

## CHAPTER 1

# Neurophysiology of voiding

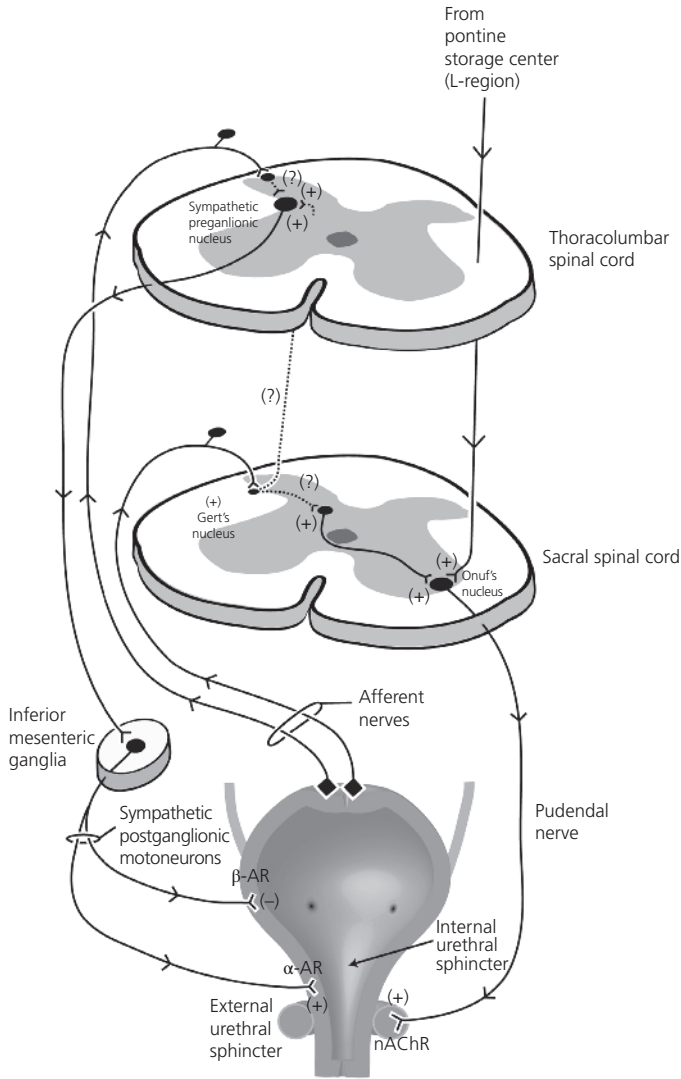
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### Anatomy of the lower urinary tract

The three main components of the bladder are the detrusor smooth muscle, connective tissue, and urothelium. The detrusor constitutes the bulk of the bladder and is arranged into inner longitudinal, middle circular, and outer longitudinal layers [1]. Elastin and collagen make up the connective tissue, which determines passive bladder compliance [2]. Elevated type III collagen and decreased elastin are associated with poorly compliant bladders [2]. Active compliance is determined by the detrusor, which is able to change its length over a wider range than skeletal muscle, allowing for a wide variation in bladder volume while maintaining a low pressure [2]. The detrusor maintains a baseline tension, which is modulated by hormones, local neurotransmitters, and the autonomic nervous system. Impedance studies reveal that compared to other smooth muscles, the detrusor is not electrically well coupled. This decreases the likelihood of detrusor overactivity (DO) during filling [2].

The urothelium has multiple layers, consisting of basal cells, intermediate cells, and luminal umbrella cells. The umbrella cells have tight junction complexes, lipid molecules, and uroplakin proteins that contribute to barrier function. A sulfated polysaccharide glycosaminoglycan layer covers the lumen of the bladder and defends against bacterial infection. Although the urothelium was previously thought to be an inert barrier, we now know that urothelial cells participate in afferent signaling. Bladder nerves terminate close to, as well as on urothelial cells. Urothelial cells have pain receptors and mechanoreceptors, which can be modulated by ATP to activate or inhibit sensory neurons. Abnormal activation of these channels by inflammation can lead to pain responses to



**Figure 1.1** Storage function.  $\alpha$ -AR,  $\alpha$  adrenergic receptor;  $\beta$ -AR,  $\beta$  adrenergic receptor; nAChR, nicotinic acetylcholine receptor. Source: Beckel and Holstege [4]. Reproduced with permission from Springer.

normally nonnoxious stimuli. Urothelial cells release factors such as acetylcholine, ATP, prostaglandins, and nitric oxide that affect sensory nerves [3].

The internal and external urethral sphincters (EUS) are vital for urinary continence. The internal urethral sphincter functions as a unit with the bladder base and trigone to store urine. The EUS is comprised of inner smooth muscle surrounded by outer skeletal muscle. It is omega shaped, with the majority of its muscle anterior to the urethra, and the opening of the omega sitting posteriorly. The smooth muscle is comprised of a thick longitudinal layer and an outer circular

layer. The smooth muscle of the female EUS has less sympathetic innervation than that of the male, and the male EUS is larger in size. The skeletal muscle of the EUS has both slow and fast twitch fibers, of which the slow twitch fibers are more important in maintaining tonic force in the urethra. Contraction of the EUS, coaptation of the mucosa, as well as engorgement of blood vessels in the lamina propria contribute to urinary continence [2].

The lower urinary tract (LUT) is innervated by both the autonomic and somatic nervous systems. Sympathetic nervous system control of the LUT travels via the hypogastric nerve ( $T_{10}$ - $L_2$ ) (Figure 1.1, sympathetic preganglionic nucleus in thoracolumbar spinal cord), while parasympathetic control travels via the pelvic nerve ( $S_{2-4}$ ) (Figure 1.1, Gert's nucleus in sacral spinal cord) [4, 5]. The somatic motor neurons control the skeletal muscle of the EUS via the pudendal nerve ( $S_{2-4}$ ) [4]. Its motor neurons are found in Onuf's nucleus (Figure 1.1, sacral spinal cord). The sympathetic and somatic nervous systems promote storage, while the parasympathetic system promotes emptying.

## Afferent mechanisms

The sensation of bladder fullness is carried by two types of afferent fibers via the pelvic, hypogastric, and pudendal nerves. A-delta ( $A\delta$ ) fibers, which are activated at low thresholds, are myelinated large diameter nerves that conduct action potentials quickly [3]. C-fibers are high threshold, unmyelinated nerves that conduct signals more slowly, and usually transmit pain sensations. Normal bladder sensations are carried by  $A\delta$  fibers, whereas C-fibers become more important in diseased bladders [3]. In humans, C-fibers are found in the urothelial and suburothelial layers, whereas  $A\delta$  fibers are found in the smooth muscle [6]. A certain population of C-fibers is called silent afferents, because they normally respond to chemical or irritative stimuli. While these stimuli are uncommon in the bladder, chemical irritation can sensitize the bladder, to cause abnormal responses to normal stretch [3]. The transient receptor potential vanilloid type 1 ( $TRPV_1$ ) receptor responds to pain, heat and acidity. Vanilloids, such as resiniferatoxin, desensitize C-fibers and suppress painful sensation [7]. Although initial studies suggested that resiniferatoxin may improve neurogenic DO, it is currently being studied as a treatment for cancer related pain [8], rather than as a treatment of DO.

Although we have long known that acetylcholine (muscarinic agonist) and ATP (purinergic agonist) act via the parasympathetic nervous system to cause bladder contraction, they have been shown to play a role in afferent sensation as well. Muscarinic acetylcholine receptors are found on urothelial cells, suburothelial interstitial cells of Cajal, and on afferent nerves. The urothelium releases acetylcholine and ATP in response to stretch, both of which enhance spontaneous activity in interstitial cells of Cajal, to cause bladder smooth muscle contractions.

This enhancement of spontaneous contractions may cause an increase in “afferent noise” that may be interpreted as urgency [9]. In spinalized rats, botulinum toxin lowers ATP release from the urothelium and blocks detrusor contraction [2]. Another mechanism of botulinum toxin’s action is by decreasing afferent firing from the bladder [10].

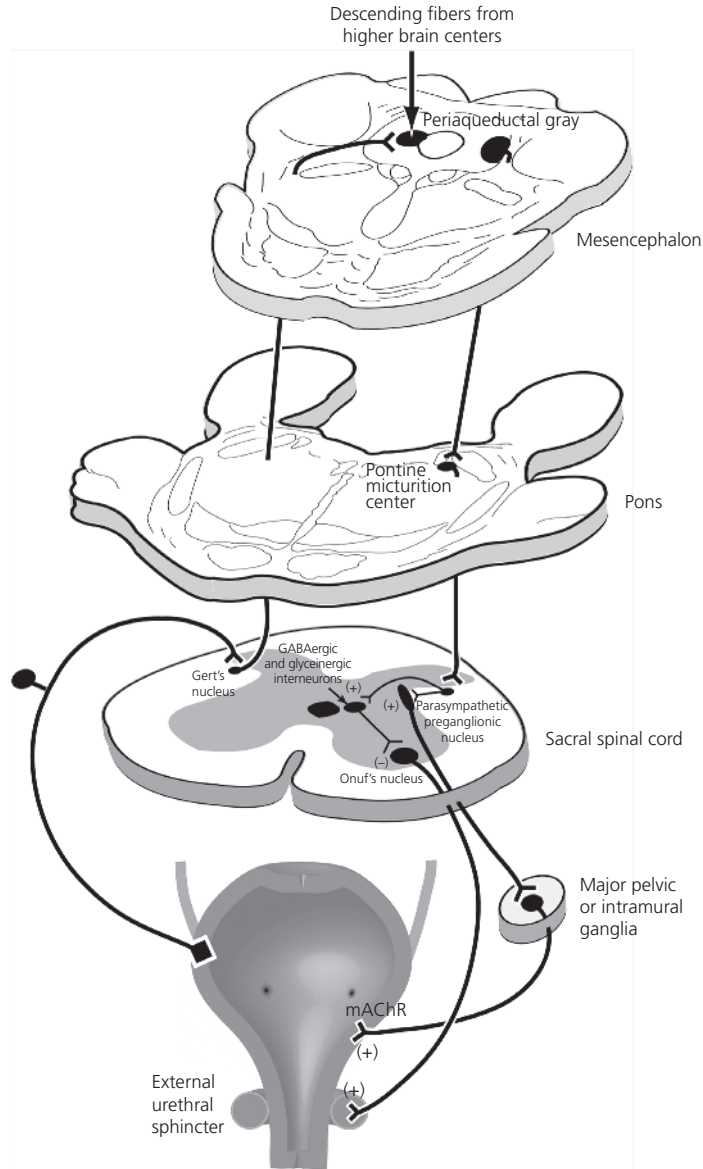
Adjacent pelvic organs such as the colon and uterus can affect urinary continence [2]. This may be due to a common afferent system via the hypogastric nerve, or intermediary neurons allowing for cross talk between pelvic organs [3]. Distension of the colon from constipation is a well-recognized cause of urinary incontinence in children. This is likely due to changes in bladder afferent signaling arising from a chronically distended colon, which prevents the child from recognizing a full bladder [11].

## **Spinal cord and brainstem**

During bladder storage, afferent signals from the hypogastric nerve and pelvic nerve travel to the thoracolumbar and sacral spinal cord, respectively (Figure 1.1). The hypogastric nerve sends signals via the sympathetic nervous system to block bladder contraction and contact the internal urethral sphincter. Onuf’s nucleus maintains contraction of the EUS, which is coordinated with bladder storage by the pontine micturition center (PMC) in the medial pons (Figure 1.1, L-region).

Once the bladder pressure threshold is exceeded, afferent signals travel via the pelvic nerve to synapse on interneurons in Gert’s nucleus in the S<sub>1-2</sub> spinal cord [4] (Figure 1.2). These interneurons send projections up to the periaqueductal gray (PAG) in the midbrain to initiate voiding, which occurs if the cerebral cortex determines that it is appropriate to void. The PAG sends caudal projections to the PMC, which is the final efferent center of the LUT. The PMC sends projections caudally to the sacral parasympathetic nucleus, activating the neurons, which cause bladder contraction and EUS relaxation [4] (Figure 1.2).

Although the mechanism of sacral or pudendal neuromodulation remains unclear, the two likely locations would be the peripheral nervous system (including the autonomic nervous system efferents) or the brainstem (PAG and PMC) and cortex [12, 13]. Positron emission tomography imaging shows that sacral neuromodulation restores normal afferent midbrain activity in women with Fowler’s syndrome, which is characterized by EUS overactivity. Prior to neuromodulation, they exhibit continuous EUS activity and lack of bladder afferent activity reaching the PAG or PMC. After neuromodulation and reestablishment of normal bladder afferent activity, they regain control of EUS activity [14]. Functional MRI evaluation confirms that neuromodulation reduced deactivation in the PAG, suggesting that exaggerated EUS



**Figure 1.2** Emptying function. mAChR, muscarinic acetylcholine receptor. Source: Beckel and Holstege [4]. Reproduced with permission from Springer.

afferent activity is capable of blocking normal bladder afferent sensation from reaching the cortex [15]. Inhibition of DO is also believed to result from inhibition of abnormal afferent activity. Pudendal nerve neuromodulation represents a more peripheral means to stimulate  $S_{2-4}$  and inhibit the voiding reflex, decreasing uninhibited detrusor contractions and increasing bladder capacity [12].

## Cortex

The role of the cerebral cortex in controlling voiding function has recently been described using PET scanning and functional MRI, to reveal differences in patients with normal LUT function, and those with DO. The PAG gathers information from the median prefrontal cortex, anterior cingulate gyrus, and insula. It is unclear whether these connections function as a looped relay system to the PAG or if they individually connect to the PAG. The median prefrontal cortex is a decision-making area, taking into account emotions and social context. The anterior cingulate gyrus generates autonomic response to stress and conflict, and the insula processes visceral sensations. Patients with DO show decreased MRI response at small bladder volumes, but have exaggerated responses in the anterior cingulate gyrus at large bladder volumes. When leakage occurs, deactivation of the anterior cingulate gyrus occurs. One hypothesis states that this is a learned response to imminent urinary leakage, which requires increased monitoring by the anterior cingulate gyrus in order to maintain continence [16–18].

## Efferent mechanisms, peripheral

The hypogastric nerve causes detrusor relaxation via  $\beta$ -adrenergic receptors and internal urethral sphincter contraction via  $\alpha$ -adrenergic receptors [4] (Figure 1.1). Both of these effects enhance storage function.  $\alpha_1$  Antagonists are used off-label to treat children with obstructive voiding patterns.  $\beta_3$  Adrenergic agonists increase bladder capacity without increasing voiding pressure or post void residual volume [19]. They are approved for treatment of DO in adults, but are not yet approved for children. In male rats, hypogastric nerve stimulation releases norepinephrine and raises urethral pressure, while in female rats the release of nitric oxide predominates over norepinephrine, resulting in a decrease in urethral pressure [20]. If this sex difference is present in humans, this would suggest that electrical stimulation of the hypogastric nerve to enhance internal urethral sphincter contraction would be more effective in males.

The axons of the pelvic nerve travel to two groups of postganglionic neurons in the detrusor and pelvic plexus [2]. The first group causes detrusor contraction by releasing acetylcholine and ATP, while the second group causes internal urethral sphincter relaxation by releasing nitric oxide [2, 4, 7] (Figure 1.2).

Muscarinic antagonists such as oxybutynin block muscarinic receptors. As previously discussed under afferent mechanisms, it is believed that muscarinic antagonists are effective at treating urgency during the storage phase by blocking the enhancement of afferent noise, which may be responsible for spontaneous contractions.  $M_3$  receptors cause detrusor contraction, while  $M_2$  receptors enhance  $M_3$  activity. Despite the relative abundance of  $M_2:M_3$  receptors in the bladder (3:1),  $M_2$  receptors play a secondary role in modulating  $M_3$  receptor

response. Although there is always a concern with causing urinary retention in patients when using muscarinic antagonists, there is a therapeutic window in which afferent signals can be blocked without affecting bladder contractility [7, 9]. There is one clinical scenario in which completely blocking bladder contractility is useful: neurogenic bladder patients who are already using clean intermittent catheterization to drain their bladders. In these cases, high doses of muscarinic antagonists or botulinum toxin can prevent bladder contractility. Botulinum toxin increases bladder capacity and decreases maximal detrusor pressure by preventing acetylcholine release [21].

ATP stimulates atropine-resistant, noncholinergic, nonadrenergic detrusor contraction, modulates urothelial signaling, and regulates afferent nerve activity. Despite these multiple mechanisms, which would predict that purinergic (P2 × 3) antagonists would be effective in the treatment of DO, they are currently being developed as treatments for pain. The main obstacle for developing a safe and tolerable purinergic antagonist is the fact that ATP is an important molecule in every organ system in the body, so developing a bladder-specific molecule has been difficult. It is proposed that purinergic signaling becomes more important in obstructed bladders [7, 22].

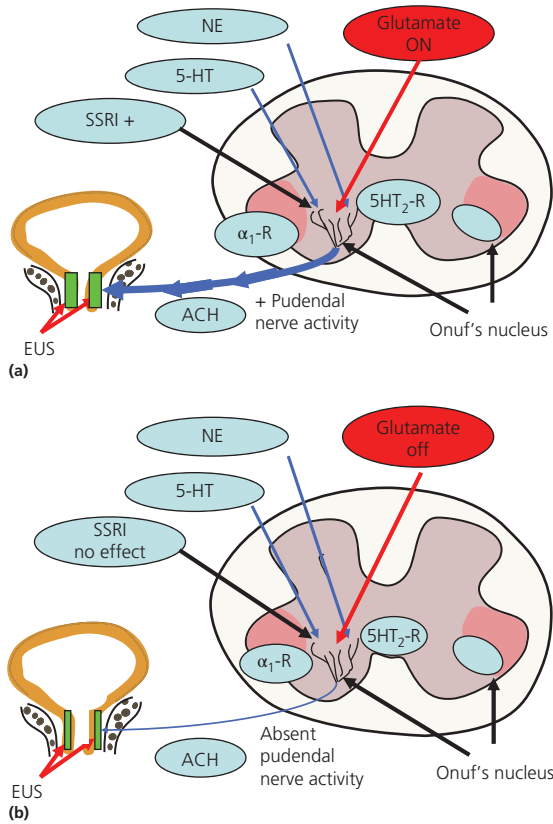
Prostaglandins facilitate voiding by altering neurotransmitter release or by inhibiting the breakdown of acetylcholine [2]. While there are multiple prostaglandins, it is believed that prostaglandin E2 (PGE2) interacts with EP1 receptors to drive DO [23]. Urinary levels of PGE2 were found to be elevated in both men and women with OAB compared to controls [24, 25]. Nonsteroidal anti-inflammatory drugs, which block prostaglandins, are commonly used after surgery to decrease bladder spasms in children.

## Efferent mechanisms, central

The pudendal nerve has small neurons with both somatic and autonomic components. The skeletal muscle component of the EUS is regulated by GABA, the major inhibitory neurotransmitter in the spinal cord [7]. Baclofen (GABA agonist) can be used to treat detrusor sphincter dyssynergia, although it is more often used to treat limb spasticity via an intrathecal route [7, 26]. Injection of botulinum toxin into the EUS decreases its contractility, and is another means to temporarily block EUS contractility.

Imipramine, a tricyclic antidepressant drug, has been used for refractory nocturnal enuresis [27]. However, its mechanism of action in treatment of DO remains unclear. It may enhance serotonin and norepinephrine (NE) reuptake inhibition, directly relax smooth muscle, or increase urethral resistance [5, 28].

Serotonin (5-HT) modulates the afferent system and may affect the bladder and EUS by supraspinal enhancement of bladder storage as well as EUS tonic activity at Onuf's nucleus (Figure 1.3a) [2, 30, 31]. Glutamate is the major spinal



**Figure 1.3** (a) Serotonin and storage function. (b) Serotonin and emptying function. 5-HT, serotonin; 5HT<sub>2</sub>-R, serotonin type 2 receptor; Ach, acetylcholine; NE, norepinephrine; SSRI, selective serotonin reuptake inhibitor;  $\alpha_1$ -R, alpha 1 adrenergic receptor. Source: Adapted from Franco [29]. © Elsevier.

cord regulator of the EUS [32] (Figure 1.3a). In the presence of glutamate, both 5-HT and NE can enhance its contraction of the EUS. However, if glutamate is absent, neither 5-HT nor NE can independently cause the EUS to relax (Figure 1.3b). Glutamate can be considered the on/off switch, while 5-HT and NE adjust the gain in the system [31]. The major problem with developing glutamate agonists to enhance continence by increasing EUS activity is that they do not penetrate the blood–brain barrier; therefore, glutamate agonists require intrathecal administration [33]. Duloxetine (a combined serotonin and norepinephrine reuptake inhibitor) decreases voiding frequency and incontinent episodes. Duloxetine reduced the motor threshold for EUS contraction and increased urethral phasic contractions in healthy women without affecting urethral resting pressure, suggesting that it both enhanced the afferent urethral response and increased signaling from the cortex to the EUS [34]. It is approved for stress incontinence in Europe, but not the United States.



## Development of urinary continence

The human neonate initially voids hourly [35], developing an increased bladder capacity in two phases: at birth and then at 3 years of age [36]. Since neonates do not have voluntary control of urination, it was believed that a local sacral reflex was responsible for emptying the bladder without involving the brain. However, electroencephalography suggests that most neonates experience cortical arousal and awaken before voiding, so the brain is always involved with voiding [37]. The interrupted infant voiding pattern results from a lack of coordination between the bladder and the external sphincter, which causes elevated voiding pressures until the child reaches the age of 18 months. One-week-old boys void with detrusor pressures of 117 cm H<sub>2</sub>O, and girls with pressures of 75 cm H<sub>2</sub>O [36, 38]. Overactive contractions are rarely seen during urodynamic studies carried out in asymptomatic infant [38, 39], indicating that the urinary frequency is due to a small bladder capacity rather than OAB.

Our understanding of neural control and smooth muscle function of the neonatal bladder is derived from animal models. Neonatal rats are unable to void spontaneously [40] and are dependent on their mother to lick the perigenital area to cause bladder emptying. By 3 weeks of age (the age of weaning), neonatal rats are able to void spontaneously in response to bladder filling [41]. At first glance, this might suggest that the neonatal nervous system is not mature, and additional maturation of the hypogastric, pelvic, or pudendal nerves needs to occur. However, this is not consistent with the fact that human and rat fetuses start to urinate by the second trimester, so the voiding reflex needs to be functional during prenatal life. What actually happens in rats is that the perigenital reflex inhibits the mature bladder emptying reflex at the sacral level, and this inhibition is removed at weaning [42, 43]. Experimental bladder distension does not change the onset of spontaneous voiding at 3 weeks, but prolongs the perigenital reflex for another 2 weeks [44]. Surgical reduction of bladder volume causes the immediate onset of spontaneous voiding in neonatal rats [45]. These findings suggest that maturation from immature and mature voiding reflexes requires central neural control, despite having afferent and efferent connections, which are ready for use at birth.

Animal studies show that the neonatal bladder smooth muscle produces more pressure per gram of tissue and is more dependent on local calcium levels than adult bladder smooth muscle [46–49]. Neonatal bladders also have large-amplitude spontaneous contractions, which are downregulated with maturation [50, 51]. The elevated voiding pressures seen in infants may represent immature smooth muscle function. Once the detrusor becomes more mature and less overactive, it becomes easier for the brain to control. Since the brain makes the decision to void by integrating emotions, social context, stress, and visceral sensation, toilet training can be derailed by multiple factors, resulting in persistent urinary incontinence.

## Conclusion

While the neurophysiology of voiding is based on a relatively simple circuit, our ability to improve the treatment of pediatric urinary incontinence will depend on finding more focused methods of regulating bladder sensation and coordinating bladder and EUS function. While there are many possible targets, most of our current therapies are aimed at peripheral efferent systems, and we are only beginning to understand and develop treatments aimed at the afferent system, such as neuromodulation. The challenges of developing treatments aimed at the cerebral cortex and central efferent systems are the next frontier for physicians and researchers treating pediatric urinary incontinence.

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