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Integrative control of the lower urinary tract: preclinical perspective

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Storage and periodic expulsion of urine is regulated by a neural control system in the brain and spinal cord that coordinates the reciprocal activity of two functional units in the lower urinary tract (LUT): (a) a reservoir (the urinary bladder) and (b) an outlet (bladder neck, urethra and striated muscles of the urethral sphincter). Control of the bladder and urethral outlet is dependent on three sets of peripheral nerves: parasympathetic, sympathetic and somatic nerves that contain afferent as well as efferent pathways. Afferent neurons innervating the bladder have A-δ or C-fibre axons. Urine storage reflexes are organized in the spinal cord, whereas voiding reflexes are mediated by a spinobulbospinal pathway passing through a coordination centre (the pontine micturition centre) located in the brainstem. Storage and voiding reflexes are activated by mechanosensitive A-δ afferents that respond to bladder distension. Many neurotransmitters including acetylcholine, norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids, adenosine triphosphate, nitric oxide and neuropeptides are involved in the neural control of the LUT. Injuries or diseases of the nervous system as well as disorders of the peripheral organs can produce LUT dysfunctions including: (1) urinary frequency, urgency and incontinence or (2) inefficient voiding and urinary retention. Neurogenic detrusor overactivity is triggered by C-fibre bladder afferent axons, many of which terminate in the close proximity to the urothelium. The urothelial cells exhibit 'neuron-like' properties that allow them to respond to mechanical and chemical stimuli and to release transmitters that can modulate the activity of afferent nerves.
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Abbreviations: ATP, adenosine triphosphate; BTX-A, botulinum toxin A; GABA, gamma-aminobutyric acid; 5-HT, 5-hydroxytryptamine; LUT, lower urinary tract; NGF, nerve growth factor; NO, nitric oxide; PAG, periaqueductal grey; PMC, pontine micturition centre; PRV, pseudorabies virus; RT PCR, real-time polymerase chain reaction; SCI, spinal cord injury; TTX, tetrodotoxin; VAChT, vesicle acetylcholine transporter

Introduction

The functions of the lower urinary tract (LUT) to store and periodically release urine are dependent upon neural circuits located in the brain, spinal cord and peripheral ganglia (see Morrison *et al*., 2005). This dependence on central nervous control distinguishes the LUT from many other visceral structures (e.g., the gastrointestinal tract and cardiovascular system) that maintain a certain level of activity even after elimination of extrinsic neural input. The LUT is also unusual in regard to its pattern of activity and the complexity of its neural regulation. For example, the urinary bladder has two principal modes of operation: storage and elimination. Thus many of the neural circuits controlling the bladder exhibit switch-like or phasic patterns of activity in contrast to tonic patterns occurring in autonomic pathways to cardiovascular organs. In addition, micturition is under voluntary control and depends upon learned behaviour that develops during maturation of the nervous system, whereas many other visceral functions are regulated involuntarily. Micturition also depends on the integration of autonomic and somatic efferent mechanisms within the lumbosacral spinal cord (see Morrison *et al*., 2005). This is necessary to coordinate the activity of visceral organs (the bladder and urethra) with that of urethral striated muscles. This paper will review the peripheral and central neural mechanisms controlling the LUT and the disruption of this control by neural injury.

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Innervation of the LUT

The storage and periodic elimination of urine is dependent upon the activity of two functional units in the LUT: (1) a reservoir (the urinary bladder) and (2) an outlet, consisting of bladder neck, urethra and striated muscles of the urethral sphincter (see Fry *et al*., 2005; Morrison *et al*., 2005). These structures are, in turn, controlled by three sets of peripheral nerves: sacral parasympathetic (pelvic nerves), thoracolumbar sympathetic nerves (hypogastric nerves and sympathetic chain) and sacral somatic nerves (pudendal nerves) (Figure 1) (see Morrison *et al*., 2005).

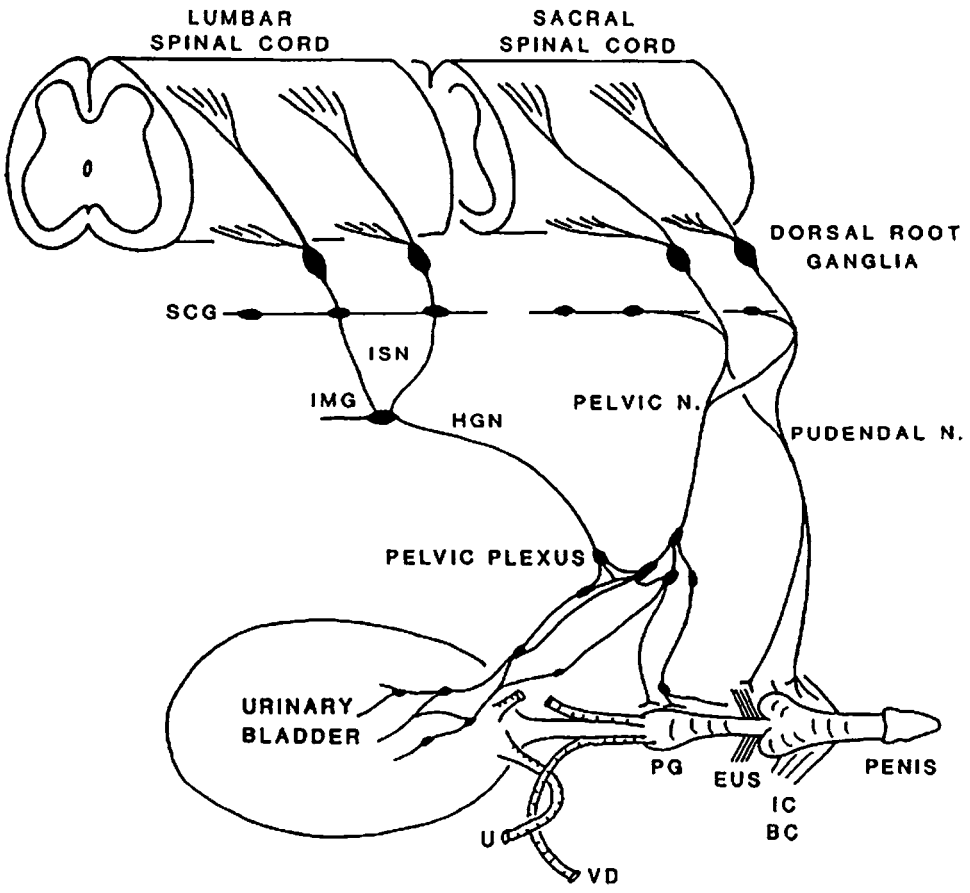

Figure 1 Diagram showing the sympathetic, parasympathetic and somatic innervation of the urogenital tract of the male cat. Sympathetic preganglionic pathways emerge from the lumbar spinal cord and pass to the sympathetic chain ganglia (SCG) and then *via* the inferior splanchnic nerves (ISN) to the inferior mesenteric ganglia (IMG). Preganglionic and postganglionic sympathetic axons then travel in the hypogastric nerve (HGN) to the pelvic plexus and the urogenital organs. Parasympathetic preganglionic axons that originate in the sacral spinal cord pass in the pelvic nerve to ganglion cells in the pelvic plexus and to distal ganglia in the organs. Sacral somatic pathways are contained in the pudendal nerve, which provides an innervation to the penis, the ischiocavernosus (IC), bulbocavernosus (BC) and external urethral sphincter (EUS) muscles. The pudendal and pelvic nerves also receive postganglionic axons from the caudal sympathetic chain ganglia. These three sets of nerves contain afferent axons from the lumbosacral dorsal root ganglia. Abbreviations: ureter (U), prostate gland (PG), vas deferens (VD).

Sacral parasympathetic pathways

The sacral parasympathetic outflow provides the major excitatory input to the urinary bladder. Cholinergic preganglionic neurones located in the intermediolateral region of the sacral spinal cord (Morgan *et al*., 1993) send axons *via* the pelvic nerves to ganglion cells in the pelvic plexus and in the wall of the bladder. Transmission in bladder ganglia is mediated by a nicotinic cholinergic mechanism, which can be modulated by activation of various receptors including muscarinic, adrenergic, purinergic, and peptidergic (Table 1) (see de Groat & Booth, 1993). Ganglia in some species (cats and rabbits) also exhibit a prominent frequency-dependent facilitatory mechanism that can amplify parasympathetic activity passing from the spinal cord to the bladder (see de Groat & Booth, 1993).

The parasympathetic ganglion cells in turn excite bladder smooth muscle *via* the release of cholinergic (acetylcholine) and nonadrenergic–noncholinergic transmitters. Cholinergic excitatory transmission in the bladder is mediated by muscarinic receptors, which are blocked by atropine (see Andersson, 1993; Andersson & Arner, 2004; Morrison *et al*., 2005), whereas noncholinergic excitatory transmission is mediated by adenosine triphosphate (ATP), acting on P2X purinergic receptors (Table 1) (Ralevic & Burnstock, 1998; Burnstock, 2001). Inhibitory input to the urethral smooth muscle is mediated by nitric oxide (NO) released by parasympathetic nerves (Andersson, 1993; Andersson & Arner, 2004). Both M_2 and M_3 muscarinic receptor subtypes are expressed in bladder smooth muscle; however, examination of subtype selective muscarinic receptor antagonists and studies of muscarinic receptor knockout mice have revealed that the M_3 subtype is the principal receptor involved in excitatory transmission (Matsui *et al*., 2000; 2002). Muscarinic receptors are also present prejunctionally on parasympathetic nerve terminals. Activation of these receptors by acetylcholine can enhance (M_1 receptors) or suppress (M_4 receptors) transmitter release, depending upon the intensity of neural firing (Somogyi *et al*., 1996; 1998; 2003; D'Agostino *et al*., 1997; see de Groat & Yoshimura, 2001). Postganglionic neurones innervating the bladder also contain neuropeptides, such as vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY) (Keast & de Groat, 1989). These substances are co-released with acetylcholine or ATP and may function as modulators of

Table 1 Receptors for putative transmitters in the lower urinary tract

Tissue	*Cholinergic*	*Adrenergic*	*Other*
Bladder body	+(M_2)	−(β_2)	+Purinergic ($P2X_1$)
	+(M_3)	−(β_3)	−VIP
			+Substance P (NK_2)
Bladder base	+(M_2)	+(α_1)	−VIP
	+(M_3)		+Substance P (NK_2)
			+Purinergic (P2X)
Urothelium	+(M_2)	α	+TRPV1
	+(M_3)	β	+TRPM8
			+P2X
			+P2Y
			+Substance P
			+Bradykinin (B2)
Urethra	+(M)	+(α_1)	+Purinergic (P2X)
		+(α_2)	−VIP
		−(β)	−Nitric oxide
Sphincter striated muscle	+(N)		
Adrenergic nerve terminals	−(M_2)	−(α_2)	−NPY
	+(M_1)		
Cholinergic nerve terminals	−(M_2)	+(α_1)	−NPY
	+(M_1)		
Afferent nerve terminals			+Purinergic ($P2X_{2/3}$)
			+TRPV1
Ganglia	+(N)	+(α_1)	−Enkephalinergic (δ)
	+(M_1)	−(α_2)	−Purinergic (P_1)
		+(β)	+Substance P

VIP, vasoactive intestinal polypeptide; NPY, neuropeptide Y; TRP, transient receptor potential. Letters in parentheses indicate receptor type, for example, M (muscarinic) and N (nicotinic). Plus and minus signs indicate excitatory and inhibitory effects.

neuroeffector transmission (Tran *et al*., 1994). It has been proposed that frequency-dependent synaptic modulatory mechanisms in parasympathetic ganglia and at postganglionic nerve terminals perform gating functions that suppress excitatory input to the bladder during urine storage when the parasympathetic preganglionic outflow from the spinal cord is low and amplify the input to the bladder during voiding when preganglionic nerve activity is high, thereby contributing to efficient bladder emptying (de Groat & Booth, 1993; Tran *et al*., 1994; Somogyi *et al*., 1996).

Thoracolumbar sympathetic pathways

Sympathetic pathways to the LUT originate in the lumbosacral sympathetic chain ganglia as well as in the prevertebral inferior mesenteric ganglia (de Groat *et al*., 1993). Input from the sacral chain ganglia passes to the bladder *via* the pelvic nerves, whereas fibres from the rostral lumbar and inferior mesenteric ganglia travel in the hypogastric nerves. Sympathetic efferent pathways in the hypogastric and pelvic nerves in cat elicit similar effects in the bladder, consisting of (1) inhibition of detrusor muscle *via* β-adrenoceptors; (2) excitation of the bladder base and urethra *via* α_1-adrenoceptors (Andersson & Arner, 2004; Morrison *et al*., 2005) and (3) inhibition and facilitation in bladder parasympathetic ganglia *via* α_2- and α_1-adrenoceptors, respectively (Table 1) (de Groat & Booth, 1993). Facilitatory α_1-adrenoceptors are also present on parasympathetic nerve terminals in the rat bladder (Szell *et al*., 2000). Adrenergic inhibition of transmission in bladder parasympathetic ganglia is prominent at low frequencies of parasympathetic nerve activity but minimal at higher frequencies. Thus, it would be expected that adrenergic inhibitory modulation of ganglionic transmission in the bladder would be effective during urine storage when parasympathetic nerve activity is low, but would not interfere with voiding when parasympathetic nerve activity is high.

Somatic efferent pathways

The efferent innervation of the urethral striated muscles in various species originates from cells in a circumscribed region of the lateral ventral horn that is termed Onuf's nucleus (de Groat *et al*., 2001). Sphincter motoneurones send their axons into the pudendal nerve and excite sphincter muscles *via* the release of acetylcholine, which stimulates postjunctional nicotinic receptors.

Afferent pathways

Afferent axons innervating the urinary tract are present in the three sets of nerves (Morrison *et al*., 2005). The most important afferents for initiating micturition are those passing in the pelvic nerves to the sacral spinal cord. These afferents are small myelinated (A-δ) and unmyelinated (C) fibres, which convey information from receptors in the bladder wall to second-order neurones in the spinal cord. A-δ bladder afferents in the cat respond in a graded manner to passive distension as well as active contraction of the bladder and exhibit pressure thresholds in the range of 5–15 mmHg, which are similar to those pressures at which humans report the first sensation of bladder filling. These fibres also code for noxious stimuli in the bladder. On the other hand, C-fibre bladder afferents in the cat have very high thresholds and commonly do not respond to even high levels of intravesical pressure (Häbler *et al*., 1990). However, activity in some of these afferents is unmasked or enhanced by chemical irritation of the bladder mucosa. These findings indicate that C-fibre afferents in the cat have specialized functions, such as the signalling of inflammatory or noxious events in the LUT. In the rat, A-fibre and C-fibre bladder afferents cannot be distinguished on the basis of stimulus modality; thus both types of afferents consist of mechanosensitive and chemosensitive populations (Morrison *et al*., 2005).

C-fibre afferents are sensitive to the neurotoxins, capsaicin and resiniferatoxin as well as to many other substances including tachykinins, NO, ATP, prostaglandins, endothelins and neurotrophic factors released in the bladder by afferent nerves, urothelial cells and inflammatory cells (Maggi, 1993; Chuang *et al*., 2001; Rong *et al*., 2002; Morrison *et al*., 2005). These substances can modulate afferent nerve excitability and change the response of afferents to mechanical stimulation. Intravesical administration of ATP enhances the firing of

bladder afferent nerves (Rong *et al*., 2002; Morrison *et al*., 2005), presumably by acting on $P2X_3$ or $P2X_{2/3}$ receptors on afferent terminals within or adjacent to the urothelium. In addition, ATP applied to afferent nerves on the surface of the rat bladder enhances the firing induced by bladder distension and reduces the threshold for electrical stimulation of A-δ and C-fibre afferent axons (Yu & de Groat, 2004). These data suggest that purinergic receptors are located on the axons of afferent nerves as well as at the nerve terminals. This raises the possibility that afferent axons may be sensitive to purinergic excitation at any site along the path of the axon as it passes through the bladder wall. Sensitivity of vagal and sural nerve C-fibre afferent axons to ATP has also been reported (Inrich *et al*., 2001; 2002; Lang *et al*., 2005).

Axonal tracing studies have revealed that a small percentage of lumbosacral afferent neurons innervate multiple pelvic organs. For example, 3–15% of dorsal root ganglion neurons were double labelled following injections of different tracers into the colon and bladder (Keast & de Groat, 1992; Christianson *et al*., 2004) The double labelling occurred more frequently in rostral lumbar (L1–L2) than in caudal lumbosacral (L6–S1) dorsal root ganglia, which provide the major innervation to the bladder and colon. It has been speculated that double labelling is due to dichotomizing afferents that send axonal branches to different target organs. This unusual anatomical arrangement has been put forward as a possible mechanism for neural cross-talk and bidirectional cross-sensitization between pelvic organs in which chemical irritation of the colon leads to enhancement of reflex bladder activity or irritation of the bladder leads to enhancement of colonic reflexes (Pezzone *et al*., 2005).

The properties of lumbosacral dorsal root ganglion cells innervating the bladder, urethra and external urethral sphincter in the rat and cat have been studied with patch-clamp recording techniques in combination with axonal tracing methods to identify the different populations of neurons (Yoshimura *et al*., 1996; 2003; Yoshimura & de Groat, 1997; 1999; Sculptoreanu *et al*., 2005a, b). Based on responsiveness to capsaicin, it is estimated that approximately 70% of bladder afferent neurons in the rat are of the C-fibre type. These neurons exhibit high threshold tetrodotoxin-resistant sodium channels and action potentials and phasic firing (one to two spikes) in response to prolonged depolarizing current pulses. Approximately 90% of the bladder afferent neurons are also excited by ATP, which induces a depolarization and firing by activating $P2X_3$ or $P2X_{2/3}$ receptors (Zhong *et al*., 2003). Bladder afferent nerves near the urothelium express $P2X_3$ and $P2Y_4$ purinergic receptors (Birder *et al*., 2004). A-fibre afferent neurons are resistant to capsaicin, have low threshold tetrodotoxin-sensitive sodium channels and action potentials and tonic firing (multiple spikes) to depolarizing current pulses. C-fibre bladder afferent neurons also express a slowly decaying A-type K^+ current that controls spike threshold and firing frequency (Yoshimura *et al*., 1996; 2003). Suppression of this K^+ current by drugs or chronic bladder inflammation induces hyperexcitability of the afferent neurons (Yoshimura & de Groat, 1999). Conversely, enhancement of A-type K^+ currents with an experimental drug (KW-7158) suppresses the excitability of cultured dorsal root ganglion neurons (Sculptoreanu *et al*., 2004) and decreases bladder hyperexcitability induced by chemical irritation of the bladder *in vivo* (Lu *et al*., 2002).

A large percentage of bladder afferent neurons contain peptides: calcitonin-gene-related peptide, vasoactive intestinal polypeptide, pituitary-adenyl cyclase activating polypeptide (PACAP), tachykinins, galanin and opioid peptides (Maggi, 1993; Morrison *et al*., 2005). Nerves containing these peptides are common in the bladder, in the submucosal and epithelial layers, and around blood vessels. Peptidergic bladder afferent neurons in the rat also express TrkA, a high-affinity receptor for nerve growth factor (NGF) and receptors for tachykinins (NK_2 and NK_3 receptors) (Sculptoreanu & de Groat, 2003; Morrison *et al*., 2005) and endothelins (Ogawa *et al*., 2004). Peptidergic afferent axons project into the lumbosacral parasympathetic nucleus in the spinal cord and application of various neuropeptides to the spinal cord influences bladder activity. These findings suggest that the neuropeptides may be important transmitters in the afferent pathways from the LUT.

Urothelium

Recent studies have revealed that the urothelium, which has been traditionally viewed as a passive barrier at the bladder luminal surface (Lewis, 2000; Apodaca, 2004), also has specialized sensory and signalling properties that allow urothelial cells to respond to their chemical and physical environment and to engage in reciprocal chemical communication with neighbouring nerves in the bladder wall (Ferguson *et al*., 1997; Birder *et al*., 1998; 2001; 2002a, b; de Groat, 2004; Stein *et al*., 2004; Beckel *et al*., 2005a, b; Chopra *et al*., 2005). These properties include: (1) expression of nicotinic, muscarinic, tachykinin, adrenergic, bradykinin and transient receptor potential receptors (TRPV1, TRPV2, TRPV4, TRPM8 and ANKTM1), (2) responsiveness to transmitters released from sensory nerves, (3) close physical association with afferent nerves and (4) ability to release chemical mediators such as ATP, ACh and NO that can regulate the activity of adjacent nerves and thereby trigger local vascular changes and/or reflex bladder contractions.

The role of ATP in urothelial-afferent communication has attracted considerable attention because bladder distension releases ATP from the urothelium (Ferguson *et al*., 1997; Sun *et al*., 2001; Birder *et al*., 2003) and intravesical administration of ATP induces bladder hyperactivity, an effect blocked by administration of P2X purinergic receptor antagonists that suppress the excitatory action of ATP on bladder afferent neurons (Morrison *et al*., 2005). Mice in which the $P2X_3$ receptor was knocked out exhibited hypoactive bladder activity and inefficient voiding (Cockayne *et al*., 2000), suggesting that activation of $P2X_3$ receptors on bladder afferent nerves by ATP released from the urothelium is essential for normal bladder function. In humans and cats with interstitial cystitis, a painful bladder condition, ATP release from urothelial cells is enhanced (Sun *et al*., 2001; Birder *et al*., 2003). Higher levels of ATP may induce abnormal afferent nerve firing and pain.

Botulinum toxin A (BTX-A), which is injected into the bladder wall to reduce neurogenic detrusor overactivity in patients (Smith & Chancellor, 2004; Schurch *et al*., 2005), not only suppresses the release of acetylcholine and norepinehrine from autonomic nerves in the rat bladder (Smith *et al*., 2003a) and inhibits neurally evoked bladder contractions (Smith *et al*., 2003b) but also reduces the release of ATP into the bladder

lumen of chronic spinal cord-injured rats (Khera *et al*., 2004). BTX-A also blocks the stretch-evoked or capsaicin-evoked release of ATP from cultured urothelial cells (Barrick *et al*., 2004) and reduces the activation of afferent nerves by bladder irritation (Chuang *et al*., 2004; Vemulakonda *et al*., 2005). Thus, the clinical efficacy of BTX-A in the treatment of bladder dysfunction may be related to its action on urothelial sensory mechanisms as well as to its effects on neurotransmitter release from efferent nerves.

NO released from the urothelium (Birder *et al*., 1998) has been implicated in an inhibitory modulatory mechanism (Ozawa *et al*., 1999). Exogenous NO inhibits Ca^{2+} channels in dissociated lumbosacral dorsal ganglion neurons innervating the urinary bladder (Yoshimura *et al*., 2001). In addition, intravesical administration of NO donors suppresses bladder hyperactivity in cyclophosphamide-induced cystitis (Ozawa *et al*., 1999), whereas intravesical administration of oxyhaemoglobin, an NO scavenger, produces bladder hyperactivity in normal rats (Pandita *et al*., 2000). These data indicate that NO released from the urothelium can suppress the excitability of adjacent afferent nerves. However, NO may also contribute to bladder overactivity. NO production and nNOS expression are increased in the urothelium of cats with IC (Birder *et al*., 2005) and high levels of NO can disrupt the urothelial passive barrier. Thus, NO produced in the urothelium may also enhance sensory mechanisms in the bladder.

The presence of muscarinic and nicotinic receptors in the urothelium has attracted interest in the role of acetylcholine as a chemical mediator of neural–urothelial interactions (Hawthorn *et al*., 2000; Templeman *et al*., 2002; Beckel *et al*., 2004; 2005a; de Groat, 2004). Cholinergic nerves staining for vesicle acetylcholine transporter (VAT) have been detected in close proximity to the urothelial cells in the rat bladder (Beckel *et al*., 2005b). Exogenous muscarinic and nicotinic cholinergic agonists applied to cultured urothelial cells can elicit an increase in intracellular Ca^{2+} concentration and evoke the release of NO and ATP (Birder *et al*., 2003; Beckel *et al*., 2005a). In bladder strips or whole bladder preparations, muscarinic agonists also stimulate the release of a smooth muscle inhibitory factor from the urothelium (Hawthorn *et al*., 2000). Electrical stimulation of the pelvic nerve or reflex activation of the autonomic nervous system by spinal cord injury (SCI) (Apodaca *et al*., 2003; Birder, 2005) can elicit changes in urothelial permeability as well as changes in the morphology of the urothelium in the rat raising the possibility that autonomic or sensory nerves make 'synaptic connections' with the urothelial cells. Further studies are needed to determine if acetylcholine is involved in these connections.

The function of cholinoceptors in the urothelium has also been evaluated by testing the effects of intravesically administered cholinergic agonists and antagonists on voiding function in cats and rats (Beckel *et al*., 2004; de Groat *et al*., 2004; Kim *et al*., 2004; Ungerer *et al*., 2005). Intravesical application of nicotine in the rat elicits two effects: a decrease in the frequency of reflex micturition in low concentrations and an increase in frequency in high concentrations. The inhibitory effect was blocked by methyllycaconitine, an antagonist of $\alpha 7$ nicotinic receptors, whereas the facilitatory effect was blocked by hexamethonium, an antagonist of $\alpha 3$-type nicotinic receptors. Methyllycaconitine alone did not alter reflex bladder activity, whereas hexamethonium alone decreased reflex bladder activity suggesting the existence of a tonically active nicotinic facilitatory mechanism. Nicotine also increased intracellular Ca^{2+} in cultured urothelial cells by activating hexamethonium-sensitive receptors. These data coupled with the results of real-time polymerase chain reaction (RT PCR) experiments that revealed the expression of multiple subtypes of nicotinic receptors in rat urothelial cells ($\alpha 3$, $\alpha 5$, $\alpha 7$, $\beta 3$ and $\beta 4$) raise the possibility that sensory mechanisms in the urothelium are modulated by complex nicotinic mechanisms (Beckel *et al*., 2005a).

In chronic spinal cord-injured cats, intravesical infusion of carbachol, a muscarinic-nicotinic agonist, as well as oxotremorine methiodide, a quaternary muscarinic agonist that should have a relatively low ability to penetrate the urothelial barrier, decreased bladder capacity and enhanced the number of premicturition contractions (Ungerer *et al*., 2005) during cystometrograms, but did not alter the amplitude of micturition contractions. These effects were blocked by intravesical administration of atropine sulphate or the quaternary analogue, atropine methylnitrate. Intravesical administration of neostigmine methylsulphate, a quaternary anticholinesterase agent, mimicked the facilitatory effects of muscarinc agonists. The effects of neostigmine were blocked by atropine. These results indicate that activation of muscarinic receptors in the urothelium or in suburothelial afferent nerves facilitates the spinal micturition reflex mediated by C-fibre afferent nerves (Cheng *et al*., 1999). In the rat, a similar facilitation of bladder activity induced by intravesically administered muscarinic agonists has been reported (de Groat *et al*., 2004; Kim *et al*., 2004).

Urothelial cells express the various proteins necessary for the synthesis and storage of acetylcholine including the plasma membrane choline transporter, choline acetyltransferase and the VAT as well as the enzyme responsible for the metabolism of acetylcholine (acetylcholinesterase) (Beckel *et al*., 2005b). In addition, there are reports that acetylcholine is released from the bladder urothelium in rats (Klapproth *et al*., 1997; Beckel *et al*., 2004) and humans (Yoshida *et al*., 2004). Synthesis and release of acetylcholine has also been reported in epithelial cells in the lung (Proskocil *et al*., 2004). Thus, acetylcholine released from urothelial cells may function as an autocrine factor that acts on cholinoceptors on the urothelial cells to release other transmitters or to modify urothelial cell functions. Alternatively, because afferent nerves express cholinoceptors, the acetylcholine released from urothelial cells may act to alter afferent nerve excitability (de Groat, 2004). The clinical effect of antimuscarinic agents to decrease sensory symptoms in overactive bladder may be related, in part, to a block of muscarinic receptors in the urothelium or afferent nerves.

Reflex control of the LUT

The neural pathways controlling LUT function are organized as simple on–off switching circuits (Figure 2) that maintain a reciprocal relationship between the urinary bladder and urethral outlet. The principal reflex components of these switching circuits are listed in Table 2 and illustrated in Figure 3. Intravesical pressure measurements during bladder filling in both humans and animals reveal low and relatively constant bladder pressures when bladder volume is below the threshold for inducing voiding (Figure 2a) (Morrison *et al*.,

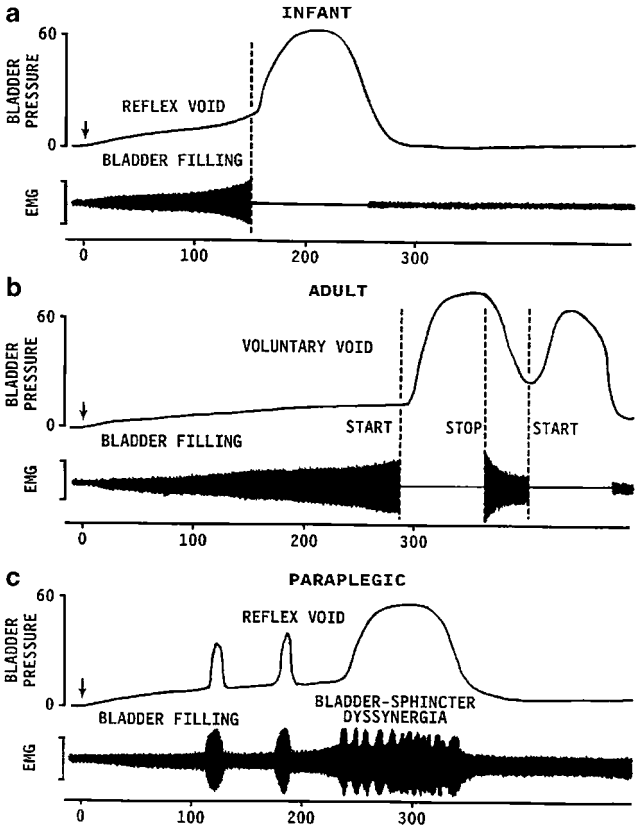

Figure 2 Combined cystometrograms and sphincter electromyograms (EMG) comparing reflex voiding responses in an infant (a) and in a paraplegic patient (c) with a voluntary voiding response in an adult (b). The abscissa in all records represents bladder volume in millilitres and the ordinates represent bladder pressure in cmH_2O and electrical activity of the EMG recording. On the left side of each trace, the arrows indicate the start of a slow infusion of fluid into the bladder (bladder filling). Vertical dashed lines indicate the start of sphincter relaxation which precedes by a few seconds the bladder contraction in (a and b). In part (b) note that a voluntary cessation of voiding (stop) is associated with an initial increase in sphincter EMG followed by a reciprocal relaxation of the bladder. A resumption of voiding is again associated with sphincter relaxation and a delayed increase in bladder pressure. On the other hand, in the paraplegic patient (c), the reciprocal relationship between bladder and sphincter is abolished. During bladder filling, transient uninhibited bladder contractions occur in association with sphincter activity. Further filling leads to more prolonged and simultaneous contractions of the bladder and sphincter (bladder–sphincter dyssynergia). Loss of the reciprocal relationship between bladder and sphincter in paraplegic patients interferes with bladder emptying.

2005). The accommodation of the bladder to increasing volumes of urine is primarily a passive phenomenon dependent upon the intrinsic properties of the vesical smooth muscle and quiescence of the parasympathetic efferent pathway. In addition, in some species, urine storage is also facilitated by sympathetic reflexes that mediate an inhibition of bladder activity, closure of the bladder neck and contraction of the proximal urethra (Table 2, Figure 3a). During bladder filling, the activity of the urethral sphincter electromyogram (EMG) also increases (Figure 2b), reflecting an increase in efferent firing in the pudendal nerve and an increase in outlet resistance that contributes to the maintenance of urinary continence.

The storage phase of the urinary bladder can be switched to the voiding phase either involuntarily (reflexly) or voluntarily (Figure 2). The former is readily demonstrated in the human infant (Figure 2a) when the volume of urine exceeds the micturition threshold. At this point, increased afferent firing from tension receptors in the bladder produces firing in the sacral parasympathetic pathways and inhibition of sympathetic and somatic pathways. The expulsion phase consists of an initial relaxation of the urethral sphincter (Figure 2a) followed by a contraction of the bladder, an increase in bladder pressure, and flow of urine. Relaxation of the urethral outlet is mediated by activation of a parasympathetic reflex pathway to the urethra (Table 2) that triggers the release of NO, an inhibitory transmitter (Andersson, 1993), as well as by removal of adrenergic and somatic excitatory inputs to the urethra.

Anatomy of central nervous pathways controlling the LUT

The reflex circuitry controlling micturition consists of four basic components: spinal efferent neurones, spinal interneurones, primary afferent neurones and neurones in the brain that modulate spinal reflex pathways. New research methods, including transneuronal virus tracing (Figures 4 and 5) (Nadelhaft *et al*., 1992; Vizzard *et al*., 1995), measurements of gene expression (Figure 5b) (Birder & de Groat, 1993; Birder *et al*., 1999) and patch-clamp recording in spinal cord slice preparations (Araki & de Groat, 1996; 1997), have recently provided new insights into the morphological and electrophysiological properties of these reflex components.

Pathways in the spinal cord

The spinal cord grey matter is divided into three general regions: (1) the dorsal horn, which contains interneurones that process sensory input; (2) the ventral horn, which contains motoneurones and (3) an intermediate region located between the dorsal and ventral horns that contains interneurones and autonomic preganglionic neurons (Figures 4 and 5). These regions are further subdivided into layers or laminae that are numbered, starting with the superficial layer of the dorsal horn (lamina I) and extending to the ventral horn (lamina IX) and the commissure connecting the two sides of the spinal cord (lamina X) (Figure 5d).

Efferent neurons Parasympathetic preganglionic neurones are located in the intermediolateral grey matter (laminae V–VII) in the sacral segments of the spinal cord (Figure 4), whereas sympathetic preganglionic neurones are located in medial (lamina X) and lateral sites (laminae V–VII) in the rostral lumbar spinal cord. EUS motoneurones are located in lamina IX in Onuf's nucleus (Thor *et al*., 1989; de Groat *et al*., 2001; Morrison *et al*., 2005). Parasympathetic preganglionic neurones and EUS motoneurones send dendrites to similar regions of the spinal cord (laminae I, V–VII and X) indicating that these sites contain important pathways for coordinating bladder and sphincter function.

Afferent projections in the spinal cord Afferent pathways from the LUT project to discrete regions of the dorsal horn

Table 2 Reflexes to the lower urinary tract

Afferent pathway	*Efferent pathway*	*Central pathway*
Urine storage		
Low-level vesical afferent activity (pelvic nerve)	1. External sphincter contraction (somatic nerves) 2. Internal sphincter contraction (sympathetic nerves) 3. Detrusor inhibition (sympathetic nerves) 4. Ganglionic inhibition (sympathetic nerves) 5. Sacral parasympathetic outflow inactive	Spinal reflexes
Afferent activity from the external urethral sphincter	6. Inhibition of parasympathetic outflow	Spinal reflex
Micturition		
High-level vesical afferent activity (pelvic nerve)	1. Inhibition of external sphincter activity 2. Inhibition of sympathetic outflow 3. Activation of parasympathetic outflow to the bladder	Spinobulbospinal reflexes
	4. Activation of parasympathetic outflow to the urethra	Spinal Reflex

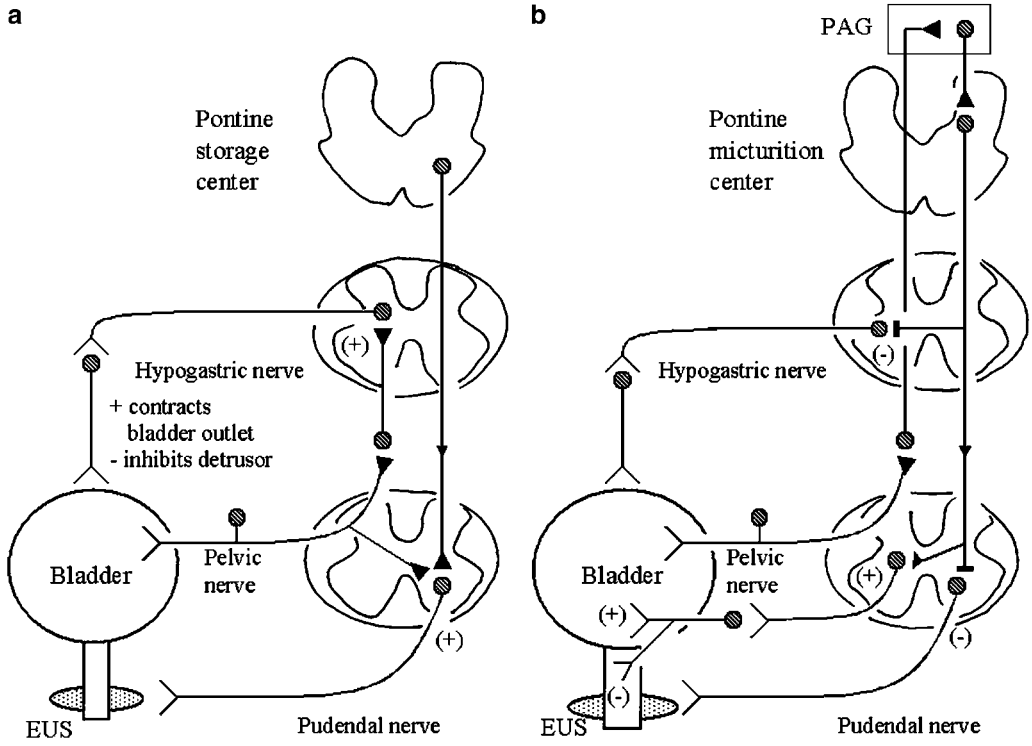

Figure 3 Diagram showing neural circuits controlling continence and micturition. (a) Urine storage reflexes. During the storage of urine, distention of the bladder produces low-level vesical afferent firing, which in turn stimulates (1) the sympathetic outflow to the bladder outlet (base and urethra) and (2) pudendal outflow to the external urethral sphincter. These responses occur by spinal reflex pathways and represent guarding reflexes, which promote continence. Sympathetic firing also inhibits detrusor muscle and modulates transmission in bladder ganglia. A region in the rostral pons (the pontine storage centre) 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 (PMC), which stimulate the parasympathetic outflow to the bladder and urethral smooth muscle and inhibit the sympathetic and pudendal outflow to the urethral outlet. Ascending afferent input from the spinal cord may pass through relay neurones in the periaqueductal grey (PAG) before reaching the PMG.

that contain interneurones and efferent neurones innervating the LUT (Morgan *et al*., 1981; Steers *et al*., 1991). Afferent pathways from the urinary bladder project into Lissauer's tract at the apex of the dorsal horn and then pass rostrocaudally, giving off collaterals that extend laterally and medially through the superficial layer of the dorsal horn (lamina I) into the deeper layers (laminae V–VII and X) at the base of the dorsal horn (Figure 5a). The lateral pathway terminates in the region of the sacral parasympathetic nucleus and also sends some axons to the dorsal commissure (Figure 5a). Pudendal afferent pathways from the urethra and urethral sphincter exhibit a similar pattern of termination in the sacral spinal cord (Thor *et al*., 1989; de Groat *et al*., 2001). The overlap of bladder and urethral afferents in the lateral dorsal horn and dorsal commissure indicates that these regions are likely to be important sites of viscerosomatic integration and involved in coordinating bladder and sphincter activity.

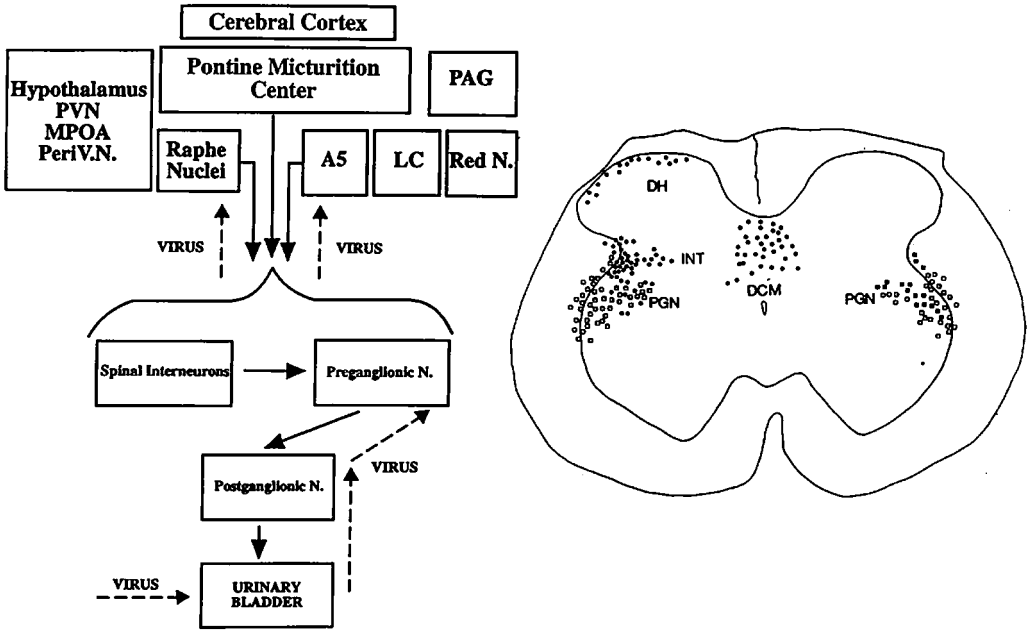

Figure 4 Transneuronal virus tracing of the central pathways controlling the urinary bladder of the rat. Injection of PRV into the wall of the urinary bladder leads to retrograde transport of virus (dashed arrows) and sequential infection of postganglionic neurones, preganglionic neurones, and then various central neural circuits synaptically linked to the preganglionic neurones. Normal synaptic connections are indicated by solid arrows. At long survival times, virus can be detected with immunocytochemical techniques in neurones at specific sites throughout the spinal cord and brain, extending to the PMC in the pons (i.e. Barrington's nucleus) and to the cerebral cortex. Other sites in the brain labelled by virus are (1) the paraventricular nucleus (PVN), medial preoptic area (MPOA) and periventricular nucleus (Peri V.N.) of the hypothalamus; (2) periaqueductal grey (PAG); (3) locus coeruleus (LC) and subcoeruleus; (4) red nucleus; (5) medullary raphe nuclei and (6) the noradrenergic cell group designated A5. L6 Spinal-cord section, showing on the left-hand side the distribution of virus-labelled parasympathetic preganglionic neurones (□) and interneurones (●) in the region of the parasympathetic nucleus, the dorsal commissure (DCM) and the superficial laminae of the dorsal horn (DH), 72 h after injection of the virus into the bladder. The right-hand side shows the entire population of preganglionic neurones (PGN) (□) labelled by axonal tracing with the fluorescent dye (fluorogold), injected into the pelvic ganglia and the distribution of virus-labelled bladder PGN (■). Composite diagram of neurones in 12 spinal sections (42 μm).

Spinal interneurones As shown in Figures 4 and 5, interneurones retrogradely labelled by injection of pseudorabies virus (PRV) into the urinary bladder or urethra of the rat are located in regions of the spinal cord receiving afferent input from the bladder (Nadelhaft *et al*., 1992; Vizzard *et al*., 1995). Large populations of interneurones are located just dorsal and medial to the preganglionic neurones as well as in the dorsal commissure and lamina I (Figure 5c).

The spinal neurones involved in processing afferent input from the LUT have been identified by the expression of the immediate early gene, c-*fos* (Figure 5b). In the rat, stimulation of the bladder and urethra increases the levels of Fos protein primarily in the dorsal commissure, the superficial dorsal horn and in the area of the sacral parasympathetic nucleus (Figure 5b) (Birder & de Groat, 1993; Birder *et al*., 1999). Some of these interneurones send long projections to the brain (Birder *et al*., 1999), whereas others make local connections in the spinal cord and participate in segmental spinal reflexes (Araki & de Groat, 1996). The former are involved in transmitting sensory input to supraspinal centres for subsequent relay to the cerebral cortex or to micturition reflex circuits in the brainstem. Ascending axons terminate in several areas, including the periaqueductal grey (PAG) (Blok *et al*., 1995) and the gracile nucleus. It is believed that neurones in the PAG relay information to the pontine micturition centre (PMC) and initiate the micturition reflex (Figure 3b). Projections to the gracile nucleus carry nociceptive signals which are eventually routed to the thalamus and cortex.

Patch-clamp recordings from parasympathetic preganglionic neurones in the neonatal rat spinal slice preparation have revealed that interneurones located immediately dorsal and medial to the parasympathetic nucleus make direct monosynaptic connections with the preganglionic neurones. Microstimulation of interneurones in both locations elicits glutamatergic, *N*-methyl-D-aspartate (NMDA), and non-NMDA excitatory postsynaptic currents in preganglionic neurones (Araki & de Groat, 1996; 1997). Stimulation of a subpopulation of medial interneurones elicits GABAergic and glycinergic inhibitory postsynaptic currents (Araki, 1994). Stimulation of neurons in the dorsal commissure also elicits monosynaptic and polysynaptic glutamatergic excitatory inputs to the preganglionic neurons (Miura *et al*., 2003). Thus, local interneurones are likely to play an important role in both excitatory and inhibitory reflex pathways controlling the preganglionic outflow to the LUT.

Pathways in the brain

In the rat, transneuronal virus tracing methods have identified many populations of neurones in the brain that are involved in the control of bladder, urethra and the urethral sphincter, including Barrington's nucleus (the PMC); medullary raphe nuclei, which contain serotonergic neurones; the locus

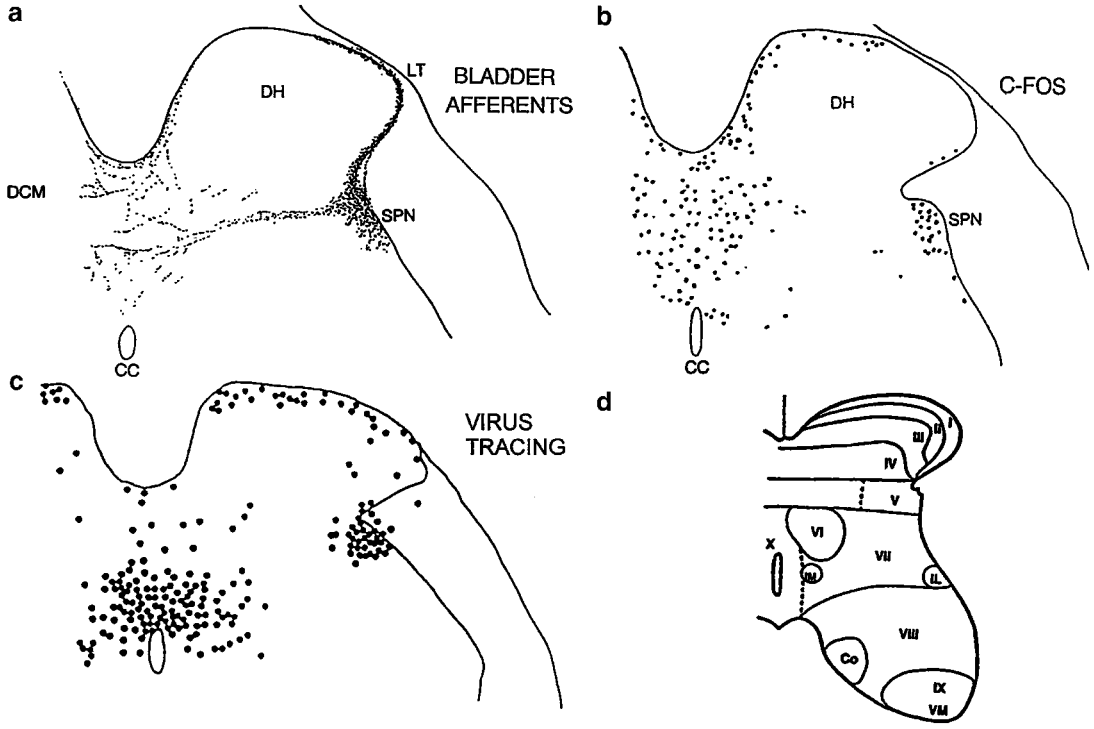

Figure 5 Comparison of the distribution of bladder afferent projections to the L6 spinal cord of the rat (a), with the distribution of c-*fos*-positive cells in the L6 spinal segment following chemical irritation of the LUT of the rat (b), and the distribution of interneurones in the L6 spinal cord labelled by transneuronal transport of PRV injected into the urinary bladder (c). Afferents labelled by WGA-HRP injected into the urinary bladder. C-*fos* immunoreactivity is present in the nuclei of cells. DH, dorsal horn; SPN, sacral parasympathetic nucleus; CC central canal. Calibration represents 500 μm. (d) The laminar organization of the cat spinal cord. These data show that spinal interneurones involved in the reflex control of the urinary bladder are concentrated in specific regions of the spinal cord that receive afferent input from the LUT. Some of these interneurones provide excitatory input to the parasympathetic preganglionic neurones and represent an essential component of the spinal micturition reflex pathway.

coeruleus, which contains noradrenergic neurones; PAG and the A5 noradrenergic cell group (Figure 4) (Nadelhaft *et al*., 1992; Vizzard *et al*., 1995; Sugaya *et al*., 1997). Several regions in the hypothalamus and the cerebral cortex also exhibited virus-infected cells. Neurones in the cortex were located primarily in the medial frontal cortex.

Other anatomical studies in which anterograde tracer substances were injected into brain areas and then identified in terminals in the spinal cord are consistent with the virus tracing data. Tracer injected into the paraventricular nucleus of the hypothalamus labelled terminals in the sacral parasympathetic nucleus as well as the sphincter motor nucleus (Holstege & Mouton, 2003). On the other hand, neurones in the anterior hypothalamus project to the PMC. Neurones in the PMC in turn project primarily in the lateral funiculus to the sacral parasympathetic nucleus and the lateral edge of the dorsal horn and the dorsal commissure, areas containing dendritic projections from preganglionic neurones, sphincter motoneurones and afferent inputs from the bladder. Patch-clamp studies revealed that lumbosacral preganglionic neurons in the neonatal rat spinal cord receive monosynaptic and polysynaptic glutamatergic excitatory inputs from axons in the lateral funiculus (Miura *et al*., 2001). Conversely, projections from neurones in the lateral pons terminate rather selectively in the sphincter motor nucleus. Thus, the sites of termination of descending projections from the PMC are optimally located to regulate reflex mechanisms at the spinal level.

Organization of urine storage reflexes

Sympathetic storage reflex

Although the integrity of the sympathetic input to the LUT is not essential for the performance of micturition, it does contribute to the storage function of the bladder. Surgical interruption or pharmacological blockade of the sympathetic innervation can reduce urethral outflow resistance, reduce bladder capacity and increase the frequency and amplitude of bladder contractions recorded under constant volume conditions (de Groat *et al*., 1993; Morrison *et al*., 2005).

Sympathetic reflex activity is elicited by a sacrolumbar intersegmental spinal reflex pathway that is triggered by vesical afferent activity in the pelvic nerves (Figure 3a) (de Groat & Lalley, 1972; de Groat & Theobald, 1976). The reflex pathway is inhibited when bladder pressure is raised to the threshold for producing micturition. This inhibitory response is abolished by transection of the spinal cord at the lower thoracic level, indicating that it originates at a supraspinal site, possibly the PMC. Thus, the vesicosympathetic reflex represents a negative feedback mechanism that allows the bladder to accommodate larger volumes (Figure 3).

Urethral sphincter storage reflex

Motoneurones innervating the striated muscles of the urethral sphincter exhibit a tonic discharge which increases during

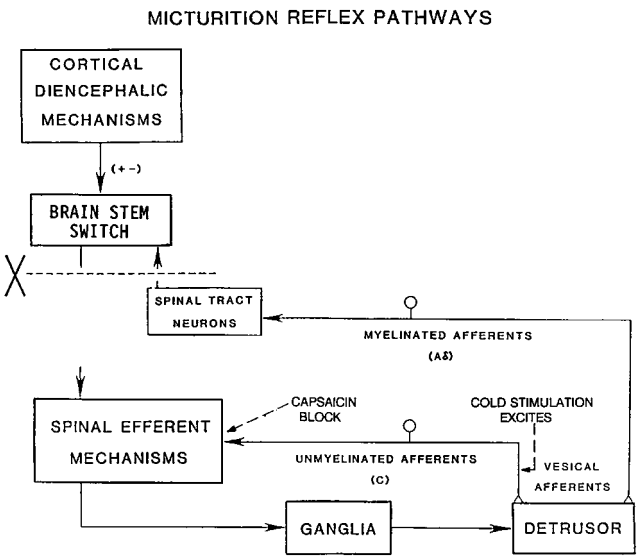

Figure 6 Diagram showing the organization of the parasympathetic excitatory reflex pathway to the detrusor muscle. Scheme is based on electrophysiologic studies in cats. In animals with an intact spinal cord, micturition is initiated by a supraspinal reflex pathway passing through a centre in the brain stem. The pathway is triggered by myelinated afferents (A-δ fibres), which are connected to the tension receptors in the bladder wall. Injury to the spinal cord above the sacral segments interrupts the connections between the brain and spinal autonomic centres and initially blocks micturition. However, over a period of several weeks following cord injury, a spinal reflex mechanism emerges, which is triggered by unmyelinated vesical afferents (C-fibres); the A-fibre afferent inputs are ineffective. The C-fibre reflex pathway is usually weak or undetectable in animals with an intact nervous system. Stimulation of the C-fibre bladder afferents by instillation of ice water into the bladder (cold stimulation) activates voiding responses in patients with SCI. Capsaicin (20–30 mg, subcutaneously) blocks the C-fibre reflex in chronic spinal cats, but does not block micturition reflexes in intact cats. Intravesical capsaicin also suppresses detrusor hyper-reflexia and cold-evoked reflexes in patients with neurogenic bladder dysfunction.

bladder filling (Figure 2). This activity is mediated in part by low-level afferent input from the bladder (Table 2, Figure 3a). During micturition, the firing of sphincter motoneurones is inhibited. This inhibition is dependent in part on supraspinal mechanisms (Figure 3b), since it is less prominent in chronic spinal animals. Electrical stimulation of the PMC induces sphincter relaxation, suggesting that bulbospinal pathways from the pons may be responsible for maintaining the normal reciprocal relationship between bladder and sphincter (Mallory *et al*., 1991).

Sphincter-to-bladder reflexes may also contribute to urine storage because afferent activity arising in the striated sphincter muscles during contractions can suppress reflex bladder activity and in turn increase bladder capacity. Studies in cats and monkeys revealed that direct electrical stimulation of the sphincter muscles or electrical stimulation of motor pathways to induce a contraction of the sphincters suppresses reflex bladder activity (McGuire *et al*., 1983; de Groat *et al*., 2001). Similar inhibitory responses are elicited by electrical stimulation of afferent axons in the pudendal nerve, some of which must arise in the sphincter muscles. Electrophysiological studies in cats showed that activation of pudendal afferent input from various sites including penis, vagina, anal canal/rectum and sphincters inhibited micturition by suppressing interneuronal pathways in the spinal cord as well as by directly inhibiting parasympathetic preganglionic neurons (de Groat, 1978; de Groat *et al*., 1981). The inhibitory responses occur at low frequencies of stimulation (1–5 Hz), whereas excitatory responses, which are due in part to activation of perineal cutaneous afferent axons, are elicited by high frequencies of stimulation (20–40 Hz) (Tai *et al*., 2005).

Organization of voiding reflexes

Spinobulbospinal micturition reflex pathway

Micturition is mediated by activation of the sacral parasympathetic efferent pathway to the bladder and the urethra as well as reciprocal inhibition of the somatic pathway to the urethral sphincter (Table 2) (Figure 3b). Studies in cats using brain-lesioning techniques revealed that neurones in the brainstem at the level of the inferior colliculus have an essential role in the control of the parasympathetic component of micturition (Figure 3b). Removal of areas of the brain above the inferior colliculus by intercollicular decerebration usually facilitates micturition by elimination of inhibitory inputs from more rostral centres (de Groat *et al*., 1993; Yokoyama *et al*., 2000; 2001; Morrison *et al*., 2005). However, transections at any point below the colliculi abolish micturition. Bilateral lesions in the rostral pons in the region of the locus coeruleus in cats, or Barrington's nucleus in rats, also abolishes micturition, whereas electrical or chemical stimulation at these sites suppresses urethral sphincter activity, triggers bladder contractions and release of urine (Noto *et al*., 1989; Mallory *et al*., 1991). These observations led to the concept of a spinobulbospinal micturition reflex pathway that passes through a centre in the rostral brainstem (the PMC) (Figures 3b and 6). The pathway functions as an 'on–off' switch (de Groat, 1975) that is activated by a critical level of afferent activity arising from tension receptors in the bladder and is, in turn, modulated by inhibitory and excitatory influences from areas of the brain rostral to the pons (e.g. diencephalon and cerebral cortex) (Figure 6).

Suprapontine control of micturition

Lesion and electrical stimulation studies in humans and animals indicate that voluntary control of micturition depends on connections between the frontal cortex hypothalamus and other forebrain structures such as anterior cingulate gyrus, amygdala, bed nucleus of the stria terminalis and septal nuclei, where electrical stimulation elicits excitatory bladder effects. Damage to the cerebral cortex due to tumours, aneurysms or cerebrovascular disease removes inhibitory control of the PMC resulting in bladder overactivity (Yokoyama *et al*., 2000; 2001).

Spinal micturition reflex pathway

SCI rostral to the lumbosacral level eliminates voluntary and supraspinal control of voiding, leading initially to an areflexic bladder and complete urinary retention followed by a slow development of automatic micturition and bladder hyperactivity (Figure 2c) mediated by spinal reflex pathways

(de Groat, 1995). However, voiding is commonly inefficient due to simultaneous contractions of the bladder and urethral sphincter (bladder–sphincter dyssynergia) (Figure 2c). Electrophysiologic studies in animals have shown that the micturition reflex pathways in spinal intact animals and in chronic spinal-injured animals are markedly different (de Groat & Ryall, 1969; Cheng *et al*., 1999). In cats with an intact spinal cord, myelinated (A-δ) afferents activate the micturition reflex, whereas in cats with chronic thoracic spinal cord transection, micturition is induced by unmyelinated (C-fibre) axons (Figure 6). In normal cats, capsaicin did not block reflex contractions of the bladder or the A-δ-fibre-evoked bladder reflex. However, in cats with chronic spinal injury, capsaicin, a neurotoxin known to disrupt the function of C-fibre afferents, completely blocked C-fibre-evoked bladder reflexes (de Groat *et al*., 1990; Cheng *et al*., 1999).

Chronic spinal injury in humans also causes the emergence of an unusual bladder reflex that is elicited in response to infusion of cold water into the bladder (Geirsson *et al*., 1993). The response to cold water does not occur in normal adults but does occur in (1) infants, (2) patients with multiple sclerosis and Parkinson's disease and (3) elderly patients with hyperactive bladders. Studies in animals indicate that cold temperature activates receptors in bladder C-fibre afferents and urothelial cells (Figure 6) (Fall *et al*., 1990; Stein *et al*., 2004). The presence of the cold reflex in infants, its disappearance with maturation of the nervous system and its re-emergence under conditions in which higher brain functions are disrupted suggests that it may reflect a primitive spinal involuntary voiding reflex activated by C-fibre afferents. Evidence of the contribution of C-fibre bladder afferents to bladder hyperactivity and involuntary voiding has been obtained in clinical studies in which capsaicin or resiniferatoxin, C-fibre afferent neurotoxins, was administered intravesically to patients with multiple sclerosis or SCIs and hyperreflexic bladders. In these patients, capsaicin increased bladder capacity and reduced the frequency of incontinence (Chancellor & de Groat, 1999; Szallasi & Fowler, 2002).

The emergence of C-fibre bladder reflexes seems to be mediated by several mechanisms including changes in central synaptic connections and alterations in the properties of the peripheral afferent receptors that lead to sensitization of the 'silent' C fibres and the unmasking of responses to mechanical stimuli (de Groat, 1995; de Groat & Yoshimura, 2005). In rats, it has been shown that bladder afferent neurons undergo both morphologic (neuronal hypertrophy) (Kruse *et al*., 1995) and physiologic changes (upregulation of tetrodotoxin (TTX)-sensitive Na^+ channels and downregulation of TTX-resistant Na^+ channels) following SCI (Yoshimura & de Groat, 1997). It has been speculated that this neuroplasticity is mediated by the actions of neurotrophic factors such as NGF released within the spinal cord or the urinary bladder. The production of neurotrophic factors including NGF increases in the bladder after SCI (Vizzard, 2005) and chronic administration of NGF into the bladder of rats induced bladder hyperactivity and increased the firing frequency of dissociated bladder afferent neurons. On the other hand, intrathecal application of NGF antibodies to neutralize NGF in the spinal cord suppressed detrusor hyper-reflexia and detrusor-sphincter dyssynergia in spinal cord-injured rats (Seki *et al*., 2002; 2004).

Neurotransmitters in central micturition reflex pathways

Excitatory neurotransmitters

Excitatory transmission in the central pathways to the LUT may depend on several types of transmitters, including glutamic acid, neuropeptides (substance P), nitric oxide and ATP (de Groat & Yoshimura, 2001; Morrison *et al*., 2005). Pharmacological experiments in rats have revealed that glutamic acid is an essential transmitter in the ascending, pontine and descending limbs of the spinobulbospinal micturition reflex pathway and in spinal reflex pathways controlling the bladder and external urethral sphincter (Yoshiyama & de Groat, 2005). NMDA and non-NMDA glutamatergic synaptic mechanisms appear to interact synergistically to mediate transmission in these pathways (Yoshiyama *et al*., 1995; Araki & de Groat, 1996).

Inhibitory neurotransmitters

Several types of inhibitory transmitters, including inhibitory amino acids (γ-aminobutyric acid (GABA), glycine) and opioid peptides (enkephalins), can suppress the micturition reflex when applied to the central nervous system. Experimental evidence in anesthetized animals indicates that GABA and enkephalins exert a tonic inhibitory control in the PMC and regulate bladder capacity (Mallory *et al*., 1991; de Groat *et al*., 1993; de Groat & Yoshimura, 2001). GABA and enkephalins also have inhibitory actions in the spinal cord.

Transmitters with mixed excitatory and inhibitory actions

Some transmitters (5-hydroxytryptamine (5-HT), noradrenaline, dopamine, acetylcholine and non-opioid peptides including vasoactive intestinal polypeptide, corticotropin-releasing factor) have both inhibitory and excitatory effects on reflex bladder activity depending on the type of receptors activated.

The recent development of duloxetine (Dmochowski *et al*., 2003; Millard *et al*., 2004), a serotonin-norepinephrine reuptake inhibitor, for the treatment of stress urinary incontinence has focused attention on the role of serotonin and norepinephrine in the control of LUT function. Nerves containing these transmitters are localized in sympathetic and parasympathetic nuclei in the lumbosacral spinal cord as well as in Onuf's nucleus indicating that they are involved in the control of the bladder and the urethral sphincter (de Groat, 2002). Studies in cats in which the bladder was irritated with intravesical infusion of acetic acid indicate that duloxetine inhibits reflex bladder activity and enhances external urethral sphincter activity (Thor & Katofiasc, 1995). The excitatory effects on the sphincter seem to be mediated by 5-HT_2 receptors and α_1 adrenoceptors, whereas the inhibitory effects on the bladder seem to be mediated by 5-HT_1 receptors.

The role of 5-HT_1 receptors in inhibiting bladder activity was confirmed by administration of 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist. This agent increased bladder capacity in chloralose anesthetized cats in which the bladder was irritated with acetic acid but had only moderate effects on bladder activity in the absence of irritation (Thor *et al*., 2002). The drug had a facilitator effect on activity of the external urethral sphincter.

8-OH-DPAT also inhibited reflex bladder activity in awake or chloralose-anesthetized, chronic spinal cord-injured (SCI) cats, but did not alter the somato-bladder excitatory reflex induced in SCI cats by tactile stimulation of the perigenital region (Miscik *et al*., 2003; Gu *et al*., 2004). The effects of 8-OH-DPAT were blocked by WAY 100635, a 5-HT_{1A} receptor antagonist which alone had no effect. These results indicate that 8-OH-DPAT acts in the spinal cord to inhibit the micturition reflex triggered by C-fibre bladder afferent axons and has much less effect on the spinobulbospinal reflex elicited by A-δ afferents. It seems likely that inhibition occurs at a proximal site on the reflex pathway at the primary afferent terminals or at an interneuronal level rather than on the efferent limb of the reflex (i.e., preganglionic and postganglionic neurons) because the efferent limb should be common to both the perigenital stimulation-evoked and bladder distension-evoked reflexes and only the latter was inhibited by 8-OH-DPAT.

In rats the modulatory effects of 5-HT on LUT function are different than those in cats (de Groat, 2002). Intravenous, intrathecal or intracerebroventricular administration of 8-OH-DPAT facilitates the micturition reflex and intravenous administration enhances spontaneous and reflex activity of the external urethral sphincter (Lecci *et al*., 1992; Conley *et al*., 2001; Chang *et al*., 2004). On the other hand, WAY 100635, a 5-HT_{1A} receptor antagonist administered *via* various routes depressed reflex bladder and sphincter activity (Testa *et al*., 1999; Conley *et al*., 2001; Kakizaki *et al*., 2001). It has been speculated that WAY 100635 blocks 5-HT_{1A} autoinhibitory receptors in raphe neurons in the brain stem and enhances raphe neuron firing which in turn increases release of 5-HT in the spinal cord (Testa *et al*., 1999; Kakizaki *et al*., 2001). In the spinal cord it is thought that 5-HT released from raphe bulbospinal axons activates 5-HT_{2C} receptors on inhibitory neurons to suppress the micturition reflex. NAD-299, another selective 5-HT_{1A} receptor antagonist, had a similar inhibitory response on micturition in the rat (Pehrson *et al*., 2002). The intracerebroventricular injection of a 5-HT_7 receptor antagonist also increased the volume threshold for triggering micturition in the anesthetized rat (Read *et al*., 2003). Thus, micturition in the rat is sensitive to both excitatory and inhibitory serotonergic mechanisms, whereas in the cat serotonin appears to act primarily to promote urine storage by enhancing sphincter activity and suppressing bladder activity. The similarities in the effects of duloxetine on sphincter activity in cat and human indicate that micturition in the cat may be a useful model for developing centrally acting serotonergic agents for the treatment of LUT dysfunction (Thor *et al*., 2002; de Groat, 2002; Burgard *et al*., 2003).

Activation of cholinoceptors in the rat brain also elicits mixed effects. Nicotinic agonists administered intracerebroventricularly suppress voiding in awake or anesthetized rats (Lee *et al*., 2003), whereas activation of muscarinic receptors stimulates bladder activity during bladder filling but suppresses voluntary voiding (Ishiura *et al*., 2001; Nakamura *et al*., 2003). Atropine blocked both the inhibitory and excitatory effects of muscarinic agonists. Intracerebroventricular administration of atropine alone increased bladder capacity and reduced voiding efficiency indicating that muscarinic excitatory mechanisms in the brain are tonically active.

The effects of dopamine on the central control of micturition are complex. Inhibitory effects of dopamine are mediated by D_1-like (D_1 and D_5), and the facilitatory effects are mediated by D_2-like (D_2, D_3 and D_4) receptor subtypes (Yoshimura *et al*., 1993; 1998; Yokoyama *et al*., 1999; 2001; 2002; de Groat & Yoshimura, 2001; Seki *et al*., 2001). Loss of forebrain dopaminergic mechanisms in patients with idiopathic Parkinson's disease is associated with bladder hyperactivity (de Groat & Yoshimura, 2001). Interactions between dopamine and NMDA glutamatergic facilitatory mechanisms also are important in the emergence of bladder overactivity after middle cerebral artery occlusion in the rat (Yokoyama *et al*., 2002).

Conclusions

The functions of the LUT to store and periodically eliminate urine are regulated by a complex neural control system that performs like a simple switching circuit to maintain a reciprocal relationship between the bladder and urethral outlet. The switching circuit is modulated by several neurotransmitter systems and is therefore sensitive to a variety of drugs and neurologic diseases. A more complete understanding of the neural mechanisms involved in bladder and urethral control will no doubt facilitate the development of new diagnostic methods and therapies for LUT dysfunction.

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