Neural Control of the Lower Urinary Tract

Naoki Yoshimura* and William C. de Groat

Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, USA

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INTRODUCTION

The urinary bladder and its outlet, the urethra, serve 2 main functions: the storage of urine without leakage, and the periodic release of urine. These 2 functions are dependent on central as well as peripheral autonomic and somatic neural pathways.[1–6] Since the lower urinary tract switches, in an all-or-none manner, between storage and elimination of urine, many of the neural circuits controlling voiding exhibit phasic patterns of activity rather than the tonic patterns occurring in autonomic pathways to other viscera. Micturition is a special visceral mechanism because it is dependent on voluntary control, which requires the participation of higher centers in the brain, whereas many other visceral functions are regulated involuntarily. Because of these complex neural regulations, the central nervous system control of the lower urinary tract is susceptible to a variety of neurologic disorders.

This paper reviews studies in animals and humans that have led to our current concepts of the neural mechanisms underlying urinary continence and micturition. In addition, the final section of the paper focuses on recent evidence indicating that plasticity in bladder afferent pathways is involved in the reorganization of the micturition reflex pathways in various pathologic conditions.

NEUROANATOMY AND NEUROPHARMACOLOGY

The storage and elimination of urine are dependent on the reciprocal activity of 2 functional units in the lower urinary tract: (1) a reservoir (the bladder); and (2) an outlet (bladder neck, smooth and striated sphincter muscles of the urethra). During urine storage, the bladder outlet is closed and the bladder smooth muscle is quiescent, allowing intravesical pressure to remain low over a wide range of bladder volumes. During voluntary voiding, the initial event is a relaxation of the pelvic floor and striated urethral sphincter muscles, followed by a detrusor muscle contraction and opening of the bladder neck. Reflex inhibition of the smooth and striated urethral sphincter muscles also occurs during micturition. This activity is mediated by 3 sets of peripheral nerves: parasympathetic (pelvic), sympathetic (hypogastric) and somatic (pudendal) nerves[1–7] (Fig. 1). These nerves also contain afferent axons terminating in the lower

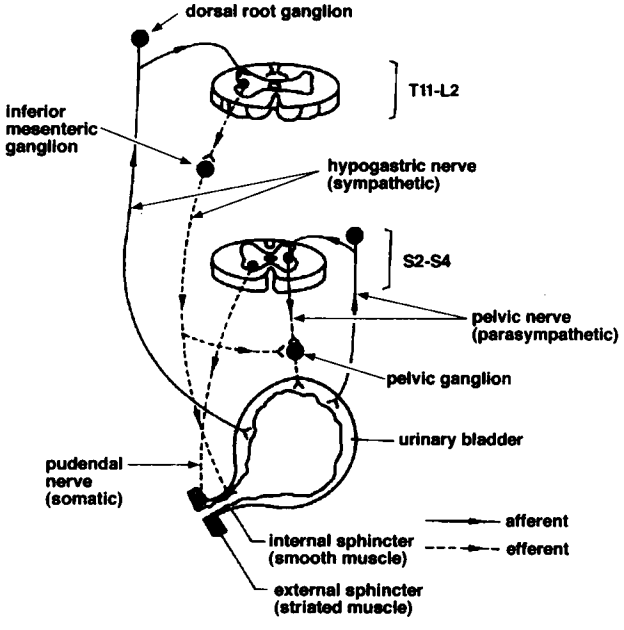

Fig. 1. Diagram showing the sympathetic, parasympathetic and somatic innervation of the lower urinary tract. Sympathetic preganglionic pathways emerge from the thoracolumbar cord (T11–L2) and pass to the inferior mesenteric ganglia. Preganglionic and postganglionic sympathetic axons then travel in the hypogastric nerve to the pelvic ganglia and lower urinary tract. Parasympathetic preganglionic axons, which originate in the sacral cord (S2–S4), pass in the pelvic nerve to ganglion cells in the pelvic ganglia, and postganglionic axons innervate the bladder and urethral smooth muscle. Sacral somatic pathways are contained in the pudendal nerve, which provides an innervation to the external urethral sphincter striated muscles. Afferent axons from the lower urinary tract are carried in these 3 nerves.

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urinary tract; the most important afferents for initiating micturition are those carried in the pelvic nerve.[1,8–13]

Efferent Pathways

The parasympathetic efferent pathway represents the major excitatory input to the bladder. Parasympathetic preganglionic axons originate in the intermediolateral column of the S2 to S4 spinal cord and terminate on postganglionic neurons in the bladder wall and in the pelvic plexus, which is a neural network located on lateral surface of the rectum in humans (Fig. 1). The parasympathetic preganglionic axons release acetylcholine, which activates postsynaptic nicotinic receptors (ganglionic type; N_2).[14,15] Nicotinic transmission at ganglionic synapses can be regulated by various modulatory synaptic mechanisms that involve muscarinic (M_1, M_2), adrenergic (α, β), purinergic, and enkephalinergic receptors[2,15–20] (Fig. 2A). Parasympathetic postganglionic neurons in turn provide an excitatory input to the bladder smooth muscle.

Parasympathetic postganglionic nerve terminals release acetylcholine, which can excite various muscarinic receptors including 2 subtypes (M_2, M_3), which are present in the detrusor muscle.[21–24] Receptor binding and molecular biological techniques indicate that M_2 receptors are predominant in detrusor muscle of animals and humans.[25] However, M_3 receptors are most important for mediating neurally evoked smooth-muscle contractions in the bladder.[25–27] It has been postulated that M_2 receptors may function to inhibit adenylate cyclase and thereby block the β-adrenoceptor signaling mechanism, which facilitates bladder relaxation during urine storage.[21] M_2 receptors are also involved in a presynaptic inhibition of acetylcholine release from postganglionic nerve terminals in the bladder. Presynaptic M_1 muscarinic autoreceptors, which are activated during high-frequency nerve firing, can facilitate acetylcholine release, amplify the parasympathetic excitatory input to the bladder, and thereby promote complete bladder emptying[28,29] (Fig. 2B).

Adenosine triphosphate (ATP), which is a cotransmitter also released from parasympathetic postganglionic terminals acts on P_{2X} purinergic receptors to induce a rapid onset, transient contraction of the bladder[15,30] (Fig. 2B). Due to the presence of adenosine triphosphate-mediated neural transmission, antimuscarinic agents do not completely abolish neurally evoked bladder contractions in animals or humans, although the contribution of the purinergic pathway in humans seems to be small.[7,30–32]

The parasympathetic input to the urethra elicits inhibitory effects mediated at least in part via the release of nitric oxide, which directly relaxes the urethral smooth muscle.[7,15,33–37] In contrast to other transmitters that are stored and released from synaptic vesicles by exocytosis, nitric oxide is not stored, but is synthesized immediately prior to release by the enzyme nitric oxide synthase (NOS). NOS is activated by calcium ion (Ca^{2+}) influx during the action potential and then generates nitric oxide from L-arginine. Nitric oxide, which is a gas, can diffuse out of the nerve terminals. NOS-containing nerve terminals are found more densely in the bladder base and urethra than in the detrusor.[38]

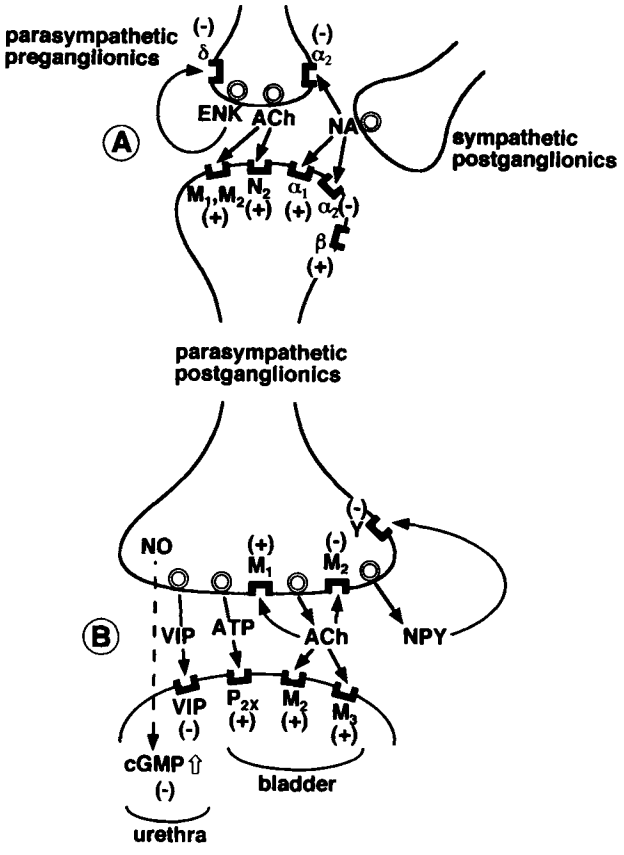

Fig. 2. Diagram of the interaction between neurotransmitters and their receptors in the parasympathetic pathways to the lower urinary tract. (**A**) ganglionic level. Homosynaptic and heterosynaptic (sympathetic) modulation of ganglionic transmission are shown. (**B**) postganglionic level. Facilitatory and inhibitory responses are indicated by plus and minus in parentheses, respectively; circles indicate exocytosis from synaptic vesicles; dotted line indicates diffusion. Abbreviations: acetylcholine (ACh), enkephalin (ENK), norepinephrine (NA), vasoactive intestinal polypeptide (VIP), adenosine triphosphate (ATP), nitric oxide (NO), neuropeptide Y (NPY), nicotinic receptor (N_2), muscarinic receptors (M_1, M_2, and M_3), adrenergic receptors (α_1, α_2, and β), purinergic receptor (P_{2X}), NPY receptor (Y), VIP receptor (VIP), cyclic guanosine monophosphate (cGMP). Note that NO, which is a gas, diffuses into the postsynaptic site and increases the concentration of intracellular cGMP, which in turn induces other actions.

Although the primary inhibitory transmitter in urethral smooth muscle seems to be nitric oxide,[7,33,34,36] another factor mediating long-lasting urethral relaxation is released during high stimulation frequency.[39] In other parasympathetic pathways such as those to vascular smooth muscle, the stomach, or the airways, vasoactive intestinal polypeptide peptide has been shown to colocalize with nitric oxide and act as a second relaxant factor.[15] Similarly, in the rabbit urethra, it was found that fast and slow components of neurally evoked relaxation were suppressed by a NOS inhibitor and a vasoactive intestinal polypeptide (VIP) antagonist, respectively.[40] Vasoactive intestinal polypeptide-containing nerve terminals are prominent in the urethra[30]; and vasoactive intestinal polypeptide, choline acetyltransferase, and NOS appear to colocalize in neurons in the major pelvic ganglia of the rat.[41] Thus, it seems reasonable to assume that the excitation of sacral parasympathetic efferent pathways induces a bladder contraction via acetylcholine/adenosine triphosphate release and urethral relaxation via nitric oxide/vasoactive intestinal polypeptide release (Fig. 2B).

Sympathetic preganglionic neurons located within the intermediolateral cell column of the T11 to L2 spinal cord make synaptic connections with postganglionic neurons in the inferior mesenteric ganglion, as well as with postganglionic neurons in the paravertebral ganglia and pelvic ganglia[1,2,14,42] (Fig. 1). Ganglionic transmission in sympathetic pathways is also mediated by acetylcholine acting on N_2 nicotinic receptors (Fig. 3A). Sympathetic postganglionic terminals, which release norepinephrine, elicit contractions of the bladder base and urethral smooth muscle, and relaxation of the bladder body mediated mainly though α_1- and β_2-adrenoceptors, respectively, although the possible involvement of α_2- and β_1-adrenoceptors has also been suggested[1,3,7,43] (Fig. 3B). In addition, postganglionic sympathetic input to bladder parasympathetic ganglia can facilitate and inhibit parasympathetic ganglionic transmission via α_1 and β adrenoceptors and α_2 adrenoceptors, respectively[2,17,18] (Fig. 2A). In urethral and prostatic smooth muscle, sympathetic excitation is mediated by an α_{1A} adrenoceptor subtype.[44]

Somatic efferent pathways that originate from the motoneurons in Onuf's nucleus of the anterior horn of the S2 to S4 spinal cord innervate the external striated urethral sphincter muscle and the pelvic floor musculature (Fig. 1). Somatic nerve terminals release acetylcholine, which acts on nicotinic receptors (skeletal muscle type; N_1) to induce a muscle contraction. The striated urethral sphincter also receives noradrenergic inputs from sympathetic nerves.[45–48] The combined activation of sympathetic and somatic pathways elevates

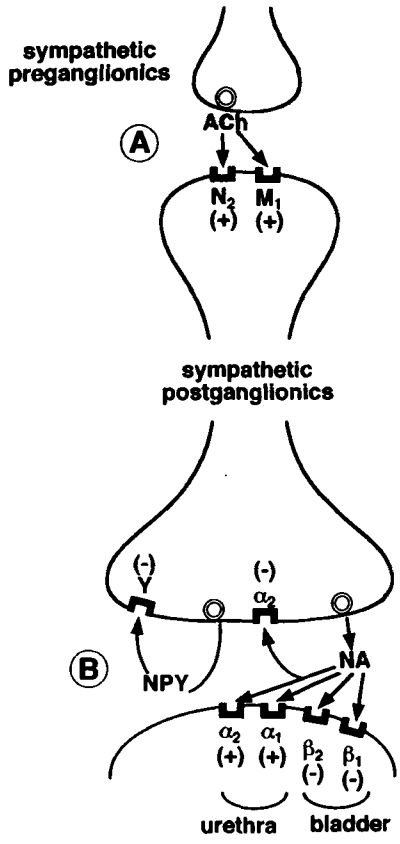

Fig. 3. Diagram of the interaction between neurotransmitters and their receptors in the sympathetic pathways to the lower urinary tract. (**A**) ganglionic level. (**B**) postganglionic level. Facilitatory and inhibitory responses are indicated by plus and minus in parentheses, respectively; circles indicate exocytosis from synaptic vesicles. Abbreviations: acetylcholine (ACh), norepinephrine (NA), neuropeptide Y (NPY), nicotinic receptor (N_2), muscarinic receptors (M_1), adrenergic receptors (α_1, α_2, β_1 and β_2), NPY receptor (Y).

bladder outlet resistance and contributes to urinary continence.

Several other nonadrenergic-noncholinergic transmitters have been identified as modulators of efferent inputs to the lower urinary tract. Leucine enkephalin released by preganglionic neurons inhibits cholinergic transmission via δ opioid receptors in pelvic ganglia[2,20,49] (Fig. 2A). In contrast, vasoactive intestinal polypeptide and substance P facilitate ganglionic transmission in pelvic ganglia.[16,50,51] Neuropeptide Y, which is contained in adrenergic and cholinergic nerve terminals, elicits a prejunctional inhibition of norepinephrine and acetylcholine release from postganglionic nerve terminals[52,53] (Figs. 2B and 3B).

Afferent Pathways

Sensory information, including the feeling of bladder fullness or bladder pain, is conveyed to the spinal cord

via afferent axons in the pelvic and hypogastric nerves.[8–13] Neuronal somata of these afferent nerves are located in the dorsal root ganglia at the S2 to S4 and T11 to L2 spinal segmental levels (Fig. 1). The afferent fibers carry impulses from tension receptors and nociceptors in the bladder wall to neurons in the dorsal horn of the spinal cord. Afferent fibers passing in the pelvic nerve to the sacral cord are responsible for initiating the micturition reflex. These bladder afferents have small myelinated (Aδ-fiber) or unmyelinated (C-fibers) axons.[54–56] Electrophysiologic studies in cats and rats have shown that the normal micturition reflex is mediated by small, myelinated Aδ-fiber afferents that respond to bladder distention.[5,8,54] In cats, the C-fiber afferents have high thresholds and are usually unresponsive to mechanical stimuli such as bladder distention; they have therefore been termed "silent C-fibers". However, many of these fibers do respond to chemical, noxious or cold stimuli.[9,10,57] A recent study in the rat using patch clamp techniques revealed that C-fiber afferent neurons are relatively inexcitable due to presence of high-threshold, tetrodotoxin-resistant sodium channels and low-threshold, A-type potassium channels.[58] Activation of C-fiber afferents by chemical irritation induces bladder hyperreflexia that is blocked by administration of capsaicin, a neurotoxin of C-fiber afferents.[1,11,59] However, since capsaicin does not block normal micturition reflexes, C-fiber afferents are not essential for normal voiding.[1,11,60–62]

Immunohistochemical studies indicate that bladder afferent neurons contain various neuropeptides such as substance P, calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide, and enkephalin (Fig. 4).[1,12,63,64] The distribution of these peptidergic afferent terminals in the spinal cord is similar to that of central projections of bladder afferent neurons.[13,65] Substance P and calcitonin gene-related peptide are present in a large percentage of C-fiber afferent neurons.[11,66] The release of these peptides in the bladder wall is known to trigger inflammatory responses, including plasma extravasation or vasodilation.[11,15]

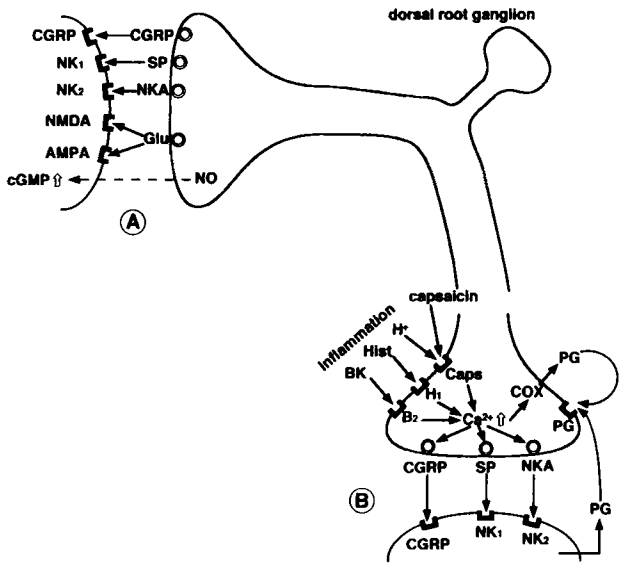

Fig. 4. Diagram showing the interaction of neurotransmitters and chemical mediators with their receptors in bladder afferent pathways (especially in C-fiber afferents): (A) spinal cord, (B) urinary bladder. Abbreviations: calcitonin gene-related peptide (CGRP), substance P (SP), neurokinin A (NKA), proton (H^+), histamine (Hist), bradykinin (BK), glutamate (Glu), nitric oxide (NO), prostaglandin (PG), CGRP receptor (CGRP), tachykinin receptors (NK_1 and NK_2), glutamatergic receptors (*N*-methyl-D-aspartate [NMDA] and α-amino-3-hydroxy-5-methylisoxazole-4-propionate [AMPA]), vanilloid receptor (Caps), histamine receptor (H_1), bradykinin receptor (B_2), prostaglandin receptor (PG), cyclic guanosine monophosphate (cGMP), cyclo-oxygenase (COX). Note that protons, histamine, and bradykinin released by inflammation induce an influx of calcium ions (Ca^{2+}), which triggers the release of neuropeptides and/or production of prostaglandin mediated by cyclo-oxygenase. Prostaglandin, which is also released from target cells (e.g., mast cells) by tachykinins, can act back on afferent terminals and sensitize afferent receptors to facilitate the release of transmitters. Circles indicate exocytosis from synaptic vesicles; dotted line indicates diffusion.

REFLEX MECHANISMS CONTROLLING MICTURITION

Storage Reflexes

The bladder functions as a low pressure reservoir during urine storage. In both humans and animals, bladder pressures remain low and relatively constant when bladder volume is below the threshold for voiding. This is mainly due to the combined effect of a passive phenomenon depending on viscoelastic properties of the bladder wall, and quiescence of the parasympathetic pathway to the bladder.[1,4,5] In addition, during bladder filling, afferent activity arising in the bladder activates a sacral-to-thoracolumbar intersegmental spinal reflex pathway, which triggers firing in sympathetic pathways to the bladder.[1,67] Activation of sympathetic efferents then mediates an inhibition of bladder activity and contraction of the bladder neck and proximal urethra.[68] Pudendal motoneurons are also activated by vesical afferent input as the bladder fills, thereby inducing a contraction of the striated sphincter muscle, which contributes to urinary continence.[69,70] Thus, urine storage is mainly controlled by reflexes integrated in spinal cord (Fig. 5A). However, it is also reported that a supraspinal

urine storage center is located in the dorsolateral pons. Descending inputs from this region activate the pudendal motoneurons to increase urethral resistance (Fig. 5A).[71,72]

Voiding Reflexes

When bladder volume reaches the micturition threshold, afferent activity originating in bladder mechanoceptors triggers micturition reflexes, which consist of firing in the sacral parasympathetic pathways and inhibition of sympathetic and somatic pathways (Fig 5B). This leads to a contraction of the bladder and a concomitant relaxation of the urethra. The afferent fibers that trigger micturition in the rat and cat are small myelinated Aδ-fibers.[54-56] These bladder afferents in the pelvic nerve synapse on neurons in the sacral spinal cord, which then send their axons rostrally to a micturition center in the dorsolateral pons. This center contains neurons that are essential for inducing voiding reflexes.[55,56,73-76] Bilateral lesions in the region of the locus coeruleus in the cat or the dorsolateral tegmental nucleus in the rat abolish micturition, while electric or chemical stimulation of this region induces a bladder contraction and a reciprocal relaxation of the urethra, leading to bladder emptying.[71,73-77] Studies in the rat and cat indicate that activity ascending from the spinal cord may pass through a relay center in the periaqueductal gray before reaching the pontine micturition center.[78-80] Thus, voiding reflexes depend on a spinobulbospinal pathway, which passes through an integrative center in the brain (Fig. 5B). This center functions as an 'on-off' switch activated by afferent activity derived from bladder mechanoceptors, and also receives inhibitory and excitatory inputs from the brain regions rostral to the pons.

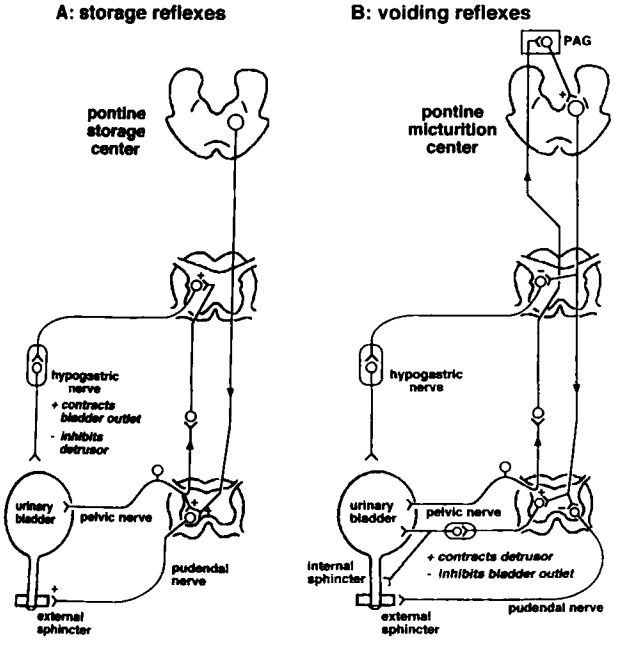

Fig. 5. Diagrams showing neural circuits controlling continence and micturition. (**A**) urine storage reflexes. During urine storage, bladder distention produces low level firing in bladder afferent pathways, which in turn stimulates (1) the sympathetic outflow to the bladder outlet (bladder base and urethra) and (2) pudendal outflow to the external sphincter muscle. These responses are elicited by spinal reflex pathways. Sympathetic firing also inhibits detrusor muscle activity and transmission in bladder ganglia. A region in the rostral pons (pontine storage center) increases external urethral sphincter activity. (**B**) Voiding reflexes. During elimination of urine, intense bladder afferent firing activates spinobulbospinal reflex pathways passing through the pontine micturition center, which stimulate the parasympathetic outflow to the bladder and internal sphincter smooth muscle and inhibit the sympathetic and pudendal outflow to the bladder outlet. Ascending afferent input from the spinal cord may pass through relay neurons in the periaqueductal gray (PAG) before reaching the pontine micturition center.

Neurotropic viruses, such as pseudorabies virus, have been particularly useful in identifying the central neural pathways involved in micturition. These viruses can be injected into a target organ (urinary bladder, urethra, or urethral sphincter), and then move intra-axonally from the periphery to the central nervous system, where they replicate and then pass retrogradely across synapses to infect second- and third-order neurons in the neural pathways. Since the pseudorabies virus can be transported across many synapses, it could sequentially infect all of the neurons that connect directly or indirectly to the lower urinary tract. The pseudorabies virus has been used in the rat[81-84] and cat[85] to identify neurons in the spinal cord and the brain involved in the control of the lower urinary tract. In the rat, transneuronal virus-tracing methods have identified many populations of central neurons that are involved in the control of bladder, urethra, and urethral sphincter. Injection of pseudorabies virus into the bladder labeled several areas of the brain stem, including the laterodorsal tegmental nucleus (the pontine micturition center); the medullary raphe nucleus, which contains serotonergic neurons; the locus coeruleus, which contains noradrenergic neurons, the periaqueductal grey, and noradrenergic cell group-A5. Several regions in the hypothalamus and the cerebral cortex also had virus-infected cells. Neurons in the cortex were located primarily in the medial frontal cortex. Similar brain areas were labeled after injection of virus into the urethra and urethral sphincter, suggesting that coordination between different parts of the lower urinary tract is mediated by similar populations of neurons in the brain.

Reflex voiding is also facilitated by afferent inputs from the urethra. This urethrovesical reflex, triggered by urine flow into the urethra, enhances bladder contractions.[6] During voiding, activity in the pudendal efferent pathway to the striated urethral sphincter is suppressed to reduce outlet resistance.[74,86,87] This mechanism is mainly due to an inhibition of the pudendal motoneurons by the descending inputs from the dorsolateral pons.[69,70] As mentioned above, an excitation of the sacral parasympathetic pathway also directly induces a relaxation of urethral smooth muscle mediated by the release of nitric oxide and vasoactive intestinal polypeptide.

Supraspinal and Spinal Neurotransmitters Controlling Micturition

Various neurotransmitters at the spinal and supraspinal level are involved in regulation of micturition and continence (Fig. 6). Glutamic acid, which is the major excitatory transmitter in the central nervous system, has an important role in the control of the micturition reflex. Both *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kainate receptors are involved in glutamatergic transmission in the micturition reflex pathway.[88-93] However, the function of glutamate seems to vary under different experimental conditions. In anesthetized rats, intravenous or intrathecal administration of NMDA or AMPA/kainate receptor antagonists suppressed the micturition reflex[88-90,92]; whereas in unanesthetized rats, an NMDA receptor antagonist had a slight facilitatory effect on bladder activity although an AMPA/kainate receptor antagonist still had a depressant effect.[91,94] However, a recent study indicated that synergic activation of both types of glutamate receptors is necessary to induce reflex activation of the bladder in unanesthetized decerebrate rats.[95] AMPA/kainate or NMDA receptor antagonists also suppress bladder contractions induced by electric stimulation of the rat pontine micturition center, indicating that AMPA/kainate and NMDA glutamatergic excitatory mechanisms are involved in the descending limb of spinobulbospinal micturition reflex.[96,97]

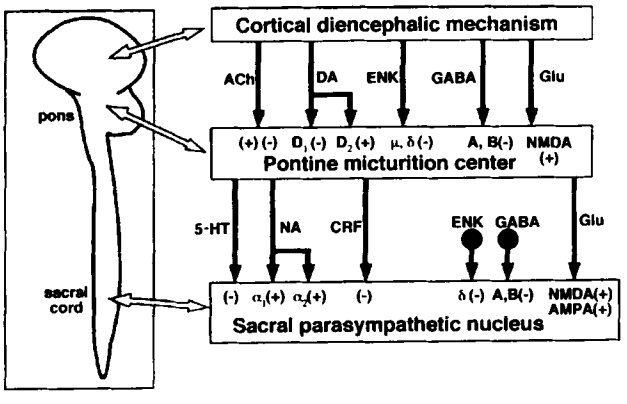

Fig. 6. Diagram of neurotransmitters in spinal and supraspinal sites. Glutamate is the major excitatory transmitter in the control of the micturition reflex. Modulation of the micturition reflex in the spinal cord occurs by segmental interneuronal mechanisms (ENK, GABA) or by descending input from the 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. Abbreviations: acetylcholine (ACh), dopamine (DA), enkephalin (ENK), glutamate (Glu), 5-hydroxytryptamine (5-HT), norepinephrine (NA), corticotropin-releasing factor (CRF), dopamine receptors (D_1 and D_2), opioid receptors (μ and δ), γ-aminobutyric acid (GABA) receptors (A and B).

Recent studies using spinal slice preparations from neonatal rats revealed that the sacral preganglionic neurons directly receive glutamatergic excitatory inputs from spinal interneurons in the region of the sacral parasympathetic nucleus.[98] These inputs are mediated by both NMDA and AMPA/kainate receptors. Interneurons in these regions also exhibit increased expression of an immediate early gene, *c-fos*, in response to stimulation of bladder afferents.[59] In addition, *c-fos* expression in the spinal cord induced by chemical bladder irritation is suppressed by the NMDA or AMPA/kainate receptor antagonists, indicating the involvement of glutamatergic transmission in bladder afferent pathways.[99,100] Thus, it is likely that the micturition reflex is dependent upon glutamatergic transmission at various sites including the descending projections from the pontine micturition center to the sacral preganglionic neurons, the spinal processing of afferent inputs from the bladder, and the synapses between spinal interneurons and preganglionic neurons.

The micturition reflex pathway can be modulated by a variety of other transmitter mechanisms at both spinal and supraspinal sites. In the spinal cord, modulation can occur by segmental interneuronal mechanisms, or by descending input from the brain (Fig. 6).

A bulbospinal noradrenergic pathway arising from the locus coeruleus in the rostral pons has a facilitatory effect on the micturition reflex. This pathway has been demonstrated in anesthetized cats by measuring bladder activity or neuronal firing in the sacral spinal cord elicited by electric stimulation of noradrenergic neurons in the locus coeruleus. The excitatory effects of locus coeruleus stimulation were blocked by intrathecal administration of prazosin, indicating that α_1-adrenergic receptors mediate the effect.[101,102] Since destruction of the noradrenergic pathway by 6-hydroxydopamine caused urinary retention, and this

effect was partially reversed by intrathecal application of the α_1-adrenoceptor agonist, phenylephrine,[103] it was concluded that this pathway must play an important role in the control of micturition. This is consistent with the results of a recent pharmacologic study in conscious rats showing that intrathecal administration of an α_1-adrenergic antagonist suppressed reflex bladder contractions in the normal rats, and that these effects were more profound in the rats with bladder hypertrophy.[104] Spinal α_2-adrenoceptors are also reported to facilitate the micturition reflex in the rat.[105,106] However, other studies revealed that α-adrenergic antagonists did not alter voiding in conscious cats or rats, suggesting spinal noradrenergic mechanisms may not be active under normal conditions.[107,108]

Sphincter function is also modulated by the spinal noradrenergic pathways. It has been shown that striated sphincter reflexes are inhibited by α_2-adrenoceptor agonists in cats and rats,[106,109] and that central sympathetic and somatic outflow to the lower urinary tract in cats is suppressed by α_1-adrenoceptor antagonists such as prazosin.[110,111] These data indicate the existence of α_1-receptor-mediated tonic facilitation of sphincter function and a putative α_2 inhibitory mechanism in the spinal cord.

In the brain stem, cholinergic mechanisms may be involved in both inhibitory and facilitatory modulation of the micturition reflex. Microinjection of acetylcholine into the pontine micturition center in cats can increase or decrease the threshold volume for inducing a reflex contraction of the bladder.[77,112] These effects were blocked by atropine indicating a role of muscarinic receptors.

Pharmacologic studies in rats revealed that activation of central D_2 dopaminergic receptors facilitates the micturition reflex pathway.[113] In cats, microinjection of dopamine to the pontine micturition center also reduced bladder capacity and facilitated the micturition reflex,[112] whereas intracerebroventricular administration of dopamine or a D_1 dopamine receptor agonist (SKF38393), inhibited the micturition reflex.[114] Experiments in monkeys with parkinsonism induced by a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), revealed that dopaminergic neurons originating in the substantia nigra tonically inhibit the micturition reflex. MPTP-treated monkeys exhibited hyperreflexic bladders as reported in patients with Parkinson's disease.[115,116] In these monkeys, stimulation of D_1 receptors with SKF38393 suppressed the detrusor hyperreflexia, whereas the nonselective dopamine receptor agonist (apomorphine) and the D_2 receptor agonist (quinpirole) reduced the bladder volume threshold.[116] Thus central dopaminergic pathways exhibit different effects on micturition via actions on multiple receptors at presumably different sites in the brain.

Enkephalinergic pathways in the central nervous system have an inhibitory effect on the micturition reflex. Enkephalinergic varicosities are found at various sites, including the pontine micturition center, the sacral parasympathetic nucleus, and the urethral sphincter motor nucleus. Administration of leucine enkephalin or opiate drugs to the brain or the spinal cord suppresses micturition and sphincter reflexes, whereas administration of an opioid receptor antagonist (naloxone) facilitates the micturition reflex in animals and humans, indicating that endogenous opioid mechanisms exert a tonic inhibition on the micturition reflex.[64,117,118] Among the 3 opiate receptors (μ, δ, and κ subtypes), the inhibition of the micturition reflex at the spinal cord level is mediated by δ receptors, whereas in the brain, μ and δ receptors are involved in enkephalin-mediated inhibition of micturition in the cat.[119,120] In addition, sphincter muscle activity is suppressed by κ receptor agonists.[121] In the rat, both μ and δ receptors mediate the inhibitory effect on the micturition reflex in the spinal cord and the brain.[64]

Inhibitory amino acids also have a role in modulating the micturition reflex. γ-Aminobutyric acid (GABA) has been implicated as an inhibitory transmitter at supraspinal and spinal sites, where it can act on both $GABA_A$ and $GABA_B$ receptors.[1,118,122] A recent study using patch clamp techniques showed that GABA released from interneurons mediates inhibitory synaptic inputs to the sacral parasympathetic preganglionic neurons in the neonatal rat spinal cord.[123] Clinical studies also revealed that intrathecal administration of a $GABA_B$ receptor agonist (baclofen) increased the bladder volume threshold for inducing the micturition reflex.[124,125] Glycine, another inhibitory amino acid, is also released from interneurons in the spinal cord and mediates recurrent inhibition on the micturition reflex pathway.[126] Glycinergic inhibition occurs on the descending limb of the micturition reflex pathway but not at the level of the preganglionic neurons in the cat,[1,86] although glycinergic inhibitory postsynaptic currents elicited by inhibitory interneuronal inputs to sacral preganglionic neurons have been detected in the neonatal rat spinal slice preparation.[123]

The sympathetic and parasympathetic autonomic nuclei, as well as the sphincter motor nuclei, receive a prominent serotonergic input from the raphe nuclei in the caudal brain stem. Activity in the serotonergic pathway generally enhances urine storage by facilitating the vesicosympathetic reflex pathway and inhibiting the parasympathetic micturition pathway.[1,6] Among the various subtypes of 5-hydroxytryptamine (5-HT) receptors, 5-HT_2 receptors mediate excita-

tory effects on sympathetic and somatic reflexes to increase outlet resistance; whereas 5-HT_{1C} or 5-HT_3 receptors are involved in inhibition of the micturition reflex.[127-130] 5-HT_{1A} autoreceptors in the raphe nucleus are likely to exert an inhibitory control over activity of raphe neurons.[127]

Corticotropin-releasing factor (CRF), which is contained in neurons within the pontine micturition center,[131] seems to be an inhibitory co-transmitter in the descending glutamatergic excitatory pathway to the sacral parasympathetic nucleus because intrathecally applied corticotropin-releasing factor inhibits the bladder contractions produced by stimulation to the pontine micturition center in the rat.[132,133]

PLASTICITY OF BLADDER AFFERENT PATHWAYS

Recent studies provide evidence that capsaicin-sensitive, C-fiber afferents innervating the bladder are at least in part responsible for changes in bladder motility induced by various pathologic conditions such as cystitis, bladder outlet obstruction, and spinal cord injury. It is also suggested that the reorganization of bladder reflex pathways in neuropathologic conditions is influenced by neural–target-organ interactions.[134,135] This section of the paper focuses on the recent findings concerning disease-specific changes in C-fiber bladder afferent pathways.

Cystitis

The effect of bladder inflammation on the micturition reflex has usually been investigated using animals in which cystitis is induced by intravesical instillation of chemical or pharmacologic agents. Electrophysiologic studies in the cat revealed that C-fiber afferents, which originally do not respond to the mechanoceptive stimuli, are sensitized to respond to those stimuli after chemical irritation of the bladder.[9] It is known that the majority of C-fiber afferents are sensitive to capsaicin, which acts on a capsaicin-binding site (vanilloid receptor) and causes dose-dependent excitation, desensitization, and neurotoxicity.[11,15] Inflammation is accompanied by a reduction in tissue pH, which may play a role in afferent sensitization. Protons (H^+) are known to activate vanilloid receptors in afferent nerve terminals, induce an influx of Ca^{2+} ions, and then release neuropeptides such as tachykinins (substance P and related substances), calcitonin gene-relating peptide, or vasoactive intestinal polypeptide, which produce local inflammatory responses including plasma extravasation or vasodilation[11,15] (Fig. 4). Other endogenous substances such as bradykinin, histamine, and prostaglandins, which are released in response to the actions of afferent neuropeptides and other chemical changes induced by inflammation also enhance the bladder motility and neuropeptide release[13,15,136,137] (Fig. 4).

These changes in target organs also affect the central processing in afferent pathways. It appears that tachykinins, which are released from central terminals, enhance afferent transmission in the spinal cord, thereby inducing bladder hyperactivity. Bladder hyperreflexia in a rat model in which cystitis is induced by intravesical instillation of capsaicin is suppressed by intrathecal application of NK_1 and NK_2 receptor antagonists.[138,139] Since tachykinins are predominantly found in C-fiber afferent nerves,[66] it is assumed that cystitis elicits bladder hyperactivity by enhancing central neurotransmission mediated by C-fiber afferents. Nitric oxide is another candidate for cystitis-induced changes in afferent neurotransmission in the spinal cord. NOS immunoreactivity in bladder afferent dorsal root ganglion neurons increases in the rat with chronic cystitis induced by cyclophosphamide.[140] In addition, intrathecally administered NOS inhibitors suppress bladder hyperactivity associated with chemically induced cystitis, but had no effect on the normal micturition in the rat.[141] Thus, plasticity in afferent neurotransmission in the spinal cord seems to play an important role in cystitis-induced bladder hyperactivity.

Bladder Outlet Obstruction

Bladder hyperactivity often occurs in the patients with bladder outlet obstruction induced by prostatic hypertrophy. Animal models of outlet obstruction produced by partial urethral ligation have been used to study mechanisms underlying bladder hyperactivity induced by obstruction.[1] It has been reported that a spinal micturition reflex is unmasked or enhanced in rats with urethral obstruction.[142] Afferent neuron hypertrophy and increased afferent projections in the spinal cord have also been detected in these animals.[65] These changes in bladder afferent pathways are due at least in part to increased levels of nerve growth factor in the hypertrophied bladder smooth muscle, since autoimmunization against nerve growth factor reduced urinary frequency and afferent neuron hypertrophy in the obstructed rat.[143,144] It has been speculated that the bladder hypertrophy and increased levels of nerve growth factor are triggered by increased bladder work in response to increased outlet resistance. A high-affinity tyrosine kinase receptor, trkA, that binds nerve growth factor is expressed in C-fiber afferents.[145] Thus, plasticity in the C-fiber afferent pathway may be involved in bladder hyperactivity associated with outlet obstruction. It has also been proposed that enhanced afferent transmission in the spinal cord mediated by tachykinins contributes to obstruction-related bladder hyperactivity, since the

effects of intrathecal administration of an NK_1 receptor antagonist on the micturition reflex were greater in the rat with urethral obstruction than in the normal rat.[139]

Spinal Cord Injury

Spinal cord transection rostral to the lumbosacral level abolishes voluntary control of voiding as well as the spinobulbospinal micturition reflex. However, after an initial period of detrusor areflexia, a spinal reflex pathway emerges that mediates automatic micturition and detrusor hyperreflexia.[134,135] Electrophysiologic and pharmacologic studies in chronically spinalized cats indicate that the spinal micturition reflex is mediated by C-fiber afferents and is blocked by systemic capsaicin administration.[55,61] Vasoactive intestinal polypeptide immunoreactivity, which is a marker for bladder C-fiber afferents,[11,146,147] is distributed in a wider area in the spinal cord in spinalized cats.[61] In spinalized animals, small doses of vasoactive intestinal polypeptide administered intrathecally facilitated the micturition reflex; whereas in normal animals, vasoactive intestinal polypeptide inhibited the reflex, suggesting that an alteration in the actions of a putative C-fiber afferent neurotransmitter is important in the emergence of C-fiber-mediated spinal micturition reflexes.[1,61,134]

In paraplegic animals and humans, reflex voiding is inefficient due to tonic activity of the urethral sphincter (detrusor-sphincter dyssynergia). In rats, this functional outlet obstruction induces bladder muscle hypertrophy similar to that occurring after outlet obstruction induced by partial urethral ligation.[148,149] Both conditions are associated with afferent neuron hypertrophy.[65,150] Electrical recordings using patch clamp techniques revealed that hypertrophied bladder afferent neurons in spinalized rats exhibit increased excitability due to a shift in expression of sodium channels from high-threshold tetrodotoxin-resistant to low-threshold tetrodotoxin-sensitive channels.[151,152] In normal animals, tetrodotoxin-resistant sodium channels are mainly expressed in capsaicin-sensitive C-fiber afferent neurons.[58,153]

Another type of plasticity in C-fiber bladder afferent neurons after spinal cord injury was evident as a change in neurofilament immunoreactivity in the cells. In normal animals, C-fiber neurons that are small in size exhibit poor or no neurofilament staining.[66] However, spinal cord injury significantly increased the number of afferent neurons with prominent neurofilament immunoreactivity.[154] In addition, the sensitivity of bladder afferent neurons to capsaicin decreased in spinalized rats.[154] Taken together, these findings indicate that spinal cord injury induces various phenotypic changes in C-fiber bladder afferent pathways, in parallel with the emergence of the C-fiber-mediated spinal micturition reflex. Since urinary diversion in spinalized rats prevented the hypertrophy of the bladder and of the bladder afferent neurons,[150] it has been suggested that neurotrophic factors released in hypertrophied bladder muscles by functional bladder outlet obstruction secondary to bladder-sphincter dyssynergia are responsible for the afferent neuron plasticity and thus contribute to the emergence of bladder hyperactivity after spinal cord injury.

Clinical Studies

The involvement of C-fiber afferents in neurogenic bladder hyperactivity in humans has also been demonstrated in clinical studies in which intravesical instillation of capsaicin suppressed bladder hyperactivity in patients with various types of neurogenic disorders of the lower urinary tract, including spinal cord injury. When administered intravesically in concentrations between 100 μmol/L and 2 mmol/L, capsaicin increased bladder capacity and reduced the number of incontinence episodes in patients with spinal cord injury or multiple sclerosis.[155–158] The effects of high concentrations of capsaicin in patients with multiple sclerosis persisted for weeks to months after treatment. Capsaicin also increased bladder capacity and reduced irritative symptoms in patients with hypersensitive bladders.[156,159]

Another example of a reorganization of C-fiber-mediated reflex pathways was obtained in studies of the cold stimulation-evoked voiding reflex. It is known that instillation of cold water into the bladder of patients with upper motoneuron lesions induces reflex voiding (the Bors ice water test).[160] This reflex does not occur in normal subjects. It has been shown in the cat that C-fiber bladder afferents are responsible for cold-induced bladder reflexes.[57] Intravesical administration of capsaicin to paraplegic patients blocks the cold-induced bladder reflexes, indicating that they are mediated by C-fiber afferents in humans as well.[159] The ice water test is also positive in patients with multiple sclerosis, cerebrovascular disease, and Parkinson's disease, as well as in infants.[161,162] Thus, it is suggested that cold-evoked bladder reflexes are enhanced or unmasked after the elimination of supraspinal controls by spinal cord injury or damage to central pathways in patients with multiple sclerosis. It is noteworthy that the cold-induced bladder reflex also has been detected in elderly patients who have uninhibited overactive bladders without any other obvious neurologic problems.[161] Taken together, these observations suggest that capsaicin-sensitive, C-fiber bladder afferents are involved in various pathologic conditions associated with neurogenic bladder hyperactivity in humans.

CONCLUSIONS

The 2 main functions of the lower urinary tract (storage and periodic elimination of urine) are controlled by a neuronal switching circuit that maintains a reciprocal relationship between the reservoir (bladder) and the urethral outlet (urethra and urethral sphincter). This switching depends upon glutamatergic excitatory transmission. Various neural pathways and transmitters in the brain and spinal cord also mediate excitatory and inhibitory influences on this switching mechanism. Studies in animals and humans indicate that changes in target organ functions that occur in various pathologic conditions have a significant effect on the bladder afferent pathway to alter spinal reflex mechanisms, resulting in bladder hyperactivity. A more precise understanding of the mechanisms responsible for these disease-specific changes will no doubt lead to new treatments of lower urinary tract dysfunction.

REFERENCES

1. de Groat WC, Booth AM, Yoshimura N. Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:227–290.
2. de Groat WC, Booth AM. Synaptic transmission in pelvic ganglia. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:291–347.
3. Tsuchida S. Nervous control of micturition. Jpn J Urol 1989;80:1257–1277.
4. Van Arsdalen K, Wein AJ. Physiology of micturition and continence. In: Krane RJ, Siroky M (eds) Clinical neuro-urology. 2nd ed. New York: Little Brown & Company, 1991:25–82.
5. Torrens M, Morrison JFB. The physiology of the lower urinary tract. Berlin: Springer-Verlag, 1987.
6. Chai TC, Steers WD. Neurophysiology of micturition and continence. Urol Clin North Am 1996;23: 221–236.
7. Andersson K-E. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev 1993;45:253–308.
8. Jänig W, Morrison JFB. Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. Prog Brain Res 1986;67:87–114.
9. Häbler HJ, Jänig W, Koltzenburg M. Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat. J Physiol (Lond) 1990;425:545–562.
10. Jänig W, Koltzenburg M. Pain arising from the urogenital tract. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:525–578.
11. Maggi CA. The dual, sensory and efferent function of the capsaicin-sensitive primary sensory nerves in the bladder and urethra. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:383–422.
12. de Groat WC. Neuropeptides in pelvic afferent pathways. Experientia 1987;43:801–812.
13. de Groat WC. Spinal cord projections and neuropeptides in visceral afferent neurons. Prog Brain Res 1986;67:165–187.
14. Jänig W, McLachlan EM. Organization of lumber spinal outflow to distal colon and pelvic organs. Physiol Rev 1987;67:1332–1404.
15. Lundberg JM. Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol Rev 1996; 48:113–178.
16. Kawatani M, Rutigliano M, de Groat, WC. Selective facilitatory effect of vasoactive intestinal polypeptide (VIP) on muscarinic firing in vesical ganglia of the cat. Brain Res, 1985;336:223–234.
17. Keast J, Kawatani M, de Groat WC. Sympathetic modulation of cholinergic transmission in cat vesical ganglia is mediated by α_1 and α_2 adrenoceptors. Am J Physiol 1990;258:R44–R50.
18. Nakamura T, Yoshimura M, Shinnick-Gallagher P, Gallagher JP, Akasu T. α_2 and α_1-adrenoreceptors mediate opposing actions on parasympathetic neurons. Brain Res 1984;323:349–353.
19. Theobald RJ, de Groat WC. The effects of purine nucleotides on transmission in vesical parasympathetic ganglia of the cat. J Auton Pharmacol 1989;9:167–182.
20. de Groat WC, Kawatani M. Enkephalinergic inhibition in parasympathetic ganglia of the urinary bladder of the cat. J Physiol (Lond), 1989;413:13–29.
21. Eglen RM, Hegde SS, Watson N. Muscarinic receptors subtypes and smooth muscle action. Pharmcol Rev 1996;48:531–565.
22. Maeda A, Kubo T, Mishina, M, Numa S. Tissue distribution of mRNA's encoding muscarinic acetylcholine receptor subtypes. FASEB Lett 1988;239: 339–342.
23. Kondo S, Morita T, Tashima Y. Muscarinic cholinergic receptor subtypes in human detrusor muscle studied by labelled and non-labelled pirenzepine, AF-DX116 and 4-DAMP. Urol Int 1995;54:150–153.
24. Yamaguchi O, Shishido K, Tamura K, Ogawa T, Fujimura T, Otsuka A. Evaluation of mRNAs encoding muscarinic receptor subtypes in human detrusor muscle. J Urol 1996;156:1208–1213.
25. Wang P, Luthin GR, Ruggieri MR. Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP proteins. J Pharmacol Exp Ther 1995;273:959–966.
26. Longhurst PA, Leggett RE, Briscoe JAK. Characterization of functional muscarinic receptors in the rat urinary bladder. Br J Pharmacol 1995;116:2279–2285.
27. Tobin G and Sjögren C. In vivo and in vitro effects of muscarinic receptor antagonists on contractions and release of [^{3}H]acetylcholine in the rabbit urinary bladder. Eur J Pharmacol 1995;281:1–8.
28. Somogyi GT, de Groat WC. Evidence for inhibitory nicotinic and facilitatory muscarinic receptors in

cholinergic nerve terminals of the rat urinary bladder. J Auton Nerv Syst 1992;37:89–97.
29. Somogyi GT, Tanowitz M, de Groat WC. M-1 muscarinic receptor mediated facilitation of acetylcholine release in the rat urinary bladder but not in the heart. J Physiol (Lond) 1994;480:81–89.
30. Hoyle CHV, Burnstock G. Postganglionic efferent transmission in the bladder and urethra. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:349–382.
31. Luheshi GN, Zar MA. Presence of non-cholinergic motor transmission in human isolated bladder. J Pharm Pharmacol 1990;42:223–224.
32. Igawa Y, Mattiasson A, Andersson K-E. Functional importance of cholinergic and purinergic neurotransmission for micturition contraction in the normal, un-anesthetized rat. Br J Pharmacol 1993;109:473–479.
33. Fraser MO, Flood HD, de Groat WC. Urethral smooth muscle relaxation is mediated by nitric oxide (NO) released from parasympathetic postganglionic neurons. J Urol 1995;153:461A.
34. Bennett, BC, Roppolo, JR, Kruse, MN, de Groat, WC. Neural control of urethral smooth and striated muscle activity in vivo: role of nitric oxide. J Urol 1995;153:2004–2009.
35. Persson K, Igawa Y, Mattiasson A, Andersson K-E. Effects of inhibition of the L-arginine/nitric oxide pathway in the rat lower urinary tract in vivo and in vitro. Br J Pharmacol 1992;107:178–184.
36. Thornbury KD, Hollywood MA, McHale NG. Mediation by nitric oxide of neurogenic relaxation of the urinary bladder neck muscle in sheep. J Physiol (Lond) 1992;451:133–144.
37. Takeda M, Lepor H. Nitric oxide synthase in the dog urethra: a histochemical and pharmacological analysis. Br J Pharmacol 1995;116:2517–2523.
38. Persson K, Alm P, Johansson K, Larsson B, Andersson K-E. Nitric oxide synthase in pig lower urinary tract: immunohistochemistry, NADPH diaphorase histochemistry, and functional effects. Br J Pharmacol 1993;110:521–530.
39. Werkström V, Persson K, Ny L, Bridgewater M, Brading A, Andersson K-E. Factors involved in the relaxation of female pig urethra evoked by electrical stimulation. Br J Pharmacol 1995;116:1599–1604.
40. Hashimoto S, Kigoshi S, Muramatsu I: Nitric oxide-dependent and -independent neurogenic relaxation in isolated dog urethra. Eur J Pharmacol 1993;231: 209–214.
41. Keast JR. Visualization and immunohistochemical characterization of sympathetic and parasympathetic neurons in the male rat major pelvic ganglion. Neuroscience 1995;66:655–662.
42. Lincoln J, Burnstock G. Autonomic innervation of the urinary bladder and urethra. In: Maggi CA (ed) The autonomic nervous system. Vol. 3. Nervous control of the urogenital system. London: Harwood Academic Publishers, 1993:33–68.
43. Levin RM, Ruggieri MR, Wein AJ. Identification of receptor subtypes in the rabbit and human urinary bladder by selective radio-ligand binding. J Urol 1988;139:844–848.
44. Testa R, Guarneri L, Ibba M, Strada G, Poggesi E, Taddei C, Simonazzi I, Leonardi A. Characterization of alpha 1-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man. Eur J Pharmacol 1993;249:307–315.
45. Gosling JA, Dixon JS, Lendon RG. The autonomic innervation of the human male and female bladder neck and proximal urethra. J Urol 1977;118:302–305.
46. Elbadawi A, Schenk EA. A new theory of the innervation of the bladder musculature. Part 4. Innervation of the vesicourethral junction and external urethral sphincter. J Urol 1974;111:613–615.
47. Elbadawi A, Atta MA. Ultrastructural analysis of vesicourethral innervation: evidence for somatomotor plus autonomic innervation of the feline rhabdosphincter. Neurourol Urodyn 1985;4:23–36.
48. Kakizaki H, Koyanagi T, Shinno Y, Kobayashi S, Matsumura K, Kato M. An electromyographic study on the urethral rhabdosphincter in normal and chronically rhizotomized cats. Analysis of electrical potentials evoked by sympathetic nerve stimulation. J Urol 1994;151:238–243.
49. Kawatani M, Shioda S, Nakai Y, Takeshige C, de Groat WC. Ultrastructural analysis of enkephalinergic terminals in parasympathetic ganglia innervating the urinary bladder of the cat. J Comp Neurol 1989; 288:81–91.
50. Akasu T, Gallagher JP, Hirai K, Shinnick-Gallagher P. Vasoactive intestinal polypeptide depolarizations in cat bladder parasympathetic ganglia. J Physiol (Lond) 1986;374:457–473.
51. Kawatani M, Whitney T, Booth AM, de Groat WC. Excitatory effect of substance P in parasympathetic ganglia of the cat urinary bladder. Am J Physiol 1989;257:R1450–R1456.
52. Tran LV, Somogyi GT, de Groat WC. Inhibitory effects of neuropeptide Y on cholinergic and adrenergic transmission in the rat urinary bladder and urethra. Am J Physiol 1994;266:R1411–R1417.
53. Zoubek JA, Somogyi GT, de Groat WC. A comparison of inhibitory effects of neuropeptide Y on rat urinary bladder, urethra and *vas* deferens. Am J Physiol 1993;265:R537–R543.
54. Mallory B, Steers WD, de Groat WC. Electrophysiological study of micturition reflexes in rats. Am J Physiol 1989;257:R410–R421.
55. de Groat WC, Nadelhaft I, Milne RJ, Booth AM, Morgan C, Thor K. Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine. J Auton Nerv Sys 1981;3:135–160.
56. Vera PL, Nadelhaft I. Conduction velocity distribution of afferent fibers innervating the rat urinary bladder. Brain Res 1990;520: 83–89.
57. Fall M, Lindström S, Mazieres L. A bladder-to-bladder cooling reflex in the cat. J Physiol (Lond) 1990;427:281–300.
58. Yoshimura N, White G, Weight FF, de Groat WC. Different types of Na^+ and A-type K^+ currents in dorsal root ganglion neurons innervating the rat urinary bladder. J Physiol (Lond) 1996;494:1–16.
59. Birder LA, de Groat WC. Increased *c-fos* expression in spinal neurons after chemical irritation of the lower urinary tract of the rat. J Neurosci 1992;12:4878–4889.
60. Cheng C-L Ma C-P, de Groat WC. The effects of capsaicin on micturition and associated reflexes in the rat. Am J Physiol 1993;265:R132–R138.

61. de Groat WC, Kawatani M, Hisamitsu T, Cheng C-L, Ma C-P, Thor K, Steers W, Roppolo JR. Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. J Auton Nerv Syst 1990;30:S71–S77.
62. Cheng C-L, Ma C-P, de Groat WC. Effect of capsaicin on micturition and associated reflexes in chronic spinal rats. Brain Res 1995;678:40–48.
63. Keast JR, de Groat WC. Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats. J Comp Neurol 1992;319:615–623.
64. Maggi CA. The role of peptides in the regulation of the micturition reflex: an update. J Gen Pharmacol 1991;22:1–24.
65. Steers WD, Ciambotti J, Etzel B, Erdman S, de Groat WC. Alterations in afferent pathways from the urinary bladder of the rat in response to partial urethral obstruction. J Comp Neurol 1991;310:401–410.
66. Lawson SN, Perry MJ, Prabhaker E, McCarthy PW. Primary afferent neurones: neurofilament, neuropeptides, and conduction velocity. Brain Res Bull 1993;30:239–243.
67. de Groat WC, Lalley PM. Reflex firing in the lumbar sympathetic outflow to activation of vesical afferent fibers. J Physiol (Lond) 1972;226:289–309.
68. de Groat WC, Theobald RJ. Reflex activation of sympathetic pathways to vesical smooth muscle and parasympathetic ganglia by electrical stimulation of vesical afferents. J Physiol(Lond) 1976;259:223–237.
69. Shimoda N, Takakusaki K, Nishizawa O, Tsuchida S, Mori S. The changes in the activity of pudendal motoneurons in relation to reflex micturition evoked in decerebrated cats. Neurosci Lett 1992;135:175–178.
70. Fedirchuk B, Shefchyk SJ. Membrane potential changes in sphincter motoneurons during micturition in the decerebrate cat. J Neurosci 1993;13:3090–3094.
71. Holstege G, Griffiths D, de Wall H, Dalm E. Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in cat. J Comp Neurol 1986;250:449–461.
72. Kohama T. Neuroanatomical studies on the urine storage facilitatory areas in the cat brain. Part I: Input neuronal structures to the nucleus subcoeruleus and the nucleus reticularis pontis oralis. Jpn J Urol 1992; 83:1469–1477.
73. Kuru M. Nervous control of micturition. Physiol Rev 1965;45:425 494.
74. Nishizawa O, Sugaya K, Noto H, Harada T, Tsuchida S. Pontine micturition center in the dog. J Urol 1988;140;872–874.
75. Mallory BS, Roppolo JR, de Groat WC. Pharmacological modulation of the pontine micturition center. Brain Res 1991;546:310–320.
76. Noto H, Roppolo JR, Steers WD, de Groat WC. Excitatory and inhibitory influences on bladder activity elicited by electrical stimulation in the pontine micturition center in rat. Brain Res. 1989;492:99–115.
77. Sugaya K, Matsuyama K, Takakusaki K, Mori S. Electrical and chemical stimulations of the pontine micturition center. Neurosci Lett 1987;80:197–201.
78. Noto H, Roppolo JR, Steers WD, de Groat WC. Electrophysiological analysis of ascending and descending components of the micturition reflex pathway in the rat. Brain Res 1991;549:95–105.
79. Blok BF, Holstege G. Direct projections from the periaqueductal gray to the pontine micturition center (M-region). An antegrade and retrograde tracing study in the cat. Neurosci Lett 1994;166:93–96.
80. Blok BF, De Weerd H, Holstege G. Ultrastructural evidence for a paucity of projections from the lumbosacral cord to the pontine micturition center or M-region in the cat: a new concept for the organization of the micturition reflex with the periaqueductal gray as central relay. J Comp Neurol 1995;359:300–309.
81. de Groat WC, Roppolo JR, Yoshimura N, Sugaya K. Neural control of the urinary bladder and colon. In: Taché Y, Wingate DL, Burks TF (eds) Innervation of the gut: pathophysiological implications. Boca Raton, FL: CRC Press, 1994;167–190.
82. Nadelhaft I, Vera PL, Card JP, Miselis RR. Central nervous system neurons labelled following the injection of pseudorabies virus into the rat urinary bladder. Neurosci Lett 1992;143:271–274.
83. Nadelhaft I, Vera P L. Central nervous system neurons infected by pseudorabies virus injected into the rat urinary bladder following unilateral transection of the pelvic nerve. J Comp Neurol 1995;359:443–456.
84. Vizzard MA, Erickson VL, Card JP, Roppolo JR, de Groat WC. Transneuronal labelling of neurons in the adult rat brainstem and spinal cord after injection of pseudorabies virus into the urethra. J Comp Neurol 1995;355:629–640.
85. Erickson V, Roppolo JR, Booth AM, Vizzard MA, Card JP, de Groat WC. Transsynaptic labeling of neurons within CNS which control the bladder or penis of the cat. Soc Neurosci Abstr 1995;21:1872.
86. Kruse MN, Noto H, Roppolo JR, de Groat WC. Pontine control of the urinary bladder and external urethral sphincter in the rat. Brain Res 1990;532:182–190.
87. Kruse MN, Mallory BS, Noto H, Roppolo JR, de Groat WC. Properties of the descending limb of the spinobulbospinal micturition reflex pathway in the cat. Brain Res 1991;556:6–12.
88. Maggi CA, Giuliani S, Giachetti A, Meli A. The effect of MK-801 on the micturition reflex in anesthetized rats. Eur J Pharmacol 1990;181:105–109.
89. Yoshiyama M, Roppolo JR, de Groat WC. The effects of MK801 on the micturition reflex in the rat: possible sites of action. J Pharmacol Exp Ther 1993;265:844–850.
90. Yoshiyama M, Roppolo JR, Thor KB, de Groat WC. The effects of LY-274614 on the micturition reflex in the urethane-anesthetized rat. Br J Pharmacol 1993; 110:77–86.
91. Yoshiyama M, Roppolo JR, de Groat WC. Interactions between glutamatergic and monoaminergic systems controlling the micturition reflex in the urethane-anesthetized rat. Brain Res 1994;639:300–308.
92. Yoshiyama M, Roppolo JR, de Groat WC. Effects of GYKI 52466 and CNQX, AMPA/kainate receptor antagonists, on the micturition reflex in the rat. Brain Res 1995;691:185–194.
93. Sugaya K, de Groat WC. Effects of MK-801 and CNQX, glutamate receptor antagonists on bladder activity in neonatal rats. Brain Res 1994;640:1–10.
94. Vera PL, Nadelhaft I. MK-801, a non-competitive NMDA receptor antagonist, produces facilitation of

the micturition reflex in awake, freely-moving rats. Neurosci Lett 1991;134:135–138.
95. Yoshiyama M, Roppolo JR, de Groat WC. Interactions between NMDA and AMPA/kainate receptors in the control of micturition in the rat. Eur J Pharmacol 1995;287:73–78.
96. Matsumoto G, Hisamitsu T, de Groat WC. Role of glutamate and NMDA receptors in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. Neurosci Lett 1995;183:58–61.
Matsumoto G, Hisamitsu T, de Groat WC. Non-NMDA glutamatergic excitatory transmission in the descending limb of the spinobulbospinal micturition reflex pathway in the rat. Brain Res 1995;693: 246–250.
98. Araki I, de Groat WC. Unitary excitatory synaptic currents in preganglionic neurons mediated by two distinct groups of interneurons in neonatal rat sacral parasympathetic nucleus. J Neurophysiol 1996;76: 215–226.
99. Birder LA, de Groat WC. The effect of glutamate antagonists on *c-fos* expression induced in spinal neurons by irritation of the lower urinary tract. Brain Res 1992;580:115–120.
100. Kakizaki H, Yoshiyama M, de Groat WC. Role of NMDA and AMPA glutamatergic transmission in spinal *c-fos* expression after urinary tract irritation. Am J Physiol 1996;270:R990–R996.
101. Yoshimura N, Sasa M, Ohno Y, Yoshida O, Takaori S. Contraction of urinary bladder by central norepinephrine originating in the locus coeruleus. J Urol 1988;139:423–427.
102. Yoshimura N, Sasa M, Yoshida O, Takaori S. α_1–Adrenergic receptor-mediated excitation from the locus coeruleus of the sacral parasympathetic preganglionic neuron. Life Sci 1990;47:789–797.
103. Yoshimura N, Sasa M, Yoshida O, Takaori S. Mediation of micturition reflex by central norepinephrine from the locus coeruleus in the cat. J Urol 1990; 143:840–843.
104. Ishizuka O, Persson K, Mattiasson A, Maylor A, Wyllie M, Andersson K-E. Micturition in conscious rats with and without bladder outlet obstruction – role of spinal α_1–adrenoceptors. Br J Pharmacol 1996; 117:962–966.
105. Kontani H, Maruyama I, Sakai T. Involvement of α_2-adrenoceptors in the sacral micturition reflex in rats. Jpn J Pharmacol 1992;60:363–368.
106. Ishizuka O, Mattiasson A, Andersson K-E. Role of spinal and peripheral alpha$_2$ adrenoceptors in micturition in normal conscious rats. J Urol 1996;156:1853–1857.
107. Duvant PAC, Lucas PC, Yaksh TL. Micturition in unanesthetized rat: spinal vs. peripheral pharmacology of the adrenergic system. J Pharmacol Exp Ther 1988; 245:426–435.
108. Espey MJ, Downie JW, Fine A. Effect of 5-HT receptor and adrenoceptor antagonists on micturition in conscious cats. Eur J Pharmacol 1992;221:167–170.
109. Galeano C, Jubelin B, Germain L, Guenette L. Micturition reflexes in chronic spinalized cats: the underactive detrusor and detrusor-sphincter dyssynergia. Neurourol Urodyn 1986;5:45–63.
110. Gajewski J, Downie JW, Awad SA. Experimental evidence for a central nervous system site of action in the effect of alpha-adrenergic blockers on the external urinary sphincter. J Urol 1984;132:403–409.
111. Danuser H, Thor KB. Inhibition of central sympathetic and somatic outflow to lower urinary tract of the cat by α_1-adrenergic receptor antagonist prazosin. J Urol 1995;153:1308–1312.
112. Roppolo JR, Noto H, Mallory BS, de Groat WC. Dopaminergic and cholinergic modulation of bladder reflexes at the level of the pontine micturition center. Soc Neurosci Abstr 1987;13:733.
113. Kontani H, Inoue T, Sakai T. Dopamine receptor subtypes that induce hyperactive bladder response in anesthetized rats. Jpn J Pharmacol 1990;54:482–486.
114. Yoshimura N, Sasa M, Yoshida O, Takaori S. Dopamine D-1 receptor-mediated inhibition of micturition reflex by central dopamine from the substantia nigra. Neurourol Urodyn 1992;11:535–545.
115. Albanease A, Jenner P, Marsden CD, Stephenson JD. Bladder hyperreflexia induced in marmosets by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neurosci Lett 1988;87:46–50.
116. Yoshimura N, Mizuta E, Kuno S, Sasa M, Yoshida O. The dopamine D_1 receptor agonist SKF 38393 suppresses detrusor hyperreflexia in the monkey with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neuropharmacology 1993;32:315–321.
117. de Groat WC, Kawatani M, Hisamitsu T, Lowe I, Morgan C, Roppolo JR, Booth AM, Nadelhaft I, Kuo D, Thor K. The role of neuropeptides in the sacral autonomic reflex pathways of the cat. J Auton Nerv Sys 1983;7:339–350.
118. de Groat WC, Kawatani M. Neural control of the urinary bladder: possible relationship between peptidergic inhibitory mechanisms and detrusor instability. Neurourol Urodyn 1985;4:285–300.
119. Hisamitsu T, de Groat WC. The inhibitory effect of opioid peptides and morphine applied intrathecally and intracerebroventricularly on the micturition reflex in the cat. Brain Res 298:51–65,1984
120. Noto H, Roppolo JR, de Groat WC, Nishizawa O, Sugaya K, Tsuchida S. Opioid modulation of the micturition reflex at the level of the pontine micturition center. Urol Int 47:19–22,1991.
121. Thor, KB, Hisamitsu T, Roppolo JR, Tuttle R, Nagel J, de Groat WC. Selective inhibitory effects of ethylketocyclazocine on reflex pathways to the external urethral sphincter of the cat. J Pharmacol Exp Ther 1989;248:1018–1025.
122. Igawa Y, Mattiasson A, Andersson K-E. Effects of GABA-receptor stimulation and blockade on micturition in normal rats and rats with bladder outflow obstruction. J Urol 1993;150:537–542.
123. Araki I. Inhibitory postsynaptic currents and the effects of GABA on visually identified sacral parasympathetic preganglionic neurons in neonatal rats. J Neurophysiol 1995;72:2903–2910.
124. Nanninga J, Frost F, Penn R. Effect of intrathecal baclofen on bladder and sphincter function. J Urol 142:101–105.
125. Steers WD, Meythaler JM, Haworth C, Herrell D, Park TS. Effects of acute bolus and chronic continuous intrathecal baclofen on genitourinary dysfunction due to spinal cord pathology. J Urol 1992;148:1849–1855.
126. de Groat WC. Mechanisms underlying recurrent inhibition in the sacral parasympathetic outflow to the urinary bladder. J Physiol (Lond) 1976;257:503–513.

127. Thor KB, Hisamitsu T, de Groat WC. Unmasking of a neonatal somatovesical reflex in adult cats by the serotonin autoreceptor agonist 5-methoxy-N,N dimethyltryptamine. Dev Brain Res 1990;54:35–42.
128. Steers WD, Albo M, van Asselt E. Effects of serotonergic agonists on micturition and sexual function in the rat. J Drug Dev Res 1992;27:361–375.
129. Espey MJ, Downie JW. Serotonergic modulation of cat bladder function before and after spinal transection. Eur J Pharmacol 1995;287:173–177.
130. Danuser H, Thor KB. Spinal 5-HT_2 receptor-mediated facilitation of pudendal nerve reflexes in the anesthetized cats. Br J Pharmacol 1996;118:150–154.
131. Vincent SR, Satoh K. Corticotropin-releasing factor (CRF) immunoreactivity in the dorsolateral pontine tegmentum: further studies on the micturition reflex system. Brain Res 1984;308:387–391.
132. Suzuki T, Kawatani M, Erdman S, de Groat WC. Role of CRF and 5-HT in central pathways controlling micturition in the rat. Soc Neurosci Abstr 1990; 16:1064.
133. Pavcovich LA, Valentino RJ. Central regulation of micturition in the rat by the corticotropin-releasing hormone from Barrington's nucleus. Neurosci Lett 1995;196:185–188.
134. de Groat WC. Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Paraplegia 1995;33:493–505.
135. de Groat WC, Kruse MN, Vizzard MA, Cheng C-L, Araki I, Yoshimura N. Modification of urinary bladder function after neural injury. In: Seil F (ed) Advances in neurology. Vol. 72. Neuronal regeneration, reorganization, and repair. New York: Lippincott-Raven Publishers, 1997:347–364.
136. Maggi CA, Santicioli P, Del Bianco E, Lecci A, Guliani S. Evidence for the involvement of bradykinin in chemically-evoked cystitis in anesthetized rats. Naunyn-Schmiedebergs Arch Pharmacol 1993;347: 432–437.
137. Ishizuka O, Mattiasson A, Andersson K-E. Prostaglandin E_2-induced bladder hyperactivity in normal, conscious rats. Involvement of tachykinins? J Urol 1995;153:2034–2038.
138. Lecci A, Giuliani S, Garret C, Maggi CA. Evidence for a role of tachykinins as sensory transmitters in the activation of micturition reflex. Neuroscience 1993; 54:827–837.
139. Ishizuka O, Igawa Y, Lecci A, Maggi CA, Mattiasson A, Andersson K-E. Role of intrathecal tachykinins for micturition in unanesthetized rats with and without bladder outlet obstruction. Br J Pharmacol 1994; 113:111–116.
140. Vizzard MA, Erdman SL, de Groat WC. Increased expression of neuronal nitric oxide synthase in bladder afferent pathways following chronic bladder irritation. J Comp Neurol 1996;370:191–202.
141. Kakizaki H, de Groat WC. Role of spinal nitric oxide in the facilitation of the micturition reflex by bladder irritation. J Urol 155:355–360,1996.
142. Steers WD, de Groat WC. Effect of bladder outlet obstruction on micturition reflex pathways in the rat. J Urol 1988;140:864–871.
143. Steers WD, Kolbeck S, Creedon D, Tuttle JB. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest 1991;88:1709–1715.
144. Steers WD, Creedon DJ, Tuttle JB. Immunity to nerve growth factor prevents afferent plasticity following urinary bladder hypertrophy. J Urol 1996;155: 379–385.
145. Verge VMK, Richardson PM, Benoit R, Riopelle RJ. Histochemical characterization of sensory neurons with high-affinity receptors for nerve growth factor. J Neurocytol 1989;18:583–591.
146. Kawatani M, Erdman SL, de Groat WC. VIP and substance P in primary afferent pathways to the sacral spinal cord of the cat. J Comp Neurol 1985;241:327–347.
147. Kawatani M, Nagel J, de Groat WC. Identification of neuropeptides in pelvic and pudendal nerve afferent pathways to the sacral spinal cord of the cat. J Comp Neurol 1986;249:117–132.
148. Kruse MN, Belton AL, de Groat WC. Changes in bladder and external urethral sphincter function following spinal cord injury in the rat. Am J Physiol 1993;264:R1157–R1163.
149. Kruse MN, Bennett BC, de Groat WC. Effect of urinary diversion on the recovery of micturition reflexes after spinal cord injury in the rat. J Urol 1994;151:1088–1091.
150. Kruse MN, Bray LA, de Groat WC. Influence of spinal cord injury on the morphology of bladder afferent and efferent neurons. J Auton Nerv Sys. 1995; 54:215–224.
151. Yoshimura N, de Groat WC. Changes in electrophysiological and pharmacological properties of rat bladder afferent neurons following spinal cord injury. J Urol 1993;149:340A.
152. Yoshimura N, Yoshida O, de Groat WC. Regional differences in plasticity of membrane properties of rat bladder afferent neurons following spinal cord injury. J Urol 1995;153:262A.
153. Arbuckle JB, Docherty RJ. Expression of tetrodotoxin-resistant sodium channels in capsaicin-sensitive dorsal root ganglion neurons of adult rats. Neurosci Lett 1995;185:70–73.
154. Yoshimura N, de Groat WC. Spinal cord injury (SCI) increases neurofilament immunoreactivity and reduces capsaicin-sensitivity of visceral afferent neurons innervating rat urinary bladder. Soc Neurosci Abstr 1996;22:1798.
155. Cruz F, Guimaraes M, Silva C, Rio ME, Coimbra A, Reis M. Desensitization of bladder sensory fibers by intravesical capsaicin has long lasting clinical and urodynamic effects in patients with hyperactive or hypersensitive bladder dysfunction. J Urol 1997;157: 585–589.
156. Fowler CJ, Jewkes D, McDonald WI, Lynn B, de Groat WC. Intravesical capsaicin for neurogenic bladder dysfunction. Lancet 1992;339:1239.
157. Fowler CJ, Beck RO, Gerard S, Betts CD, Fowler CG. Intravesical capsaicin for treatment of detrusor hyperreflexia. J Neurol Neurosurg Psychiatry 1994; 57:169–173.
158. Geirsson G, Fall M, Sullivan L. Clinical and urodynamic effects of intravesical capsaicin treatment in patients with chronic traumatic spinal detrusor hyperreflexia. J Urol 1995;154:1825–1829.
159. Maggi CA, Barbanti G, Santicioli P, Beneforti P, Misuri D, Meli A, Turini D. Cystometric evidence that capsaicin-sensitive nerves modulate the afferent branch of micturition reflex in humans. J Urol 1989; 142:150–154.

160. Bors E, Comarr AE. Neurological urology, physiology of micturition, its neurological disorders and sequelae. Baltimore: University Park Press, 1971.
161. Geirsson G, Fall M, Lindström S. The ice-water test--a simple and valuable supplement to routine cystometry. Br J Urol 1993;71:681–685.
162. Geirsson G, Lindström S, Fall M, Hermansson GG, Hjalmas K. Positive bladder cooling test in neurologically normal young children. J Urol 1994;151: 446–448.