Pharmacology of the Lower Urinary Tract

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Abstract

The functions of the lower urinary tract, to store and periodically release urine, are dependent on the activity of smooth and striated muscles in the urinary bladder, urethra, and external urethral sphincter. This activity is in turn controlled by neural circuits in the brain, spinal cord, and peripheral ganglia. Various neurotransmitters, including acetylcholine, norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids, adenosine triphosphate, nitric oxide, and neuropeptides, both in the periphery and the central nervous system have been implicated in the neural regulation of the lower urinary tract. Injuries or diseases of the nervous system, as well as drugs and disorders of the peripheral organs, can produce lower urinary tract dysfunctions such as urinary frequency, urgency, pain and incontinence or inefficient voiding and urinary retention. This chapter will review recent advances in our understanding of the pharmacology in the control of lower urinary tract function and the targets for drug therapy.

8.1 Peripheral Nervous System

8.1.1 Muscarinic Mechanisms

8.1.1.1 Efferent Function and Detrusor Muscle

Excitation of parasympathetic postgangliomic nerves in the bladder releases acetylcholine (ACh) from nerve terminasl to induce detrusor muscle contractions during the voiding phase. ACh released form parasympathetic nerve terminals binds to muscarinic ACh receptors located on detrusor smooth muscles (Fig. 8.1).

There are at least five receptor subtypes based on molecular cloning and four different receptor subtypes based on pharmacology (M1–M5) [1–5]. In the human bladder, M1, M2, and M3 receptor subtypes have been found by receptor binding assays [6]; whereas all M1 to M5 receptor mRNAs are detected by reverse transcription–polymerase chain reaction assays [7, 8]. Although ligand receptor binding studies revealed that M2 receptors predominate, M3 receptors mediate cholinergic contractions [4, 5, 9–12]. (Fig. 8.2).

Stimulation of M3 receptors by acetylcholine leads to IP3 hydrolysis due to phospholipase C activation and then to the release of intracellular calcium and a smooth muscle contraction [10, 13]. The involvement of transmembrane flux of calcium ions through nifedipine-sensitive L-type Ca^{2+} channels has also been indicated in M3 receptor–mediated detrusor muscle contractions [7, 12, 14–17]. (Fig. 8.3). In addition, since the inhibition of Rho kinase reportedly suppresses carbachol-induced detrusor contractions in rats and humans, muscarinic receptor activation in detrusor smooth muscles is likely to stimulate the Rho kinase pathway, leading to a direct inhibition of myosin phosphatase that induces calcium sensitization to enhance the ability of the muscle to generate the same contractile force with lower levels of intracellular calcium [7, 15–17]. (Fig. 8.3).

It has also been proposed [5, 18] that coactivation of M2 receptors could enhance the response to M3 stimulation by (1) inhibition of adenylate cyclase, thereby suppressing sympathetically mediated depression of detrusor muscle; (2) inactivation of K^+ channels; or (3) activation of nonspecific cation channels. It has also been reported that the muscarinic receptor subtype–mediated detrusor contractions shift from M3 to M2 receptor subtype in certain pathologic conditions, such as obstructed or denervated hypertrophied bladders in rats [19–21], as well as in bladder muscle specimens from patients with neurogenic bladder dysfunction [22]. (Fig. 8.3).

Studies using mutant mice lacking the M3 receptor or the M2 and M3 receptors have demonstrated that this subtype plays key roles in salivary secretion, pupillary constriction, and detrusor contractions [23–25]. However, M3-mediated signals in digestive and reproductive organs are dispensable, probably because of redundant mecha-

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L. Liao, H. Madersbacher (eds.), *Neurourology*, https://doi.org/10.1007/978-94-017-7509-0_8

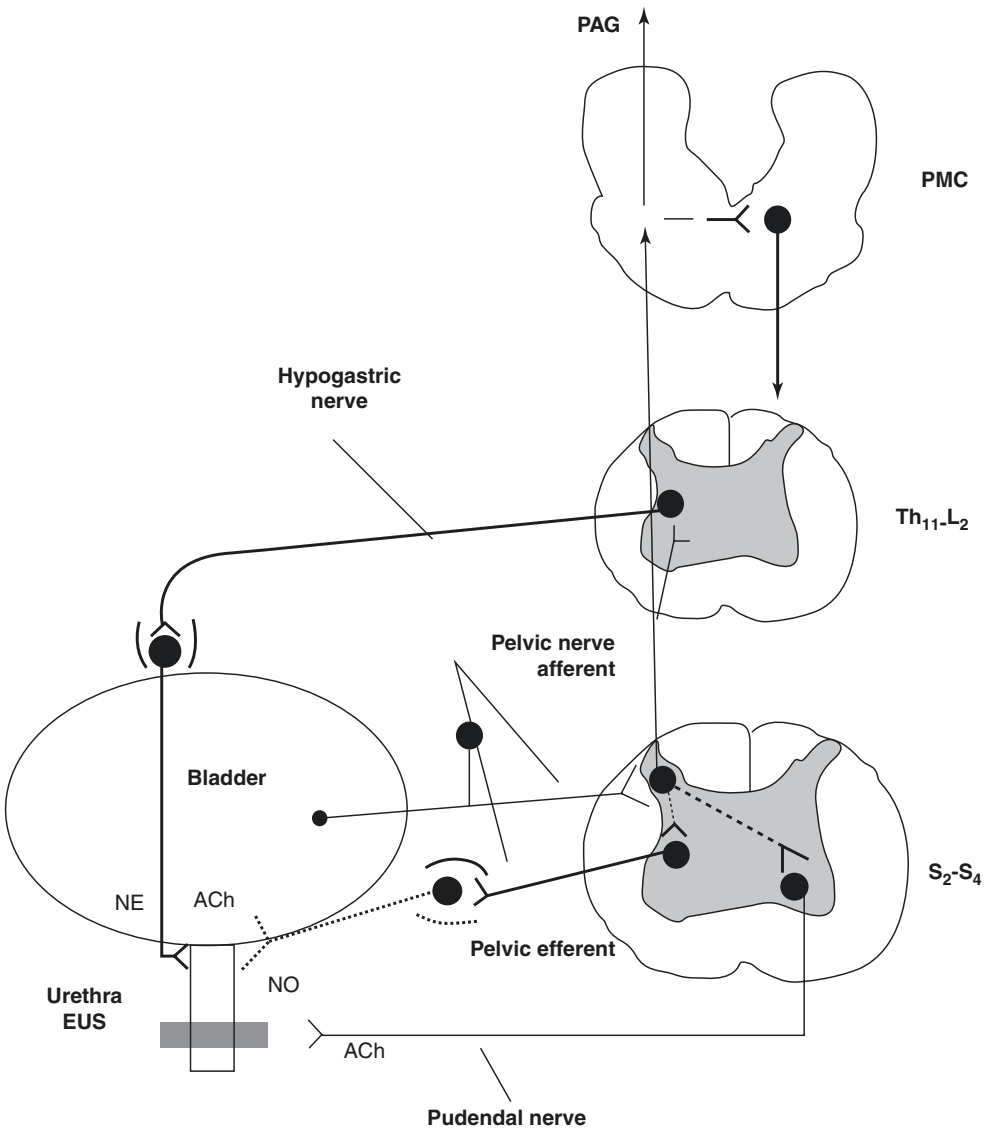

Fig. 8.1 Efferent pathways. Major preganglionic and postganglionic neural pathways from the spinal cord to the lower urinary tract: The sympathetic hypogastric nerve, emerging from the inferior mesenteric ganglion, stimulates urethral smooth muscle. The parasympathetic pelvic nerve, emerging from the pelvic ganglion, stimulates bladder detrusor muscle and inhibits urethral smooth muscle. The somatic pudendal nerve stimulates striated muscle of the EUS. Afferent pathways. Ascending afferent inputs from the spinal cord passes through neurons in the PAG to upper brain regions and the PMC. *ACh* acetylcholine, *NE* norepinephrine, *NO* nitric oxide, *S2-S4* sacral segments of the spinal cord, *T10-L2* thoracolumbar segments of the spinal cord; EUS, external urethral sphincter, *PMC* pontine micturition center, *PAG* periaqueductal gray

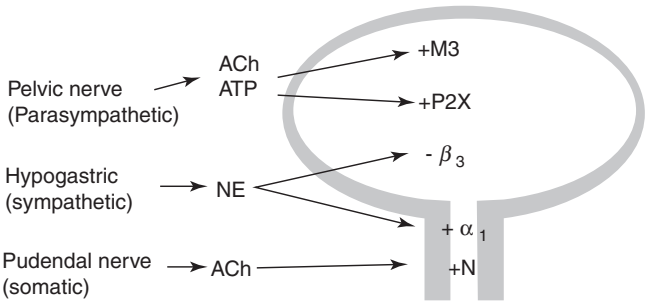

Fig. 8.2 Innervation of the lower urinary tract: The parasympathetic pelvic nerve stimulates the bladder detrusor muscle, mediated by muscarinic receptors (M3) being activated by ACh. The sympathetic hypogastric nerve stimulates urethral smooth muscle and inhibits bladder detrusor, mediated by α_1-adrenergic and β_3-adrenergic receptors, respectively. The somatic pudendal nerve stimulates striated muscle of the external urethral sphincter, mediated by ACh activating nicotinic (N) receptors. *ACh* acetylcholine, *NE* norepinephrine. Plus and minus signs indicate neural stimulation and inhibition, respectively

nisms through other muscarinic acetylcholine receptor subtypes or other mediators such as ATP [23]. In addition, it has also been found that male M3 knockout mice had the distended bladder and larger bladder capacity compared with females, indicating a considerable sex difference in the micturition mechanism [24, 25].

Muscarinic receptors are also located prejunctionally on cholinergic nerve terminals in the bladder [26–31]. Activation of M1 prejunctional receptors facilitates acetylcholine release [27, 28], whereas activation of M2–M4 receptors inhibits the release [12, 29, 30]. It has been proposed that inhibitory M2–M4 receptors are preferentially activated by autofeedback mechanisms during short periods of low-frequency nerve activity and thereby suppress cholinergic transmission during urine storage [27]. Conversely, M1 receptors are activated during more prolonged, high-frequency nerve firing that would occur during voiding and

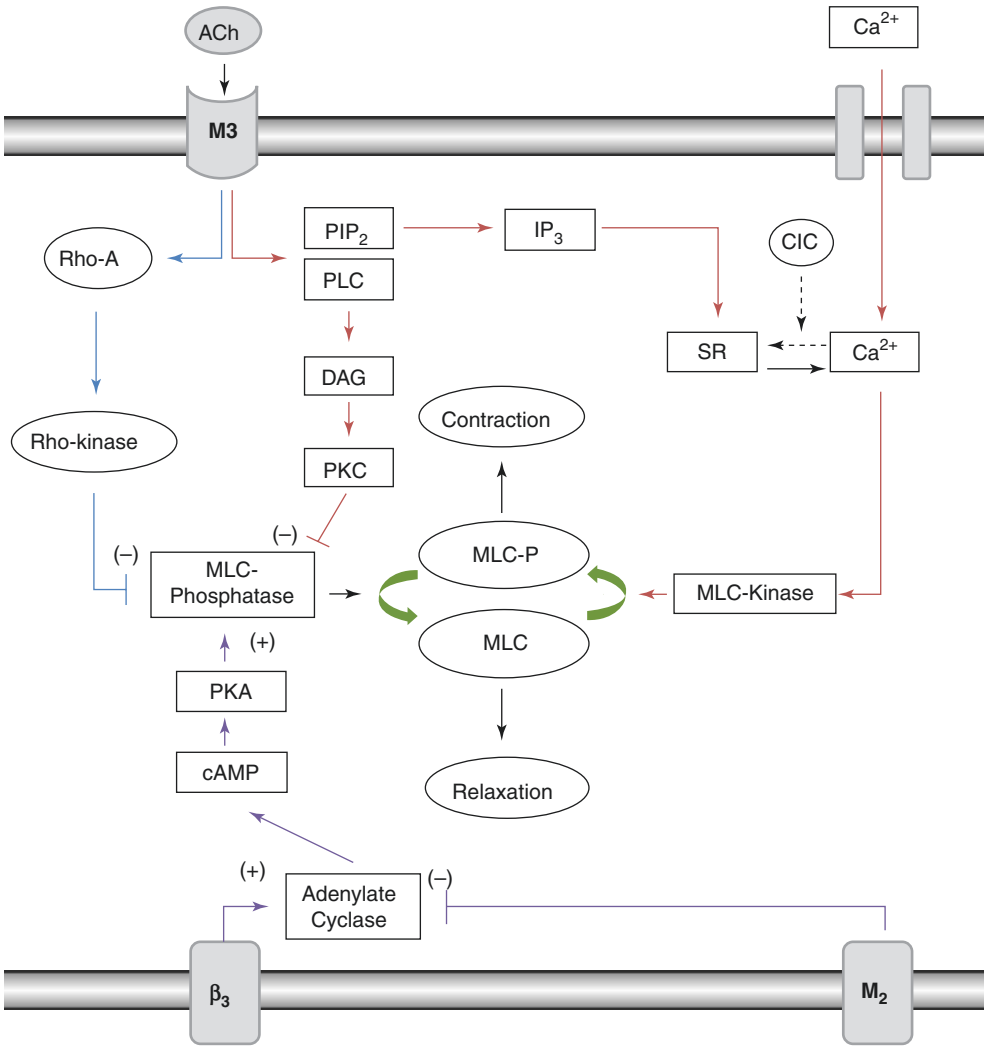

Fig. 8.3 Intracellular signaling pathways involved in activation and relaxation of detrusor contractions via M2 and M3 muscarinic and ß$_3$ adrenergic receptors, respectively. *ACh* acetylcholine, *PLC* phospholipase C, *DAG* diacylglycerol, *PKC* protein kinase C, *PKA* protein kinase A, *MLC* myosin light chain, *IP3* inositol trisphosphate, *PIP2* phosphatidylinositol 4,5-bisphosphate, *SR* sarcoplasmic reticulum, *CIC* calcium-induced calcium release. Intracellular Ca^{2+} release and Ca^{2+} influx contribute to contractions. Activation of M2 muscarinic receptors inhibits adenylate cyclase and reduces ß$_3$ adrenergic receptor mediated relaxation

thus participate in an amplification mechanism to promote complete bladder emptying. M1-mediated facilitation of transmitter release involves the activation of a phospholipase C–protein kinase C signaling cascade that appears to facilitate the opening of L-type Ca^{2+} channels that are necessary for prejunctional facilitation of acetylcholine release from parasympathetic nerve terminals [28, 32]. Inhibitory and facilitatory muscarinic receptors are also present in bladder parasympathetic ganglia, where they modulate nicotinic transmission [33].

8.1.1.2 Bladder Urothelium, Afferent Nerves and Interstitial Cells

Previous studies have shown that the bladder urothelium is a non-neuronal source of ACh release, which is induced by stretch of the urothelium by using vesicular storage and exocytosis mechanisms different from those in neuronal release of ACh [12]. The bladder urothelium of many species including humans also expressed multiple muscarinic receptors, with M2 andM3 receptors being most abundant at the mRNA and protein levels [34]. Activation of the muscarinic receptors in the urothelium releases substances (e.g., ATP) that modulate afferent nerves and smooth muscle activity [35, 36].

In bladder afferent pathways, it has been shown that dorsal root ganglion (DRG) neurons innervating the bladder express M2, M3 and M4 ACh receptors [37]. Systemic application of muscarinic receptor antagonists such as oxybutynin and darifenacin reportedly attenuates the afferent activity in response to bladder filling in rats [38, 39]. Also, intravesical administration of a muscarinic receptor agonist (oxotremorin-M) induces bladder overactivity, which is blocked by M2 receptor antagonists [40, 41]. These data suggest that activation of muscarinic receptors in the bladder has an excitatory effect on afferent nerve activity; however, it is not known due to the nature of in vivo studies if the facilitatory effects are mediated by direct interaction with muscarinic receptors expressed on afferent nerves or indirectly via the substances (e.g., ATP) released from the urothelium upon stimulation of urothelial muscarinic receptors.

Furthermore, muscarinic receptors such as M2 and M3 are also expressed in interstitial cells (IC) located in the suburothelial and detrusor layers (Fig. 8.4). Recent studies have revealed that bladder IC can modulate the bladder functions of filling and voiding in addition to sensory transduction by both excitatory and inhibitory mechanisms [42]. It has been shown that large Ca^{2+}-transients in detrusor IC induced by cholinergic receptor agonist (carbachol) are blocked by M3 antagonists with some sensitivity to M2 antagonists in mice and guinea pigs, raising the possibility that bladder IC can modulate the detrusor activity [43, 44].

Overall, the peripheral muscarinic receptor systems control lower urinary tract (LUT) function through multiple mechanisms that include not only direct smooth muscle activation, but also indirect ones via the urothelium and IC, which may help to explain in part the mechanism of action for muscarinic antagonists in reducing symptoms of bladder disorders such as overactive bladder (OAB).

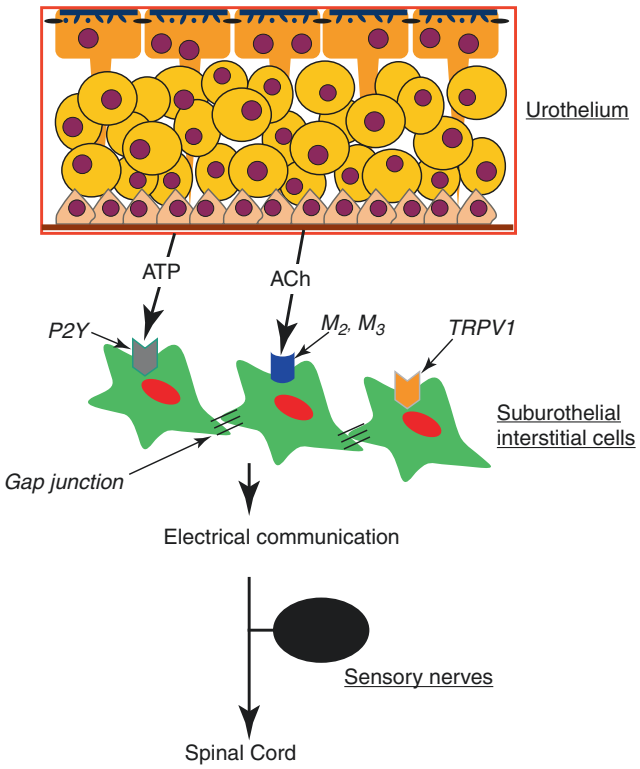

Fig. 8.4 Schematic representation of suburothelial interstitial cells (IC), which are also called myofibroblasts. Substances released from the basolateral surface during stretch, such as adenosine triphosphate (ATP) and acetylcholine (ACh), activate afferents in the suburothelial layer through the intermediation of suburothelially located interstitial cells, which express purinergic P2Y receptors, muscarinic M2 and M3 receptors or capsacin TRPV1 receptors, and are connected each other by gap junction proteins

8.1.2 Purinergic Mechanisms

8.1.2.1 Efferent Function and Detrusor Muscle

Purinergic contribution to parasympathetic stimulation has been shown to exist in a variety of species including rat, rabbit, and guinea pig [45–47]. However, there is less evidence for the contribution of purinergic neurotransmission in humans, at least in the normal micturition although it may play a role in pathologic conditions such as detrusor overactivity or bladder outlet obstruction [48–50].

ATP acts on two families of purinergic receptors: an ion channel family (P2X) and a G protein–coupled receptor family (P2Y) [51–53]. Seven P2X subtypes and eight P2Y subtypes have been identified. Immunohistochemical experiments with specific antibodies for different P2X receptors showed that $P2X_1$ receptors are the dominant subtype in membranes of rat detrusor muscle and vascular smooth muscle in the bladder [54] (Fig. 8.2). Clusters of $P2X_1$ receptors were detected on rat bladder smooth muscle cells, some of which were closely related to nerve varicosities. Northern blotting and in situ hybridization revealed the presence of $P2X_1$ and $P2X_4$ mRNA in the bladder [55]. The predominant expression of $P2X_1$ receptors has also been confirmed in the human bladder [50, 56]. Investigators also found that the amount of $P2X_1$ receptors was increased in the obstructed bladder compared with the control bladder, suggesting upregulated purinergic mechanisms in the overactive bladder due to bladder outlet obstruction [50]. In addition, ATP also seems to act through P2Y receptors in the smooth muscle to suppress cholinergic and purinergic contractions [54, 57].

Purinergic nerves are also involved in the modulation of synaptic transmission in parasympathetic ganglia [58–61] because excitatory purinergic receptors in pelvic ganglia have been demonstrated in the cat [58], rabbit [59], and rat [60, 61].

8.1.3 Bladder Urothelium and Afferent Nerves

ATP is also released from urothelial cells during stretch and by chemical stimuli, and can activate a population of suburothelial bladder afferents expressing $P2X_2$ and $P2X_3$ receptors, signaling changes in bladder fullness and pain [62] (Fig. 8.5). Accordingly, $P2X_2$ or $P2X_3$ null mice exhibit bladder hyporeflexia, suggesting that this receptor as well as neural-epithelial interactions are essential for normal bladder function [63, 64] . However, recent studies reported that the $P2X_3$ receptor-mediated urothelial-afferent interaction is more important in bladder pathological conditions such as cystitis because, in mice, a lack of $P2X_2$ or $P2X_3$ receptors

did not show any changes in normal micturition, but attenuated bladder overactivity induced byipopolysaccharide (LPS) treatment in the bladder [65, 66]. ATP released from the urothelium or surrounding tissues may also play a role in the regulation of membrane trafficking. This is supported by studies in the urinary bladder in which urothelium-derived ATP release purportedly acts as a trigger for exocytosis—in part by autocrine activation of urothelial purinergic (P2X, P2Y) receptors [67]. These findings suggest a mechanism whereby urothelial cells sense or respond to ATP and thereby translate extracellular stimuli into functional processes (Fig. 8.5).

$P2X_3$ receptors that have been identified in small-diameter afferent neurons in DRG have also been detected immunohistochemically in the wall of the bladder and ureter in a suburothelial plexus of afferent nerves [54]. Studies using patch clamp recordings in rats have also demonstrated that the majority (90%) of bladder afferent neurons projecting via pelvic nerves responded to α,β-methylene ATP and ATP with persistent currents, suggesting that bladder afferent pathways in the pelvic nerve express predominantly $P2X_{2/3}$ heteromeric receptors rather than $P2X_3$ homomeric receptors [68, 69]. Intravesical or intra-arterial administration of ATP or 2-methylthio-ATP activated bladder afferent fibers and enhanced reflex bladder activity [70–74]. Intra-arterial injection of ATP can also activate bladder afferent nerves [70], whereas suramin, an antagonist of certain types of ATP receptors (P2X purinergic receptors), given intravesically reduces by 50% the firing of bladder mechanoreceptors induced by bladder distention [75].

In addition, adenosine, which can be formed by the metabolism of ATP, can depress parasympathetic nerve-evoked bladder contractions by activating P1 inhibitory receptors in parasympathetic ganglia [76], in postganglionic nerve termi-

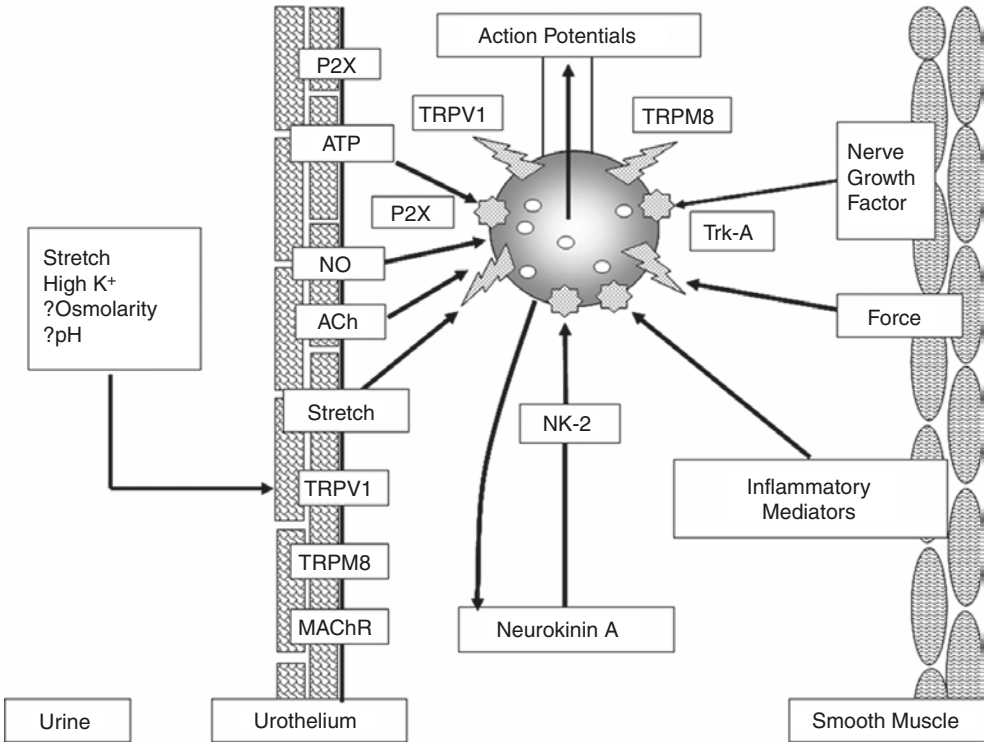

Fig. 8.5 Diagram showing: (1) receptors present in the urothelium (left side) and in sensory nerve endings in the bladder mucosa (center) and (2) putative chemical mediators that are released by the urothelium, nerves or smooth muscle (right side) that can modulate the excitability of sensory nerves. Urothelial cells and sensory nerves express common receptors (P2X, TRPV1 and TRPM8). Distension of the bladder activates stretch receptors and triggers the release of urothelial transmitters such as ATP, ACh and NO that may interact with adjacent nerves. Receptors in afferent nerves or the urothelium can respond to changes in pH, osmolality, high K^+ concentration, chemicals in the urine or inflammatory mediators released in the bladder wall. Neuropeptides (neurokinin A) released from sensory nerves in response to distension or chemical stimulation can act on neurokinin 2 (NK-2) autoreceptors to sensitize the mechanosensitive nerve endings. The smooth muscle can generate force which may influence some mucosal endings. Nerve growth factor released from muscle or urothelium can exert an acute and chronic influence on the excitability of sensory nerves via an action on Trk-A receptors. *ACh* acetylcholine, *MAChR* muscarinic acetylcholine receptor, *TRPV1* transient receptor potential vanilloid receptor 1 that are sensitive to capsaicin, *TRPM8* menthol/cold receptor, *NO* nitric oxide, *Trk-A* tropomyosin receptor kinase A receptor

nals, and in the bladder muscle [57, 64]. Adenosine P1 receptors have been further classified into a number of subtypes (i.e., A1, A2A, A2B, and A3) [77]. A study has demonstrated that adenosine reduces the force of nerve-mediated contractions by acting predominantly at presynaptic sites at the nerve-muscle junction through a subtype of an adenosine receptor (the A1 receptor in guinea pigs), although these actions of adenosine are less evident in human detrusor muscles [78]. Adenosine is also produced and released by the urothelium, and may contribute to the modulation of sensory afferent function and smooth muscle contraction [79]. A recent study also demonstrated that other purines including nicotinamide adenine dinucleotide (NAD+), ADP-ribose, and cADP-ribose content are released urothellially at different ratios during bladder filling although the functional role of these purines are not yet elucidated [80].

8.1.4 Adrenergic Mechanisms

8.1.4.1 β-Adrenergic

Stimulation of β_2- and β_3-adrenergic receptors that exist in the human detrusor results in the direct relaxation of the detrusor smooth muscle [81–84]. In addition, β-adrenergic–stimulated relaxation is mediated through the stimulation of adenylate cyclase and the accumulation of cyclic AMP (cAMP) [7, 13, 81]. Because β adrenoceptor–mediated relaxation of the human detrusor was not blocked by selective β_1 or β_2 adrenoceptor antagonists such as dobutamine and procaterol but was blocked by selective β_3 adrenoceptor antagonists, the relaxation induced by adrenergic stimulation of the human detrusor is mediated mainly through β_3 adrenoceptor activation [13, 85, 86]. A quantitative analysis by reverse transcription–polymerase chain reaction has also confirmed that the β_3-adrenergic receptor is the most highly expressed subtype among α and β adrenoceptor subtypes at the mRNA level in human bladders [87]. Thus, it has been considered that the major site of action of β_3-adrenoceptors is the detrusor muscle (Fig. 8.2); however, recent studies raised the possibility that other sites such as cholinergic nerve terminals and urothelium are involved in the β_3-adrenoceptor-mediated control of bladder activity. For example, β_3-adrenoceptors are abundantly located in ACh-containing nerve fibers in the mucosa and muscular layers of the human bladder [88]. Recent studies demonstrated that activation of prejunctional β_3-adrenoceptors can decrease ACh release from cholinergic nerve terminals via the adenosine-A1 receptor system, thereby leading to an inhibitory control of bladder activity during filling, in human and rat urinary bladders [89], β_3-Adrenoceptors are also shown to be expressed in the urothelium although the role of urothelial β_3-ARs in bladder relaxation has not yet to be fully elucidated [90, 91].

The β_3-receptor agonist mirabegron has been approved as a new treatment option for OAB with symptoms of urge incontinence [13, 92]. This agent has been shown to provide an alternative for patients with contraindications or intolerance to existing therapy, although combination therapy (mirabegron and the antimuscarinic solifenacin) has also been shown to be effective [93] The mechanism of action may be related to effects on multiple cell types including bladder afferent activity [94]. Findings in rodents have revealed that β_3- adrenoceptor stimulation with mirabegron increased bladder compliance and shortened the intervoid interval; this regulation may be a result of the effect at multiple sites including reduction of nonvoiding contractions and decreased afferent nerve activation [95, 96].

8.1.4.2 α-Adrenergic

Although α-adrenergic stimulation is not prominent in the normal bladder, under pathologic conditions such as detrusor overactivity associated with bladder outlet obstruction, the α-adrenergic receptor density, especially the α_{1D} receptor subtype, increases to such an extent that the norepinephrine-induced responses in the bladder are converted from relaxation to contraction [13]. In rats with bladder outlet obstruction, the proportion of α_{1D} receptor subtype in the total α_1 receptor mRNA in the bladder is increased to 70% from 25% in the normal rat bladders [97], and urinary frequency is suppressed by an inhibition of α_{1D} and α_{1A} receptors by tamsulosin whereas α_{1A} receptor suppression by 5-methyl-urapidil has no effects. Moreover, α_{1D} receptor knockout mice have larger bladder capacity and voided volumes than do their wild-type controls, supporting an important role of α_{1D} receptors in the control of bladder function [98]. However, in humans, there is the predominant expression of α_{1D} receptors already in the normal bladder [99], and the level of expression of α adrenoceptor mRNA, which is considerably low compared with β_3 adrenoceptors in normal bladders, was not increased in the bladder with outflow obstruction [87]. Thus, the contribution of α_{1D} receptors to detrusor overactivity observed in a variety of pathologic conditions including obstructive uropathy and incontinence still needs to be established [13].

α-Adrenergic mechanisms are more important in urethral function. Substantial pharmacologic and physiologic evidence indicates that urethral tone and intraurethral pressure are influenced by α-adrenergic receptors. The presence of α_1 and α_2 adrenoceptors has been shown in the urethra od various species including humans. Among α_1 adrenoceptors, the α_{1A} adrenoceptor is the major subtype expressed in urethral smooth muscles at the mRNA and protein levels [100, 101] (Figs. 8.1 and 8.2). Isolated human urethral smooth muscle contracts in response to α-adrenergic agonists [102–105]. It is also reported in the rabbit that the urethral contraction is mediated by the α_{1A} adrenoceptor subtype [101, 106]. Likewise, hypogastric nerve stimulation and α-adrenergic agonists produce a rise in intraurethral pressure, which is blocked by α_1-adrenergic antagonists [102, 107].

Conversely, α adrenergic receptor antagonists facilitate urine release in conditions of functionally increased urethral resistance, such as benign prostatic hyperplasia. In accordance with the α_{1A} adrenoceptor being the major subtype in the prostate and urethra, highly-selective α_{1A} adrenoceptor antagonists (e.g. silodosin) significantly improve lower urinary tract symptom (LUTS) scores in men with BPH [108]. In addition, α_1 adrenoceptor antagonists that contain α_{1D} adrenoceptor blocking activity also improve bladder-based symptoms in humans [109], suggesting that the α_{1D} adrenoceptors contribute to storage symptoms associated bladder outlet obstruction, which are potentially located at the bladder or the spinal cord [110].

α_2-Adrenergic antagonists increase the release of norepinephrine from urethral tissues through a presynaptic mechanism, but this does not affect the contractility of urethral smooth muscle in vitro [101, 105, 111]. The human urethra lacks postjunctional α_2-adrenergic receptors, although in vitro prejunctional activation of these receptors produces a feedback inhibition of norepinephrine release. Pharmacologic and electrophysiologic data suggest that adrenergic nerves influence excitatory cholinergic transmission in pelvic ganglia. It has been shown in the cat that hypogastric nerves inhibit excitatory cholinergic transmission in vesical ganglia by activation of α_2-adrenergic receptors [112].

8.1.5 Nitric Oxide

8.1.5.1 Efferent Function

Nitric oxide (NO) has been identified as a major inhibitory transmitter mediating relaxation of the urethral smooth muscle during micturition [81, 113–115] (Fig. 8.1). In the rat, NO is released by postganglionic nerves arising from neurons in the major pelvic ganglia [116]. These neurons contain nitric oxide synthase (NOS), the enzyme that synthesizes NO, as well as nicotinamide adenine dinucleotide phosphate–diaphorase, a marker for NOS [117]. Electrophysiologic studies in female rats showed that electrical stimulation of the lumbosacral (L6-S1) spinal roots elicits simultaneous bladder contractions and urethral relaxation [116]. The urethral relaxation was inhibited by NOS inhibitors, which did not alter the bladder responses. The inhibition was reversed by administration of L-arginine, a precursor of NO. The electrically evoked urethral relaxation was abolished by ganglionic blocking agents, indicating that it was mediated by stimulation of preganglionic parasympathetic axons in the lumbosacral roots.

NO-mediated smooth muscle relaxation is due to stimulation NO-sensitive guanylyl cyclase (NO-GC), resulting in increased production of intracellular cyclic guanosine monophosphate (cGMP). NO-GC is found in urethral smooth muscles, but not in bladder detrusor muscle; therefore, NO can induce urethral relaxation, but does not relax detrusor smooth muscle, indicating a minor role of NO-mediated relaxation mechanisms in the bladder, although NO-GC is expressed in vascular smooth muscle or interstitial cells in the bladder [118].

cGMP is inactivated by PDEs by hydrolytic cleavage of the phosphodiester bond. Therefore, the level of intracellular second messengers can be regulated by PDE isoenzymes [119, 120]. Because of their central role in regulating smooth muscle tone and the considerable variation of PDE isoenzymes among species and tissues, PDEs have become an attractive target for drug development.

8.1.5.2 Afferent Nerves and Urothelial Function

NO is also involved in controlling bladder afferent nerve activity. Inhibitors of NOS, given systemically or intrathecally, do not affect normal micturition in conscious or anesthetized rats. However, detrusor overactivity that accompanies irritation with turpentine, acetic acid or cyclophosphamide is ameliorated by spinal application of NOS inhibitors [121–123]. However, intravesically administered capsaicin induces detrusor overactivity that is not influenced by an intrathecally applied NOS inhibitor, although the behavioral effects of the irritation are reduced [124]. It is shown that NO is involved in mediating *N*-methyl-D-aspartate (NMDA) receptor–dependent effects but not those involving neurokinin 2 (NK2) receptors. Overall, the NO mechanism at the spinal level has an excitatory effect on the micturition reflex.

In contrast, NO seems to have an inhibitory effect in the bladder. NO can be released by the urothelium, particularly during inflammation [125]. The release of NO may be evoked by the calcium ionophore, norepinephrine, and capsaicin. Substance P also acts on receptors on urothelial cells to release NO. Bladder afferent nerves can also release NO because NOS expression is found in bladder afferent neurons and increased in chronic bladder inflammation or bladder outlet obstruction [126, 127]. Intravesical application of NO can suppress bladder overactivity due to cyclophosphamide-induced bladder irritation in rats [128]. Intravesical oxyhemoglobin, an NO scanvenger, also induces bladder overactivity as evidenced by reductions in bladder capacity and micturition volume, which is prevented by L-arginine or enhanced by the guanylate cyclase inhibitor in rats. Previous studies also showed that bladder overactivity induced by intravesical capsaicin instillation was enhanced by a NOS inhibitor (L-NAME) administered intravenously or intravesically and that these l-NAME-induced excitatory effects were significantly suppressed by desensitization of C-fiber afferent pathways by capsaicin pretreatment [129, 130]. Thus, NO released locally in the bladder can mediate inhibitory effects by modulation of bladder afferent activity [131].

8.1.5.3 PDE Inhibitor Treatment of Male LUTS

PDE5 inhibitors, which increases the tissue cGMP concentration by inhibiting degradation, has been approved and

shown to be effective for the treatment of male LUTS due to BPH [132]. It has been shown that the improvement of LUTS is associated with increased urinary flow, suggesting the urethral smooth muscle relaxation; however, the underlying mechanisms of drug efficacy seem to be multifactorial, which include the improvement of bladder ischemia due to vascular smooth muscle relaxation, inhibition of RhoA/Rho-kinase pathway activation in detrusor muscle and suppression of bladder/prostate afferent activity in addition to cGMP-mediated smooth muscle relaxation [133].

8.1.5.4 Possible Gender Difference in Cholinergic, Nitrergic and Adrenergic Innervation in the Urethra

A parasympathetic cholinergic excitatory input to the urethra has been identified in male but not in female rats [134, 135]. This was demonstrated by measuring intraurethral pressure during voiding after blockade of striated external urethral sphincter activity with a neuromuscular blocking agent. Under these conditions, urethral pressure increased during micturition in male rats. This urethral reflex was blocked by hexamethonium (a ganglionic blocking agent), markedly reduced by atropine, and increased by an NOS inhibitor. However, it was not changed by transection of sympathetic nerves or administration of an α_1-adrenergic blocking agent (prazosin). These results indicate that in male rats, parasympathetic nerve activity induces a predominant cholinergic muscarinic contraction of the urethra and a subordinate NO-mediated relaxation. These studies implicate possible gender differences in parasympathetic and especially nitrergic pathways in the human urethra. Furthermore, there also seems to be a gender difference in the α_1 adrenoceptor expression in urethra because α_{1A} adrenoceptor-induced contractions and α_{1A} adrenoceptor expression in the proximal urethra of male mice and marmoset monkeys are significantly greater than in the female counterpart [136].

8.1.6 Afferent Neuropeptides

Afferent neurons innervating the lower urinary tract exhibit immunoreactivity for various neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide (PACAP), leucine enkephalin, corticotropin releasing factor and vasoactive intestinal polypeptide (VIP) [137–142], as well as growth associated protein-43 (GAP43), nitric oxide synthase (NOS) [126], glutamic acid and aspartic acid [143]. These substances have been identified in many species and at one or more locations in the afferent pathways including: (1) afferent neurons in lumbosacral DRG, (2) afferent nerves in the peripheral organs and (3) afferent axons and terminals in the lumbosacral spinal cord [144–148]. The majority (>70%) of bladder DRG neurons in rats appear to contain multiple neuropeptides, CGRP, substance P or PACAP being the most common. In cats VIP is also contained in a large percentage of bladder DRG neurons [138]. Many of these peptides, which are contained in capsaicin-sensitive, C-fiber bladder afferents, are released in the bladder by noxious stimulation and contribute to inflammatory responses by triggering plasma extravasation, vasodilation, and alterations in bladder smooth muscle activity [140, 149, 150]. These peptides also function as transmitters at afferent terminals in the spinal cord.

8.1.6.1 Tachykinins

Tachykinins are a family of small peptides sharing a common C-terminal sequence Phe-Xaa-Gly-Leu-Met-NH2 whose main members are SP, neurokinin A, and neurokinin B. Tachykinins are found in both central and peripheral nervous systems. In the peripheral nerves, tachykinins are predominantly located in the terminals of nonmyelinated C fiber afferent pathways. The diverse biologic effects of the tachykinins are mediated through three receptors, designated NK1, NK2, and NK3, which belong to the superfamily of seven transmembrane–spanning G protein–coupled receptors [151]. Substance P is the most potent tachykinin for the NK1 receptor, whereas neurokinin A exhibits the highest affinity for the tachykinin NK2 receptor and neurokinin B for the tachykinin NK3 receptor. All receptor subtypes have been identified in the bladder in humans and animals such as rats, mice, and dogs [13, 152].

Tachykinins released from capsaicin-sensitive sensory C fibers in response to irritation in the bladder can act on (1) NK1 receptors in blood vessels to induce plasma extravasation and vasodilation, (2) NK2 receptors to stimulate bladder contractions, and (3) NK2 receptors on primary afferent terminals to increase the excitability during bladder filling or during bladder inflammation [81, 138, 148, 152, 153] (Fig. 8.5). It has also been demonstrated that activation of NK3 receptors on capsaicin-sensitive C-fiber afferents in the rat bladder can increase the excitability during bladder filling [154].

Intrathecal administration of NK1 antagonists (RP 67580 and CP 96345) or systemic application of centrally acting NK1 antagonists (GR 205171 and CP 99994) increased bladder capacity in normal rats and guinea pigs, respectively, without changing voiding pressure, whereas NK2, NK3, or peripherally acting NK1 antagonists were ineffective [155, 156]. Detrusor overactivity in rats induced by chemical cystitis, intravesical administration of capsaicin, or intravenous injection of L-dopa was also suppressed by intrathecal injection of NK1 antagonists [149, 157, 158]. Detrusor overactivity induced by capsaicin was reduced by an NK2 antagonist (SR 48965) that did not influence normal voiding [159]. In the anesthetized guinea pigs, TAK-637, an NK1 receptor antagonist, administered orally or intravenously, also increased the volume threshold for inducing micturition and

inhibited the micturition reflex induced by capsaicin applied topically to the bladder [160]. In a clinical study, an NK1 receptor antagonist, aprepitant, is also shown to effectively decrease the average daily number of micturitions and urgency episodes compared with placebo in women with idiopathic overactive bladder [161] although a later clinical study using another NK1 receptor antagonist showed that the reduction in the average daily number of micturitions was significantly greater compared with placebo; but not as good as the efficacy of tolterodine in patients with OAB [162]. These results indicate that sensory inputs to the spinal cord from non-nociceptive bladder afferents is mediated by tachykinins acting on NK1 receptors, whereas input from nociceptive afferents in the bladder can be mediated by NK1, NK2, and NK3 receptors. In addition, tachykinin NK3 receptor activation in the spinal cord can inhibit the micturition reflex through an activation of the spinal opioid mechanism [154]. Furthermore, autofeedback mechanisms may be important at afferent nerve terminals because sensory neurons obtained from rat DRG can be excited by NK2 agonists and inhibited by NK3 agonists through modulation of Ca2+ channel activity mediated by protein kinase C activation [163]. NK2 receptor activation also leads to PKC-induced phosphorylation of TRPV1 channels, resulting in an increase in capsaicin-evoked currents in rat DRG neurons [163, 164].

8.1.6.2 Other Neuropeptides

Other afferent neuropeptides have effects on the peripheral organs or the central reflex pathways controlling the lower urinary tract. However, the effects can vary in different species and at different sites in the lower urinary tract. CGRP applied exogenously or released from primary afferents relaxes smooth muscle and produces vasodilation. The effect of CGRP on bladder is prominent in the guinea pig and dog but is absent in the rat and human bladder [81]. VIP, which is contained in C-fiber afferents as well as in postganglionic neurons [112], inhibits spontaneous contractile activity in isolated bladder muscle from several species, including humans. However, VIP usually has little effect on bladder contractions induced by muscarinic receptor agonists or nerve stimulation [81]. In vivo studies in the cat revealed that VIP facilitates muscarinic but not nicotinic transmission in bladder parasympathetic ganglia and also depresses neurally evoked contractions of the bladder [30].

In the spinal cord, VIP-containing afferent pathways have been implicated in the recovery of bladder reflexes after spinal injury. In cats with chronic spinal injury, VIP immunoreactivity, which is a marker for C-fiber afferent terminals, is distributed over a wider area of the lateral dorsal horn, suggestive of afferent axonal sprouting after spinal injury [160, 165]. In addition, the effects of intrathecal administration of VIP are changed after spinal injury. In normal cats, VIP inhibits the micturition reflex; whereas in spinalized cats, VIP facilitates the micturition reflex, suggesting that the action of a putative C-fiber afferent transmitter may underlie the emergence of C-fiber bladder reflexes after spinal injury. In the normal rat, VIP and PACAP, another member of the secretin-glucagon-VIP peptide family, also facilitate the micturition reflex by actions on the spinal cord [158, 166, 167]. A study using PACAP null mice showed that PACAP gene disruption induces changes in bladder morphology, bladder function and somatic and visceral hypoalgesia [168]. In rats with spinal cord injury, increase in expression of PACAP-immunoreactivity in bladder DRG neurons and expansion of PACAP-IR afferent axons in the lumbosacral spinal cord are observed and intrathecal administration of PACAP6-38, a PAC1 PACAP receptor antagonist, reduces premicturition contractions during bladder filling and reduces maximal voiding pressure, suggesting that activation of PAC1 receptors by endogenous PACAP contributes to the micturition reflex and bladder overactivity in spinalized rats [169, 170]. Chemical inflammation of the rat bladder also increases PACAP expression in bladder afferent neurons [149, 171]. In addition, patch clamp studies in the neonatal rat spinal slice preparation also revealed that PACAP has a direct excitatory action on parasympathetic preganglionic neurons due in part to blockade of K^+ channels [172].

8.1.7 Prostanoids and Endothelins

8.1.7.1 Prostanoids

Prostanoids (prostaglandins and thromboxanes), which comprise a family of oxygenated metabolites of arachidonic acid via the enzymatic activity of cyclooxygenases 1 and 2, are manufactured throughout the lower urinary tract and have been implicated in bladder contractility, inflammatory responses, and neurotransmission. Biopsy specimens of human bladder mucosa contain prostaglandin (PG) I2, PGE2, PGF2α, and thromboxane A. The actions of prostanoids are mediated by specific receptors on cell membranes, which include the DP, EP, FP, IP, and TP receptors that preferentially respond to PGD2, PGE2, PGF2α, PGI2, and thromboxane A2, respectively. Furthermore, EP is subdivided into four subtypes: EP1, EP2, EP3, and EP4 [173, 174]. EP receptors are reportedly found in the urothelium, detrusor smooth muscle and intramural ganglia [175, 176]. In the guinea pig bladder, the major production of prostaglandins occurs in the urothelium and where production increases greatly with inflammation [177]. In mice PGE2 provokes ATP release from cultured urothelial cells, which express EP1 receptors; and bladder overactivity induced by intravesical application of PGE2 is prevented in EP1 receptor-knockout mice, suggesting the involvement of EP1 receptors in the PGE2-mediated urothelial-afferent interaction and bladder overactivity [178, 179]. Thus, EP1-selective antagonists may improve bladder storage function; however, the EP1 receptor antagonist ONO-8359 failed to

show the therapeutic efficacy compared with placebo for the treatment of patients with overactive bladder (OAB) [180].

The EP3 receptor is also involved in the modulation of bladder function in the normal condition as well as bladder overactivity induced by enhanced PGE2 production evoking DO because EP3 receptor null mice have a reduction in bladder overactivity in response to bladder PGE2 infusion and demonstrate a larger bladder capacity than wild type mice under the normal condition [181]. The EP4 receptor is also another candidate for the treatment of bladder overactivity because of the findings that; (1) intravenous application of an EP4 antagonist (AH23848) reduced bladder overactivity induced by cyclophosphamide without affecting normal micturition in rats [182], and (2) intravesical infusion of another EP4 antagonist (ONO-AE1-329) significantly decreased KCl-induced contraction of bladder strips and increased bladder capacity in rats with bladder outlet obstruction without changes in controls [176].

Despite that PGE2 can enhance the micturition reflex, clinical attempts to use prostaglandins to facilitate voiding have had mixed results. Intravesical PGE2 has been shown to enhance bladder emptying in women with urinary retention and patients with neurogenic voiding dysfunction [183–185]. Others have failed to find PGE2 useful to facilitate complete evacuation of the bladder [186, 187]. Intravesical PGE2 does produce urgency and involuntary bladder contractions [188]. However, more recently, the EP2 and EP3 receptor dual agonist (ONO-8055), which induces EP3-mediated detrusor contraction and EP2-mediated urethral relaxation, has been shown to improve inefficient voiding in animal models of detrusor underactivity induced by lumbar spinal canal stenosis (rat) [189] and hysterectomy (monkey) [119].

8.1.7.2 Endothelins

Endothelins (ETs), a family of 21–amino acid peptides originally isolated from bovine aortic endothelial cells, include ET-1, ET-2, and ET-3, which are encoded by separate genes and mediate a variety of biologic actions through two distinct G protein–coupled receptor subtypes, the endothelin-A (ET_A) and the endothelin-B (ET_B) receptor [190, 191]. The ET_A receptor subtype has a higher affinity for ET-1 and ET-2 than for ET-3; the ET_B receptor subtype binds all ETs with equal affinity [192]. ET-1, which is known to be primarily produced by human endothelial cells, can induce prolonged contractile responses in isolated urinary bladder muscle strips in various species [193, 194]. In humans and rabbits, ET-like immunoreactivity is identified in almost all cell types in the bladder, including bladder epithelium, vascular endothelium, detrusor and vascular smooth muscles, and fibroblasts; it plays a role in control of bladder smooth muscle tone, regulation of local blood flow, and bladder wall remodeling in pathologic conditions [195]. In a rabbit model of bladder outlet obstruction, ET-1 and ET_A receptor binding sites in detrusor smooth muscle and urothelium as well as ET_B receptor binding sites in detrusor smooth muscle were significantly increased [193, 196]. In addition, the endothelin-converting enzyme inhibitor WO-03028719, which suppresses ET-1 production, can improve voiding efficiency and suppress detrusor overactivity in a rat model of bladder outlet obstruction [197]. YM598, a selective ET_A receptor antagonist, also reduces detrusor overactivity in urethral obstructed rats [198]. These results suggest that the increase in ET-1 expression and ET receptors could be involved in detrusor hyperplasia and overactivity seen in patients with bladder outlet obstruction resulting from benign prostatic hyperplasia.

There is also evidence that ETs have a role in modulation of sensory function in the peripheral and central nervous system. The activation of ET_A receptors in capsaicin-sensitive C-fiber afferents in the bladder induces detrusor overactivity, whereas ET_A receptor activation in the spinal cord can inhibit the micturition reflex through activation of a spinal opioid mechanism in rats [199]. In spinal cord injured rats, the bladder ET-1 level was increased, and the application of ABT-627, an ET_A antagonist, suppresses C-fiber-mediated detrusor overactivity. Taken together, modulation of ET_A receptor activity in bladder afferent pathways or the spinal cord could be effective in treating bladder overactivity or painful conditions [200].

8.1.8 Serotonin

Serotonin (5-HT) has been found in neuroendocrine cells along the urethra and in the prostate [201]. More recently, these cells are characterized as serotonergic paraneurons in the female mouse urethra, which show the close proximity to putative C-fiber afferent nerve fibers positive for CGRP, substance P and TRPV1 [202]. Intraurethral perfusion of serotonin also induced excitation of urethral afferent neurons and increased pain sensitivity during urethral distention, suggesting that irritative symptoms such as the urethral syndrome may arise because of urethral serotonergic mechanisms. The close proximity of CGRP-immunoreactive nerve fibers and 5-HT-positive endocrine cells has also been demonstrated in the prostatic urethra of male rats [203].

5-HT also has several pharmacologic effects on mammalian urinary bladders, both in vitro and in vivo. Human and pig isolated detrusor muscles are known to contract in a concentration-dependent manner in response to 5-HT [204]. In human isolated urinary bladder, there was potentiation of the contractions induced by electrical field stimulation, mediated by the $5\text{-}HT_4$ receptor subtype [205, 206]. A similar response is present on guinea pig detrusor muscle through $5\text{-}HT_{2A}$ and $5\text{-}HT_4$ receptors, whereas in the rabbit and the rat, the receptors involved are the $5\text{-}HT_3$ and $5\text{-}HT_7$ subtypes, respectively [207]. Furthermore, contractile responses of bladder strips are reportedly enhanced and significantly reduced by a $5\text{-}HT_{2A}$ antagonist, in association of upregula-

tion of 5-HT_{2A} and 5-HT_{2B} receptors in detrusor muscle, in rats with bladder outlet obstruction [208, 209].

8.1.9 TRP Channels

The superfamily of TRP (transient receptor potential) channels expressed in mammals are subdivided into six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin) groups, which are Ca^{2+} permeable cation channels and activated by physical (depolarization, hot/cold temperature, mechanical stress) or chemical (pH, osmolality) stimuli and binding to specific ligands (vanilloids. menthol). The available evidence suggests that TRP channels have a four-subunit combination, in either a homotetrameric or heterotetrameric complex, to form functional ion permeation complexes [210].

8.1.9.1 TRPV1

TRPV1, the most extensively studied TRP channel, is expressed on capsaicin-sensitive afferent pathways, predominantly C-fiber nociceptors, and responds to increases in temperature to the noxious range (>43°C) and by protons, suggesting that it functions as a transducer of painful thermal stimuli and acidity in vivo. When it is activated, the channel opens, allowing an influx of Ca^{2+} and Na^{+} ions that depolarizes the nociceptive afferent terminals, initiating a nerve impulse that travels through afferent nerves into the central nervous system. Noxious temperature uses the same elements, which explains why the mouth feels hot when eating chili peppers [211]. In the lower urinary tract, TRPV1 is expressed in suburothelial afferent fibers, urothelium, detrusor smooth muscle and other non-neuronal cells such as suburothelial interstitial cells (Fig. 8.5). Studies using TRPV1 knock out mice provide the evidence showing that TRPV1 receptors are not essentially involved in conscious voiding, but have a role in afferent sensitization due to cystitis because bladder overactivity induced by chemical cystitis using cyclophosphamide or acrolein was not observed in TRPV1 knock-out mice [212–214]. In addition, TRPV1 expressed in the bladder urothelium may function as a stretch sensor because the release of ATP and NO from cultured urothelial cells during hypotonic stretch is reduced in TRPV1 knockout mice compared with the wild type [212].

In patients with spinal cord injury–induced detrusor overactivity, clinical response to intravesical therapy with RTX led to a marked decrease of nerve fibers positively stained for PGP9.5, a neuronal marker, and TRPV1. Six of 17 patients in this investigation showed a satisfactory clinical response to RTX treatment, with marked improvements on cystometry and other parameters [215]. Spinal-injury patients who did not respond to RTX showed no decrease in nerve fiber population, similar to controls. In addition, intravesical RTX administered to patients with idiopathic detrusor overactivity delayed or suppressed involuntary detrusor contractions during filling cystometry. The mean interval to the first involuntary contraction more than doubled vs. baseline at 30 and 90 days; mean maximal cystometric capacity increased; the mean number of episodes of urinary incontinence daily fell to fewer than one; and mean daily frequency also decreased significantly [216]. It has also been reported that C-fiber desensitization induced by intravesical application of high-dose capsaicin and resiniferatoxin (RTX) is effective for treating painful symptoms in IC patients [217, 218] although a prospective, randomized clinical trial using intravesical RTX application showed no effect in patients with IC [219].

In addition, targeting TRPV1 receptors using selective TRPV1 antagonists is being evaluated for the treatment of bladder dysfunction. A oral TRPV1 antagonist (GRC-6211), has been shown to decrease bladder overactivity and noxious bladder input in cystitis animal models [220] and bladder contraction frequency [221]. It has also shown that a selective TRPV1 antagonist (JTS-653) significantly suppressed the capsaicin-induced increase in afferent nerve discharge and reduced bladder overactivity induced by intravesical infusion of resiniferatoxin or acetic acid, without affecting normal micturition [222]. Furthermore, herpes simplex virus (HSV) vector-mediated gene therapy against TRPV1 receptors in the bladder and afferent pathways suppressed bladder overactivity and enhanced bladder pain sensitivity in rats with resiniferatoxin-induced bladder inflammation [223]. These results suggest the possibility of TRPV1 antagonists for the treatment of bladder pain/overactivity.

8.1.9.2 TRPM8

TRPM8 is a member of the temperature sensitive TRP channels, which responds to cold temperature less than 23°C. Pharmacological agents that evoke cool sensation such as menthol and ilicin can activate TRPM8. In sensory pathways, TRPM8 is expressed in DRG and trigeminal ganglion neurons that do not express TRPV1, isolectin-B4, or CGRP, which are usually markers of C-fiber afferents. Thus, it seems that TRPM8 is expressed in a subpopulation of thermoceptive and nociceptive afferents, which are different from the TRPV1 expressing subpopulation. In the human lower urinary tract, TRPM8 expression is found the prostate, the testes, scrotal skin, and bladder [224]. In addition, although in the study by Stein et al., expression in the human bladder was limited to the urothelium (Fig. 8.5), Mukerji et al. showed TRPM8 immunoreactivity in the bladder urothelium as well as in fine nerve fibers in the suburothelial layer and that the number of TRPM8 positive C-fibers in the bladder suburothelium is increased in patients with idiopathic detrusor overactivity [225]. In animal studies, activation of TRPM8 channels in the guinea pig bladder by intravesical application of menthol reduces volume threshold for micturition and increases sensitivity to bladder cooling [226] while a TRPM8 antagonist, AMTB, decreases bladder contraction frequency without affecting contraction ampli-

tude in cystometry as well as the visceromotor reflex of abdominal muscle in response to noxious urinary bladder distension in rats [227]. More recently, intravenous application of a selective TRPM8 antagonist (RQ-00203078) is shown to increase bladder capacity and voided volume and decrease nerve firing activity of mechanosensitive C-fiber afferents in the normal condition, and reduced bladder overactivity and increased afferent firing induced by intravesical menthol in rats [228]. In another study in rats, intravesical application of a TRPM8 antagonist (DFL23448) increases micturition intervals, micturition volume and bladder capacity in the normal condition and reduced PGE2-induced bladder overactivity [229]. Furthermore, TRPM8 expression in bladder afferent neurons is increased in rats with bladder outlet obstruction [230]. Therefore, TRPM8 in bladder afferent pathways and urothelium could be involved in modulation of sensory function of the lower urinary tract. In addition, TRPM8 channels expressed in the skin have been shown to be involved in cold stress-induced bladder overactivity because a TRPM8 channel antagonist (BCTC) inhibited bladder overactivity induced by menthol applied to the leg skin or by an exposure to low-temperature environment [231]. These results raise the possibility that the TRPM8 channel can be a therapeutic target for certain types of bladder overactivity.

8.1.9.3 TRPA1

TRPA1 is the only member of the Ankyrin TRP channel, and a receptor for several pungent chemicals that evoke pain such as allyl-isothiocyanate (the pungent compound in mustard oil), allicin (garlic), cinnamaldehyde (in cinnamon) and acrolein (the metabolite of cyclophosphamide). TRPA1 also functions as a receptor-operated channel that can be activated by growth factors or proinflammatory peptides such as bradykinin, which increases intracellular Ca^{2+} levels via G protein-coupled receptors. TRPA1 is expressed in sensory neurons, in which it is co-expressed with TRPV1, but not with TRPM8. Although TRPA1 can be activated by cold (<17°C) via an increase in intracellular Ca^{2+} concentration when expressed in heterologous systems, its role as a cold sensor in native peripheral sensory neurons including DRG cells remains uncertain. In mice, cooling does not evoke unspecific rises in Ca^{2+} concentration in DRG neurons while visceral sensory neurons in nodose ganglia exhibit a strong correlation between cold sensitivity and TRPA1 expression [232], suggesting that TRPA1 may contribute to cold transduction in visceral sensory neurons rather than somatic neurons [233]. In the bladder, TRPA1 is expressed in the urothelium, TRPV1 and CGRP-positive suburothelial afferent nerves and detrusor muscles in mice, rats and humans [234, 235]. TRPA1 receptor activation by intravesical application of hydrogen sulfide, allyl isothiocyanate and cinnamaldehyde induces frequent voiding as evidenced by a reduction in intercontraction intervals, which is suppressed by capsaicin-induced C-fiber desensitization in rats [235, 236]. Additionally, intravenous administration of a TRPA1 antagonist (HC-030031) reduced the single unit mechanosensitive afferent activity during bladder filling and prevented the increase in afferent activity during TRPA1 channel stimulation in rats [237]. Furthermore, TRPA1 mRNA expression in the bladder mucosa from male patients with lower urinary tract symptoms due to bladder outlet obstruction has shown to be significantly increased compared with control subjects [235]. In rats with spinal cord injury, intravenous administration of a TRPA1 antagonist (HC-030031) or intrathecal treatment with antisense oligodeoxynucleotide of TRPA1 receptors is effective in suppressing detrusor overactivity whereas the TRPA1 expression is increased in the bladder and L6-S1 dorsal root ganglia (DRG) in these animals [238]. Overall, TRPA1 channels expressed in the bladder urothelium and sensory pathways may have a role in sensory transduction in pathological conditions including overactive bladder.

8.1.9.4 TRPV4

TRPV4 is a member of vanilloid TRPV channels and a nonselective cation channel activated by mechanical pressure, osmolality (hypotonicity), moderate warmth (>27 °C) and chemical stimuli such as phorbol derivates. Its expression has been detected in urothelial cells and detrusor muscle, but not in the suburothelial layer, in the bladder of mice, rats and guinea pigs [239–243]. The TRPV4 agonist, 4α-phorbol 12,13-didecanoate, and hypotonic cell swelling promote Ca^{2+} influx and evokes ATP release in cultured urothelial cells from mice or rats [239, 240]. In cultured urothelial cells from TRPV4 knockout mice, the intracellular Ca^{2+} increase and ATP release in response to stretch stimulation were significantly attenuated compared to the wild type mice [244]. Cystometric experiments revealed that TRPV4 knockout mice exhibit a lower frequency of voiding contractions as well as a higher frequency of nonvoiding contractions [240] and that intravesical application of TRPV4 agonists induces bladder overactivity as evidenced by increased micturition pressure in rats [239] or reduced contraction frequency in mice [241]. In addition, intravesical application of a TRPV4 agonist (GSK1016790A) is shown to induce P2X receptor-mediated bladder overactivity by activation of mechanosensitive, capsaicin-insensitive C-fiber afferents in rats [245]. Furthermore, intravesical application of a TRPV4 antagonist (HC067047) reduced bladder overactivity observed after repeated variate stress, which is associated with increased urothelial TRPV4 expression [246]. These results suggest that urothelial TRPV4 channels act as an important molecule to enhance bladder activity, predominantly through activation of bladder afferent pathways via urothelially released ATP.

In addition to the functional role of urothleilal TRPV4 channels, recent studies suggest that TRPV4 channels in the forebrain is involved in the decision of early timing of voiding in mice [247] and that activation of TRPV4 channels in the detrusor muscle suppresses spontaneous contractions through activation of BK channels, which is likely to function as a self-limiting mechanism for reducing bladder contractility during bladder filling in guinea pigs [248]. The latter finding is in line with the observation in decerebrated TRPV4 null mice, which showed the increase of non-voiding contractions during bladder filling [247].

8.1.10 Cannabinoides

Cnnabinoid (CB) is the general term of bioactive substances contained in cannabis, and more than 60 kinds of CBs are found in cannabis [249, 250]. Of the more than 60 different CBs, tetrahydrocannabinol (THC), which is a major active ingredient of the drug marijuana, can induce mind-nerve reactions such as euphoria and relaxation, followed by drowsiness, sedation, and depression [249]. Effects of CBs are mediated of two types of G protein-coupled receptors; CB1 and CB2, which are expressed throughout the lower urinary tract including bladder urothelium, submucosal afferent nerves and detrusor muscle [251]. Pharmacological experiments using exogenous application of CB agonists revealed that activation of both CB1 and CB2 receptors increases threshold pressure and micturition intervals while minimally affecting voiding function, suggesting that CB receptor activation mainly inhibits the afferent limb of the micturition reflex [251]. At the spinal cord level, CB1 and CB2 receptor activation by intrathecal applications of their ligands is effective to reduce bladder pain sensitivity in animal models of cystitis [252, 253]. Furthermore, it has been shown that inflammatory changes in the bladder can be improved by CB2 receptor activation in rats with chemically-induced cystitis [254].

These data indicate that CB receptor modulation could be a new modality for the treatment of bladder overactivity and pain conditions. However, because the exocannbinoid therapy can induce the side effects in the central nervous system, modulation of the endocannabinoid system may be an alternative and attractive option. Endocannabinoids are endogenously generated substances that are degraded by two enzymes: fatty-acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) [255]. While the latter is less investigated, the FAAH and its target endocannabinoid, anandamide, have been studied to elucidate their roles in the control of lower urinary tract function. Application of FAAH inhibitors such as URB937 or OEA reduces PGE2-induced firing of C-fiber afferents and frequent urination in normal rats [96, 256] and attenuates referred hyperalgesia in rats with experimental cystitis [257]. These results suggest that the endocannabinoid system could be a therapeutic target for OAB and hypersensitive bladder disorders such as interstitial cystitis/bladder pain syndrome (IC/BPS).

8.1.11 Botulinum Toxin

There has been increasing evidence for the therapeutic efficacy of botulinum neurotoxin (BoNT) for the treatment of various lower urinary tract dysfunctions [258–261].

Botulinum toxins act by inhibiting acetylcholine release at the presynaptic cholinergic nerve terminal, thereby inhibiting striated and smooth muscle contractions. The toxins are synthesized as single-chain polypeptides with a molecular weight of about 150 kD [262]. Initially, the parent chain is cleaved into its active dichain polypeptide form, consisting of a heavy chain (approximately 100 kD) connected by a disulfide bond to a light chain (approximately 50 kD) with an associated zinc atom [263]. Four steps are required for toxin-induced paralysis: binding of the toxin heavy chain to a receptor, synaptic vesicle protein 2 (SV2) on nerve terminals, internalization of the toxin within the nerve terminal, translocation of the light chain into the cytosol, and inhibition of neurotransmitter release. Vesicle docking requires the interaction of various cytoplasm, vesicle, and target membrane proteins (i.e., synaptosome-associated membrane receptor [SNARE] proteins), some of which are specifically targeted with clostridial neurotoxins. For example, BoNT-A cleaves the cytosolic translocation protein SNAP-25, thus preventing vesicle fusion with the plasma [260, 264].

Seven immunologically distinct neurotoxins are designated types A, B, C, D, E, F, and G. Clinically, the urologic community has used commercial preparations of BTX-A to treat patients with neurogenic and idiopathic detrusor overactivity [262, 265–270]. Although ACh release from bladder parasympathetic efferent terminals is the primary target of BoNT treatment, suppression of bladder afferent activity with BoNT treatment is also evident because the reduction of urgency symptom in patients with neurogenic and idiopathic detrusor overactivity is associated with reduced expression of the capsaicin receptor (TRPV1) and the ATP receptor ($P2X_3$) in C-fibers [271]. In addition, in basic research, botulinum toxins are shown to suppress not only efferent nerve activity by inhibition of the release of acetylcholine but also afferent nerve activity by release inhibition of neurotransmitters such as substance P and CGRP from sensory terminals [272, 273]. Incubation of rat bladder strips with the botulinum toxin A for 3 h *in vitro* also reportedly reduce detrusor contractions induced by electrical field stimulation or capsaicin application, suggesting the dual toxin effects on efferent and afferent nerve terminals [274], although an earlier study with a shorter toxin incubation time for 10 min showed the negative results in mice and guinea pigs [275].

There is also evidence that the toxin can reduce the release of ATP from urothelial cells in normal and spinalized rats [276–279]. Thus, the use of the toxins has been expanded to treat patients with neurogenic or non-neurogenic overactive bladder and even IC/BPS [258, 261, 280, 281].

8.2 Central Nervous System

8.2.1 Spinal Ascending and Descending Pathways

8.2.1.1 Glutamate

Intrathecal or intravenous administration of glutamatergic NMDA or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) antagonists in urethane-anesthetized rats depressed reflex bladder contractions and electromyographic activity of the EUS in animals with an intact spinal cord as well as in animals with chronic spinal injury [282, 283]. Studies in rats also indicate that activation of bladder preganglionic neurons (PGN) by input from the pontine micturition center (PMC) can be blocked by inotropic glutamate receptor antagonists, suggesting that the descending pathways from the PMC utilize glutamate as a neurotransmitter [284]. These results indicate that spinal reflex pathways controlling bladder and sphincter function utilize NMDA and AMPA glutamatergic transmitter mechanisms (Figs. 8.6 and 8.7). In spinal cord-injured rats, external sphincter muscle activity was more sensitive than bladder reflexes to glutamatergic antagonists, raising the possibility that the two reflex pathways might have different glutamatergic receptors [285]. This was confirmed with in situ hybridization techniques, which revealed that sacral parasympathetic PGN in the rat express high mRNA levels of GluR-A and GluR-B AMPA receptor subunits and NR1 but not NR2 NMDA receptor subunits [286]. Conversely, motoneurons in the urethral sphincter nucleus express all four AMPA receptor subunits (GluR-A, -B, -C and -D) in conjunction with moderate amounts of NR2A and NR2B as well as high levels of NR1 receptor subunits. It seems likely that this difference in expression accounts for the different sensitivity of bladder and sphincter reflexes to glutamatergic antagonists.

Glutamate also plays a role as an excitatory transmitter in the afferent limb of the micturition reflex. C-fos expression induced in spinal interneurons by activation of bladder afferents is suppressed by the administration of both NMDA and non-NMDA glutamatergic receptor antagonists [287–289]. Additionally, the spinal glutamatergic pathway is shown to

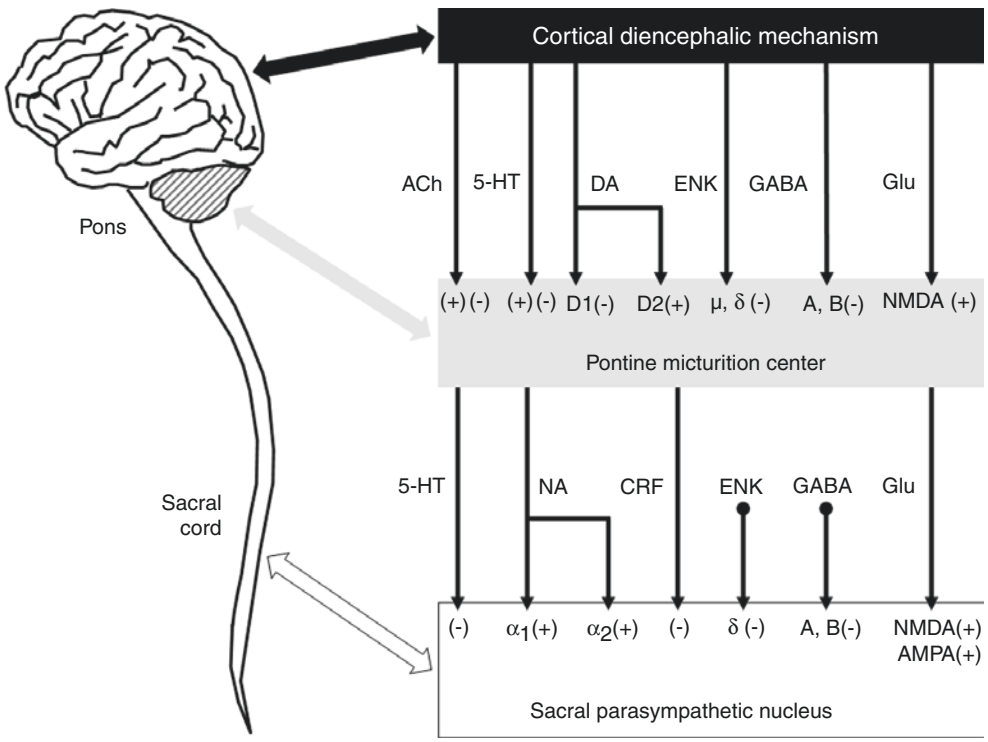

Fig. 8.6 Diagram of neurotransmitters at spinal and supraspinal sites. Glutamate is the major excitatory transmitter in control of the micturition reflex. Modulation of the micturition reflex in the spinal cord occurs by segmental interneuronal mechanisms (ENK, GABA) or by descending input from the brain (5-HT, NA, CRF). Modulation in the pontine micturition center can be activated in part by input from cortical–diencephalic areas. Facilitatory and inhibitory responses are indicated by plus and minus in parentheses, respectively. *ACh* acetylcholine, *CRF* corticotrophin releasing factor, *DA D1 and D2* dopamine receptors, *ENK* enkephalin, *GABA* gamma-aminobutyric acid receptors (A and B), *Glu* glutamate, *NA* norepinephrine, *μ* opioid receptors; *5-HT* 5-hydroxytryptamine

be involved in the external urethral sphincter (EUS) contraction reflex during sneezing via spinal AMPA receptor activation in rats. Intrathecal application of an AMPA receptor antagonist (NBQX) decreased the sneeze-induced urethral pressure responses without affecting urethral baseline pressure, and caused stress urinary incontinence during sneezing [290].

In contract to excitatory effects of glutamate via ionotropic glutamate receptors (NMDA and AMPA/kinate), activation metabotropic glutamatergic receptors (mGluRs) in the spinal cord has inhibitory effects on the descending limb of the micturition reflex because a group I/II mGluR agonist applied to the spinal cord at the lumbosacral level suppressed reflex bladder contractions as well as those induced by PMC stimulation in rats [291]. It has also been reported that mGluRs are involved in inhibition of the excitatory pathway to the external urethral sphincter (EUS) because a group I/II mGluR antagonist applied into the lumbosacral intrathecal space significantly facilitated the electromyogram activity of the EUS in rats [292].

In the synaptic transmission, glutamate released from presynaptic nerve terminals is cleared from synaptic clefts into presynaptic nerve terminals and adjacent astrocytes, via glutamate transporters. A previous study demonstrated that intrathecal application of a non-selective inhibitor of glutamate transporters, L-trans-pyrrolidine-2,4-dicarboxylic acid (L-trans-PDC) that increases endogenous glutamate concentration at nerve terminals, delayed the onset of micturition by increasing inter-micturition intervals and pressure thresholds in rats under urethane anesthesia [293].

8.2.1.2 Inhibitory Amino Acids (GABA, Glycine, Glycine Transporter)

Intrathecal injection of either $GABA_A$ or $GABA_B$ agonists increases bladder capacity and decreases voiding pressure and efficiency in normal rats [294, 295] and also suppress detrusor overactivity in rats with intravesical application of oxyhemoglobin, an NO scavenger [294] or spinal cord injury [296] (Figs. 8.6 and 8.7). In addition, intravenous or intrathecal application of a GABA re-uptake inhibitor (tiagabine)

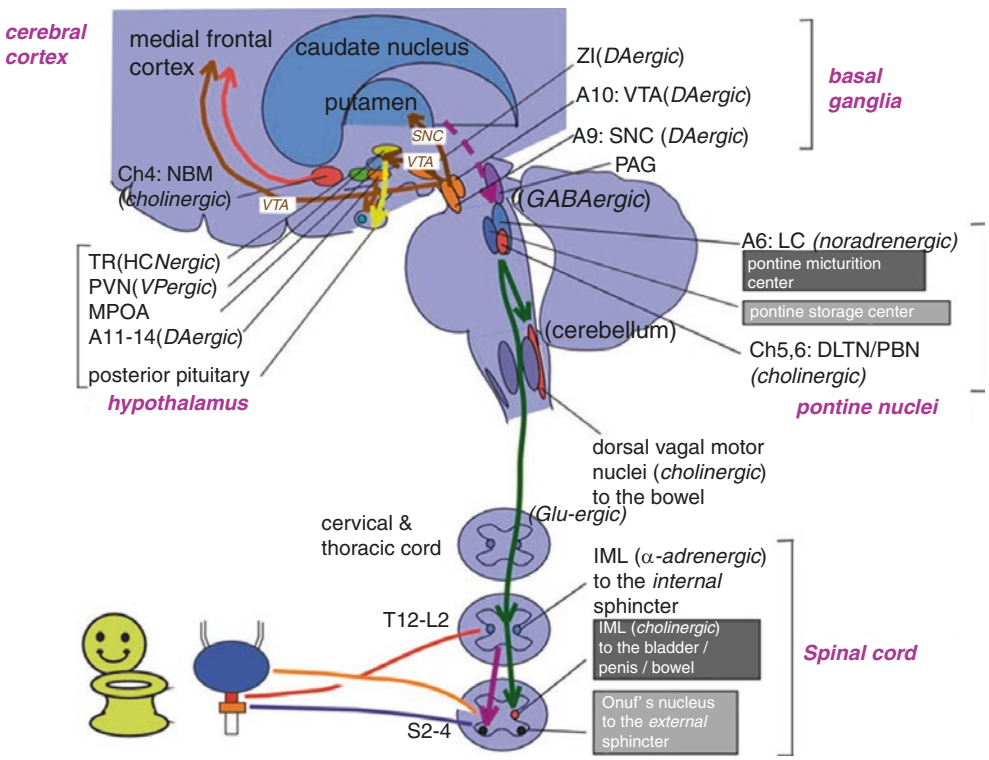

Fig. 8.7 Neural circuitry and neurotransmitteres relevant to micturition. The lower urinary tract consists of two major components, the bladder and the urethra. The bladder is mainly innervated by the parasympathetic pelvic nerve. The urethra is innervated by the sympathetic hypogastric nerve and somatic pudendal nerve, respectively. Urinary storage is dependent on the reflex arc of the sacral spinal cord. The storage reflex is thought to be tonically facilitated by the brain, particularly the pontine storage center. The storage function is thought to be further facilitated by the hypothalamus, cerebellum, basal ganglia, and frontal cortex. Central cholinergic fibers from the nucleus basalis Meynert (NBM, also called the Ch4 cell group) seem to facilitate urinary storage. Micturition is dependent on the reflex arc of the brainstem and spinal cord, which involves the midbrain periaqueductal gray (PAG) and the pontine micturition center (located in or adjacent to the locus coeruleus [LC]). The voiding function is thought to be initiated by the hypothalamus and prefrontal cortex, which overlap the storage-facilitating area. *PVN* paraventricular nucleus, *MPOA* medial preoptic area, *A* adrenergic/noradrenergic, *ZI* zona incerta, *VTA* ventral tegmental area, *SNC* substantia nigra pars compacta, *DLTN* dorsolateral tegmental nucleus, *PBN* parabrachial nucleus, *TR* tuberous region, *HCN* hypocretinergic, *DLTN* dorsolateral tegmental nucleus, *IML* IML cell column, *GABA* γ-aminobutyric acid, *T* thoracic, *L* lumbar, *S* sacral

that increases endogenous GABA concentrations reportedly inhibits normal micturition in rats [297]. In a small clinical study in three subjects, intrathecal administration of a $GABA_B$ receptor agonist (baclofen) increased the volume threshold for inducing the micturition reflex [298]. Intrathecally administered baclofen also produced phaclofen-sensitive inhibition of distention-evoked micturition in conscious rats that appears to be resistant to capsaicin (substance P depletion) and parachlorophenylalanine (5-hydroxytryptamine depletion) pretreatment [299]. Because baclofen also inhibits field stimulation–evoked release of calcitonin gene-related peptide from primary afferent terminals in dorsal horn slices, one possible site of action of suppression by baclofen is transmitter release from primary afferent terminals in the spinal cord.

Previous studies also showed that glycine, another inhibitory amino acid, acting on strychnine-sensitive receptors exerts an inhibitory effect on the micturition reflex pathway [300, 301] and is also involved in the inhibition of sphincter motoneurons during micturition [302]. Glycine and GABA inhibitory mechanisms have also been identified in the neonatal rat spinal cord in local intraneuronal inhibitory pathways projecting directly to the PGN [303]. Application of $GABA_A$ agonists to sacral parasympathetic PGN inhibits reflex firing, opens chloride channels, and hyperpolarizes the cells and b Baclofen, a $GABA_B$ agonist, suppresses Ca^{2+} channels in sacral preganglionic neurons in the rat [304].

In addition, some studies have revealed that the level of glycine in the spinal cord is decreased by approximately 50% in rats with detrusor overactivity induced by chronic spinal cord injury, compared with spinal intact rats [301, 303] and that dietary supplement of glycine can restore bladder function along with an increase in the serum level of glycine in spinal cord injured rats [304]. The level of glutamic acid decarboxylase (GAD), the GABA synthetic enzyme, is also reduced in the spinal cord and lumbosacral dorsal root ganglia in spinal cord–injured rats with detrusor overactivity and sphincter-detrusor dyssynergia, and both impaired functions are improved by intrathecal application of $GABA_A$ or $GABA_B$ receptor agonists [305, 306]. These results suggest that downregulation of spinal glycinergic and GABAergic mechanisms may contribute to the emergence of neurogenic detrusor overactivity associated with spinal cord injury.

The extracellular concentration of glycine at synapses is regulated by two types of Na^+/Cl^--dependent glycine transporters (GlyTs): GlyT1 and GlyT2 [307]. GlyT1 is widely distributed in the CNS and predominantly expressed in glial cells near both excitatory and inhibitory neurons, while GlyT2 is specifically distributed in the spinal cord, cerebellum, and brainstem, and localized in the presynaptic terminals of inhibitory glycinergic neurons [308]. A previous study reported that intrathecal application of a selective GlyT2 inhibitor, ALX-1393, but not a GlyT1 inhibitor, sarcosine, produced significant increases in inter-micturition intervals and pressure thresholds in rats with cyclophosphamide-induced cystitis [309], suggesting that inhibition of GlyT2 is a new approach to enhance the spinal glycinergic inhibitory mechanism controlling the micturition reflex.

8.2.1.3 Adrenergic

In the spinal cord, descending pathways form noradrenergic brainstem nuclei such as the locus coeruleus (LC) can mediate excitatory and inhibitory influences on the lower urinary tract via adrenoceptors (Figs. 8.6 and 8.7). In anesthetized cats, α_1-adrenoceptors were implicated in a bulbospinal noradrenergic excitatory pathway from the LC to the sacral parasympathetic outflow to bladder [310–312], although subsequent studies could not confirm these findings in conscious cats [313].

Experiments in conscious or anesthetized rats revealed that intrathecal administration of an α_1-adrenergic antagonist (doxazosin) decreased the amplitude of bladder contractions [314, 315]. The bladder inhibitory effect of intrathecal α_1-adrenergic antagonist was more prominent in animals with chronic outlet obstruction [314]. It was also found that intrathecal administration of doxazosin suppressed detrusor overactivity (unstable bladder contractions) in spontaneously hypertensive rats [124]. Although intrathecal injection of doxazosin suppressed the amplitude of reflex bladder contractions in anesthetized rats, it increased the frequency of isovolumetric contractions, indicating the presence of a tonic adrenergic inhibitory mechanism [316]. This was supported by the finding that phenylephrine, an α_1-adrenergic agonist, applied intrathecally, decreased the frequency of bladder contractions without changing contraction amplitude [316]. Overall, it appears that the spinal noradrenergic system has a modulatory role in the control of the micturition reflex and that efferent and afferent limbs of the micturition reflex receive excitatory and inhibitory input, respectively, from this system. Also, it has been reported that intrathecal injection of tamsulosin, an α_{1A}-selective adrenergic antagonist, or naftopidil, an selective $\alpha_{1A/D}$- adrenergic antagonist, transiently abolished isovolumetric rhythmic bladder contraction in normal rats [317] and that intrathecal injection of naftopidil prolonged the interval between voiding contractions and decreased the maximum voiding contraction pressure and the number of non-voiding contractions in spinal rats [318]. Intrathecal application of silodosin, a selective α_{1A} adrenergic antagonist, or naftopidil is also shown to increase bladder capacity in a rat model of cerebral infarction induced by middle cerebral occlusion [319]. These results suggest that α_{1A} and/or α_{1D} adrenoceptor subtypes are involved in the spinal excitatory mechanism controlling micturition in rats.

Evidence for a role of α_2 adrenoceptors in micturition is conflicting because both facilitatory and inhibitory roles of α_2-adrenoceptors have been documented [314, 316].

Atipamezole, an α_2-adrenergic antagonist given intrathecally, can increase micturition pressure in the conscious rat, implying that there is a tonic inhibitory adrenergic control [314]. However, yohimbine, an α_2-adrenergic antagonist, inhibits micturition in anesthetized rats [320]. In paraplegic patients, intrathecal injection of clonidine suppressed detrusor overactivity [321]. Conversely, in conscious spinal cats, clonidine, an α_2-adrenergic agonist, increased bladder pressures and facilitated voiding [322].

It is also known that locus coeruleus (LC) noradrenergic neurons are activated by visceral stimuli such as bladder and colon distension, and then modulate arousal and attention [323, 324]. Previous studies showed that the excitatory response of LC neurons to bladder distention was strongly affected by the state of anesthesia and that the response was accompanied by lightening of the anesthesia, indicative of arousal, detected by EEG recordings in rats [325]. Valentino et al. also reported that neurons containing corticotrophin-releasing factor in Barrinton's nucleus (i.e. the pontine micturition center) relay input from pelvic visceral afferents to the LC and may serve as a coordinating center of central and peripheral responses to pelvic visceral stimuli [324, 326].

Pharmacologic experiments showed that the bladder-to-sympathetic reflex pathway is also modulated by spinal noradrenergic mechanisms [316, 327, 328]. In the chloralose-anesthetized cat, prazosin or doxazosin, α_1-adrenergic antagonists, suppressed spontaneous firing [329] or the reflex discharge recorded on the hypogastric nerve in response to pelvic nerve afferent stimulation [327]. Administration of α_2-adrenergic agonists also suppresses reflex sympathetic activity [327]. These observations suggest that bulbospinal noradrenergic pathways provide a tonic α_1-excitatory control of the bladder-sympathetic reflex in the spinal cord. α_2-Adrenergic inhibitory mechanisms are not active under control conditions in anesthetized animals but can be up-regulated by elevating endogenous norepinephrine levels with an inhibitor (tomoxetine) of norepinephrine reuptake [327]. These results suggest that the lumbar sympathetic outflow is controlled by α_1-excitatory and α_2-inhibitory mechanisms.

The activation of urethral sphincter motoneurons by stimulation of bladder (pelvic nerve) or urethral/perineal (pudendal nerve) afferents is part of a continence-maintaining mechanism. These reflexes recorded as efferent discharges on the pudendal nerve in chloralose-anesthetized cats were suppressed by the α_1-adrenoceptor antagonist prazosin [327, 330], but not by the α_2 blocker idazoxan [327]. Using whole-cell patch clamp techniques in rat neonatal spinal cord slices, norepinephrine is shown to depolarize urethral sphincter motoneurons and evoke their action potentials, and these effects are blocked by prazosin, suggesting that there is a direct facilitatory mechanism increasing urethral sphincter motoneuron excitability by norepinephrine via α_1-adrenoceptors [331].

Conversely, clonidine, an α_2-adrenoceptor agonist, suppressed the reflex in anesthetized cats [332]. The norepinephrine uptake blocker tomoxetine produced a slight inhibition alone and only a slightly greater inhibition after prazosin. However, it greatly facilitated the reflex when given after idazoxan [327]. These data indicate the existence of α_2-adrenoceptor--mediated inhibitory and α_1 adrenoceptor--mediated tonic facilitation of sphincter function and that the α_2 adrenoceptor--dependent inhibitory mechanism is the dominant adrenergic modulator of the pudendal nerve reflex [333]. These α_1 and α_2 adrenoceptor--mediated facilitatory and inhibitory mechanisms, respectively, also contribute to the urethral continence reflex that prevents stress urinary incontinence because previous studies using a norepinephrine reuptake inhibitor nisoxetine or a norepinephrine/serotonin reuptake inhibitor, duloxetine, induces α_1-adrenoceptor activation in the lumbosacral spinal cord to enhance reflex contractions of the external urethral sphincter during sneezing [334, 335] and that α_2 adrenergic antagonists, yohimbine or idazoxan, enhances the duloxetine-induced urethral sphincter contraction during sneezing or abdominal compression in rats [336, 337].

8.2.1.4 Serotonergic

Neurons containing 5-HT in the raphe nucleus of the caudal brain stem send projections to the dorsal horn, as well as to the autonomic and sphincter motor nuclei in the lumbosacral spinal cord (Fig. 8.6). In cats, activation of raphe neurons or 5-HT receptors in the spinal cord inhibits reflex bladder contractions and firing of the sacral efferent pathways to the bladder [338–341] and also inhibits firing of spinal dorsal horn neurons elicited by stimulation of pelvic nerve afferents [342]. Extracellualar recordings of neuronal activity in the raphe nucleus in response to storage/voiding cycles under the isovolumetric condition have revealed that the most common (~50%) were tonic storage neurons that exhibited increased firing at an interval between reflex bladder contractions in cats [341].

In rats, the administration of m-chlorophenylpiperazine (mCPP), which is an agonist for 5-$HT_{2A/C}$ receptors, suppressed efferent activity on bladder nerves and reflex bladder contractions [343]. These effects were blocked by mesulergine, a 5-HT_2 receptor antagonist [343, 344]. Intrathecal administration of methysergide, a 5-$HT_{1/2}$ antagonist, or zatosetron, a 5-HT_3 antagonist, decreased the micturition volume threshold in cats [345], implying that descending serotonergic pathways tonically depress the afferent limb of the micturition reflex through $5HT_2$ and/or $5HT_3$ receptors.

The role of 5-HT_1 receptors in bladder activity seems different in cats and rats. In cats, administration of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a 5-HT1A receptor agonist increased bladder capacity in chloralose anesthetized cats, in which the bladder was irritated with

acetic acid, but had only moderate effects on bladder activity in the absence of irritation [346]. The drug also had a facilitatory effect on activity of the external urethral sphincter. 8-OH-DPAT also inhibited reflex bladder activity in awake or chloralose-anesthetized, chronic spinal cord-injured cats, but did not alter the somato-bladder excitatory reflex induced in spinal cats by tactile stimulation of the perigenital region [347]. The effects of 8-OH-DPAT were blocked by WAY 100635, a 5-HT_{1A} receptor antagonist, which alone had no effect. These results indicate that 8-OH-DPAT acts in the spinal cord to inhibit the micturition reflex triggered by C-fiber bladder afferent axons and has much less effect on the spino-bulbo-spinal reflex elicited by Aδ-afferents.

In contrast, 8-OH-DPAT administered intrathecally facilitated bladder activity in both normal and spinal cord-injured rats but not in rats in which bladder afferents were damaged by treatment with capsaicin at birth [299]. Conversely, administration of the 5-HT_{1A} receptor antagonist WAY 100635, which increases the firing rate of raphe neurons by blocking 5-HT_{1A} inhibitory autoreceptors, inhibits reflex bladder contractions in rats [348]. The inhibition is antagonized by pretreatment with mesulergine, a 5-HT_2 receptor antagonist, indicating that 5-HT_2 receptors are involved in descending raphe/spinal inhibitory mechanisms [348]. Similar inhibitory effects of another 5-HT_{1A} receptor antagonist, NAD-299, on the micturition reflex have been reported in rats [349].

When the effects of intrathecal administration of WAY 100635 on the ascending and descending limbs of the micturition reflex pathway were examined in anesthetized rats, WAY 100635 depressed bladder contractions evoked by electrical stimulation of the pontine micturition center, but did not alter the evoked field potentials in the region of during electrical stimulation of afferent axons in the pelvic nerve, indicating that the drug suppresses the pathway from the brainstem to the spinal cord but does not alter the afferent pathway from the bladder to the pontine micturition center [350, 351]. Thus, micturition in the rat is facilitated by stimulation of $5HT_1$ inhibitory autoreceptors, whereas in the cat $5HT_1$ receptor activation appears to act primarily through postsynaptic mechanisms to promote urine storage by enhancing sphincter activity and suppressing bladder activity [352].

The sympathetic autonomic nuclei as well as the sphincter motor nuclei also receive a serotonergic input from the raphe nucleus [333, 345, 353]. Serotonergic activity mediated via 5-HT_2 and 5-HT_3 receptors enhances urine storage by facilitating sphincter reflexes in cats [345, 354]. Another study in rats also reported that activation of $5HT_{2C}$ receptors enhances the urethral closure reflex induced by pudendal nerve-mediated urethral striated muscle contraction during sneezing at the spinal level whereas $5HT_{1A}$ receptors inhibit it because intrathecally applied 8-OH-DPAT (a $5HT_{1A}$ agonist) decreases urethral contractile responses during sneezing and that mCPP (a $5HT_{2B/2C}$ agonist) increases them, and the effects of 8-OH-DPAT and mCPP are antagonized by intrathecal applications of WAY-100635, a selective $5HT_{1A}$ antagonist, and RS-102221, a selective $5HT_{2C}$ antagonist, respectively [355].

Duloxetine, a combined norepinephrine/serotonin reuptake inhibitor has been shown, in a bladder-irritated cat model, to increase the neural activity of both the urethral sphincter and the bladder [333, 356]. Duloxetine appears to have effects on both the bladder and the sphincter has been proposed as a treatment for both stress and urge incontinence [333, 357]. Duloxetine increases the neural activity to the EUS via 5-HT_2 receptors and α_1 adrenoceptors and decreases bladder activity via 5-HT_1 receptors in the spinal cord [333]. Clinical trials have also shown the efficacy of duloxetine for the treatment of stress urinary incontinence, and the drug has been approved in Europe and is already available in several countries [358] although it was withdrawn from the FDA approval process in the US by the manufacturer.

8.2.1.5 Acetylcholine

Muscarinic acetylcholine (mACh) receptors have an inhibitory effect on the micturition reflex in the spinal cord. In the rat, intrathecal application of an ACh receptor agonist, oxotremorine-M, or a cholinesterase inhibitor, neostigmine, increases bladder capacity and pressure thresholds, and these effects are atropine-sensitive, indicating the mACh receptor-mediated inhibitory action in the spinal cord [359–361]. Since intrathecal application of atropine by itself has no effects on the micturition reflex in normal rats, but decreases inter-micturition intervals in rats with cyclophosphamide-induced cystitis, the endogenous mACh mechanism for the inhibitory modulation of micturition, which is not tonically active in the normal condition, might be up-regulated after bladder inflammation [361]. Nicotinic receptors are also involved in the control of voiding function since intrathecal application of nicotine have an facilitatory effect on the micturition reflex in the rat [362].

Spinal mAChR also modulate the urethral continence reflex that prevents stress urinary incontinence since a cholinesterase inhibitor, neostigmine, administered intrathecally reduces the urethral closure reflex induced by pudendal nerve-mediated urethral striated muscle contraction during sneezing. The neostigmine-induced decrease in sneeze-induced urethral responses was reversed by pretreatment with atropine (nonselective mACh antagonist), methoctramine (M2 receptor antagonist) or 4-DAMP (M3 receptor antagonist), but not with pirenzepine (M1 receptor antagonist), tropicamide (M4 receptor antagonist), or mecamylamine (nicotinic receptor antagonist), suggesting the involvement of M2 and M3 mACh in muscarinic receptor-mediated modulation of urethral function [363].

8.2.1.6 Opioid Peptides

Opioid peptides have an inhibitory action on reflex pathways in the spinal cord. In the cat spinal cord, inhibition of reflex bladder activity is mediated by μ receptors whereas inhibition of sphincter activity is mediated by κ receptors [165, 328, 340]. In the rat, both μ and δ receptors mediate bladder inhibition [328, 364, 365]. The spinal opioid inhibitory system can also be activated by tachykinins via NK3 receptors [366] and by endothelins via endothelin A receptors to inhibit the micturition reflex [199].

Opioid receptors also seem to be involved in pudendal or tibial nerve neuromodualtion, which has been shown to be effective for the treatment of overactive bladder symptoms, because naloxone, an opioid receptor antagonist, reverses the increasing effect of pudendal or tibial nerve stimulation on bladder capacity during intravesical saline infusion or bladder overactivity induced by intravesical acetic acid infusion, respectively, in cats [366–369]. However, the site of action for opioid receptor activation during neuromodulation may not be limited in the spinal cord as naloxone was administered systemically in these studies.

8.2.2 Pontine Micturition Center and Supraspinal Pathways

8.2.2.1 Glutamate

Glutamic acid also has a role in excitatory transmission at supraspinal sites in the micturition reflex pathway (Fig. 8.6). A recent study has confirmed that the majority of corticotrophin-releasing factor (CRF)-positive neurons in the PMC, which send axons to the lumbosacral spinal cord, are glutamatergic cells positive for vesicular glutamate transporters [370]. Exogenous L-glutamate or its analogue injected at sites such as PMC or parabrachial nucleus in the brain stem of supracollicular decerebrate or chloralose anesthetized cats where electrical stimulation evoke bladder contractions [371], elicits voiding when the bladder is partially filled or increased frequency and amplitude of rhythmic bladder contractions when the bladder is filled above the micturition threshold volume and maintained under isovolumetric conditions [339, 372]. On the other hand, injections of glutamic acid at some sites in the PMC elicits inhibition of isovolumetric contractions or initial excitation followed by inhibition [373].

Administration of glutamatergic agonists into the region of the PMC in rats also elicits voiding or increases frequency and amplitude of bladder contractions [373, 374], whereas injection of agonists in the brain of rats and cats at other sites known to have inhibitory functions in micturition elicits inhibitory effects [339, 375–378]. Intracerebroventricular injection of AMPA or NMDA receptor antagonists blocks reflex bladder contractions in anesthetized rats, indicating that glutamatergic transmission in the brain is essential for voiding function [283].

In rat brain slices patch clamp recordings from pre-parasympathetic output (PPO) and pre-sympathetic output (PSO) neurons projecting, respectively, to the sacral parasympathetic and thoraco-lumbar sympathetic intermediolateral nuclei revealed that spontaneous EPSCs recorded after blocking GABAergic and glycinergic inhibitory receptors with bicuculline and strychnine were blocked by the AMPA glutamatergic receptor antagonist CNQX. This indicates that the neurons receive excitatory inputs from glutamatergic neurons located in the slice [378]. Blocking AMPA and NMDA ionotropic glutamate receptors also decreased the spontaneous firing of PSO neurons but paradoxically increased the firing of PPO neurons indicating that the latter neurons receive a tonic inhibitory input triggered by a glutamatergic mechanism. This is consistent with the observation mentioned above that injections of glutamate at some sites in the cat PMC unexpectedly inhibited reflex bladder activity [368].

A previous study also showed that a non-selective inhibitor of glutamate transporters, L-trans-PDC, administered into the lateral ventricle increased inter-micturition intervals and pressure thresholds in anesthetized rats, suggesting that activation of the overall glutamatergic system at the supraspinal site has an inhibitory effect on micturition, possibly via activation of glutamate-mediated inhibitory pathways [291].

8.2.2.2 Acetylcholine

Excitatory and inhibitory cholinergic influences on the micturition pathway have been identified at the supraspinal level using various techniques (Fig. 8.6). In the rat brain, muscarinic receptor-mediated cholinergic mechanisms may be involved in both inhibitory and facilitatory modulation of the micturition reflex [359, 379, 380], and the muscarinic inhibitory mechanism seems to involve an activation of M1 muscarinic receptors [379] and protein kinase C [381]. One site of action can be localized to the midbrain-pons region because cholinergic agonists are effective after supracollicular decerebration in rats [382]. In the brain stem, microinjection of acetylcholine to the PMC in cats increased or decreased the threshold volume for inducing a reflex contraction of the bladder [165, 383]. These effects were blocked by atropine, indicating a role of muscarinic receptors. Nicotinic receptors are also involved in the control of voiding function since nicotinic receptor agonists, epibatidine or nicotine, injected into the lateral ventricle have an inhibitory effect on the micturition reflex in the rat [362, 384]. A decreased volume threshold and increased micturition pressure were detected after administration of bethanechol, a muscarinic agonist, into the central circulation of the cross-perfused dog [385].

8.2.2.3 GABA and Glycine

GABA has been implicated as an inhibitory transmitter at supraspinal sites where it can act on both $GABA_A$ and $GABA_B$ receptors [165, 316, 328, 340, 386] (Figs. 8.6 and 8.7). As mentioned in an earlier section of this paper, injection of $GABA_A$ receptor agonists, into the PMC of decerebrate cats or into the PAG of rats suppresses reflex bladder activity and increases the volume threshold for inducing micturition [372]. These effects are reversed by bicuculline, a $GABA_A$ receptor antagonist; and bicuculline alone stimulates bladder activity and lowers the volume threshold for micturition, indicating that the micturition reflex pathway in the PMC and PAG is tonically inhibited by a GABAergic mechanism. Intracerebroventricular administration of melatonin increases bladder capacity in rats; and this effect is blocked by bicuculline indicating that melatonin activates a GABAergic inhibitory mechanism in the brain [387]. Intracerebroventricular injection of baclofen, a $GABA_B$ agonist, suppresses distention-evoked micturition in urethane-anesthetized rats but unexpectedly this effect is not blocked by phaclofen, a $GABA_B$ receptor antagonist [316, 328].

Patch clamp recordings in rat brain slices showed that blocking $GABA_A$ receptors with bicuculline increases the excitability of both pre-parasympathetic output (PPO) and pre-sympathetic output (PSO) neurons in the PMC, which are labeled by injecting fluorescent tracers into the intermediolateral region of the spinal cord at T13-L1 and S1-S2 levels, respectively, while blocking glycine receptors with strychnine increases the firing of only PPO neurons [388]. Blocking ionotropic glutamatergic receptors which increases firing of PPO neurons in untreated slices does change firing in the presence of strychnine, indicating that glutamatergic excitatory transmission generates the tonic glycinergic inhibitory input to PPO neurons.

8.2.2.4 Dopamine

In the central nervous system, dopaminergic pathways exert inhibitory and facilitatory effects on the micturition reflex through D1-like (D1 or D5 subtypes) and D2-like (D2, D3, or D4 subtypes) dopaminergic receptors, respectively [389–397] (Figs. 8.6 and 8.7). In anesthetized cats, activation of dopaminergic neurons in the substantia nigra inhibits reflex bladder contractions via D1-like receptors [391]. In awake rats a D1 dopaminergic antagonist (SCH 23390) facilitates the micturition reflex whereas a D1 agonist (SKF 38393) does not alter reflex bladder contractions, suggesting that D1 receptor--mediated suppression of bladder activity is tonically active in the normal awake state [396]. Conversely, activation of central D2-like dopaminergic receptors with quinpirole or bromocriptine facilitates the micturition reflex pathway in rats, cats, and monkeys [390, 392, 394, 395, 398]. D2-like receptor-mediated facilitation of the micturition reflex may involve actions on spinal cord as well as actions on the brain stem because microinjection of dopamine to the PMC reduced bladder capacity and facilitated the micturition reflex in cats [340].

It is also known in cats that neurons in the substantia nigra pars compacta and the ventral tegmental area, which are the major dopamine-containing nuclei in the midbrain, respond to the storage/micturition cycles of isovolumetric cystometry [399] and that dopamine levels in the striatum, where nigrostiatal dopaminergic nerves terminate, increase during the storage phase of the micturition cycle [400]. Thus, central dopaminergic pathways appear to be involved in the control of the bladder function through actions on multiple receptors at different sites in the brain.

Activation of D2-like receptors at a supraspinal site suppresses the activity of the striated sphincter muscle and reduces intraurethral pressure; whereas inhibition of dopamine D1- or D2-like receptors has a minimal effect on urethral function in anesthetized rats, suggesting the dopaminergic control of urethral function is minimally active in the normal condition [401].

Parkinson's disease (PD) is a degenerative disorder of central nervous system caused by the insufficient formation and action of dopamine, which is produced in the pathways from the substantia nigra to the striatum in the mid-brain. Clinical studies have demonstrated that patients with PD often have lower urinary tract symptoms such as nocturia, increased urinary frequency and urinary incontinence with he reported incidence ranging between 27–63.9% across different studies [402]. The most common finding in the urodynamic study is detrusor overactivity (DO) shown by uninhibited contractions during bladder filling [402]. In monkeys, disruption of nigrostriatal dopaminergic pathways induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces PD-like motor symptoms accompanied by bladder overactivity shown by frequent urination with reduced voided volume [389, 392, 402]. A rat model of PD induced by a unilateral 6-hydroxydopamine injection into the substantia nigra exhibits a similar type of bladder overactivity [394, 403]. In these animal models, bladder overactivity was suppressed by enhancement of D1-like receptors with SKF 38393 or pergolide, suggesting that bladder overactivity in PD is primarily induced by disruption of D1-like dopamine receptor-mediated inhibition of the micturition reflex [392, 394, 398] (Fig. 8.8). In addition, in a rat model of PD, bladder overactivity was suppressed by an adenosine A2A receptor antagonist, ZM241385, suggesting that enhanced activity of the adenosine A2A system in the brain contribute to bladder overactivity associated with PD [403]. The adenosine A2A receptor-expressing neural pathways are very likely located downstream of D1 receptor expressing pathways in the control of micturition

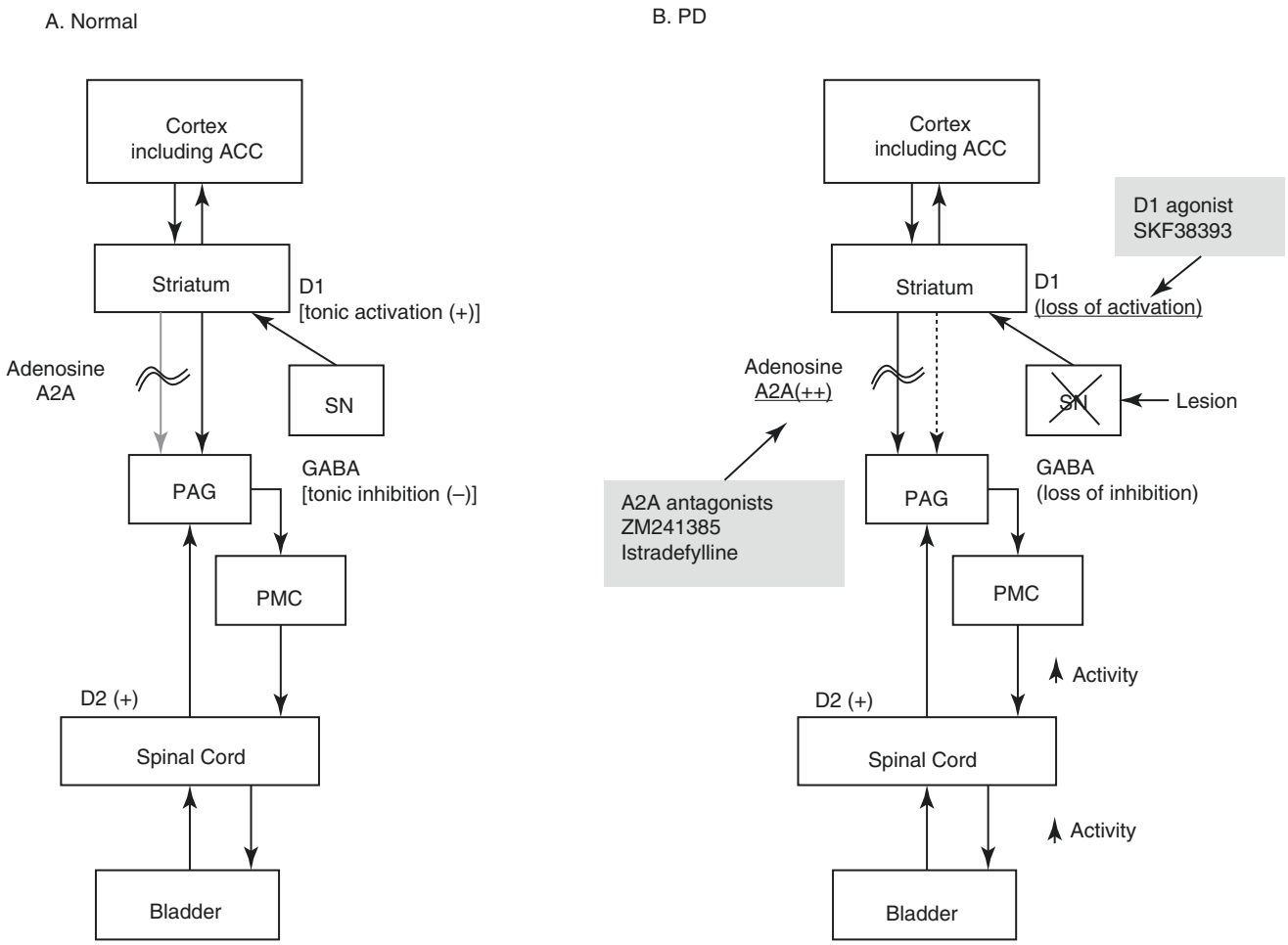

Fig. 8.8 A hypothetical diagram demonstrates working model of bladder dysfunction in Parkinson's disease (PD). This figure was adopted from ref. 61, and modified. Micturition reflex is controlled by spinobulbospinal pathways through PAG in midbrain and PMC in brainstem. This neural circuit is under control of higher centers including ACC and other cortex regions. A, under normal conditions tonic inhibition from ACC suppress micturition reflex. Tonic firing (+) of dopaminergic neurons in SN activates dopamine D1 receptors expressed on GABAergic inhibitory neurons in the striatum to induce tonic GABAergic inhibition (−) of the micturition reflex at the level of PAG. At the same time, D1 receptor stimulation suppresses the activity of adenosinergic neurons, which exert an excitatory effect on micturition via adenosine A2A receptors (+). B. In PD, dopaminergic neurons in the SN are lost (lesion), leading to the loss of dopamine D1 receptors activation [D1 (loss of activation)], which results in reduced activation inhibitory GABAergic neurons in the striatum [GABA (loss of inhibition)]. At the same time, reduced D1 receptor stimulation enhances the adenosinergic mechanism to stimulate adenosine A2A receptors [Adenosine A2A (++)], leading to facilitation of the spinobulbospinal pathway controlling the micturition reflex pathway. Administration of dopamine D1 receptor agonist (SKF38393) can restore the GABAergic nerve activity and suppress A2A receptor-mediated activation to reduce bladder overactivity in PD. Also, administration of adenosine A2A antagonists (ZM241385 or Istradefylline) can suppress A2A receptor-mediated activation of the micturition reflex to reduce bladder overactivity in PD. Dopamine D2 receptors [D2 (+)] expressed in the spinal cord enhances the micturition reflex. *ACC* anterior cingulate cortex, *GABA* gamma-aminobutyric acid, *PAG* periaqueductal gray, *PMC* pontine micturition center, *SN* substantia nigra pars compacta

because inhibition of bladder activity by D1 receptor activation can induce the partial suppression of adenosine A2A receptor-mediated excitatory mechanisms in the rat model of PD [403] (Fig. 8.8). To support this assumption, a recent open-labeled clinical study reported that treatment with istradefylline, a selective adenosine A2A receptor antagonist, for 12 weeks significantly improved lower urinary tract symptoms in 13 male PD patients although a larger-sized, placebo-controlled randomized study is needed to confirm the results [404].

8.2.2.5 Serotonin

The serotonergic system (5-HT) in the supraspinal site seems to also contribute to the modulation of the micturition reflex. A rat model of depression induced by clomipramine administration, which depletes the brain 5-HT concertation, exhibits frequent urination with bladder overactivity that is improved by a 5HT reuptake inhibitor (fluoxetine) [384]. These results suggest that the central 5-HT system exerts the inhibitory effect on micturition. More recently, increased concertation of 5-HT in the prefrontal cortex after fluoxetine

treatment has an inhibitory effect on the micturition reflex, which is blocked by a 5-HT_{1A} receptor antagonists in rats [405]. Because the prefrontal cortex is shown to be one of the major brain sites involved in the voluntary control of micturition in human brain imaging studies [406], it is likely that the brain 5-HT system is involved in the modulation of the prefrontal cortex activity to exert the inhibitory effects on micturition.

In contrast, the brain 5-HT system can be excitatory to induce bladder overactivity in the psychological stress condition. Recent studies demonstrated that bladder overactivity induced by intracerebroventricular (i.c.v.) application of bombesin, a stress-related neuropeptide, is suppressed by pretreatment with a 5-HT synthesis inhibitor (p-chlorophenylalanine) or i.c.v. application of a 5-HT_7 receptor antagonist (SB269970) [407, 408]. These results suggest that the 5-HT_7 receptor-mediated serotonergic mechanism may contribute to the emergence of bladder overactivity in the psychological stress condition.

8.2.2.6 Opioid Peptides

Intracerebroventricularly administered morphine suppresses isovolumic bladder contractions in rats and cats, and this effect is blocked by naloxone [364, 365, 409, 410] (Fig. 8.6). Naloxone administered intracerebroventricularly also reversed the effects of systemically administered morphine. Naloxone administered alone intracerebroventricularly or injected directly into the PMC facilitates the micturition reflex, indicating that micturition is tonically inhibited by a supraspinal opioid mechanism [410, 411]. Both μ and δ opioid receptors mediate inhibitory effects that are blocked by naloxone [372, 410]. In addition, activation of μ and δ1, but not δ2 opioid receptors in the brain increases bladder capacity in both normal rats and rats with cerebral infarction that exhibit frequent voiding; however, κ receptor activation increases bladder capacity only in rats with cerebral infarction [412]. In rat brain slices application of a specific μ opioid receptor agonist (DAMGO) suppresses the firing of pre-parasympathetic output (PPO) and pre-sympathetic output (PSO) neurons in the PMC, which are labeled by injecting fluorescent tracers into the intermediolateral region of the spinal cord at T13-L1 and S1-S2 levels, respectively [388].

8.3 Conclusion

During the past few decades, research in the field of neurourology has led to the emergence of new concepts regarding the neural control of the lower urinary tract and the etiology of lower urinary tract dysfunction. This has stimulated the search for new therapies to treat voiding disorders. In addition to traditional drugs, which target the smooth muscle or postjunctional muscarinic and adrenoceptors, it is now clear that targets at other sites such as afferent neurons, efferent nerve terminals, urothelial cells, and the central nervous system are equally important for drug development. Because micturition is controlled by complex neural circuits distributed throughout the central and peripheral nervous systems that utilize a wide variety of neurotransmitters, it is probable that many different classes of drugs will eventually be used to treat voiding problems. The major challenge is to identify drugs which exhibit "uroselectivity," i.e. affect the lower urinary tract without eliciting undesirable side effects. We hope that this chapter helps understand and update the readers' knowledge for the pharmacology of the lower urinary tract, thereby leading to the future development of new therapeutic modalities of lower urinary tract dysfunction.

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