7 Anatomy, Physiology and Pharmacology of the Lower Urinary Tract

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Introduction

Storage and elimination of urine requires a regulated interplay of reciprocal contraction and relaxation of bladder and outflow region and these structures are working as a functional unit [1, 2]. The interaction is controlled by neural circuits in the brain and spinal cord, which coordinate the activity of the detrusor smooth muscle as well as of the smooth and striated muscles of the outflow region [2, 3]. The peripheral nervous mechanisms for this control involve a complex pattern of efferent and afferent signaling in parasympathetic, sympathetic, and somatic nerves. Even if vesical and urethral functions are dependent on autonomic reflexes, the voluntary control of micturition, regulated by higher cortical centers, differentiates these organs from other viscera innervated by the autonomic nervous system. This review will briefly discuss the anatomy of the lower urinary tract and the principles of nervous control of micturition, and then focus on the peripheral, physiological and pharmacological mechanisms involved in the contraction and relaxation of bladder and urethra.

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Anatomy

The gross anatomy of the bladder and urethra is shown in Fig. 7.1. Main bladder components are the base (fundus), body and the trigone. The smooth muscle of the bladder (detrusor) remains continuous and inseparable from the urethra at the urethra-vesical junction. Details on the morphology of these structures can be found in many reviews and textbooks [1, 4–7].

Bladder

Bladder wall. The bladder wall has three well-defined layers: the mucosa (innermost portion), the muscularis propria, and the adventitia/serosa [8]. The mucosa defined as urothelium, basement membrane and lamina propria, also contains within the lamina propria some smooth muscle cells, muscularis mucosae. These cells are sometimes used to separate the mucosa from the "submucosa". Since the muscularis mucosa cells often do not form a distinct layer and is not very well defined in the human bladder (and sometimes seems to be absent), it may be questioned whether the human bladder, unlike the gut, has

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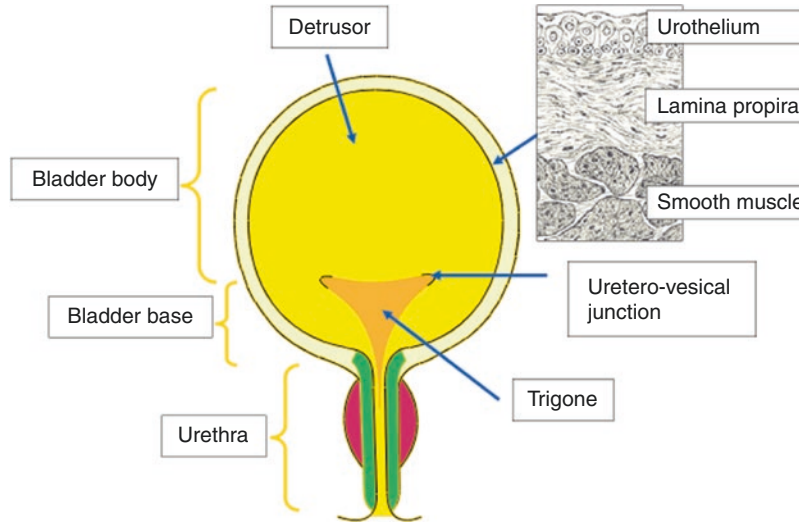

Fig. 7.1 The gross anatomy of the bladder and urethra (from Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev. 2004;84(3):935–86)

a true "submucosal" layer. However, the term is sometimes used to denote the part of the lamina propria closest to the muscularis propria.

Urothelium. The uroepithelium, or urothelium, lines the renal pelvis, ureters, bladder, upper urethra, and glandular ducts of the prostate, and forms the interface between the urinary space and the underlying vasculature, connective, nervous, and muscular tissues [8]. There are at least three urothelial lineages consisting of the ureter/renal pelvis, detrusor/trigone, and bladder neck/proximal urethra. The functional significance of these findings has yet to be determined. The urothelium of the detrusor/trigone is a transitional epithelial tissue, composed of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial or apical layer composed of large hexagonal cells (diameters of 25–250 μm) known as "umbrella cells" [9, 10]. The apical surface of umbrella cells possesses a unique asymmetric unit membrane (AUM), whose protein components (uroplakins) have been well studied. Tight junctions, localized between the superficial umbrella cells, are composed of multiple proteins such as the occludins and claudins. These proteins, along with uroplakins, which are crystalline proteins that assemble into hexagonal plaques, contribute to the urothelial barrier function. There appears to be little difference between the urothelium of the trigone and the detrusor.

In the proximal urethra, the urothelium transitions to a stratified or columnar epithelium accompanied by a lack of urothelial-specific differentiation markers. Urethral epithelial cells express microvilli on the apical surface. The presence of cilia or microvilli may have a number of functions including ability to increase the cell surface area, as well as affect bacterial adherence and fluid transport.

A urothelial glycosaminoglycan (GAG) layer covers the umbrella cells and has been suggested to contribute to urothelial barrier function [11].

Lamina propria. The lamina propria (LP) lies between the basement membrane of the mucosa and the muscularis propria (detrusor muscle) and is composed of an extracellular matrix containing several types of cells, including fibroblasts, adipocytes, interstitial cells, and sensory nerve endings [12]. In addition, LP contains a rich vascular network, lymphatic channels, elastic fibers, and smooth muscle fascicles (muscularis mucosae). Notably, the thickness of the LP varies within the bladder. The morphological characteristics of the LP, muscularis mucosae, and the detrusor muscle are important for pathological tumor staging of bladder cancer. However, LP is not only a landmark, but also a functionally active structure.

The roles of the LP and its components in bladder function have not been definitively established [13], although it has been suggested to be the capacitance layer of the bladder, determin-

ing bladder compliance and enabling adaptive changes to increasing volumes. However, the bladder LP may also serve as a communication center, with an important integrative role in signal transduction to the central nervous system (nociception, mechano-sensation). The LP may also, by means of its different components, make it possible for the urothelium to transmit information to other components of the bladder wall, contributing to activation of the detrusor muscle. In addition, the LP may serve as a source for production of factors influencing the growth of both the overlying urothelium and the underlying detrusor muscle.

A dense layer of spindle-shaped cells has been described in bladder upper lamina propria in both humans and animals [14]. These cells have been categorized heterogeneously as interstitial cells (ICs), interstitial cells of Cajal (ICC), interstitial Cajal-like cells (ICLC) cells, myofibroblasts, or telocytes. Even if significant progress has been made in the study of bladder ICs' cellular markers, ion channels and receptor expression, electrical and calcium signaling, their specific functions in normal bladder filling and emptying remain elusive.

Different types of nerves have been described in the LP. The highest density of mucosal innervation was found in the neck and the initial part of the urethra. In human bladder, intramural ganglion cells were demonstrated both in the LP or embedded among the detrusor muscle bundles. The majority of the ganglia were small in size and contained from one to six neurons. These ganglion cells possessed fine structural characteristics of parasympathetic nerve cells. Smet et al. [15] showed that in the human bladder, peptidergic (CGRP; tachykinin) nerves are localized mainly within the sub-epithelium, surrounding the vasculature as well as intramural ganglia. While these nerves have not been detected within the detrusor smooth muscle, vasoactive intestinal polypeptide (VIP)-containing nerves have been localized within both the sub-urothelial plexus as well as the detrusor muscle bundles.

Detrusor muscle. The detrusor is a smooth muscle layer, comprising interlacing muscle fibres running randomly in all directions [4, 5]. Only close to the internal urethral meatus do the fibres orientate themselves into three specific layers (inner longitudinal, middle circumferential, outer longitudinal), thus forming the proximal bladder neck sphincter. The detrusor muscle in the male is better developed than in the female as greater pressure needs to be generated to overcome the resistance posed by the longer male urethra. Detrusor muscle remains continuous and inseparable from the urethra at the urethra vesical junction. Its smooth fibers form the bladder neck and the internal urethral sphincter (IUS).

Trigone. The trigone consists of the triangular region between the ureteral orifices and the bladder outlet [4, 16]. Muscular extensions of the two distal ureters blend to form a thin triangular muscular sheet, designated as the trigonal muscle (so-called *superficial trigone*). This muscle is spread over the base detrusor and tapers off at the vesicourethral junction. The trigonal muscle and the similarly innervated, predominantly longitudinally helical muscularis of the distal ureters have been designated as the uretero-trigonal muscle. It contracts during bladder filling to keep the ureteral orifices opened and the bladder neck closed and relaxes during micturition to help funnel urine into the outlet and prevent ureteric reflux.

Vasculature. The arterial supply to the bladder is primarily from the superior, middle and inferior vesical arteries which arise from the hypogastric (anterior) trunk of the internal iliac artery [13]. Small branches also arise from the obturator and inferior gluteal arteries and in females also from the uterine and vaginal arteries, to provide a contribution to the lower bladder. A plexus of veins surrounds the bladder and in the male form a vesico-prostatic (Santorini's) plexus between the bladder and the prostate, which empties into the hypogastric (internal iliac) veins.

Lymphatics. Lymphatics that drain the bladder begin in mucosal, intermuscular and serosal plexuses. There are three sets of collecting vessels (the trigone, superior and inferolateral surface of the bladder) draining lymph into the para/vesical, hypogastric (internal iliac), external iliac and common iliac lymph nodes. Minute nodules of lymphoid tissue may occur along the vesical lymph vessels. Using antibodies against

the lymphatic vessel endothelial hyaluronan receptor (LYVE-1), Matsumoto et al. [17] demonstrated the distribution of lymphatic vessels in the human bladder. Small lymphatics expressing LYVE-1 were distributed in all layers of the normal bladder except for the urothelium. The border areas—the LP and detrusor or the detrusor and adventitia—showed the greatest distribution of these vessels. The small vessels were irregular in shape and without thick walls. The density of the lymphatics in the detrusor was significantly greater than in other parts of the bladder wall.

Urethra

Internal urethral smooth muscle sphincter (IUS). At the level of the bladder neck, the IUS surrounds the proximal urethra and is seen as a continuation of the detrusor smooth muscle, therefore favoring proximal urethral closure by constricting its lumen [18]. Smooth muscle fibers within the IUS are arranged in a horse-shoe shaped arrangement, but Wallner et al. [19] describe the superior part of the urethra to have a completely circular arrangement of smooth muscle. Layers of striated muscle, arranged in a circular configuration and thought to be derived from levator ani, surround the smooth muscle layer of the IUS in the midportion of the urethra [18, 20].

External urethral striated muscle sphincter (EUS.) Skeletal muscle, derived from the inner fibers of the levator ani muscle, surrounds the urethra as it traverses the deep perineal pouch therefore forming the EUS. In males, the EUS covers the inferior side of the prost ate and is located at the level of the membranous urethra [20] where fibers are oriented in a horse-shoe shape and without anatomical fixation to the levator ani muscle. This implies that voluntary closure of the urethra in males is executed by the EUS alone, without any involvement of the levator ani muscle [21]. The EUS is under voluntary control via the pudendal nerve. In females, the EUS begins at the inferior end of bladder and includes (1) sphincter urethrae muscle, (2) the compressor urethrae muscle, and (3) the urethrovaginal sphincter [20, 22]. Dorsolateral extensions of the inferior portion of the sphincter urethrae muscle are continuous with compressor urethrae muscle, whose contraction causes compression of the ventral part of urethra. The urethrovaginal sphincter is a thin, broad and flat muscle. As the inferior portion of EUS, the urethrovaginal sphincter encircles both the anterolateral parts of urethra and lateral aspect of vagina [20]. Based on their findings from fetal pelves, Wallner et al. [19] observed the following urethral closure mechanism in females: (1) the con- traction of levator ani muscle compresses the vagina against the posterior urethra above the level of EUS, (2) the simultaneous contraction of EUS and levator ani muscle induces an anteriorly convex bend in the midurethra, (3) the contraction of the inferior part of the EUS induces a posteroinferior force on the urethra as a result of the tendinous connection between the inferior part of the EUS and the puborectalis portion of levator ani [19]. Histological [23] and magnetic resonance imaging [22] studies have demonstrated the smooth muscle component of the IUS and the striated muscle component of the EUS to be maximally thick in the middle third of the urethra, therefore forming the true annular sphincter surrounding the urethra.

Basic Bladder Physiology/ Pharmacology

Details of the autonomous nervous control of the bladder storage and emptying functions have been discussed in several reviews [1, 2, 24, 25]. The bladder and the outflow region work as a functional unit and micturition requires the integration of autonomic and somatic efferent mechanisms to coordinate the activity of the bladder and urethral smooth muscle with that of urethral striated muscles. Micturition is under voluntary control and depends on learned behavior whereas many other visceral functions are regulated involuntarily. Despite extensive research, a number of both central and peripheral nervous control mechanisms are yet incompletely understood.

Nervous Control Mechanisms

Central control. The central nervous mechanisms for regulation of micturition are still not completely known. The normal micturition reflex is mediated by a spinobulbospinal pathway, passing through relay centers in the brain. In principle, the central pathways are organized as on-off switching circuits [3, 26, 27]. The reflex circuits involved consist of five basic components. Studies in humans and animals have identified three areas in the brainstem and diencephalon that are specifically implicated in micturition control: (1) The Barrington's nucleus or the pontine micturition center (PMC) in the dorsomedial pontine tegmentum directly excites bladder motoneurons and indirectly inhibits urethral sphincter motoneurons via inhibitory interneurons in the medial sacral cord. (2) The periaqueductal grey (PAG) receives bladder-filling information, and (3) the pre-optic area of the hypothalamus is assumed to be involved in determining the beginning of micturition. According to PET-scan studies in humans, these supraspinal regions are active during micturition [3, 26, 27].

Peripheral control (Fig. 7.2). The peripheral nervous mechanisms for bladder emptying

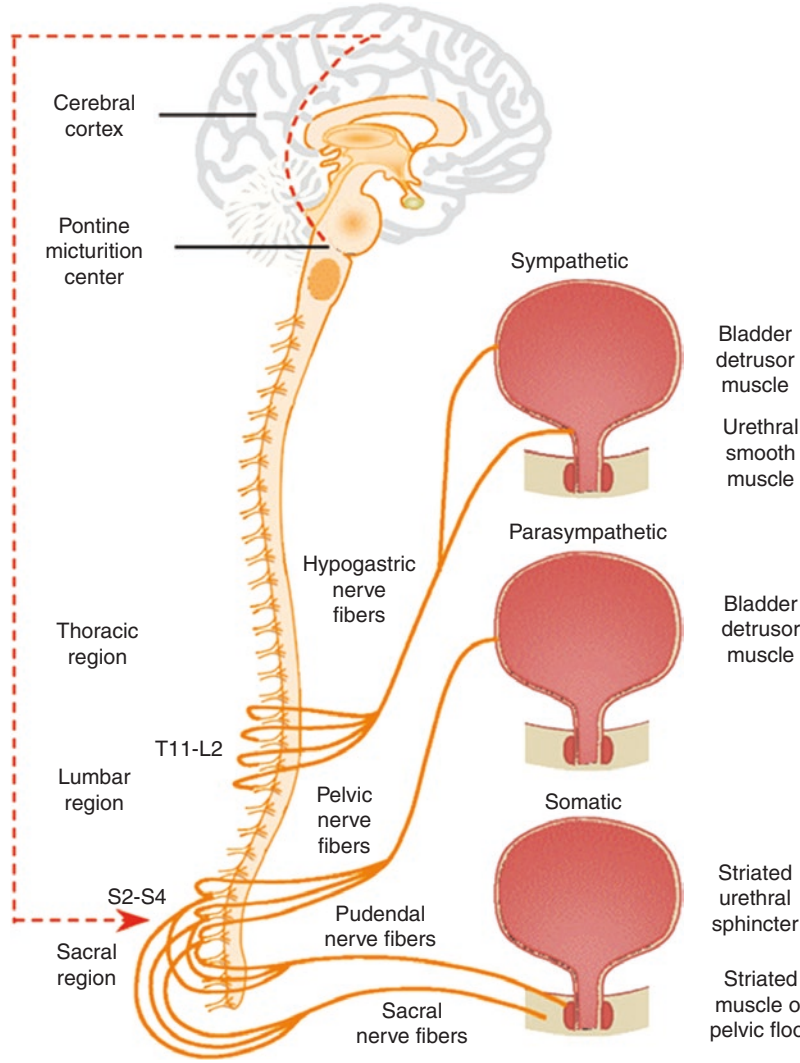

Fig. 7.2 Peripheral nervous control (from Penn Clinical Manual of Urology, Hanno P et al. 2014, Elsevier (Saunders), Philadelphia, p. 341)

and urine storage involve a complex pattern of efferent and afferent signaling in three sets of peripheral nerves: the p*arasympathetic*, *sympathetic* and *somatic* nerves. These nerves activate or deactivate bladder and outflow region in a reciprocal order, coordinated by reflex pathways. They either maintain the bladder in a relaxed state, while the outflow region is activated and enable urine storage at low intravesical pressure, or they initiate micturition by relaxing the outflow region and contracting the bladder smooth muscle. Parasympathetic action excites the bladder and relaxes the outflow region, sympathetic activation inhibits the bladder body and excites bladder outlet and urethra. Somatic nerves activate the external sphincter. The *sensory* innervation transmits information about bladder filling and contractions to the spinal cord.

Parasympathetic nerves. Parasympathetic neurons, mediating contraction of the detrusor smooth muscle and relaxation of the outflow region, are located in the sacral parasympathetic nucleus in the spinal cord at the level of S2-S4 [28]. The axons pass through the pelvic nerve and synapse with the postganglionic nerves in either the pelvic plexus, in ganglia on the surface of the bladder (vesical ganglia), or within the walls of the bladder and urethra (intramural ganglia) [29]. The preganglionic neurotransmission is predominantly mediated by acetylcholine (ACh) acting on nicotinic receptors. The transmission can be modulated by adrenergic, muscarinic, purinergic, and peptidergic presynaptic receptors [28]. The postganglionic neurons in the *pelvic nerve* mediate the excitatory input to the human detrusor smooth muscle by releasing ACh acting on muscarinic receptors. However, an atropine-resistant component, which is not mediated by cholinergic receptors has been demonstrated, particularly in functionally and morphologically altered human bladder tissue (see below). The pelvic nerve also conveys parasympathetic fibres to the outflow region and the urethra. These fibres exert an inhibitory effect and thereby relax the outflow region. This is mediated partly by nitric oxide [30], although other transmitters might be involved [31–33].

Sympathetic nerves. Most of the sympathetic innervation of the bladder and urethra originates from the intermediolateral nuclei in the thoracolumbar region (T10-L2) of the spinal cord. The axons travel either through the inferior mesenteric ganglia and the *hypogastric nerve* or pass through the paravertebral chain and enter the pelvic nerve. Thus, sympathetic signals are conveyed in both the hypogastric and pelvic nerves.

There are well-known anatomical differences between the male and female urethra, and this is also reflected in the innervation. In the human male, the smooth muscle surrounding the pre/prostatic part of the urethra is richly innervated by both cholinergic and adrenergic nerves [34]. This part is believed to serve as a sexual sphincter, contracting during ejaculation and thus preventing retrograde transport of sperm. The role of this structure in maintaining continence is unclear, but probably not essential. In the human female, there is no anatomical urethral smooth muscle sphincter, and the muscle bundles run obliquely or longitudinally along the length of the urethra. In the whole human female urethra, and in the human male urethra below the preprostatic part, there is a scarce supply of adrenergic nerves [34, 35]. Fine varicose terminals can be seen along the bundles of smooth muscle cells, running both longitudinally and transversely. Adrenergic terminals can also be found around blood vessels. Colocalization studies in animals have revealed that adrenergic nerves, identified by immunohistochemistry (tyrosine hydroxylase) also contain NPY [36]. Chemical sympathectomy (6-OH-dopamine) in rats resulted in a complete disappearance of tyrosine hydroxylase-immunoreactive (IR) nerves, whereas NOS-containing nerve fibers did not appear to be affected by the treatment [37]. This suggests that NOS is not contained within adrenergic nerves.

The predominant effects of the sympathetic innervation of the lower urinary tract in man are inhibition of the parasympathetic pathways at spinal and ganglion levels, and mediation of contraction of the bladder base and the urethra. However, in several animals, the adrenergic innervation of the detrusor is believed to inactivate the contractile mechanisms in the detrusor directly (see [24]).

Noradrenaline is released in response to electrical stimulation of detrusor tissues *in vitro*, and the normal response of detrusor tissues to released noradrenaline is relaxation (see [24]).

Somatic motoneurons. The innervation of the striated muscle of the external urethral sphincter (EUS), commonly referred to as Onuf's nucleus, originates in a specific region of the lateral ventral horn of the sacral spinal cord, generally centered in the human at the S2 segment, but also in the caudal end of the S1 segment and the middle of S3 [38]. Motoneurons in Onuf's nucleus send axons through the pudendal nerve to the pelvic floor muscles, including the external anal and external urethral sphincter [3]. These neurons are cholinergic, releasing acetylcholine to activate postjunctional nicotinic receptors on the sphincter striated muscle fibers. EUS also receives adrenergic innervation and is the only one to receive both autonomic and somatic stimuli [34, 39, 40]. Like all autonomic, but not somatic, EUS motoneurons also receive afferents from the paraventricular nucleus of the hypothalamus [41].

Afferent pathways. The *sensory* nerves monitor the urine volume and amplitude of bladder contractions during urine storage by afferent axons, which transmit the information to the lumbosacral cord [2, 40, 42]. Most of the sensory innervation of the bladder and urethra reaches the spinal cord via the pelvic nerve and dorsal root ganglia. In addition, some afferents travel in the hypogastric nerve. The sensory nerves of the striated muscle in the rhabdosphincter travel in the pudendal nerve to the sacral region of the spinal cord [2, 29]. Bladder afferent nerves are comprised of two types, myelinated Aδ fibers and unmyelinated C-fibers. Aδ fibers, located primarily in the detrusor smooth muscle layer, respond to detrusor stretching during bladder filling and convey fullness sensations. Thus, Aδ fibers respond to stretch of the bladder wall as the bladder fills with urine and to bladder contraction when voiding occurs [43]. They have a relatively low threshold pressure, approximately 5–15 mmHg [44], which corresponds to the pressure in the bladder when most humans first report sensations of bladder filling. Unmyelinated sensory C fibers are more widespread than Aδ fibers and reside in the detrusor muscle, close to the urothelium in the lamina propria and directly adjacent to urothelial cells. They have very high thresholds for firing and are not activated by physiologically relevant bladder pressures and are generally labeled as silent. C-fibers respond to nociceptive stimulation by chemicals, such as capsaicin or menthol, cold [45] or in response to inflammation [46].

Local Bladder Control

Cholinergic Mechanisms

The parasympathetic part of the autonomic nervous system is composed of neurons arising from the brainstem and sacral spinal cord. The main transmitter is acetylcholine (ACh), which is released at both ganglionic synapses and at postganglionic neuroeffector junctions. Nerves containing ACh are called cholinergic, a term introduced by Dale to describe neurons that liberate ACh. It should be remembered, however, that such nerves may contain other transmitters and that they can also be found postjunctionally in the sympathetic part of the autonomic nervous system (sweat glands and prostate). In all ganglia, released ACh stimulates nicotinic receptors. However, the postjunctional effects of ACh released from cholinergic nerves, mediating important functional actions in smooth muscle and other structures of the urogenital region, are mediated via muscarinic receptors [24, 25]. ACh may be released not only from cholinergic nerves; in isolated human bladder tissue, a basal ACh release of nonneuronal origin has also been demonstrated [47]. The released ACh was at least partly generated by the urothelium.

Cholinergic nerves. Although histochemical methods that stain for ACh-esterase (AChE) are not specific for ACh-containing nerves [29], they have been used as an indirect indicator of cholinergic nerves. The vesicular ACh transporter (VAChT) is a marker specific for cholinergic nerve terminals [48]. In e.g., rats, bladder smooth muscle bundles were supplied with a very rich number of VAChT- positive terminals also containing NPY, NOS and VIP [49]. Similar findings have been made in human bladders of

neonates and children [50]. The muscle coat of the bladder showed a rich cholinergic innervation and small VAChT-immunoreactive neurons were found scattered throughout the detrusor muscle. VAChT-immunoreactive nerves were also observed in a suburothelial location in the bladder. The function of these nerves is unclear, but a sensory function or a neurotrophic role with respect to the urothelium cannot be excluded [50].

Muscarinic receptors. Muscarinic receptors comprise five subtypes, encoded by five distinct genes [51, 52]. The five gene products correspond to pharmacologically defined receptors, and M1 through M5 are used to describe both the molecular and pharmacological subtypes. Muscarinic receptors are coupled to G-proteins, but the signal transduction systems vary. M1, M3, and M5 receptors couple preferentially to Gq/11, activating phosphoinositide hydrolysis, in turn leading to mobilization of intracellular calcium. M2 and M4 receptors couple to pertussis toxin-sensitive Gi/o, resulting in inhibition of adenylyl cyclase activity. In the human bladder, the mRNAs for all muscarinic receptor subtypes have been demonstrated [52, 53], with a predominance of mRNAs encoding M2 and M3 receptors [53, 54]. In most animal species, detrusor smooth muscle contains muscarinic receptors of the M2 and M3 subtypes [55–57].

The M3 receptors in the human bladder are believed to be the most important for detrusor contraction and to cause contraction through phosphoinositide hydrolysis [58, 59]. The main pathway for muscarinic receptor activation of the detrusor via M3 receptors may be calcium influx via L-type calcium channels, and increased sensitivity to calcium of the contractile machinery produced via inhibition of myosin light-chain phosphatase through activation of Rho-kinase. The functional role for the M2 receptors has not been clarified, but it has been suggested that M2 receptors may oppose sympathetically mediated smooth muscle relaxation, mediated by β-ARs [60]. M2 receptor stimulation may also activate nonspecific cation channels [61] and inhibit KATP channels through activation of protein kinase C [62, 63].

Muscarinic receptors may also be located on the presynaptic nerve terminals and participate in the regulation of transmitter release. The inhibitory prejunctional muscarinic receptors have been classified as M2 in the rabbit [64, 65] and rat [66], and M4 in the guinea pig [67], rat [68], and human [69] bladder. Prejunctional muscarinic facilitation has also been detected in human bladders [70].

The muscarinic receptor functions may be changed in different urological disorders, such as outflow obstruction, neurogenic bladders, bladder overactivity without overt neurogenic cause, and diabetes [52]. However, it is not always clear what the changes mean in terms of changes in detrusor function.

Urothelial cells and cells in the lamina propria express several types of muscarinic receptors, and stimulation of these receptors may affect detrusor function [8]. The porcine urothelium was found to express a high density of muscarinic receptors, even higher than the bladder smooth muscle [71], and, in the rat and human urothelium, the receptor proteins and mRNAs, respectively, for all muscarinic receptor subtypes (M1–M5) were demonstrated [72]. In these studies, not only the urothelium but also part of the lamina propria was included in the tissues investigated. However, the expression pattern of the different subtypes in the human urothelium was reported to differ: the M1 receptors on basal cells, M2 receptors on umbrella cells, M3 and M4 receptors homogenously, and M5 receptors with a decreasing gradient from luminal to basal cells [73]. Mansfield et al. [74] found, using Real Time-Polymerase Chain Reaction (RT-PCR) analysis, an abundant expression of muscarinic M2 receptors in the human bladder mucosa (urothelium/lamina propria). Some of these receptors may occur at other locations than the urothelium, e.g., on suburothelial interstitial cells of Cajal (ICC; [75–77]). The physiological significance of what appears to be a cholinergic signaling system in the mucosa is unclear. Ikeda and Kanai [78] suggested that muscarinic receptors within the mucosa were involved in urotheliogenic signaling, enhancing intrinsic detrusor contractions. Isolated strips of porcine urothelium with lamina

propria were shown to exhibit spontaneous contractile activity that was increased by stretch. The mechanism appeared to involve endogenous ACh release acting on M3 receptors [79]. It has also been suggested that cholinergic mechanisms may be involved in the urothelial release of an unknown inhibitory factor [71, 80].

Adrenergic Mechanisms

Fluorescence histochemical studies have shown that the body of the detrusor receives a relatively sparse innervation by noradrenergic nerves. The density of noradrenergic nerves increases markedly towards the bladder neck, where the smooth muscle receives a dense noradrenergic nerve supply, particularly in the male [34, 35]. The importance of the noradrenergic innervation of the bladder body has been questioned since patients with a deficiency in dopamine β-hydroxylase, the enzyme that converts dopamine to noradrenaline (NA), void normally [81]. Noradrenergic nerves also occur in the lamina propria of the bladder, only some of which are related to the vascular supply. Their functional significance remains to be shown.

α-Adrenoceptors. The human detrusor contains both α1-ARs and α2-ARs [82]. β-ARs dominate over α-ARs and the normal detrusor response to NA is relaxation [24]. α2-ARs, mainly their α2A-subtype, are expressed in bladder, urethra and prostate. They mediate prejunctional inhibition of neurotransmitter release and also a weak contractile effect in the urethra of some species, but not humans. Their overall post-junctional function in the lower urinary tract remains largely unclear.

α1-ARs are activated by adrenaline and NA. They mediate smooth muscle contraction and other functions through members of the Gq/11 family of G proteins that stimulate the hydrolysis of inositol phosphate, liberation of calcium from the endoplasmic reticulum, and activation of genes [83].

α1-ARs have been identified and characterized extensively by functional, radioligand-binding, and molecular biological techniques. Molecular clones have been isolated and characterized for three α1-subtypes (α1a, α1b, and α1d) [84]. The subtypes can be distinguished pharmacologically on the basis of their affinity for a1-adrenoceptor antagonists [83]. The α1A-subtype generally regulates smooth muscle tone in the prostate and bladder neck, whereas the α1B-subtype contributes to regulate blood pressure via contraction of the small resistance vessels. The α1D-subtype may be involved in the bladder function and spinal cord innervations [83].

In the human detrusor α1-ARs are only poorly expressed and play a limited functional role [83, 85]. Levin et al. [86] found that in the human bladder neck region, the predominating postjunctional α-AR subtype seemed to be α1. Walden et al. [87] reported a predominance of α1A-AR mRNA in the human bladder dome, trigone, and bladder base. This contrasts with the findings of Malloy et al. [88], who found that among the high affinity receptors for prazosin, only α1A and α1D-mRNAs were expressed in the human bladder. The total α1-AR expression was low, 6.3 ± 1.0 fentomol/mg, but very reproducible. The relation between the different subtypes was α_{1D}: 66 % and α1A: 34 % with no expression of α1B.

Even if α1-ARs probably play a limited functional role in the normal detrusor they seem to be involved in the peripheral control of sympathetic supply to the bladder [82]. Thus, stimulation of the hypogastric nerve has been shown to facilitate cholinergic transmission at the level of the pelvic ganglia by the actions of α1-adrenoceptors [89] and thus also bladder contractions. Intravenous injection of α1-AR antagonists inhibited the sympathetic control of the bladder by reducing hypogastric nerve activity [90, 91] and somatic activity to the urethra [92].

Okutsu et al. [93] evaluating the effects of tamsulosin on bladder blood flow (BBF) in the normal and outflow-obstructed rats found that α1-ARs expressed in the vesical artery were α1A- > α1D with almost no expression of the α1B-subtype. Experimental findings in humans have indicated involvement of the a1D-adrenoceptor subtype in storage symptoms [83]. Thus, the α1-AR is considered responsible for the dynamic component of voiding and storage symptoms.

Expression of α-ARs in the urothelium has been well documented. Ishihama et al. [94] found

the presence of α1D-adrenoceptors in the rat urothelium and suggested that activation of these adrenoceptors facilitates the micturition reflex. They suggested that endogenous catecholamines act on α1D-receptors in the urothelium to facilitate mechanosensitive bladder afferent nerve activity and reflex voiding.

Even if the α-ARs have no significant role in normal bladder contraction, there is evidence that this may change after, e.g., bladder outlet obstruction, parasympathetic decentralization, and in hyperactive bladders [1].

β-Adrenoceptors. The β-ARs of the human bladder were shown to have functional characteristics typical of neither β_1-, nor β_2- ARs [95, 96]. Normal (as well as neurogenic) human detrusors are able to express β_1-, β_2-, and β_3-AR mRNAs and selective β_3-AR agonists effectively relaxed both types of detrusor muscle [97, 98]. An investigation comparing the subpopulation of β-AR in research animals revealed significant differences amongst species [99]. Based upon quantitative PCR experiments, it appears that the β3-AR accounts for more than 95% of all β-AR mRNA in the human bladder [100]. If the amount of mRNA reflects the population of receptor protein, β_3 ARs should mediate bladder relaxation). This is in accordance with several in vitro studies [97, 98] and it seems that atypical β-AR-mediated responses reported in early studies of β-AR antagonists are mediated by β_3-ARs. It may be speculated that in bladder overactivity, there is a lack of an inhibitory β-AR-mediated noradrenaline response.

The physiological role of the sympathetic system for bladder relaxation remains unclear, however, β-AR stimulation is an effective mechanism to increase bladder capacity, as illustrated by the clinical use of the β3-AR agonist mirabegron for treatment of the overactive bladder [101]. At the mRNA level, all three subtypes are detectable in the bladder. Whereas in the rat bladder, the abundance of the three subtypes appears similar [102], in the human bladder, >95% of all β-AR mRNA belongs to the β3 subtype [100]. At the protein level, β1- and β2-ARs have been identified by radioligand binding in the bladder of humans [85] and several animals species [83], whereas a lack of β3-ARs detection is primarily attributable to a lack of suitable tools rather than a lack of presence of this subtype. Bladder smooth muscle relaxation upon β-AR stimulation has been demonstrated in many species including rats, rabbits, guinea pigs, ferrets, cats, dogs, pigs, monkeys, and humans; the maximum relaxation appears similar across species, but agonist potency may differ between them [83]. Moreover, efficacy and potency of β-AR agonists depends markedly on the stimulus used to induce bladder contraction [83, 103]. Of note, bladder relaxation occurs mainly in the detrusor and not necessarily in the bladder neck.

Non-adrenergic, Non-cholinergic Mechanisms (NANC)

In most mammalian species, part of the neuronally-induced bladder contraction is *resistant to atropine*, which blocks cholinergic muscarinic receptors [24, 52]. The proportion of NANC-mediated response to the total contraction seemed to vary with species and the frequency of stimulation. Thus, in rats and guinea-pigs, atropine has little effect on the response to single nerve stimuli, but at 20 Hz, it inhibits about 25% of the response. Corresponding figures for rabbit and pig were 40% and 75%, respectively. In strips of normal human bladders, the reported degrees of atropine resistance have varied from a few % to up to 50 % ([104]; see [25]). Luheshi and Zar [105] investigated whether the full atropine-sensitivity of the human detrusor, reported by some investigators, was due to a genuine absence of a non-cholinergic element in its motor transmission, or if it was dependent on the experimental protocols. Using a specially designed stimulation protocol, they found that part of the electrically induced response (about 30%) was resistant to atropine. Most probably, normal human detrusor muscle exhibits little atropine resistance. This does not exclude that atropine resistance can increase in morphologically and/or functionally changed bladders, and that it plays a role in the activation of the bladder.

Adenosine 5′-triphosphate. Evidence has been presented [24] that the atropine-resistant contractile component evoked in human detrusor

by electrical stimulation can be abolished by α,β-methylene ATP, suggesting that the NANC mediator is ATP. Husted et al. [106] showed that ATP produced a concentration-dependent contraction in isolated human detrusor muscle, but also that ATP influenced the responses to transmural nerve stimulation, probably by both prejunctional and postjunctional effects. The contractile effects of ATP are mediated through stimulation of P_{2X} receptors.

Two P_{2X} receptor subtypes are suggested to play a role in the bladder, P_{2X1} and P_{2X3}. Using RT-PCR, Hardy et al. [107] demonstrated the presence of the P_{2X1} receptor subtype in the human bladder, and confirmed that activation of purinergic P_{2X} receptors, putatively P_{2X1}, may be important in the initiation of contraction in human detrusor. Purinergic transmission seemed to be more important in muscle taken from patients with bladder instability. Their results also indicated the possibility that human bladder expresses multiple isoforms of the P_{2X1} receptor which may be potential sites for modifying or regulating putative purinergic activation of the human bladder. Supporting such a concept, mice deficient in P_{2X3} receptors exhibited a marked urinary bladder hyporeflexia, characterized by decreased voiding frequency and increased bladder capacity, but normal bladder pressures [108–110]. This could be caused by decreased afferent and/or efferent signaling. Immunohistochemical studies localized P_{2X3} receptors to nerve fibres innervating the urinary bladder of wild-type mice and showed that loss of this receptor subtype did not alter sensory neuron innervation density. P_{2X3} receptors thus seemed to be critical for peripheral afferent pathways (urothelial signaling) controlling urinary bladder volume reflexes. Available results suggest that ATP may contribute to excitatory neurotransmission in the bladder, both by stimulation of the detrusor and afferent nerves. The importance of this for the emptying contraction of the human bladder under normal and pathophysiological conditions remains to be established.

However, ATP is released not only from parasympathetic nerves, but also from the urothelium [109, 111]. During bladder filling, the urothelium is stretched and ATP is released from the umbrella cells thereby activating mechanotransduction pathways. ATP release can also be induced by various mediators present in the urine and and/or released from nerves or other components of the lamina propria. Urothelial release of ATP is mainly attributable to vesicular transport or exocytosis and, to a smaller extent, to pannexin hemichannel conductive efflux. After release, ATP acts on P2X3 and P2X2/3 receptors on suburothelial sensory nerves to initiate the voiding reflex and to mediate the sensation of bladder filling and urgency. ATP also acts on suburothelial interstitial cells/myofibroblasts generating an inward Ca2+ transient that via gap junctions could provide a mechanism for long-distance spread of signals from the urothelium to the detrusor muscle.

Neuropeptides. The functional roles of the many neuropeptides that have been demonstrated to be synthetized, stored, and released in the human lower urinary tract [112–115] have not been established. Neuropeptide-containing, capsaicin-sensitive primary afferents in the bladder and urethra may not only have a sensory function ("sensory neuropeptides"), but also a local effector or efferent function. In addition, they may play a role as neurotransmitters and/or neuromodulators in the bladder ganglia and at the neuromuscular junctions. As a result, the peptides may be involved in the mediation of various effects, including micturition reflex activation, smooth muscle contraction, potentiation of efferent neurotransmission, and changes in vascular tone and permeability. Evidence for this is based mainly on experiments in animals. Studies on isolated human bladder muscle strips have failed to reveal any specific local motor response attributable to a capsaicin-sensitive innervation [114]. However, cystometric evidence that capsaicin-sensitive nerves may modulate the afferent branch of the micturition reflex in humans has been presented [116]. In a small number of patients suffering from bladder hypersensitivity disorders, intravesical capsaicin produced a long-lasting, symptomatic improvement [117].

Tachykinins are fast-acting peptides. Three endogenous tachykinins, Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are widely distributed in the central and peripheral nervous system and bind to the three tachykinin-receptors (NK_1, NK_2 and NK_3). Nociceptive transmission is mainly mediated through NK_1-receptors. In the urinary tract they are suggested to act in afferent as well as efferent pathways [112].

Tachykinins have contractile effects in the human bladder [112, 118]. The potency of neurokinins was shown to be NKA > NKB » SP. This, and results with subtype selective agonists [118], suggested that the tachykinin receptor mediating contraction in the human bladder is of the NK_2 type.

Substance P (SP) and various related peptides were shown to have contractile effects in isolated bladder smooth muscle from various species. The potential role of SP in the atropine resistant component of the contractile response induced by electrical stimulation has been studied by several investigators (see [24]). With few exceptions, these studies did not favor the view that SP, released from postganglionic nerve-terminals, has an excitatory transmitter role. Evidence has been presented, on the other hand, that SP may play a role in the afferent, sensory branch of the micturition reflex [24, 115].

*Vasoactive intestinal polypeptide (VIP)*was shown to inhibit spontaneous contractile activity in isolated detrusor muscle from several animal species and from humans, but to have little effect on contractions induced by muscarinic receptor stimulation or by electrical stimulation of nerves (see [24, 115]). In isolated rat bladder, VIP had no effect, and in isolated guinea-pig bladder, VIP produced contraction. Stimulation of the pelvic nerves in cats increased the VIP output from the bladder, and increased bladder blood flow, although moderately [119]. VIP injected i.v. induced bladder relaxation in dogs [120]. On the other hand, VIP given i.v. to patients in a dose causing increases in heart rate, had no effect on cystometric parameters [121]. Plasma concentrations of VIP were obtained which, in other clinical investigations, had been sufficient to cause relaxation of smooth muscle [121].

Calcitonin gene-related peptide (CGRP) is a widely distributed in nerve endings in the bladder and considered a sensory neuromodulator [114, 115]. However, the role of CGRP in control of bladder motility is controversial. In pig detrusor CGRP did not alter the response to potassium, carbachol, substance P, or EFS [122]. In hamsters, CGRP caused dose-dependent inhibition of the response to EFS, but about 20% of the preparations were non-responders [123]. In human detrusor strips the relaxing effect of CGRP on carbachol-induced contraction was negligible, despite a slight increase in cGMP levels [124].

Neuropeptide Y (NPY). The human bladder is richly endowed with NPY containing nerves [125, 126]. NPY and noradrenaline are stored in separate vesicles at sympathetic nerve terminals, NPY is preferentially released at high frequency stimulation. It seems as if NPY can be found in adrenergic as well as cholinergic nerves.

The presence of functional NPY receptors in human bladder was investigated by [127]) using peptide YY (PYY) as the agonist and [^{125}I] PYY as the radioligand, and they found that human bladder expresses only very few if any functional NPY receptors. In neonates and children, Dixon et al. [50] found small ganglia scattered throughout the detrusor muscle of urinary bladder. Approximately 75% of the intramural neurons were VAChT immunoreactive, whereas approximately 95% contained NPY and approximately 40% contained NOS. VAChT-immunoreactive nerves were also observed in a sub-epithelial location in all the organs examined, the majority containing NPY, whereas a small proportion contained NOS. In animal bladders, NPY-containing nerves were shown to be present in abundance in the rat detrusor (see [24]). Available in formation on the effects of NPY on detrusors from different species is conflicting [128–130]. Even if it has been suggested that NPY may have an important role in the neural control of the lower urinary tract in the rat [131], there is no convincing information that this is the case in humans.

Prostanoids. Prostaglandin synthesis in detrusor and mucosa is initiated by several stimuli, as stretch, injury, nerve stimulation, and mediators of inflammation. PGE_2 has been shown

to mediate bladder contraction and increase bladder pressure after intra-arterial as well as intravesical application in rats and humans [24, 132, 133]. The mechanism of action is still not fully established, but most likely the effects are caused via influences on neurotransmission. It appears that prostanoids cause the release of tachykinins from nerves, which stimulate NK-1 and NK-2 receptors, as the above effects were blocked by selective NK-1 and NK-2 receptor antagonists [132].

Nitric oxide. Evidence has accumulated that L-arginine-derived nitric oxide (NO) is responsible for the main part of the inhibitory NANC responses in the lower urinary tract [24, 30]. In biopsies taken from the lateral wall and trigone regions of the human bladder, a plexus of NADPH-diaphorase containing nerve fibers was found [134]. Samples from the lateral bladder wall contained many NADPH-reactive nerve terminals, particularly in the subepithelial region immediately beneath the urothelium; occasionally they penetrated into the epithelial layer. Immunohistochemical investigations of pig bladder revealed that the density of NO synthase (NOS)-immunoreactivity was higher in trigonal and urethral tissue than in the detrusor [30].

In small biopsy preparations of the human detrusor James et al. [135] found that electrical stimulation evoked relaxations sensitive to N^G-nitro-L-arginine, but insensitive to tetrodotoxin. They suggested that NO might be generated from the detrusor and an important factor for bladder relaxation during the filling phase. However, others have been unable to obtain relaxation in rat isolated detrusor muscle precontracted by carbachol or potassium in response to electrical field stimulation [136, 137].

In the pig detrusor, the NO-donor, SIN-1, and NO relaxed carbachol and endothelin-1 contracted preparations by approximately 60%, but isoprenaline was about 1000 times more potent than SIN-1 and NO and caused complete relaxation. Nitroprusside, SIN-1, and NO were only moderately effective in relaxing isolated rat, pig, and rabbit detrusor muscle, compared to their effects on the urethral muscle [138]. These results agree well with those of [139], who found that in rabbits, cyclic GMP is mainly related to urethral relaxation and cyclic AMP to urinary bladder relaxation. Thus, it is unlikely that NO has a role as a neurotransmitter causing direct relaxation of the detrusor smooth muscle, since the detrusor sensitivity to NO and agents acting via the cyclic GMP system is low. This does not exclude that NO may modulate the effects of other transmitters, or that it has a role in afferent neurotransmission.

TRP Channels. Detailed information on TRP channels and LUT function can be found in several recent reviews [140–144]. These studies have indicated that several transient receptor potential (TRP) channels, including TRPV1, TRPV2, TRPV4, TRPM8 and TRPA1, are expressed in the bladder and may act as sensors of stretch and/or chemical irritation. They are highly expressed in, but not restricted to, primary afferent neurons. Thus, the urothelium [145], some interstitial cells and detrusor muscle also express several TRP channels [143, 146, 147]. There seem to be several links between activation of these channels and bladder dysfunction, and the therapeutic potential for TRPV1 channel agonists (capsaicin, resiniferatoxin) has been convincingly demonstrated. Animal studies have shown that inhibition of these pathways can be effective for the reduction in bladder activity. However, the roles of these channels for normal function and in pathological states have not been established. Nevertheless, TRP channels still may be most exciting targets for future LUT drugs. LUT dysfunction may not have been given the highest priority in TRP drug development, but research carried out for nonbladder diseases may be possible to apply also to LUT disorders.

TRPV1. TRPV1 is the best-characterized member of the TRPV subfamily TRPV1-6) in terms of expression pattern, properties, and clinical translation of its manipulation [148]. It is a non-selective cationic channel with high Ca^{2+} permeability allowing the passage of cations, mainly calcium, upon activation by vanilloids, noxious heat and low pH [149, 150]. TRPV1 expression has been observed in neuronal and non-neuronal human and rat LUT tissues including the urothelium, suburothelial nerve plexus, detrusor smooth

muscle, ICC, and sensory afferent neurons. There is evidence for TRPV1 expression in small diameter bladder afferent fibres in close proximity to the urothelium and in bladder sensory neurons in the dorsal root ganglia (DRG). However, the expression in the bladder particularly in the urothelium, has been controversial [143].

Despite extensive information on morphology and function in animal models, the role of TRPV1 in normal human bladder function is still controversial. However, its role in the pathophysiology and treatment of particularly neurogenic DO (NDO) is well established [140–142, 144].

TRPV2. TRPV2 is a nonselective cation channel with high Ca2+ permeability; it acts as a heat sensor with a temperature threshold of 50–52 8C and is activated by agonists such as 2-aminoethoxydiphenyl borate and D9-tetrahydrocannabinol (THC) [151]. In vascular smooth muscle cells TRPV2 is stretch-activated channel and can increase stretch-induced [Ca2+] i [152]. In rat urinary bladder TRPV2 mRNA is expressed in urothelial and smooth muscle cells and the channel is also functionally expressed in mouse urothelial cells [153, 154]. In the human bladder, Caprodossi et al. [155] found TRPV2 expression in normal human urothelial cells and bladder tissue specimens. The TRPV2 channel is also highly expressed in sensory DRG neurons. Even if TRPV2 channels are expressed in different parts of the bladder, its functional significance is still unclear. It has been suggested that TRPV2 has a role as a sensor of urothelium stretch and a pivotal role in bladder cancer development [146].

TRPV4. TRPV4 is a Ca^{2+}-permeable stretch-activated cation channel, which is expressed in rat and mouse urothelial and detrusor muscle cells. The activation of TRPV4 induces significant increases in $[Ca2+]_i$ in rat urothelial cells, leading to ATP release and modulation of afferent nerve activity in response increases in intravesical pressure. Ca2+ influx through TRPV4 appears to activate BK channels to suppress spontaneous contractions and thus a functional coupling of TRPV4 with BK channels may act as a self-limiting mechanism for bladder contractility during its storage phase [156]. Supporting this, Lee et al. [157] demonstrated that Ca^{2+} influx throughTRPV4 channels can activate SK channels in PDGFRα+ cells and prevent bladder overactivity during filling.

TRPV4 has been suggested to be an important urothelial mechanosensor for bladder distension [146, 153, 154]. Gevaert et al. [158] raised the possibility that TRPV4 plays a critical role in urothelium-mediated transduction of intravesical mechanical pressure. Mochizuki et al. [159] suggested that the TRPV4 channel participates in the mechanosensory pathway in urinary bladder and that mechanical stimulus-dependent activation of TRPV4 in urothelial cell layers is a key event for ATP signaling in the micturition reflex pathway.

Takaoka et al. [160] using rats with bilateral pelvic nerve crush and showing characteristics of detrusor underactivity (DU), and this model intravesical application of the TRPV4 agonist GSK1016790A significantly decreased ICI, bladder capacity, voided volume, and PVR without increasing non-voiding contractions (NVCs), and these effects were blocked by the TRPV4 antagonist RN1734. In contrast, GSK1016790A had no significant effects on CMG parameters in normal rats. Deruyver et al. [161], using female wild-type and Trpv4 knockout rats that underwent sham surgery or bilateral pelvic nerve injury. Rats with nerve injury showed DU with low-amplitude voiding contractions, decreased voiding frequency, and increased postvoid residual. Intravesical application of GSK1016790A increased voiding frequency and reduced postvoid residual in wild-type, but not Trpv4−/−, rats. In isolated bladder strips, GSK1016790A did not induce relevant contractions. These studies suggested a potential for TRPV4 for treatment of DU.

TRPA1. TRPA1 is the only mammalian member of the Ankyrin TRP subfamily. TRPA1-channels are predominantly expressed in sensory afferent nerve endings [162], but their expression and sensory function in the epithelial cells is species-specific, with a virtual absence of TRPA1 expression in the detrusor smooth muscle (DSM) cells [163]. In addition to being present in nerve endings, TRPA1-channels can be found in the mucosa of the human bladder. Agonists of TRPA1-channels are known to induce

concentration-dependent contraction of isolated muscle strips of the rat bladder via stimulation of TRPA1-expressing sensory fibers [164]. The contractile effect of TRPA1 on detrusor smooth muscle (DSM) may to be due to release from sensory afferents of inflammatory factors—tachykinins and prostaglandins. Streng et al. [165] investigated the effects of H_2S and known TRPA1 activators on micturition in conscious rats. The found that intravesical TRPA1 activators initiate detrusor overactivity indicating that TRPA1 may have a role in sensory transduction in this organ.

TRPM8. TRPM8 channels are known to be activated by low temperatures (<18–28 °C) and chemical agents such as menthol [166]. It has been reported that TRPM8 channels are expressed in urothelial cells and in sensory nerve fibres located in the urothelium and suburothelial space of the bladder and L6 dorsal root ganglia (DRG) of the rat and guinea-pig. TRPM8 may have a role in activation of bladder afferent pathways during filling of the bladder in the normal rat [167], an effect that at least partlyseems to be mediated via mechanosensitive C-fibres. TRPM8 channels may be involved in the bladder cooling reflex I humans [146, 168], and TRPM8 channels may have a role in activation of bladder afferent pathways during filling of the bladder in the normal rat. The positive correlation between the density of TRPM8 in the bladder mucosa and voiding frequency in detrusor overactivity, and also increased TRPM8 expression in bladder pain patients, led to the suggestion that this channel was involved in the symptomatology and pathophysiology of these disorders [75, 77].

Basic Urethral Physiology/ Pharmacology

Under normal conditions, there is a reciprocal relationship between the activity in the detrusor and the activity in the outlet region. Sufficient contraction of the urethral smooth muscle is an important key function in order to provide continence during the storage phase of the micturition cycle. Equally important is a coordinated and complete relaxation during the voiding phase. During voiding, contraction of the detrusor muscle is preceded by a relaxation of the outlet region, thereby facilitating bladder contraction. In humans, the normal pattern of voiding is characterized by an initial drop in urethral pressure followed by an increase in intravesical pressure [24].

Many factors have been suggested to contribute to urethral relaxation and to urethral closure, including urethral smooth muscle tone, and the properties of the urethral lamina propria [169, 170]. The role of NA as a main contractant factor is well established. In contrast, the mechanisms involved in urethral relaxation seem to be more complicated and several factors may contribute. One possibility is that the fall in intraurethral pressure is caused by stimulation of muscarinic receptors on noradrenergic nerves diminishing NA release and thereby tone in the proximal urethra. Another is that contraction of longitudinal urethral smooth muscle in the proximal urethral, produced by released ACh, causes shortening and widening of the urethra, with a concomitant decrease in intraurethral pressure. A third possibility is that a NANC mechanism, including NO, mediates this response [24, 169, 170].

Adrenergic Mechanisms

Adrenergic nerves. The well-known anatomical differences between the male and female urethra are also reflected in the innervation. In the human male, the smooth muscle surrounding the preprostatic part of the urethra is richly innervated by both cholinergic and adrenergic nerves [34], and considered the "sexual sphincter", contracting during ejaculation and thus preventing retrograde transport of sperm. The role of this structure in maintaining continence is unclear, but probably not essential.

In the human female, the muscle bundles run obliquely or longitudinally along the length of the urethra, and in the whole human female urethra, as well as in the human male urethra below the pre-prostatic part, there is only a scarce supply of adrenergic nerves [35, 171]. Fine varicose terminals can be seen along the bundles of smooth muscle cells, running both longitudinally

and transversely. Adrenergic terminals can also be found around blood vessels. Colocalization studies in animals have revealed that adrenergic nerves, identified by immunohistochemistry (tyrosine hydroxylase; TH) also contain neuropeptide Y (NPY) [36]. Chemical sympathectomy (6-OH-dopamine) in rats resulted in a complete disappearance of TH-immunoreactive (IR) nerves, while NO synthase (NOS)-containing nerve fibres were not affected by the treatment [37]. This suggests that NOS is not contained within adrenergic nerves.

α_1-Adrenoceptors. In humans, up to about 50% of the intraurethral pressure is maintained by stimulation of α-ARs, as judged from results obtained with α-AR antagonists and epidural anesthesia in urodynamic studies [172, 173]. In human urethral smooth muscle, both functional and receptor binding studies have suggested that the α_1-AR subtype is the predominating postjunctional α-AR (see [24, 174]). Most in vitro investigations of human urethral α-ARs have been carried out in the male, and the results support the existence of a sphincter structure in the male proximal urethra, which cannot be found in the female. Other marked differences between sexes in the distribution of α_1-and α_2-ARs (as can be found in e.g., rabbits), or in the distribution of α_1-AR subtypes, do not seem to occur [175]. Separating the entire length of the isolated female human urethra into seven parts, from the external meatus to the bladder neck, it was found that NA (α_1-and α_2), but not clonidine (α_2), produced concentration-dependent contractions in all parts, with a peak in middle to proximal urethra [176].

Among the three high-affinity α_1-AR subtypes (α_{1A}, α_{1B}, $\alpha_{1\Delta}$) identified in molecular cloning and functional studies, α_{1A} seems to predominate in the human lower urinary tract [175, 177, 178].

Urethral α_2-ARs are able to control the release of NA from adrenergic nerves as shown in in-vitro studies. In the rabbit urethra, incubated with [^{3}H]NA, electrical stimulation of nerves caused a release of [^{3}H] which was decreased by NA and clonidine, and increased by the α_2-AR antagonist rauwolscine [179]. Clonidine was shown to reduce intraurethral pressure in humans [180], an effect that may be attributed partly to a peripheral effect on adrenergic nerve terminals. More probable, however, this effect is exerted on the central nervous system with a resulting decrease in peripheral sympathetic nervous activity. The subtype of prejunctional α_2-AR involved in [^{3}H] NA secretion in the isolated guinea-pig urethra was suggested to be of the α_{2A}-subtype [181].

Prejunctional α_2-AR regulation of transmitter release is not confined to adrenergic nerves (see [24]). It was found that electrical field stimulation (EFS; frequencies above 12 Hz) of spontaneously contracted smooth muscle strips from the female pig urethra, evoked long-lasting, frequency-dependent relaxations in the presence of prazosin, scopolamine, and the NOS inhibitor, N^G-nitro-L-arginine (L-NOARG), suggesting the release of an unknown relaxation-producing mediator [182]. Treatment with the selective α_2-AR agonist UK-14 304 markedly reduced the relaxations evoked by EFS at all frequencies tested (16–30 Hz). The inhibitory effect of UK-14 304 was completely antagonized by rauwolscine, and the results suggested that the release of the unknown mediator in the female pig urethra can be modulated via α_2-ARs.

β-Adrenoceptors. The presence of β-AR protein in the urethra of rabbits and humans has been reported by radioligand binding studies [83]. In pigs, urethral relaxation by isoprenaline is mediated by both β2- and β3-ARs but the predominant β-AR subtype present in both the bladder and urethra was the β3-AR [183]. β-AR agonists can reduce urethral pressure in vivo in rats [184], cats [185], and humans [186]. While such urethral relaxation and hence reduction of bladder outlet resistance may be undesirable in the treatment of overactive bladder and urgency incontinence, the smaller magnitude of effects in the urethra as compared to those in the bladder make it unlikely that direct effects on the urethra limit the usefulness of β-AR agonists, specifically β3-selective drugs, in the treatment of bladder dysfunction. Alexandre et al. [187] investigated the effects of β3-AR agonist mirabegron in mouse urethra and found that effects were the result of β3-ARagonism together with α1A and α1D-AR antagonism. Although this effect might be an interesting pharmacological in vitro observation,

it seems to have no relevance for the clinical use of mirabegron in the treatment of the overactive bladder [142, 144].

Although the functional importance of urethral β-ARs has not been established, they have been targets for therapeutic interventions. Selective β_2-AR agonists have been shown to reduce intraurethral pressure [188–190], but β-AR antagonists have not been shown to influence intraurethral pressure acutely [186]. The theoretical basis for the use of β-AR antagonists in the treatment of stress incontinence is that blockade of urethral β-ARs may enhance the effects of NA on urethral α-ARs. Even if propranolol has been reported to have beneficial effects in the treatment of stress incontinence, this does not seem to be an effective treatment. Selective β_2-AR antagonists have been used as a treatment of stress incontinence, it seems paradoxical that the selective β_2-AR agonist, clenbuterol, was found to cause significant clinical improvement and increase in maximal urethral pressure in women with stress incontinence. The positive effects were suggested to be a result of an action on urethral striated muscle and/or the pelvic floor muscles [191].

Cholinergic Mechanisms

Cholinergic nerves. The urethral smooth muscle receives a rich cholinergic innervation of which the functional role is largely unknown. Most probably, the cholinergic nerves cause relaxation of the outflow region at the start of micturition by releasing NO and other relaxant transmitters. Co-localization studies in the pig urethra revealed that ACh esterase (AChE) positive and some NOS-IR nerves had profiles that were similar. These nerves also contained NPY and VIP immunoreactivity. NOS-containing nerves were present in a density lower than that of the AChE positive nerves, but higher than the density of any peptidergic nerves [192]. Coexistence of ACh and NOS in the rat major pelvic ganglion was demonstrated by double immunohistochemistry using antisera raised against NOS and choline acetyltransferase [49]. In the rat urethra, co-localization studies using VAChT antibodies confirmed that NOS and VIP are contained within a population of cholinergic nerves. Investigating the distribution of immunoreactivities to neuronal NOS (nNOS), heme oxygenases (HO), and VIP, HO-2 immunoreactivity was found in all nerve cell bodies of intramural ganglia, localized between smooth muscle bundles in the detrusor, bladder base and proximal urethra [193]. About 70% of the ganglionic cell bodies were also NOS-immunoreactive, whereas a minor part was VIP-immunoreactive.

Muscarinic receptors. The distribution and number of muscarinic receptors in different parts of the urethra seems to vary. Compared to the bladder, the number of muscarinic receptor binding sites in the rabbit urethra was lower [194], and by autoradiography, it was demonstrated that muscarinic receptors were abundant in the outer parts of the urethral wall and decreased in density in luminal direction [195]. Muscarinic receptor agonists contract isolated urethral smooth muscle from several species, including humans, but these responses seem to be mediated mainly by the longitudinal muscle layer [24, 35]. Taki et al. [176], investigating the whole length of the female human urethra, found that ACh contracted only the proximal part and the bladder neck. If this contractile activation is exerted in the longitudinal direction, it should be expected that the urethra is shortened and that the urethral pressure decreases. Experimentally, in vitro resistance to flow in the urethra was increased only by high concentrations of ACh [196, 197]. Prejunctional muscarinic receptors may influence the release of both noradrenaline and ACh in the bladder neck/urethra. In urethral tissue from both rabbit and humans, carbachol decreased and scopolamine increased concentration dependently the release of [^{3}H] noradrenaline from adrenergic and of [^{3}H] choline from cholinergic nerve terminals [179]. At least theoretically, this would mean that released ACh could inhibit noradrenaline release, thereby decreasing urethral tone and intraurethra l pressure. However, in humans, tolerable doses of the muscarinic receptor agonist, bethanechol [198], and the antagonist, emeprone [199], had little effect on intraurethral pressure. The muscarinic receptor subtypes involved in contractile effects

on smooth muscle, or controlling transmitter release in the urethra, have not been established. It has been reported that M1, M2, and M3 receptors all mediate contraction of the circular muscle of the rabbit urethra after stimulation with carbachol [200, 201].

Non-adrenergic, Non-cholinergic Mechanisms (NANC)

The mechanical responses of the cat urethra to autonomic nerve stimulation and to intraarterial ACh injection were analysed by Slack and Downie [202]. Sacral ventral root stimulation produced an atropine-sensitive constriction when basal urethral resistance was low, but dilatation when resistance was high. The latter response was reduced, but not abolished, by atropine. When urethral constriction had been produced by phenylephrine, injection of ACh produced a consistent decrease in urethral resistance, which was not reduced by atropine. It was suggested that parasympathetic dilatation of the urethra may be mediated by an unknown NANC transmitter released from postganglionic neurons. There are reasons to believe that this transmitter is NO.

Nitric oxide. NO appears to be an important inhibitory neurotransmitter in the lower urinary tract [24, 203]. NO-mediated responses in smooth muscle preparations are found to be linked to an increase in guanosine 3′,5′-cyclic monophosphate (cyclic GMP) formation, which has been demonstrated in the rabbit and pig urethra [139, 204–206]. Subsequent activation of a cyclic GMP-dependent protein kinase (cGK) has been suggested to hyperpolarize the cell membrane, probably by causing a leftward shift of the activation curve for the K^+-channels, thus increasing their open probability [207, 208]. There have also been reports suggesting that NO in some smooth muscles, might act directly on the K^+-channels [209, 210]. Other mechanisms for NO-induced relaxations, mediated by cyclic GMP, might involve reduced intracellular Ca^{2+} levels by intracellular sequestration, or reduced sensitivity of the contractile machinery to Ca^{2+} [211], both mechanisms acting without changing the membrane potential.

Electrophysiological registrations from urethral smooth muscle are scarce, probably due to the technical difficulties caused by the large amounts of connective tissue. However, Ito & Kimoto reported a hyperpolarization following NANC-stimulation in some preparations of urethral smooth muscle from male rabbits [212]. Furthermore, KRN 2391, a combined NO-donor and K^+-channel opener, had a pronounced relaxant effect accompanied by a hyperpolarization in the female rabbit urethra [213]. These effects were suggested being mediated predominantly through NO-dependent mechanisms, since the relaxant effect was less sensitive to K^+-channel blockade. However, it cannot be excluded that the hyperpolarization was a pure K^+-channel opening effect, and not mediated by NO.

It appears reasonable to believe that the relaxant effect of NO in rabbit and pig urethra is mediated by increased levels of cyclic GMP. Evidence for this has been demonstrated by several investigators [139, 204–206]. Accordingly, the cyclic GMP-analog, 8-Br-cyclic GMP, was able to induce relaxation of rabbit urethra, further supporting the view of cyclic GMP as a mediator of relaxation also in this tissue. The mechanisms by which cyclic GMP induces relaxation probably involves stimulation of a cGK, which phosphorylates K_{Ca} channels and thus increases their open probability, leading to an hyperpolarization [208]. Furthermore, cyclic GMP might affect the sequestering of intracellular Ca^{2+}, affect Ca^{2+} extrusion pumps and/or decrease the sensitivity for Ca^{2+} [211]. The latter may occur without changing the membrane potential. Thus, cyclic GMP may be able to induce relaxation in different ways in different tissues.

The role of NO for urethral relaxation was further investigated in mice lacking cGK type I (cGKI) [214]. In urethral preparations of cGKI+/+ mice, EFS elicited frequency-dependent relaxations. The relaxations were abolished by L-NOARG, and instead a contractile response to stimulation was generally found. In cGKI−/− urethral strips, the response to EFS was practically abolished, but a small relaxation generally appeared at high stimulation frequencies (16–32 Hz). This relaxant response was not

inhibited by L-NOARG, suggesting the occurrence of additional relaxant transmitter(s).

The rich occurrence of NOS-IR nerve fibres also supports the present view of NO as the main inhibitory NANC-mediator in rabbit urethra [205]. To localize target cells for NO, cyclic GMP antibodies have been used and Waldeck et al demonstrated spindle-shaped cyclic GMP-IR cells, distinct from the smooth muscle cells, forming a network around and between the urethral smooth muscle bundles [215]. These results confirmed the findings of Smet et al. [216] who found similar cyclic GMP-IR interstitial cells in the guinea-pig and human bladder/urethra. In contrast to the results of Waldeck et al. [215], Smet et al. [216] also found smooth muscle cells with cyclic GMP-immunoreactivity in the urethra. The occurrence of cyclic GMP-immunoreactivity in smooth muscle cells seems logical, since NO is believed to stimulate guanylyl cyclase with subsequent cyclic GMP-formation in the cells. The function of the interstitial cells has not been established, but since they have morphological similarities to the interstitial cells of Cajal in the gut, it has been speculated that they also may have a similar function. There seems to be no experimental basis for this speculation.

Carbon monoxide. The role of CO in urethral function is still controversial. It has been assumed, but not proven, that CO causes relaxation through the cGMP pathway [217]. Waldeck et al. [215] found only weak relaxant effects of exogenous CO in the rabbit urethra, compared to NO, concluding that CO is not an important mediator of relaxation in this tissue [215]. Nonetheless there are known interspecies differences of urethral relaxant responses to CO. In guinea pigs the maximal relaxant response to CO did not exceed 15 ± 3%, compared to 40 ± 7% in pigs [193, 206]. The distribution of the CO producing enzymes haem oxygenases, HO-1 and –2, was studied by immunohistochemistry in the pig lower urinary tract [182]. HO-2 immunoreactivity was observed in coarse nerve trunks in the urethra, and HO-1 immunoreactivity was seen in nerve cells, coarse nerve trunks and varicose nerve fibres within urethral smooth muscle. In urethral smooth muscle preparations, exogenously applied CO evoked a small relaxation associated with a small increase in cyclic GMP, but not cyclic AMP, content. CO-evoked relaxations were not significantly reduced by treatment with methylene blue, or by inhibitors of voltage-dependent (4-aminopyridine), high (iberiotoxin, charybdotoxin) and low (apamin) conductance Ca(2+)-activated, and ATP-sensitive (glibenclamide) K+ channels.

The inhibitory innervation of guinea-pig urethral smooth muscle was investigated histochemically and functionally [193]. HO-2 immunoreactivity was found in all nerve cell bodies of intramural ganglia, localized between smooth muscle bundles in the detrusor, bladder base and proximal urethra. In rabbit urethral smooth muscle, CO produced a small relaxant effect without change in membrane potential, and it was concluded that CO is less likely to be involved in the inhibitory neurotransmission in this tissue [215].

Taken together, available results do not support a transmitter role of CO in urethral smooth muscle. However, a messenger function CO cannot be excluded. Naseem et al. [217] found a weak relaxation by CO (compared to NO) in the rabbit urethra. In the presence of hydrogen peroxide, the urethral relaxation responses to both CO and NO were significantly increased, and it was suggested that hydrogen peroxide may amplify NO and CO-mediated responses. In the female pig urethra an even more pronounced increase in relaxant response to CO was found, using YC-1, a stimulator of sGC, suggesting a possible role for CO as possible messenger function for urethral relaxation [206].

Adenosine 5′-triphosphate. ATP is widely considered is an inhibitory neurotransmitter in the urethra since EFS of intramural nerves in strips of urethra or bladder neck smooth muscle produced relaxation that was mimicked by ATP and inhibited by purinergic receptor antagonists. ATP was suggested to cause smooth muscle relaxation via G-protein coupled P2Y receptors [177] and in hamster urethra, Western blotting analysis showed expression of both P2Y1and P2Y2. The relaxant response to ATP in this preparation could be mediated via these receptors [218] but

ATP may also induce relaxation via breakdown to adenosine [218–222].

Spontaneous myogenic tone in the urethra were shown to be associated with spontaneous transient depolarizations and large, regularly occurring slow waves [223, 224]. Sergeant et al. [225] suggested that this activity originated in specialized pacemaker cells (interstitial cells of Cajal: ICC). The frequency of pacemaker activity in ICC was increased by noradrenaline [226] and decreased by NO leading to the suggestion that ICC may also act as targets for neurotransmitters in the urethra [227].

Contractile responses to ATP or related compounds have also been described [228, 229]. Bradley et al. [229], found that exogenous application of ATP evoked robust contractions of strips of rabbit proximal urethral smooth muscle. These contractions were inhibited by the P2 blocker suramin and the selective P Y1 receptor antagonist MRS2500 and the authors suggested that they were mediated by the activation of P2Y receptors on interstitial cells of Cajal. Further study showed that stimulating purinergic nerves in rabbit urethral smooth muscle induced contractions via the activation of P2X receptors on smooth muscle cells [230].

Neuropeptides. *[*231*]* found abundant VIP-, CGRP-, SP-, and NPY-immunoreactive nerve fibers in the adventitia, muscularis, and lamina propria of proximal and distal segments of the mouse urethra. A proportion of fibers were closely associated with blood vessels, glands, and cells immunoreactive for PGP9.5. The epithelium contained abundant nerve fibers immunoreactive for CGRP and/or SP. Abundant fibers were traced from L5-S2 DRG to all urethral regions and the authors concluded that spinal afferent endings in the urethra would impact urethral function. However, the functional importance of most of the peptides described has not been established.

Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are expressed in the neural pathways regulating the lower urinary tract [115, 232]. VIP-immunoreactivity (IR) is present in afferent and autonomic efferent neurons innervating the bladder and urethra, whereas PACAP-IR is present primarily in afferent neurons.

In various species, VIP-containing urethral ganglion cells have been demonstrated, and numerous VIP-IR nerve fibres have been observed around ganglion cells, in the bladder neck, in the urethral smooth muscle layers, in lamina propria, and in association with blood vessels [29]. Several investigators have shown that VIP is able to relax urethral smooth muscle from various species, including man, and the peptide was suggested to be responsibe for NANC-mediated urethral relaxation (see [24]). Sjögren et al. [233] showed that VIP had a marked inhibitory effect on the isolated female rabbit urethra contracted by NA or EFS. No effect was found on NA release. In the pig urethra, VIP and NOS seem to be partly co-localized within nerve fibres (Persson et al., 1995). Waldeck et al. [215] showed that VIP-IR nerve fibres occurred throughout the smooth muscle layer of the rabbit urethra, although the number of nerves was not as high as that of NOS-IR structures. Marked relaxation of the isolated rabbit urethral muscle was reported [215], and the relaxant mechanism for VIP seemed to be independent of changes of the membrane potential. The ability of VIP to relax K^+-contracted preparations strengthens the hypothesis that the relaxant mechanism is independent of hyperpolarization [215].

Both pelvic and hypogastric nerve stimulation in dogs increased the bladder venous effluent VIP concentration [120], which supports the view that VIP can be released also from urethral nerves. In human urethral smooth muscle, relaxant responses were less consistent, but a modulatory role in neurotransmission could not be excluded [233]. Infusion of VIP in humans in amounts that caused circulatory side effects, had no effects on urethral resistance [121]. Plasma concentrations of VIP were obtained which, in other clinical investigations, had been sufficient to cause relaxation of the lower esophageal sphincter and to depress uterine contractions [121]. Therefore, the physiological importance of VIP for the lower urinary tract function in humans was questioned [121], and it is still unclear whether or not VIP contributes to NANC-mediated relaxation of the urethra.

Overview of the Micturition Cycle: Simplified Summary [234]

Bladder accommodation during normal filling is a primarily passive phenomenon, dependent on the elastic and viscoelastic properties of the bladder wall and the lack of parasympathetic excitatory input. An increase in outlet resistance occurs via the striated sphincter somatic "guarding reflex". In some species a sympathetic reflex contributes to storage by (1) increasing outlet resistance by increasing tension on the smooth sphincter, (2) inhibiting bladder contractility through an inhibitory effect on parasympathetic ganglia, and (3) causing a decrease in tension of bladder body smooth muscle. Continence is maintained during increases in intraabdominal pressure (IAP) by intrinsic competence of the bladder outlet and urethral compression against a suburethral supporting layer. A further increase in striated sphincter activity, on a reflex basis, is also contributory during increases in IAP (e.g. by coughing or straining). Emptying (voiding) can be voluntary or involuntary and normally involves an inhibition of the spinal somatic and sympathetic reflexes and activation of the vesical parasympathetic pathways, the organizational center for which is in the brainstem. Initially, there is a relaxation of the outlet musculature, mediated not only by the cessation of the somatic and sympathetic spinal reflexes but probably also by a relaxing factor, very possibly NO, released by parasympathetic stimulation or by some effect of bladder smooth muscle contraction itself.

A highly coordinated parasympathetically induced contraction of the bulk of the bladder smooth musculature occurs, with shaping or funneling of the relaxed outlet, due at least in part to a smooth muscle continuity between the bladder base and proximal urethra. With amplification and facilitation of the bladder contraction from other peripheral reflexes and from spinal cord supraspinal sources, and the absence of anatomic obstruction between the bladder and the urethral meatus, complete emptying will occur. Whatever disagreements exist regarding the anatomic, morphologic, physiologic, pharmacologic, and mechanical details involved in both the storage and expulsion of urine by the LUT, agreement is found regarding certain principles. First, the micturition cycle involves two relatively discrete processes: bladder filling/urine storage and bladder emptying/voiding. Second, whatever the details involved, these processes can be summarized succinctly from a conceptual point of view.

Bladder filling/urine storage require the following:

1. Accommodation of increasing volumes of urine at a low intravesical pressure (normal compliance) and with appropriate sensation
2. A closed bladder outlet at rest which remains so during increases of IAP.
3. Absence of involuntary bladder contractions (DO)

Bladder emptying/voiding requires the following:

1. Sustained coordinated contraction of the bladder smooth musculature of adequate magnitude and duration
2. Concomitant lowering of resistance at the level of the smooth and striated sphincter (absence of functional obstruction)
3. Absence of anatomic obstruction

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