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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, 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 voiding dysfunctions such as urinary frequency, urgency, and incontinence or inefficient voiding and urinary retention. This chapter will review recent advances in our understanding of the pathophysiology of voiding disorders and the targets for drug therapy.

INTRODUCTION

Anatomy and Innervation of the Lower Urinary Tract

The storage and periodic elimination of urine are dependent on the reciprocal activity of two functional units in the lower urinary tract: (*a*) a reservoir (urinary bladder) and (*b*) an outlet (bladder neck and smooth and striated muscles of the urethra) (1–6). During urine storage, the outlet is closed, and the bladder smooth muscle is quiescent, allowing intravesical pressure to remain low over a wide range of bladder volumes. During voiding, the muscles of the outlet relax and the bladder smooth muscles contract, raising intravesical pressure and inducing urine flow. These changes are coordinated by three sets of nerves (parasympathetic, sympathetic, and somatic) emerging from the sacral and thoracolumbar levels of the spinal cord (1–6). Sacral parasympathetic (pelvic) nerves provide an excitatory input (cholinergic and purinergic) to the bladder and inhibitory input (nitrergic) to the urethra (2, 3, 7–9). Thoracolumbar sympathetic pathways which release norepinephrine provide an excitatory input to the bladder neck and urethra, facilitatory and inhibitory input to parasympathetic ganglia (6), and, in some species, inhibitory input to the bladder smooth muscle (1–3, 5). Lumbosacral efferent

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pathways in the pudendal nerves provide a cholinergic excitatory input to the urethral sphincter striated muscle.

Afferent activity arising in the bladder and urethra is conveyed to the central nervous system by the three sets of nerves (2–4, 10). The most important afferents for initiating micturition are those passing in the pelvic nerves to the sacral spinal cord. These afferents consist of small myelinated (Aδ) and unmyelinated (C) fibers which convey impulses from tension and volume receptors and nociceptors in the bladder wall (3, 10). Afferent nerves are located in the serosal and muscle layers as well as adjacent to and within the epithelial lining (the urothelium) of the bladder and urethra (3, 10, 12). Epithelial afferents can respond to changes in the chemical composition of the urine or to chemicals [nitric oxide (NO), prostaglandins, and adenosine triphosphate (ATP)] released from the urothelial cells (3, 10, 13–18). Electrophysiological studies in cats have shown that Aδ bladder afferents respond in a graduated manner to passive distension as well as active contraction of the bladder (3, 10) and therefore trigger sensations of bladder filling. C-fiber bladder afferents, which appear to function primarily as nociceptors (11), are normally mechanoinsensitive (i.e. silent C-fibers) but can be activated by noxious stimuli (3, 10). In the rat, Aδ and some C-fiber afferents are mechanosensitive (3, 12).

Neural Control of Voiding

The central nervous system control of the lower urinary tract involves neural pathways at various levels of the neuraxis (2–4). In infants, voiding occurs via reflexes that seem to be organized as simple on-off switching circuits. However, after 4–6 years of age, voiding is initiated voluntarily by the cerebral cortex. Urine storage mechanisms mediated by sympathetic and somatic inputs to the urethral outlet are dependent upon spinal reflex circuits, which are activated by sacral afferent nerve activity during bladder filling (2–4). However, voiding reflexes involving activation of the parasympathetic input to the bladder and inhibition of the sympathetic and somatic inputs to the urethra are mediated by a spinobulbospinal reflex pathway passing through a coordinating center in the rostral brain stem [the pontine micturition center (PMC)] (2–4). Modulation of the spinobulbospinal reflex circuit by higher centers in the cerebral cortex and diencephalon presumably underlies the voluntary control of voiding.

Studies in animals have identified numerous transmitters in the central pathways controlling lower urinary tract function. The major excitatory transmitter in the micturition reflex pathway in rats is glutamic acid (19–23). Other putative excitatory transmitters include norepinephrine, dopamine, substance P, ATP, and acetylcholine (2–4, 19, 24). Inhibitory transmitters include 5-hydroxytryptamine (5-HT), opioid peptides, gamma-aminobutyric acid (GABA), glycine, and dopamine.

Pathophysiology

Voiding dysfunction can be divided into two general categories: (*a*) failure to store and (*b*) failure to empty (25). Storage problems can occur as a result of weakness

or anatomical defects in the urethral outlet, causing a condition known as stress urinary incontinence (25). Failure to store also occurs if the bladder is unstable or overactive. This can occur as a result of hyperactivity of the bladder smooth muscle or various neurological disorders, such as multiple sclerosis, cerebrovascular disease, Parkinson's disease, brain tumors, and spinal cord injury (25). Urinary urgency, frequency, and incontinence also occur in elderly patients due to changes in the brain or bladder during aging. Urinary retention occurs in patients with urethral outlet obstruction (e.g. prostate enlargement), neural injury, and/or diseases that damage nerves (e.g. diabetes mellitus) or in those who are taking drugs that depress the neural control of the bladder (2, 3, 25).

Various types of therapy are used to treat lower urinary tract disorders, including surgery, drugs, biofeedback, electrical nerve stimulation, pelvic floor exercises, and catheterization (9, 25, 26). This chapter will review the mechanisms underlying the pharmacological modulation of lower urinary tract function.

CHOLINERGIC MECHANISMS

Muscarinic receptor antagonists are the most widely prescribed drugs for the treatment of bladder hyperactivity (25–27). These drugs act at least in part by blocking postjunctional muscarinic receptors in the detrusor muscle. Two muscarinic receptor subtypes (M_2 and M_3) have been identified in the human bladder muscle (27–30). Although the M_2 receptor is the predominant subtype (~80%), bladder contractions are mediated by M_3 receptors (27–30). Stimulation of M_3 receptors by acetylcholine leads to phosphoinositol hydrolysis and then to the release of intracellular Ca^{2+} and a smooth muscle contraction (29). It has been proposed (30) that coactivation of M_2 receptors could enhance the response to M_3 stimulation by (*a*) inhibiting adenylate cyclase and thereby suppressing sympathetically mediated depression of detrusor muscle, (*b*) inactivation of K^+ channels, or (*c*) activation of nonspecific cation channels.

Muscarinic receptors are also located prejunctionally on cholinergic nerve terminals in the bladder (31–37). Activation of M_1 prejunctional receptors facilitates acetylcholine release (31–34), whereas activation of M_2/M_4 receptors inhibits the release (35–37). It has been proposed that inhibitory M_2/M_4 receptors are preferentially activated by autofeedback mechanisms during short periods of low-frequency nerve activity and thereby suppress cholinergic transmission during urine storage (31). On the other hand, M_1 receptors are activated during more prolonged, high-frequency nerve firing that would occur during voiding, and thus they participate in an amplification mechanism to promote complete bladder emptying (31). M_1-mediated facilitation of transmitter release involves the activation of a phospholipase C/protein kinase C signaling cascade which appears to facilitate the opening of L-type Ca^{2+} channels that are necessary for prejunctional facilitation (32, 33). Inhibitory and facilitatory muscarinic receptors are also present in the central nervous system (2–4) and

in bladder parasympathetic ganglia, where they modulate nicotinic transmission (6).

Urothelial cells in the rat bladder respond to cholinergic agonists by releasing NO (38). Both muscarinic and nicotinic receptors have been implicated in this action. Although the physiological role of these receptors is uncertain, it is possible that they are activated in an autocrine manner by acetylcholine released from the urothelial cells (39) or by a transmitter released from cholinergic nerves located in the mucosa adjacent to the urothelium.

Although antimuscarinic agents that act nonselectively on muscarinic receptors have been used for many years to treat bladder hyperactivity, the clinical effectiveness of these agents has been limited by side effects such as dry mouth, blurred vision, and constipation (25–27). Newer approaches to improve the tolerability of standard antimuscarinic drugs, such as oxybutynin, include alternative routes of administration and extended-release oral formulations (27, 40). Intravesical administration of oxybutynin may increase tolerability by allowing absorption directly into the bladder and/or by allowing the drug to bypass the portal circulation and reduce first-pass metabolism to the metabolite *N*-desethyl oxybutynin, which appears to be responsible in part for side effects of the drug (27). Extended-release, once daily formulations of oxybutynin also have been reported to reduce the side effects of the drug compared to the standard three-times-a-day dosing regimen (27, 40). This has been attributed to more constant plasma levels and reduced first-pass metabolism.

Certain antimuscarinic drugs seem to exhibit bladder selectivity. For example, tolterodine, a competitive antagonist that binds to all muscarinic receptor subtypes, was shown in anesthetized cats to depress neurally evoked bladder contractions in doses that did not reduce salivary secretion (41). Subsequent clinical studies revealed that the therapeutic efficacy of tolterodine was equivalent to that of oxybutynin in reducing micturition frequency and urge incontinence episodes in patients with overactive bladder, but the former produced a lower incidence and severity of dry mouth (42). These results are consistent with radioligand binding studies that showed that the two drugs had a similar K_i for muscarinic receptors in the bladder (human and guinea pig) but that tolterodine had an eightfold lower affinity than oxybutynin for M_3 receptors in the guinea pig parotid gland. Oxybutynin also has a greater efficacy in blocking M_1 and M_3 receptors than M_2 receptors. Thus, the apparent bladder selectivity of tolterodine might be due, in part, to its effectiveness in blocking M_2 receptors in the bladder. Alternatively, it is possible that M_3 receptors are heterogeneous and that M_3 receptors in the bladder are more sensitive to blockade by tolterodine (27).

Differences in the sensitivity of muscarinic receptors in bladder and salivary glands have also been detected in studies with M_3-selective antagonists darifenacin and zamifenacin (27, 43). Darifenacin, which has an 11-fold-higher affinity for M_3 than for M_2 receptors, was similar in potency to atropine in blocking acetylcholine-induced contractions of the guinea pig urinary bladder but had a fivefold lower affinity than atropine for M_3 receptors in the parotid gland (43). Similarly, in the

anesthetized dog, darifenacin was 8.6-fold more potent in blocking pelvic nerve-evoked bladder contractions than in suppressing trigeminal nerve-evoked salivation (27).

PURINERGIC MECHANISMS

Although it had been known for many years that stimulation of parasympathetic nerves induces atropine-resistant contractions of the urinary bladder, it was not until the studies of Burnstock & coworkers in 1972 (44) that evidence was presented to support the view that ATP was the mediator of the atropine-resistant, noncholinergic, nonadrenergic (NANC) contractions. Subsequent papers showed that: (*a*) exogenous ATP-induced contractions mimicked the NANC contractions; (*b*) desensitization by exogenous purinergic agonists (e.g. α,β-me-ATP) suppressed ATP-induced and NANC contractions; (*c*) various drugs (quinidine, suramin, ANAPP3, PPADS) blocked ATP-induced and NANC contractions without depressing the responses to acetylcholine; (*d*) ATP-induced and NANC contractions were enhanced by ATPase inhibitors; and (*e*) ATP-induced and NANC contractions were dependent on extracellular Ca^{2+}, whereas cholinergic contractions were dependent on intracellular Ca^{2+} release (7, 8).

ATP acts on two families of purinergic receptors: an ion channel family (P2X) and a G-protein-coupled receptor family (P2Y) (7). Seven P2X subtypes and eight P2Y subtypes have been identified. Analysis of the structure-activity relationships of a series of excitatory purinergic agonists on the guinea pig bladder revealed an order of potency consistent with $P2X_1$ or $P2X_2$ receptors (8). In other species (rabbit, cat, and rat), various studies suggested that multiple purinergic excitatory receptors are present in the bladder (8). For example, in the rat bladder, the responses to ATP and α,β-me-ATP were fast and transient; whereas the responses to adenosine diphosphate-β-S and uridine triphosphate were slower, sustained, and relatively unaffected by pretreatment with α,β-me-ATP (45). According to current concepts, the fast response elicited by ATP would be mediated by activation of a ligand-gated cation channel (P2X receptor) that promotes the influx of extracellular Ca^{2+}, whereas uridine triphosphate and adenosine diphosphate-β-S would be expected to act through G protein coupled receptors ($P2Y_2$ or $P2Y_4$) to induce smooth muscle contractions via a PLC/IP3 signaling pathway (8) and release of intracellular Ca^{2+}. Similarly, in the rabbit bladder the response to ATP is normally biphasic, but after desensitization with α,β-me-ATP, the response is monophasic, also suggesting two different types of receptors (46).

Ligand receptor binding studies (rabbit and rat) revealed high-affinity binding of [^{3}H]ATP and [^{3}H]α,β-me-ATP to bladder membranes (8). Displacement experiments with various agonists and antagonists indicated the presence of a P2X receptor (47, 48). 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 (49).

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 $P2X_4$ messenger RNA in the bladder (50, 51).

Purinergic nerves are likely to have other functions in the lower urinary tract because excitatory receptors for ATP are present in parasympathetic ganglia (52–55), afferent nerve terminals (7, 8, 17, 18), and urothelial cells (56). Excitatory purinergic receptors in pelvic ganglia have been demonstrated in the cat (52), rabbit (55), and rat (53, 54). In the pelvic ganglia of the rat, in situ hybridization and immunohistochemical studies coupled with patch clamp recording techniques revealed that $P2X_2$ receptors are the predominant subtype in ~40% of the neurons (53). On the other hand, in the guinea pig pelvic ganglia, at least three distinct P2X receptors are present in different subpopulations of neurons (54). $P2X_2$ and $P2X_3$ homomultimers are present in 5% and 70% of the neurons, respectively, and 25% of the neurons expressed heteromeric $P2X_{2/3}$ receptors.

$P2X_3$ receptors, which have been identified in small-diameter afferent neurons in dorsal root ganglia (7), have also been detected immunohistochemically in the wall of the bladder and ureter in a suburothelial plexus of afferent nerves (49). Intravesical or intra-arterial administration of ATP or 2-me-*S*-ATP activated bladder afferent fibers and enhanced reflex bladder activity (17, 18). On the other hand, desensitization of purinergic receptors by intravesical administration of α,β-me-ATP or administration of a receptor antagonist, suramin, depressed bladder afferent activity (17, 18). In $P2X_3$ knockout mice, afferent activity induced by bladder distension was significantly reduced (8). These data indicate that purinergic receptors are involved in mechanosensory signaling in the bladder.

In addition to having a direct action on afferent terminals, ATP might also have an indirect action via the release of chemical mediators from the urothelium. It has been shown that activation of purinergic receptors on cultured urothelial cells can increase intracellular Ca^{2+} (56) and that ATP releases prostanoids from urothelial cells (16). Prostanoids are known to have a sensitizing action on bladder afferent nerves (3, 10, 11). ATP is also released from urothelial cells in response to stretch (14, 57, 58). Thus, $P2X_3$ purinergic receptors in the subepithelial afferent plexus might be activated by chemical messengers, including ATP released from the urothelium during bladder filling. Conversely, ATP and other transmitters released from afferent nerves could act on the urothelial cells to modulate the properties of the urothelium (56). Chemical dialogue between these two cell types in the bladder wall (i.e. afferent-urothelial interactions) may play an important role in both sensory nerve activation and epithelial barrier functions in the bladder.

Two types of purinergic inhibitory mechanisms are present in the bladder. 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 (59), in postganglionic nerve terminals, and in the bladder muscle (7, 8). These inhibitory effects are antagonized by methylxanthines. In addition, ATP also seems to act via P2Y receptors in the smooth muscle to suppress

cholinergic and purinergic contractions (8). Structure-activity relationships for a series of purinergic agonists (2-me-*S*-ATP > ATP > ADP > UTP) indicate that relaxation is mediated by $P2Y_1$ receptors (8).

MONOAMINERGIC MECHANISMS

Adrenergic Receptors

Various types of α- and β-adrenergic receptors (ARs) have been identified in the lower urinary tract (60–68). Activation of α_1-AR promotes closure of the bladder outlet (9, 25, 26), whereas activation of β_2- or β_3-AR relaxes the detrusor muscle (1, 9, 25, 26). Thus, α_1 and β_2 or β_3 agonists have been used to facilitate urine storage, and α_1-AR antagonists have been used to facilitate voiding (3, 9, 25).

Because stimulation of prostatic/urethral α_1-AR contributes to the increased outlet resistance in patients with benign prostatic hypertrophy, α_1-AR-blocking agents have been useful in promoting bladder emptying and reducing symptoms in patients with benign prostatic hypertrophy (25). However, side effects such as dizziness that may be due to blockage of α_1-AR in the cardiovascular system limit the use of these blocking agents. Because the α_{1A}-AR is the major subtype in the prostate and urethra and all three α_1-AR subtypes (α_{1A}, α_{1B}, and α_{1D}) are present in blood vessels, selective α_{1A} antagonists are being tested clinically in patients with benign prostatic hypertrophy to determine whether side effects can be reduced while efficacy is maintained (26). Although the α_{1A}-selective drugs reduce outlet resistance, there are no reports indicating that they relieve symptoms such as urinary urgency, frequency and nocturia. A failure of selective α_{1A} antagonists to reduce symptoms may reflect the contribution of other receptor subtypes to lower urinary tract dysfunction in benign prostatic hypertrophy. For example, postjunctional α_1-AR (α_{1B} and α_{1D}) can mediate excitatory effects in the bladder smooth muscle (69) and are reportedly upregulated after chronic outlet obstruction (26). Blockade of these receptors might contribute to relief of symptoms.

Excitatory α_1-AR are also present in the urothelium, where stimulation evokes the release of NO (70), and at various sites in the nervous system, including parasympathetic nerve terminals in the bladder (69, 71, 72), parasympathetic ganglia (6), and the spinal cord (73–78). In the rat bladder, different AR subtypes are found in pre- and postjunctional locations (69). α_{1A}-ARs mediate an enhancement of acetylcholine release; whereas α_{1B}/α_{1D} receptors mediate the direct excitatory effect on the bladder smooth muscle (69, 71, 72). The postjunctional excitatory effects are not detectable in young rats but become prominent in older animals, suggesting that the expression of adrenergic receptors in the bladder is plastic and may change with disease or age.

In the spinal cord, α-AR can mediate excitatory and inhibitory influences on the lower urinary tract. In anesthetized cats, α_1-ARs were implicated in a bulbospinal noradrenergic excitatory pathway from the locus coeruleus to the sacral

parasympathetic outflow to the bladder (73–75). Although subsequent studies could not confirm these findings in conscious cats (76), experiments in conscious and anesthetized rats (72, 77) revealed that intrathecal administration of an α_1-AR antagonist (doxazosin) decreased the amplitude of bladder contractions (77, 78) and that this effect was more prominent in animals with chronic outlet obstruction (77). It was also found that intrathecal administration of doxazosin suppressed bladder hyperactivity (unstable bladder contractions) in spontaneously hypertensive rats (79). 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 (72). This was supported by the finding that phenylephrine, an α_1-AR agonist, applied intrathecally, decreased the frequency of bladder contractions without changing contaction amplitude (72). Thus, it appears that efferent and afferent limbs of the micturition reflex receive excitatory and inhibitory input, respectively, from spinal noradrenergic systems.

The function of α_2-AR in the spinal cord is less clear. Studies in rats have indicated both facilitatory and inhibitory roles of α_2-AR in the control of voiding function (2–4, 80). In conscious spinal cats, clonidine, an α_2-AR agonist, increased bladder pressure and facilitated voiding (81). On the other hand, in paraplegic patients, intrathecal injection of clonidine suppressed detrusor hyperreflexia (82).

Sphincter function is also modulated by the spinal noradrenergic pathways. Striated sphincter reflexes in cats, humans, and rats are inhibited by α_2-AR agonists such as clonidine (80–82). In addition, sympathetic and somatic pathways to the lower urinary tract in cats are suppressed by α_1-AR antagonists such as prazosin (83, 84). These data indicate the existence of α_2-AR-inhibitory and α_1-AR-mediated tonic facilitation of sphincter function.

Dopaminergic Receptors

In the central nervous system, dopaminergic pathways exert inhibitory and facilitatory effects, respectively, on the micturition reflex through D_1-like (D_1 or D_5) and D_2 -like (D_2, D_3, or D_4) dopaminergic receptors (85–92). In anesthetized cats, activation of dopaminergic neurons in the substantia nigra inhibits reflex bladder contractions via D_1-like receptors (85). A recent study (86) also revealed that a D_1 dopaminergic antagonist (SCH 23390) facilitated the micturition reflex, while a D_1 agonist (SKF 38393) had no effect on the reflex bladder contractions in awake rats, suggesting that D_1-receptor-mediated suppression of bladder activity is tonically active in the normal awake state. In monkeys, disruption of this tonic dopaminergic inhibition by destroying the nigrostriatal pathway with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces Parkinson-like motor symptoms accompanied by hyperreflexic bladders (87–89) as reported in patients with Parkinson's disease (25, 87, 88). The bladder hyperreflexia was suppressed by stimulation of D_1-like receptors with SKF 38393 or pergolide (88, 89). Conversely, activation of central D_2-like dopaminergic receptors with quinpirole or bromocriptine facilitates

the micturition reflex pathway in rats, cats, and monkeys (88–92). D_2-like receptor-mediated facilitation of the micturition reflex may involve actions on brain stem and spinal cord, given that microinjection of dopamine to the pontine micturition center reduced bladder capacity and facilitated the micturition reflex in cats (2), and intrathecal application of quinpirole induced bladder hyperactivity in normal rats and rats with 6-hydroxydopamine-lesioned nigrostriatal pathways (91). D_2-like receptors are also involved in the bladder hyperactivity induced by middle cerebral artery occlusion in rats (92). Thus, central dopaminergic pathways exhibit different effects on micturition via actions on multiple receptors at different sites in the central nervous system.

Serotonergic Receptors

5-HT-containing neurons 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. 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 (2, 93, 94) and also inhibits firing of spinal dorsal horn neurons elicited by stimulation of pelvic nerve afferents (95). In rats, the administration of *m*-chlorophenylpiperazine, which is an agonist for 5-HT_{2C} receptors, suppressed efferent activity on bladder nerves and reflex bladder contractions (96). These effects were blocked by mesulergine, a 5-HT_2 receptor antagonist (96–98). Intrathecal administration of methysergide, a 5-$HT_{1/2}$ antagonist, or zatosetron, a 5-HT_3 antagonist, decreased the micturition volume threshold in cats (99), implying that descending serotonergic pathways tonically depress the afferent limb of the micturition reflex.

8-OH-DPAT, a 5-HT_{1A} agonist, 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 (100). 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 (101). 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 (101). The sympathetic autonomic nuclei as well as the sphincter motor nuclei also receive a serotonergic input from the raphe nucleus. Serotonergic activity mediated via 5-HT_2 and 5-HT_3 receptors enhances urine storage by facilitating sphincter reflexes (99, 102).

In detrusor smooth muscle, activation of postjunctional 5-HT_2 receptors induces a bladder contraction (5) whereas activation of prejunctional 5-HT_4 receptors facilitates cholinergic transmission (103). In human bladder strips, 5-HT_4 agonists facilitate acetylcholine release (104). It is also been reported that the 5-HT_4 agonist cisapride induces bladder hyperactivity (105) and that another 5-HT_4 agonist, metoclopramide, reduces residual urine in patients with diabetic cystopathy

(106). Thus, 5-HT_4 agonists may be effective in treating patients with impaired detrusor contractility (107).

AMINO ACIDS

Glutamatergic Excitatory Mechanisms

Glutamic acid plays an essential role as an excitatory transmitter in the central pathways controlling the lower urinary tract of the rat (2, 3). It is involved at the level of the lumbosacral spinal cord in processing afferent input from the bladder (108, 109), at interneuronal synapses on parasympathetic preganglionic neurons (110, 111), and in the descending pathway from the PMC to the sacral parasympathetic nucleus (22, 112). Glutamate also functions as an excitatory transmitter in the micturition reflex pathways in the brain (2, 3). Although both *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamatergic [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)] receptors are involved in micturition, the latter are most important for the control of voiding function.

Intrathecal or intravenous administration of NMDA or AMPA antagonists in urethane-anesthetized rats depressed reflex bladder contractions and electromyographic activity of the external urethral sphincter in animals with an intact spinal cord as well as in chronic spinal-cord-injured animals with the cord transected at the midthoracic level (20, 23). These results indicate that spinal reflex pathways controlling bladder and sphincter functions utilize NMDA and AMPA glutamatergic transmitter mechanisms. In spinal-cord-injured rats, external urethral sphincter-electromyographic activity was more sensitive than bladder reflexes to glutamatergic antagonists, raising the possibility that the two reflex pathways have different glutamatergic receptors (23). This was confirmed with in situ hybridization techniques, which revealed that sacral parasympathetic preganglionic neurons (PGNs) in the rat express high messenger RNA levels of GluR-A and GluR-B AMPA receptor subunits and NR1 but not NR2 NMDA receptor subunits (113). On the other hand, 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 sensitivities of bladder and sphincter reflexes to glutamatergic antagonists.

The properties of glutamatergic synapses in parasympathetic reflex pathways have been studied using patch clamp recording in spinal cord slice preparations from neonatal rats (109, 110). These studies showed that sacral parasympathetic PGNs in the L6-S1 spinal cord receive glutamatergic excitatory inputs from interneurons in the region of the sacral parasympathetic nucleus. These inputs are mediated by AMPA receptors that elicit fast excitatory postsynaptic currents and NMDA receptors that elicit more prolonged excitatory postsynaptic currents. Interneurons in this region of the spinal cord are involved in lower urinary tract function, as evidenced by the fact that they are labeled in a retrograde manner by transneuronal virus transport after injection of pseudorabies virus into the bladder

or urethra (3) and also exhibit increased expression of an immediate-early gene (c-*fos*) after stimulation of bladder afferents (108, 109). The expression of c-*fos* in the spinal cord induced by chemical irritation of the bladder is suppressed by NMDA or AMPA receptor antagonists, indicating the involvement of glutamatergic transmission in bladder afferent pathways (108). Combined administration of low doses of NMDA and AMPA antagonists which alone had no effect on c-*fos* expression depressed expression, suggesting that these receptors play a synergistic role in visceral afferent processing in the spinal cord (109).

Glutamic acid also has a role in excitatory transmission at supraspinal sites in the micturition reflex pathway. Administration of glutamatergic agonists into the region of the PMC in cats and rats elicits voiding or increases the frequency and amplitude of bladder contractions (3, 114), whereas injection of agonists at other brain stem nuclei known to have inhibitory functions in micturition elicits inhibitory effects (94). 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 (115).

NMDA glutamatergic mechanisms also play a role in the bladder hyperactivity induced by cerebral infarction in rats. Permanent occlusion of the middle cerebral artery produces a dramatic and persistent reduction in bladder capacity in conscious rats (92, 116, 117). This bladder hyperactivity can be prevented by pretreatment with MK-801, an NMDA receptor antagonist, prior to occlusion of the middle cerebral artery or can be transiently reduced by MK-801 administered after occlusion of the middle cerebral artery. The results indicate that bladder hyperactivity induced by cerebral infarction occurs in two phases, both of which depend on activation of NMDA glutamatergic receptors. An initiation phase that occurs at the time of infarction seems to function like long-term potentiation to induce a persistent facilitation of micturition (the second phase). The initial phase is not affected by pretreament with an AMPA glutamatergic antagonist but can be blocked by injecting an NMDA antagonist (MK-801) directly into the PMC (O. Yokoyama, personal communication), suggesting that plasticity at glutamatergic synapses in the PMC can trigger this form of neurogenic bladder dysfunction.

GABAergic Inhibitory Mechanisms

GABA has been implicated as an inhibitory transmitter at spinal and supraspinal sites, where it can act on both $GABA_A$ and $GABA_B$ receptors (2–4). Injection of GABA or muscimol, a $GABA_A$ receptor agonist, into the PMC of decerebrate cats suppressed reflex bladder activity and increased the volume threshold for inducing micturition (114). These effects were reversed by bicuculline, a $GABA_A$ receptor antagonist. Because bicuculline alone stimulated bladder activity and lowered the volume threshold for micturition, the micturition reflex pathway in the PMC must be tonically inhibited by a GABAergic mechanism.

Intrathecal administration of $GABA_A$ or $GABA_B$ agonists increases bladder capacity and decreases voiding pressure and efficiency in rats and cats (118–121). Stimulation of local interneurons near the sacral parasympathetic nucleus induces

inhibitory postsynaptic currents mediated by $GABA_A$ receptors (122). Application of $GABA_A$ agonists to sacral parasympathetic PGN inhibits reflex firing, opens chloride channels, and hyperpolarizes the cells (3, 122). Baclofen, a $GABA_B$ agonist, suppresses Ca^{2+} channels in sacral PGN in the rat (122). Baclofen increases the volume threshold for inducing the micturition reflex in rats (3) and is useful for treating bladder hyperactivity in patients with neurological disorders such as multiple sclerosis (123–125). GABA has also been implicated in interneuronal inhibitory pathways that control the external urethral sphincter (126, 127).

Glycinergic Inhibitory Mechanisms

Glycine, another inhibitory amino acid, is also released from interneurons in the spinal cord and in some instances is coreleased with GABA at synapses on PGN (122). Glycine acting on strychnine-sensitive receptors mediates recurrent inhibition in the micturition reflex pathway (128) and is also involved in the inhibition of sphincter motoneurons during micturition (129, 130).

PEPTIDERGIC MECHANISMS

Opioid Peptides

Enkephalinergic pathways in the central nervous system exert an inhibitory control on the micturition reflex (2–4). Enkephalinergic varicosities have been demonstrated immunocytochemically at various sites, including the PMC, the sacral parasympathetic nucleus, and the urethral sphincter motor nucleus in the spinal cord. Administration of opioid drugs or enkephalins to the brain or spinal cord suppresses micturition and sphincter reflexes (130–133). In the brain, both μ and δ opioid receptors mediate inhibitory effects which are blocked by naloxone (114, 120, 130). Naloxone administered alone intracerebroventricularly or injected directly into the PMC facilitates the micturition reflex. In the cat spinal cord, δ opioid receptors mediate inhibition of bladder activity, and κ receptors mediate inhibition of sphincter activity (132). In the rat spinal cord, δ and μ receptors but not κ receptors are involved in the suppression of bladder reflexes (2, 130, 131). In conscious dogs, intrathecal administration of morphine increases the volume threshold for inducing micturition without altering voiding pressure. This effect is blocked by naloxone. These observations indicate that spinal opioid mechanisms can control the afferent limb of the micturition reflex (130).

Afferent Neuropeptides

Immunocytochemical studies have revealed that bladder afferent neurons contain various neuropeptides, including substance P, neurokinin A, calcitonin-gene-related peptide, vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide, and enkephalins (2–4, 11, 134). 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 (10). These agents may also function as transmitters at afferent terminals in the spinal cord. Tachykinins released in the bladder can act on: (*a*) NK_1 receptors in blood vessels to induce plasma extravasation and vasodilation, (*b*) NK_2 receptors to stimulate the bladder contractions, and (*c*) NK_2 receptors on primary afferent terminals to increase the excitability during bladder filling or during bladder inflammation (3, 5, 11). Substance P also acts on receptors on urothelial cells to release NO.

Intrathecal administration of NK_1 antagonists (RP 67580 and CP 96345) increased bladder capacity in normal conscious rats without changing voiding pressure, whereas NK_2 antagonists were ineffective (135). Bladder hyperactivity in rats induced by chemical cystitis, intravesical administration of capsaicin, or intravenous injection of L-3,4-dihydroxyphenylalanine was also suppressed by intrathecal injection of NK_1 antagonists (136–138). Bladder hyperactivity induced by capsaicin was reduced by an NK_2 antagonist (SR 48965) that did not influence normal voiding (139). In the anesthetized guinea pig, TAK-637, an NK_1 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 (140). These results indicate that sensory input to the spinal cord from non-nociceptive bladder afferents is mediated by tachykinins acting on NK_1 receptors, whereas input from nociceptive afferents can be mediated by NK_1 and NK_2 receptors.

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/or at different sites in the lower urinary tract. Calcitonin-gene-related peptide applied exogenously or released from primary afferents relaxes smooth muscle and produces vasodilation. The effect of calcitonin-gene-related peptide on the bladder is prominent in the guinea pig and dog, but it is absent in the rat and human (5, 11). VIP, which is contained in C-fiber afferents (141) as well as in postganglionic neurons in the cat (6), inhibits spontaneous contractile activity in isolated bladder muscle from several species, including humans, but usually has little effect on bladder contractions induced by muscarinic receptor agonists or nerve stimulation (5). 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 (6). In the spinal cord, VIP-containing afferent pathways have been implicated in the recovery of bladder reflexes after spinal injury. In chronic spinal cats, 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 (4, 141). In addition, the effects of intrathecal administration of VIP are changed. In normal cats, VIP inhibits the micturition reflex, whereas in paraplegic cats, VIP facilitates the micturition reflex. These findings suggest that the action of a putative C-fiber afferent transmitter may underlie the emergence of C-fiber bladder reflexes in the paraplegic cat. In the normal rat, VIP and pituitary adenylate cyclase-activating peptide, another member of the secretin/glucagon/VIP peptide family, also facilitate

the micturition reflex by actions on the spinal cord (142). Patch clamp studies of the neonatal rat spinal slice preparation revealed that pituitary adenylate cyclase-activating peptide has a direct excitatory action on parasympathetic PGN due in part to blockade of K^+ channels (143). In addition, pituitary adenylate cyclase-activating peptide has an indirect action by activating excitatory interneurons.

Other Peptides

Endothelins (ETs) induce prolonged contractile responses in isolated lower urinary tract smooth muscles from various species (9). Ligand receptor binding studies in the rabbit revealed regional differences in the density and subtype of ET receptors (144). The highest density of receptors is present in the ureter, which exhibits only the ET_A receptor subtype. The bladder dome, which has more ET_A than ET_B receptors, has a higher density of receptors than the base or urethra, where the two subtypes are equally expressed. ET-like immunoreactivity was detected in detrusor smooth muscle, epithelium, and vascular endothelium (145). In the rabbit, the ET-induced bladder contractions were abolished in Ca^{2+}-free media and markedly reduced by nifedipine, a Ca^{2+} channel blocker; whereas in human bladder strips, the ET contractions were resistant to nifedipine, indicating species differences in contractile mechanisms (146). It has been suggested that ET may have an autocrine function to modulate smooth muscle tone in the lower urinary tract (146). Because ET_B receptors are upregulated in the urethral smooth muscle following outlet obstruction, ET_B antagonists might be useful in treating prostatism (26).

Parathyroid hormone-related protein (PTHrP) is expressed in various types of smooth muscle, including smooth muscle of the urinary bladder (147, 148). Exogenous PTHrP induces a relaxation of isolated bladder strips and suppresses the contractions elicited by a muscarinic receptor agonist. However, the sensitivity to PTHrP is influenced by the state of the bladder (147). Strips from empty bladders are very sensitive to the peptide; whereas strips from distended bladders are insensitive. The resistance of muscle strips from distended bladders might be related to desensitization of the PTHrP receptors by prolonged exposure to endogenously released peptide because bladder distension increases the levels of PTHrP, messenger RNA, and PTHrP immunoreactivity in the bladder smooth muscle (147) and stretching of cultured bladder smooth muscle cells releases PTHrP (148). These results have raised the possibility that PTHrP functions in an autocrine fashion to suppress contractility and improve bladder compliance during urine storage and also has a paracrine action on blood vessels to regulate blood flow in the distended bladder.

NITRIC OXIDE

Three types of NO synthase (nNOS, eNOS, and iNOS) have been identified in the lower urinary tract and/or the neural pathways controlling voiding function (1, 3, 5). nNOS is most prominent in the parasympathetic postganglionic innervation of the

urethra (149). This is consistent with the findings that exogenous NO or parasympathetic nerve stimulation relaxes the urethral smooth muscle but does not relax the urinary bladder (1, 5, 150). Stimulation of bladder afferent nerves with capsaicin releases NO, and chronic irritation of the bladder, as well as exposure to capsaicin, increases nNOS expression in dorsal root ganglion cells (151, 152). Bladder hyperactivity induced by intravesical irritants or capsaicin can be suppressed by intrathecal injection of NOS inhibitors, indicating that NO release in the spinal cord facilitates the micturition reflex pathway (153–155).

Immunocytochemical studies revealed that uroepithelial cells contain NOS (rats, cats, and rabbits) and that exposure of epithelial strips or cultured uroepithelial cells to various chemicals, including capsaicin, nicotine, Ca^{2+} ionophores, and neurotransmitters (acetylcholine, norepinephrine, calcitonin-gene-related peptide, and SP), evokes NO release (38, 70). NO release from rat uroepithelial cells is reduced by nonselective NOS inhibitors (70) or by a selective nNOS inhibitor. NO release is also suppressed by reducing extracellular Ca^{2+} or by L-type Ca^{2+} channel blockers (nifedipine), indicating that influx of extracellular Ca^{2+} plays an important role in the activation of NOS. Ca^{2+}-independent basal release of NO was detected in bladder epithelial strips removed from spinal-cord-injured rats or rats in which the bladder was irritated with cyclophosphamide (156, 157). Basal release was not detected in epithelial strips from normal animals.

Release of NO in the urinary tract of humans has been estimated by measuring nitrite levels in the urine (158). A finding of reduced levels of nitrite in patients with interstitial cystitis, a condition characterized by chronic bladder pain and increased voiding frequency, has prompted the speculation that decreased epithelial NO production might be associated with the condition (158). A clinical trial in which L-arginine was administered orally to increase NO production resulted in reduced symptoms (159). Thus, NO release from afferent nerves or the urothelium may play a role in sensory mechanisms in the lower urinary tract. This concept receives support from studies showing that intravesical administration of an NO donor depresses reflex bladder hyperactivity in rats with cyclophosphamide-induced cystitis (160).

PROSTANOIDS

In the urinary bladder, both the urothelium and the smooth muscle express cyclooxygenase (COX) enzymes, which are responsible for the synthesis of prostanoids (5, 10, 15, 16, 161). Two types of COX enzymes have been identified: (*a*) COX-1, which is constitutively expressed and responsible for the production of prostanoids involved in physiological processes, and (*b*) COX-2, which is induced by inflammatory stimuli. Prostanoids are generated during bladder stretching, in response to injuries to the mucosa, by nerve stimulation, and by neurotransmitters (ATP) and inflammatory mediators (e.g. bradykinin). Prostaglandins (PGs) are released from smooth muscle and from the epithelium and are thought to

contribute to the basal tone of the bladder smooth muscle and to modulate afferent nerve activity and efferent neurotransmission (5, 11, 161). PGF_{2a}, PGE_1, PGE_2, and thromboxane A_2 (TXA_2) contract human and animal isolated detrusor muscle. PGF_{2a} contracts but PGE_1 and PGE_2 relax or have no effect on urethral smooth muscle (5). The contractile responses of prostanoids in bladder smooth muscle are much slower than neurally mediated responses and are not likely to be directly involved in bladder emptying. Topical application of prostanoids to the mucosal or serosal surface of the bladder stimulates the micturition reflex (11, 161). This effect seems to be due to activation of capsaicin-sensitive afferent nerves because the effect can be blocked by pretreatment with large doses of capsaicin (11, 161). The effect may be due to a direct action of PGs on afferent receptors because PGs are known to have a facilitatory action on Na^+ and vanilloid receptor (VR1) channels in DRG cells. In addition, PGs may have an indirect effect mediated by the release of tachykinins, which are known to sensitize bladder afferent nerves (10). The facilitatory effect of PGs on the micturition reflex is depressed by pretreatment with tachykinin receptor antagonists (162).

In rats with cystitis induced by cyclophosphamide or lipopolysaccharide, bladder hyperactivity was decreased by dexketoprofen, a nonselective COX-1/COX-2 inhibitor, or by NS-398, a selective COX-2 inhibitor (163). On the other hand, in rats with nonirritated bladders, dexketoprofen but not NS-398 decreased the frequency of reflex micturition and increased the pressure threshold for inducing micturition. Based on these results, it was proposed that prostanoids generated by COX-1 are involved in the control of normal micturition whereas prostanoids formed by COX-2 mediate the urodynamic changes occurring during bladder inflammation. Expression of COX-2 is also increased by outlet obstruction (164, 165). Thus, COX-2 inhibitors may be useful in the treatment of various types of abnormal voiding function.

VOLTAGE-GATED ION CHANNELS

Ca^{2+} Channels

Neurally mediated contractions of the bladder and urethral smooth muscle require mobilization of intracellular Ca^{2+} as well as influx of extracellular Ca^{2+} (1, 5). Voltage-clamp studies of bladder myocytes revealed a depolarization-evoked inward current that was mediated by an L-type Ca^{2+} channel (166). The current was sensitive to nifedipine, an L-type channel blocker, and was activated at potentials positive to -40 mV with a peak at about $+10$ mV. Ca^{2+} entry through L-type Ca^{2+} channels can contribute to muscle contractions by triggering the intracellular release of Ca^{2+} that opens ryanodine-sensitive Ca^{2+} release channels in the sarcoplasmic reticulum (167). Opening of L-type Ca^{2+} channels in bladder muscle is also important to replenish intracellular Ca^{2+} stores after contractions. Thus, blockade of L-type Ca^{2+} channels with dihydropyridines, such as nifedipine, depresses neurally and agonist-evoked contractions of bladder strips and also depresses bladder contractility recorded in vivo in animals and patients with detrusor

hyperactivity (1, 3, 9). However, even though experimental data provide a theoretical basis for the use of Ca^{2+} antagonists in the treatment of bladder hyperactivity, available information does not indicate that oral therapy with these drugs is effective (9), possibly owing to the use of low doses to limit cardiovascular side effects.

Ca^{2+} channels located in afferent and efferent nerve terminals in the lower urinary tract are important for regulation of neurotransmitter release (33, 166, 168). Neurally evoked bladder contractions elicited by low frequencies of stimulation are markedly suppressed by ω-conotoxin GVIA, indicating that Ca^{2+} influx through N-type Ca^{2+} channels in the nerve terminals is primarily responsible for triggering the release of ACh (168). The residual bladder contractions are dependent on P- and Q-type channels, which are blocked by ω-conotoxin MVIIC and ω-agatoxin IVA, respectively. Under certain conditions, L-type Ca^{2+} channels can also contribute to transmitter release (33). Recent studies in the rat revealed that ACh release from cholinergic nerve terminals can be facilitated by prolonged periods of high frequency stimulation (31). The facilitation is dependent upon the activation of L-type Ca^{2+} channels by presynaptic muscarinic M_1 receptors (32, 33). Facilitated ACh release was suppressed by concentrations of nifedipine, which had no effect on nonfacilitated release (33). The facilitated release was upregulated in bladder strips obtained from spinal-cord-injured rats with hyperactive bladders (169).

K^+ Channels

The frequency of spontaneous action potentials and the probability of Ca^{2+} channel opening in bladder smooth muscle are voltage dependent (1, 5). Therefore, membrane hyperpolarization is an effective mechanism to depress the contractility of bladder smooth muscle. Drugs such as cromakalim, pinacidil, and ZD6169, which open K^+ channels and produce membrane hyperpolarization, are very effective at suppressing spontaneous action potentials and contractions of isolated bladder smooth muscle (1, 3, 5). These drugs activate ATP-sensitive K^+ channels (K_{ATP}) in the bladder, and their effects are antagonized by K_{ATP} channel blockers such as glibenclamide and glyburide (5). K_{ATP} channel openers are less effective in blocking neurally evoked vs spontaneous bladder contractions and therefore should be more active in suppressing unstable bladder contractions during bladder filling and not interfere with normal voiding. K_{ATP} channel openers (pinacidil and cromakalim) have been effective in suppressing bladder activity in animal models, but cardiovascular side effects such as hypotension limit clinical use (1, 5, 9). New K_{ATP} channel openers, such as ZD6169, have been shown to be more selective for the bladder (170). Oral administration of ZD6169 reduces voiding frequency in rats and dogs without lowering blood pressure (170). Intravesical administration in rats increases the bladder volume for inducing a micturition reflex and also decreases the frequency and amplitude of spontaneous bladder contractions and decreases voiding pressure in both normal and outlet-obstructed animals (171–174). It has been suggested that the drug acts not only on bladder smooth muscle but also on capsaicin-sensitive bladder afferents to reduce afferent firing induced by bladder distension or chemical irritation of the mucosa (172, 173).

In bladder afferent neurons, two A-type K^+ channels with different kinetics (quickly and slowly decaying) have been identified. The slowly decaying A-type K^+ current is mainly expressed in capsaicin-sensitive C-fiber bladder afferent neurons and appears to control spike threshold and firing frequency (175). Suppression of this slow A-type K^+ current contributes to hyperexcitability of C-fiber bladder afferent neurons in rats with spinal cord injury (176) and chronic bladder irritation induced by cyclophosphamide (177).

NEUROTROPHIC FACTORS

Various trophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor, glial-derived neurotrophic factor, and ciliary neurotrophic factor, are synthesized in the lower urinary tract (178–180). The expression of these substances can change in pathological conditions and in turn alter the functions of nerves and target organs (178–181). NGF, a target and nerve cell-derived polypeptide that influences the survival and properties of specific subsets of peripheral neurons (181), has been studied extensively in the bladder. TrkA, a high-affinity receptor for NGF, is expressed in ~80% of lumbosarcal DRG neurons innervating the urinary bladder (182). NGF increases in the bladder smooth muscle after urethral obstruction, spinal cord injury, and chronic cystitis, all of which induce bladder hyperactivity (3, 178). Spontaneously hypertensive rats that exhibit a hyperactive voiding condition also have increased levels of NGF in the bladder (179). Autoimmunization against NGF suppresses the bladder hyperactivity and the morphological changes in bladder afferent neurons that occur in rats with partial urethral obstruction (178). Bladder hyperactivity induced by inflammation can be inhibited by a fusion protein that prevents the interaction between NGF and its receptor (183). Recent studies also demonstrated that acute or chronic exposure of the bladder to exogenous NGF induces hyperexcitability of bladder afferent neurons/nerves and hyperactivity of the bladder (183, 184). These data indicate that NGF may play a role in the initiation of various types of bladder dysfunction. The expression of other trophic factors, such as brain-derived neurotrophic factor, NT3, NT4/5, and glial-cell-derived neurotrophic factor, which are known to influence afferent properties, also increases in the bladders after spinal cord injury and cyclophosphamide-induced cystitis in rats (180). This raises the possibility that multiple chemical signals underlie the plasticity of bladder afferent neurons in pathological conditions.

TOXINS

Capsaicin

Capsaicin, the pungent ingredient in hot pepper, is a neurotoxin that activates vanilloid receptors (VR1) in a subpopulation of small-diameter sensory neurons to induce an initial excitation that produces a feeling of warmth and/or pain. After

the initial excitatory response, afferent nerves are desensitized or damaged, and painful sensations are suppressed (186). The VR1 receptor in cutaneous sensory nerves is thought to function as a transducer of painful thermal stimuli (7, 185–187). In the urinary bladder, VR1 receptors are also expressed in small-diameter sensory nerves (188) and in epithelial cells (13). Although it is presumed that these receptors are involved in the detection of noxious stimuli and in the triggering of painful sensations, the stimuli that normally activate VR1 in the urinary tract are unknown.

Nevertheless, capsaicin has been clinically useful in the treatment of neurogenic disorders of the lower urinary tract (189–193). For this purpose, the toxin is administered intravesically to desensitize afferent nerve endings in the mucosa. The bladder afferent pathways consist of myelinated Aδ-fibers and unmyelinated C-fibers. Aδ-fibers transmit signals from mechanoceptors that initiate the normal micturition reflex (2, 3), whereas C-fibers do not respond to bladder distension but do respond to noxious stimuli (2, 10, 11). The majority of C-fiber bladder afferent neurons are capsaicin sensitive, but only a few Aδ-fiber neurons respond to capsaicin (176, 194). The mechanoinsensitive C-fiber bladder afferent nerves become responsive to bladder distension and contribute to bladder hyperactivity and/or bladder pain under pathological conditions such as spinal cord injury or cystitis (141, 195, 196, 197). The first evidence for emergence of C-fiber-mediated neurogenic bladder hyperactivity was obtained in chronic spinal-cord-injured cats, in which systemic capsaicin treatment suppressed reflex bladder contractions (141, 196). Capsaicin did not depress bladder activity in normal cats. Subsequently, C-fiber-mediated bladder hyperactivity was demonstrated in humans (189–193). In patients with multiple sclerosis or spinal cord injury who exhibited detrusor hyperreflexia, intravesical capsaicin (1–2 mM) significantly reduced the bladder hyperactivity and/or the number of episodes of urinary incontinence (189–193). Intravesical capsaicin also suppressed autonomic dysreflexia, which is a sympathetically mediated hypertensive response induced by C-fiber bladder afferents in spinal-cord-injured patients (189). Intravesical capsaicin treatment has also been used in treatment of patients with hypersensitive bladders (interstitial cystitis) who experience urinary frequency, urgency, and pain (10). However, the use of capsaicin has been limited by the initial excitatory effect on C-fiber afferents that induces burning pain (189).

Resiniferatoxin (RTX)

RTX, a pungent substance from a cactus plant (*Euphorbia resinifera*), is 1000 times more potent than capsaicin in interacting with VR1. However, RTX in comparison to capsaicin has weaker initial excitatory effects on bladder afferents and therefore elicits less discomfort. This drug seems to be a promising alternative to capsaicin for use in patients with bladder hyperreflexia (189, 198–200). Park et al (200) compared intravesical capsaicin and RTX in 13 spinal-cord-injured patients with detrusor hyperreflexia. Seven patients treated with capsaicin showed a 44% increase in bladder capacity at 4 weeks, with 57% stating they had significant improvement in voiding symptoms. However, after capsaicin administration,

autonomic dysreflexia was observed in four patients, suprapubic pain was observed in three patients, and hematuria was observed in one patient. In contrast, six patients treated with intravesical RTX showed a 93% increase in bladder capacity at 4 weeks, with 83% describing significant improvement in voiding symptoms without side effects.

Botulinum Toxin

Botulinum toxin, a substance that inhibits acetylcholine release from cholinergic nerve terminals, is now commonly used for the local treatment of skeletal muscle spasticity. Botulinum toxin has been injected into the external urethral sphincter in spinal-cord-injured men to reduce detrusor-sphincter dyssynergia and thereby decrease urethral resistance and improve bladder emptying (201, 202). Injection of botulinum toxin into the bladder wall has also been utilized recently for the treatment of bladder hyperreflexia in spinal-cord-injured patients, indicating that it is effective in suppressing the autonomic pathways to smooth muscle (203).

FUTURE DIRECTIONS

During the past few years, 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 voiding 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 adrenergic receptors, 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 system 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. In addition, it will be important to develop drugs that modulate abnormal bladder and/or urethral activity and diminish symptoms without altering normal voiding function.

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