological targets can be found at the levels of the urothelium, detrusor muscles, autonomic and afferent pathways, spinal cord and brain [8]. The aim of this study was to carry out an overview of the insight into these potential targets for the treatment of OAB and DO. Thus, a narrative review was done in order to reach this goal.

**Muscarinic Receptors**

The rationale for antimuscarinic treatment is the blockade of muscarinic receptors (MRs) at both detrusor and non-detrusor sites. The mechanism by which antimuscarinic drugs improve the symptoms of OAB remains to be elucidated [8]. M1–M5Rs mediate the metabotropic actions of acetylcholine (Ach) in the nervous system as well as the autocrine functions of this molecule [9]. The autocrine functions of MRs broadly fall into two areas: control of cell growth or proliferation, and mediation of the release of chemical mediators from epithelial cells, ultimately causing muscle relaxation [9]. The former is involved in embryological development, oncogenesis, keratinocyte function and immune responsiveness. The latter regulates contractility of smooth muscle in the vasculature, airways and urinary bladder [9]. M1Rs and M3Rs mediate lymphocyte immunoresponsiveness, cell migration and release of smooth muscle relaxant factors; M2Rs are implicated in stem cell proliferation and development. The autocrine functions of Ach, like those in the nervous system in which it has both excitatory and inhibitory effects on the micturition reflex, involve activation of several MR subtypes [2, 3]. Consequently, the role of these subtypes in autocrine as well neuronal cholinergic systems significantly expands their importance in physiology and pathophysiology.

Release of Ach during the storage phase could be expected to enhance the myogenic contractile activity of the detrusor, which can generate afferent signals. It is suggested that antimuscarinics can decrease bladder afferent activity by blocking MRs in these sites, thereby improving OAB symptoms [10]. The basal non-neuronal release of Ach in the human bladder, at least partly generated by the urothelium, was shown to increase when bladder strips with intact urothelium were stretched, implying that shear stress of the urothelium during distension of the bladder may be one of the releasing mechanisms [11]. Moreover, this non-neuronal Ach release seems to be age-dependent and significantly higher in bladders from old (>65 years) than from young (<65 years) patients [11]. These age-related changes in Ach release may contribute to the increased prevalence of OAB in the elderly. The MR subtypes identified by radioligand binding have been demonstrated to be predominant M2Rs, with a minor population of M3Rs and M1Rs [12]. A significant increase in M2R and M3R immunoreactivity in myofibroblast-like cells was found in bladder specimens from patients with idiopathic DO (IDO) as compared with that in controls, furthermore showing a significant correlation with the urgency score [13]. It has been also reported that afferent nerves are located adjacent to the urothelium, and stimulation of MRs expressed on the urothelium may contribute to the activation of afferent nerves via non-neuronal ATP release, thus reducing urgency [14]. Activation of MRs in the urothelium releases substances that modulate afferent nerves [15]. Ach and ATP released from the urothelium can activate MRs and P2Y receptors, respectively on myofibroblasts that may be involved in the transfer of information between the urothelium and suburothelial afferent nerves [16]. In addition, Ach could enhance myogenic localized activity which may increase firing of afferent nerves [17].

Based on this evidence, it can be assumed that an increase in Ach release from the urothelium and/or upregulation of MRs in the urothelium as well as in suburothelial myofibroblasts may increase afferent nerve activity and contribute to the development of DO. In animal models bladder afferent neurons were found to express different subtypes of MRs (M2R, M3R and M4R), suggesting that processing of sensory information from the bladder appears to be under direct cholinergic control [18]. The differential roles of MR subtypes in local bladder afferent activation remain unclear. An important role in the local cholinergic modulation of bladder afferent activity in normal rats with oxotremorine-M urethane-induced OAB is played by the M2R subtype [19]. Moreover, the stimulation of MR pathways can depress sensory transduction by a mechanism independent of changes in bladder tone, suggesting that Ach could contribute to normal or pathologic bladder sensation [20].

The introduction of selective M3R antagonists has not improved clinical efficacy compared with the old non-
selective antimuscarinics, but has reduced the rate of adverse events mediated by the blockade of cardiac M2Rs (tachycardia) and central M1Rs (cognitive impairment) [2]. There is still a need for safer M3 antagonists for the treatment of OAB. New selective M3R antagonists currently in early discovery and under development have been designed to address these issues [21].

**Ion Channels and Receptors Mechanisms**

The exact mechanisms that underline mechanosensory transduction in bladder afferent terminals remain ambiguous; however, a wide range of ion channels (e.g. TTX-resistant Na⁺ channels, K⁺ channels and hyperpolarization-activated cyclic nucleotide-gated cation channels) and receptors (e.g. TRPV1, TRPM8, TRPA1, P2X(2/3), etc.) have been identified at bladder afferent terminals and implicated in the generation and modulation of afferent signals [3, 22]. Experimental investigations have revealed that expression and/or function of these ion channels and receptors may be altered in animal models and patients with OAB. Some of these ion channels and receptors may be potential therapeutic targets for bladder diseases. In the urinary bladder, activation of K⁺ channels, in particular the large-conductance Ca²⁺-activated K⁺ (BK) channels, opposes increases in excitability and contractility of urinary bladder smooth muscle. The BK channel seems to have a very significant role in reducing both cholinergic- and purinergic-induced contractility and it has been suggested that alterations in BK channel expression or function could contribute to OAB occurrence [23].

It has been shown that phasic contractions of human detrusor are dependent on calcium entry through L-type calcium channels; moreover, BK(Ca) and SK(Ca) channels can modulate human detrusor smooth muscle phasic contractility [24]. Thus, an increasing conductance through K(ATP), BK(Ca²⁺) and SK(Ca²⁺) channels may represent attractive pharmacologic targets for decreasing phasic contractions of detrusor smooth muscle in OAB. A-251179, a potent novel K(ATP) channel opener, was found to increase bladder capacity and to prolong the time between voids without affecting voiding efficiency in a rat model [25]. It can represent an interesting characteristic to be explored for further investigations of K(ATP) channel openers for the treatment of OAB. In in vitro models using guinea pig bladder smooth muscle cells, A-151892, another potent and efficacious K channel opener, was found to suppress phasic, carbachol-evoked and electrical field stimulus-evoked contractility in a glibenclamide-reversible manner [26]. Moreover, A-151892 was found to potently suppress DO in obstructed models of both pigs and rats [26].

Nicorandil, a K(ATP) channel opener with a nitric oxide (NO) donor property, was found to suppress OAB from both neurogenic and myogenic causes in animal models [27]. Thus, nicorandil, currently used clinically to treat ischemic heart disease, appears to be a promising candidate for clinical use in patients with OAB [27].

ZD0947, a novel urinary bladder selective K(ATP) channel opener, was found to cause a significant decrease in carbachol-induced contractions of isolated guinea pig urinary bladder strips [28]. In patients with OAB, ZD0947 showed disappointing results [29].

In the storage phase, mechanical stretch stimulates bladder afferents. For mechanosensory transduction, the presence of mechanosensors is essential in the peripheral sensory systems including sensory nerve endings, urothelium and others. There is increasing evidence that mechanosensitive ion channels, such as degenerin/epithelial Na⁺ channel and transient receptor potential (TRP) channel families, play key roles in the mechanosensory transduction of the urinary bladder. Pharmacological interventions targeting mechanosensitive ion channels may provide a new strategy for the treatment of bladder dysfunction [30]. The mammalian TRP family consists of 28 channels that can be subdivided into 6 different classes: TRPV (vanilloid), TRPC (canonical), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin) [31]. TRP channels are activated by a diversity of physical (voltage, heat, cold, mechanical stress) or chemical (pH, osmolality) stimuli and by binding of specific ligands, enabling them to act as multifunctional sensors at the cellular level. In urology, there is a growing conviction that disturbances in afferent mechanisms are highly important in the pathogenesis of functional problems. Therefore, the TRP family forms an interesting new target to focus on. The activation of the TRPM8 channel, a member of the large class of TRP ion channels, has been reported to be involved in OAB [32], although an endogenous activator has not been identified. In a rat model, a TRPM8 channel blocker (AMTB) was found to decrease the frequency of volume-induced bladder contractions without reducing the amplitude of contraction [33]. These results demonstrated that TRPM8 channel blocker could act on the bladder afferent pathway to attenuate the bladder micturition reflex in the rat. A reduction of the bladder expression of tachykinins and
TRPV1 and P2X3 purine receptors could explain the beneficial effects of some traditional Chinese herbal mixture extracts used for the treatment of OAB symptoms [34, 35].

It has been found that intrathecal administration of resiniferatoxin can effectively reduce DO in animal models of complete chronic spinal cord transection by suppressing the activity of TRPV1 expressing afferent fibers [36]. These findings might be relevant for the management of patients with spinal cord injuries.

A suburothelial layer of myofibroblasts (interstitial cells) that form a functional syncytium through C-43 gap junction can be identified in the bladder wall [37, 38]. These myofibroblasts make close appositions to unmyelinated C-fiber nerves [37]. Studies investigating human myofibroblasts showed that the cells can respond to ATP by generating an intracellular Ca^{2+} transient, which is mediated by a P2Y receptor, most likely including a P2Y6 [15, 39]. It has been hypothesized that the close relation between nerves and myofibroblasts allows for an amplification of the afferent system in its response to stimulatory mediators such as ATP.

Recently, overexpression of P2X2 receptors in patients with OAB was demonstrated, indicating that purinergic innervation can play an important role in the pathophysiology of IDO. Pannek et al. [40] evaluated the expression of P2X2 receptors in patients with spinal cord lesions and gave a first hint that the P2X2 expression in patients with suprasacral spinal cord injury seems to be comparable to the expression in patients with idiopathic OAB. More recently Kaan et al. [41] applied a novel selective P2X3 and P2X2/3 antagonist, AF-792 ((5-(5-ethyl vinyl-2-isopropyl-4-methoxy-phenoxy)-pyrimidine-2,4-diamine), previously known as RO-5), intrathecally in vivo, in order to assess changes in the micturition reflex contractions after drug treatment. They found that AF-792 inhibited micturition reflex activity significantly. Furthermore, inhibition of P2X3 and P2X2/3 receptors in the spinal cord significantly attenuated spinal activation of extracellular signal-regulated kinases induced by acute peripheral stimulation of the bladder with 1% acetic acid [41]. These data suggest that afferent signals originating from the bladder are regulated by spinal P2X3 and P2X2/3 receptors and establish directly an endogenous central presynaptic purinergic mechanism to regulate visceral sensory transmission [41]. Identification of this spinal purinergic control in visceral activities may help the development of P2X3 and P2X2/3 antagonist to treat urological dysfunction such as OAB.

**Beta-Adrenoceptors**

Beta-adrenoceptor agonists are currently in clinical development as treatments for OAB. Recently Michel and Sand [42] explored the ability of the beta-adrenoceptor agonist isoprenaline to induce rat isolated bladder strip relaxation on precontraction with the muscarinic agonist carbachol as compared to bladder tone induced by several non-cholinergic stimuli. They concluded that beta-adrenoceptor agonists can induce rat bladder relaxation against a wide range of contractile stimuli and are more potent and/or effective against non-cholinergic stimuli than against muscarinic agonism. This profile appears desirable for agents intended for the treatment of OAB.

**Serotonin and Noradrenaline**

Depending on the predominant receptor subtype at the site of action, serotonin (5-hydroxy tryptamine, 5-HT) can either inhibit or facilitate micturition [2]. However, descending pathway is essentially an inhibitory circuit with serotonin as a key neurotransmitter [43], and an electrical stimulation of serotonin-containing neurons in the caudal raphe nucleus causes inhibition of bladder contractions [44, 45]. Experimental studies in animals have revealed that spinal reflex circuits involved in voiding function exhibit a dense serotonergic innervation, multiple 5-HT receptors, and sensitivity to 5-HT receptor agonists and antagonists as well as 5-HT reuptake inhibitors. Activity in the serotonergic pathway generally enhances urine storage by facilitating the vesical sympathetic reflex pathway and inhibiting the parasympathetic voiding pathway. Thus, 5-HT receptor antagonists and reuptake inhibitors represent important targets for the development of new treatments of OAB and DO [46].

Beside 5-HT receptors, α1- and α2-adrenoceptors also seem to be involved in micturition control. An evidence for the involvement of a spinal α1-adrenoceptor in micturition control has been provided studying spontaneously hypertensive rats. They showed increased noradrenergic bladder innervation, increased voiding frequency [47] and pronounced DO [48], which were abolished by only intrathecally administering α1-adrenoceptor antagonists. It has been hypothesized that DO might be produced also via a α2-adrenoceptor stimulation both at spinal and supraspinal sites [49]. Actually, the administration of α2-adrenoceptor agonists such as...
clonidine and oxymetazoline to conscious rats intrathecally as well as intracerebroventricularly resulted in DO. This induced DO might be prevented by the selective α2-adrenoceptor antagonist idazoxan [49]. Recently, prevalent bladder dysfunction, produced by partial obstruction in rat, was found to have an enduring disruptive impact on cortical activity through this circuit [50]. Within 2 weeks of partial bladder obstruction, the activity of locus coeruleus was tonically elevated. A selective lesion of the locus coeruleus-norepinephrine system significantly attenuated these cortical effects. These data imply that pharmacological manipulation of central norepinephrine function may alleviate central sequelae of bladder disorders.

Cizolirtine citrate is a substance P- and calcitonin gene-related peptide (CGRP) release modulator at the spinal cord level. This modulation on substance P and CGRP is probably related to the increase of extracellular levels of norepinephrine and serotonin. The effect of these neurotransmitters on the noradrenergic pathway induces in the primary fibers a decrease in the release of substance P and CGRP at the supraspinal level [51–53]. These effects suggest its beneficial potential in urinary bladder inflammation processes, where tachykinin control would facilitate visceral motility regulation and therefore could be potentially useful with certain forms of urinary incontinence [54]. The activity of cizolirtine on OAB has been demonstrated at the preclinical level. Cizolirtine reduced the width of vesical contractions without modifying their rhythm in a model of increased intraluminal pressure in rats. In addition, a model of isovolumetric rhythmic bladder contractions in rats showed that cizolirtine produces a clear effect on urodynamic function. In a pilot dose-finding study, the therapeutic potential of cizolirtine citrate 400 mg b.i.d. in OAB was documented [55]. In a clinical setting, cizolirtine, compared to oxybutynin, showed significant superiority over placebo to improve urodynamic parameters, causing fewer antimuscarinic but more gastrointestinal (nausea) adverse events than oxybutynin [56].

**Glutamate**

Glutamate is probably the main excitatory neurotransmitter involved in the supraspinal control circuitry, in the ascending and descending limbs of the micturition reflex pathway between the pontine micturition center and the preganglionic neurons [57, 58], as well as in the reflex pathway controlling sphincter function. Antagonists of glutamatergic receptors in rats with experimental cerebral infarction were effective in abolishing stroke-mediated DO [59, 60]. Moreover, in rats with bladder outlet obstruction, they are able to significantly and dose-dependently decrease the amplitude and number of premicturition contractions during the filling phase, suggesting peripheral neurogenic components of bladder outlet obstruction linked with glutamatergic receptors [61].

**Botulinum Toxins**

Neurotoxins such as botulinum neurotoxins (BoNTs) are useful in treating disorders of the lower urinary tract. The exact mechanism of action of BoNTs in the bladder is controversial, although evidence suggests that apart from preventing the presynaptic release of Ach from the parasympathetic innervation to the bladder, it might have an effect on sensory mechanisms. The latter hypothesis could in part explain its effect on symptoms such as urgency. BoNT type A (BoNTA) is produced by Clostridium botulinum and consists of a 150-kDa neurotoxic protein that has the ability to cleave proteins within the nerve terminal. BoNTA is internalized by presynaptic neurons after binding to an extracellular receptor (ganglioside and presumably synaptic vesicle protein 2C). In the neuronal cytosol, BoNTA disrupts fusion of the Ach-containing vesicle with the neuronal wall by cleaving the SNAP-25 protein in the synaptic fusion complex. The net effect is selective paralysis of the low-grade contractions of the overactive detrusor while still allowing high-grade contraction that initiates micturition [62]. Additionally, BoNTA seems to have effects on afferent nerve activity by modulating the release of ATP in the urothelium [63], blocking the release of substance P, CGRP and glutamate from afferent nerves and reducing levels of nerve growth factor (NGF). These effects on sensory feedback loops might help explain the mechanism of BoNTA in relieving symptoms of OAB. Thus, BoNTA intradetrusor injections might be an alternative to invasive surgery for patients in whom conservative measures and anticholinergic treatment have failed. Clinical studies with different dosages and injection protocols show success rates of 60–96% for neurogenic DO (NDO) and IDO, with wide variations in the duration of response [64].

According to the most recent European recommendations [65], the use of BoNTA is recommended in the treatment of intractable symptoms of NDO or IDO in adults;
caution is recommended in IDO because the risk of voiding difficulty, urinary retention and duration of effect have not yet been accurately evaluated. Repeated treatment can be recommended in NDO. The depth and location for bladder injections should be within the detrusor muscle outside the trigone [65]. Dosage in children should be determined by body weight, with caution regarding total dose if also being used for treatment of spasticity, and minimum age. The use of BoNTA in the lower urinary tract with the current dosages and techniques is considered to be safe overall [65].

Other Targets for the Treatment of OAB and DO

NO has been identified as an important neurotransmitter involved in the control of the human urinary tract. It has been suggested that NO is one of the factors keeping the bladder relaxed during the filling phase. This function might be mediated by the NO-induced elevation of intracellular cyclic GMP (cGMP). It has been found that compounds activating the NO/cGMP pathway inhibited DO, whereas compounds inhibiting the NO/cGMP pathway increased it [66]. Recently a relaxation of human detrusor smooth muscle induced by phosphodiesterase 5 inhibitors through the involvement of cGMP-, cAMP- and K+ channel-dependent signaling pathways, with a minor contribution from NO, has been demonstrated [67]. Certainly this effect deserves further investigation.

Evidence has shown that urinary proteins such as NGF and prostaglandin E-2 levels increase in patients with OAB. Urinary NGF level increases physiologically in normal subjects at urge to void, but increases pathologically in OAB patients at a small bladder volume and with a sensation of urgency. Recent studies have shown that patients with OAB dry and OAB wet have significantly higher urinary NGF levels compared with control groups and patients with increased bladder sensation [68]. Urinary NGF levels decrease after antimuscarinic therapy and further decrease after detrusor botulinum toxin injections in refractory OAB. Urinary NGF level could be a potential biomarker for diagnosis of OAB and assessment of the therapeutic effect of antimuscarinic therapy [69–71].

Conclusion

At present, antimuscarinics represents the first-line treatment for patients with OAB/DO. When this therapy fails, the only codified and effective pharmacological therapeutic option is BoNTA (grade A level of recommendation for the treatment of intractable symptoms of NDO or IDO) [65]. Caution is recommended in the context of OAB/IDO because patients treated with BoNTA should accept beforehand the possible need to perform clean intermittent catheterization because the increase of residual/retention is the most frequent complication [65]. Improvement of the knowledge about BoNTA in terms of mechanism of action, dose efficacy, site and depth of injection and long-term follow-up results should be the focus of future research as well as preclinical and clinical investigations of other potential therapeutic targets for the treatment of OAB/DO at the level of the urothelium, detrusor muscles, autonomic and afferent pathways, spinal cord and brain.

References

Review

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