Pharmacology of the Lower Urinary Tract: Basis for Current and Future Treatments of Urinary Incontinence

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Abstract——The lower urinary tract constitutes a functional unit controlled by a complex interplay between the central and peripheral nervous systems and local regulatory factors. In the adult, micturition is controlled by a spinobulbospinal reflex, which is under suprapontine control. Several central nervous system transmitters can modulate voiding, as well as, potentially, drugs affecting voiding; for example, noradrenaline, GABA, or dopamine receptors and mechanisms may be therapeutically useful. Peripherally, lower urinary tract function is dependent on the concerted action of the smooth and striated muscles of the urinary bladder, urethra, and periurethral region. Various neurotransmitters, including acetylcholine, noradrenaline, adenosine triphosphate, nitric oxide, and neuropeptides, have been implicated in this neural regulation. Muscarinic receptors mediate normal bladder contraction as well as at least the main part of contraction in the overactive bladder. Disorders of micturition can roughly be classified as disturbances of storage or disturbances of emptying. Failure to store urine may lead to various forms of incontinence, the main forms of which are urge and stress incontinence. The etiology and pathophysiology of these disorders remain incompletely known, which is reflected in the fact that current drug treatment includes a relatively small number of more or less well-documented alternatives. Antimuscarinics are the mainstay of pharmacological treatment of the overactive bladder syndrome, which is characterized by urgency, frequency, and urge incontinence. Accepted drug treatments of stress incontinence are currently scarce, but new alternatives are emerging. New targets for control of micturition are being defined, but further research is needed to advance the pharmacological treatment of micturition disorders.

I. Introduction

The pharmacological control of the lower urinary tract is exerted both in the central nervous system (CNS[1]) and

[1]Abbreviations: CNS, central nervous system; 5-HT, 5-hydroxytryptamine, serotonin; OAB, overactive bladder; PMC, pontine micturition center; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; SSRI, selective serotonin reuptake inhibitor; AR, adrenoceptor; SHR, spontaneously hypertensive rats; GAT, GABA transporter; SP, substance P; SSP-SAP, SP-saponin conjugate; CGRP, calcitonin gene-related peptide; NO, nitric oxide; AChE, acetylcholine esterase; VAChT, vesicular acetylcholine transporter; NPY, neuropeptide Y; VIP, vasoactive intestinal polypeptide; IP$_3$, ino-

sitol tris phosphate; NANC, nonadrenergic, noncholinergic; BK$_{Ca}$, large-conductance calcium-activated potassium (channels); RT-PCR, reverse transcription-polymerase chain reaction; PDE, cyclic nucleotide phosphodiesterase; TTX, tetrodotoxin; iNOS, inducible NO synthase; ET, endothelin; SIN-1, morpholinosydnonimine; cGK, cyclic GMP-dependent protein kinase; PYY, peptide YY; ATI, angiotensin I, ATII, angiotensin II; ACE, angiotensin-converting enzyme; DADLE, [*d*-Ala$_2$,*d*-Leu$_5$]enkephalin; OP, opioid-like receptor; PTHrP, parathyroid hormone-related protein; COX, cyclo-oxygenase; PG, prostaglandin; TXA$_2$, thromboxane A$_2$; LT, leukotriene; NS-8, 2-amino-3-cyano-5-(2-fluorophenyl)-4-methylpyrrole; SK$_{Ca}$, small-conductance calcium-activated potassium channels; IR, immunoreactive; HO, heme oxygenase; EFS, electrical field stimulation; L-NOARG, N^G-nitro-L-arginine; GYKI52466, 1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-

TABLE 1
Some transmitters and modulators producing contraction or relaxation of the bladder

Transmitter/Receptor	Effect on the Bladder	Site of Action
ACh, muscarinic M_3, M_2	Contraction	Detrusor
ACh, muscarinic M_2, M_4	Inhibitory, relaxation	Prejunctional
ACh, muscarinic M_1, M_3	Excitatory, contraction	Prejunctional
ACh, nicotinic	Excitatory, contraction	Intramural ganglia
NA, β_3, β_2	Relaxation	Detrusor
NA, α_1	Contraction	Trigone (bladder base)
NA, α_2	Inhibitory, relaxation	Cholinergic nerve terminals
ATP, $P2X_1$	Contraction	Detrusor
ATP, $P2X_3$	Excitatory, contraction	Afferent nerves
Nitric oxide	Inhibitory, relaxation	Afferent nerves
5-HT, $5-HT_2$	Contraction	Detrusor
Histamine, H_1	Contraction	Detrusor
Prostanoids, EP, TP	Contraction	Detrusor
Prostanoids, EP, TP	Excitatory, contraction	Afferent nerves
Leukotrienes, LTB_4	Contraction	Detrusor
Angiotensins, AT1	Contraction	Detrusor
Bradykinin, B2	Contraction	Detrusor
Endothelins, ET_A	Contraction	Detrusor
Tachykinins, NK2	Contraction	Detrusor
Vasopressin, V1	Contraction	Detrusor
VIP, $VPAC_1$/VPC_2	Relaxation	Detrusor
PTHrP	Relaxation	Detrusor

ACh, acetylcholine.

peripherally (Andersson, 1993, 1999b; de Groat et al., 1993, 1999; de Groat and Yoshimura, 2001). In the fetus and neonate, micturition is basically a spinal reflex, which during development becomes a spinobulbospinal reflex under suprapontine control (de Groat et al., 1999). However, information remains fragmentary on supraspinal (inter)connections and their function in the regulation of micturition, and there is a marked discrepancy between neuroanatomic knowledge and the functional description of the micturition cycle. Several CNS transmitters can modulate lower urinary tract storage and emptying, including glutamic acid, glycine, enkephalins, serotonin (5-HT), noradrenaline, dopamine, and GABA, but for many of them, a defined site of action in the micturition control has not yet been demonstrated (de Groat and Yoshimura, 2001; Andersson, 2002a).

The activity of the smooth and striated musculature in the urinary bladder, urethra, and periurethral sphincteric area is affected by various neurotransmitters, including acetylcholine, noradrenaline, ATP, nitric

oxide, and neuropeptides (Table 1). Muscarinic receptors mediate normal and at least the main part of involuntary bladder contractions, but the role of other mechanisms for bladder control has not yet been established.

Injuries or diseases of the nervous system, as well as drugs and disorders of the peripheral organs, can produce voiding dysfunctions, which roughly can be classified as disturbances of storage or disturbances of emptying (Wein, 1981, 2002). Failure to store urine may lead to various forms of incontinence (mainly urge and stress incontinence), and failure to empty can lead to urinary retention, which may result in overflow incontinence. Disturbances of bladder function leading to symptoms of urgency, frequency, and eventually incontinence have been termed overactive bladder (OAB) syndrome (Abrams et al., 2002), defined as the symptoms of urgency, with or without urge incontinence, usually with frequency and nocturia. OAB syndrome has been estimated to occur in nearly 17% of the population in the United States and Europe, including Scandinavia, and the prevalence of the syndrome increases with age (Milsom et al., 2001; Stewart et al., 2003).

Theoretically, a disturbed storage function can be improved by agents that decrease detrusor activity, increase bladder capacity, and/or increase outlet resistance. Many drugs have been tried, but the results are often disappointing, partly due to poor treatment efficacy and partly due to poor tolerability in the form of side effects (Kelleher et al., 1997). The development of pharmacological treatment has been slow, and use is based on results from controlled clinical trials for only a few drugs (Andersson, 1988; Andersson et al., 1999, 2002; Wein and Rovner, 2002; Yoshimura and Chancellor, 2002).

This review will cover recent advances in our understanding of the normal physiology of the lower urinary

5*H*-2,3-benzodiazepine hydrochloride; MK801, 5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine; LY215490, ±6-(2-(1*H*-tetrazol-5-yl)ethyl) decahydroisoquinoline-3-carboxylic acid; LY274614, 6-substituted decahydroisoquinoline-3-carboxylic acid; WAY100635, [*O*-methyl-3*H*]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; SB-269970, [(*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol; CGP55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenyl-methyl)phosphinic acid; SKF38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine; Y27632, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl)cyclohexane carboxamide; HA 1077, fasudil; LOE 908, (*R,S*)-(3,4-dihydro-6,7-dimethoxy-isochinolin-1-yl)-2-phenyl-*N,N*-di[2-(2,3,4-trimethoxyphenyl)ethyl]acetamide mesylate; U-73122, 1-[6-[[17*β*-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; CL-316243, 5-[(2*R*)-2-[[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylic acid disodium salt; PD123319, *S*-(+)-1-([4-(dimethylamino)-3-methylphenyl]methyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo(4,5-*c*)pyridine-6-carboxylic acid; UK-14,304, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine; YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole; ZD6169, (S)-*N*-(4-benzoylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide.

tract and of the pathophysiology of some voiding disorders as a pharmacological basis for current and emerging drug therapies. The terminology used to describe lower urinary tract function follows the recommendations of the International Continence Society (Abrams et al., 2002).

II. Central Nervous System Targets

A. Central Nervous Control

The normal micturition reflex in the adult is mediated by a spinobulbospinal pathway, which passes through relay centers in the brain (Fig. 1). Micturition occurs in response to afferent signals from the lower urinary tract, and distension of the bladder wall is considered the primary stimulus (de Groat et al., 1999; de Groat and Yoshimura, 2001). However, signals generated in the urothelium and involving suburothelial nerves may be of importance both normally and in different disorders of micturition (Andersson, 2002b).

During bladder filling (Fig. 2), once threshold tension is achieved, afferent impulses, conveyed mainly by the pelvic nerve, reach centers in the CNS. It has been proposed that the afferent neurons send information to the periaqueductal gray, which in turn communicates with the pontine tegmentum, where two different regions involved in micturition control have been described (Griffiths et al., 1990). One is a dorsomedially located M-region, corresponding to Barrington's nucleus or the pontine micturition center (PMC). A more laterally located L-region may serve as a pontine urine storage center, which has been suggested to suppress blad-
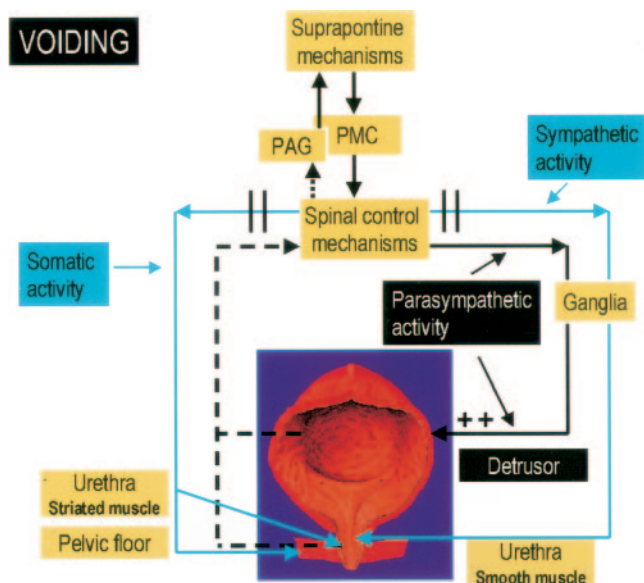

FIG. 1. Voiding reflexes involve supraspinal pathways and are under voluntary control. During bladder emptying, the spinal parasympathetic outflow is activated (++), leading to bladder contraction. Simultaneously, the sympathetic outflow to urethral smooth muscle and the somatic outflow to urethral and pelvic floor striated muscles are turned off, and the outflow region relaxes. PAG, periaqueductal gray; PMC, positive micturition center.
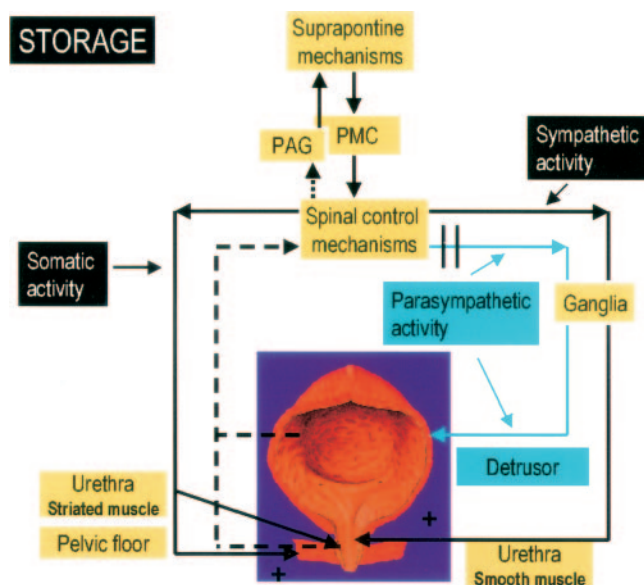

FIG. 2. During filling, there is continuous and increasing afferent activity from the bladder. There is no spinal parasympathetic outflow that can contract the bladder. The sympathetic outflow to urethral smooth muscle (α-adrenoceptors, α^+), and the somatic outflow to urethral and pelvic floor striated muscles (nicotinic receptors, N^+) keep the outflow region closed. Whether or not the sympathetic innervation to the bladder contributes to bladder relaxation during filling (β-adrenoceptors, β^+) in humans has not been established. PAG, periaqueductal gray; PSC, pontine storage center.

der contraction and to regulate the activity of the striated musculature of the bladder outlet during urine storage. The M- and L-regions may represent separate functional systems that act independently (Blok and Holstege, 1999a).

Centers rostral to the pons determine the beginning of micturition. Thus, even if the forebrain is not essential for the basic micturition reflex, it plays a role in making the decision when and where micturition should take place (Blok and Holstege, 1999b). Recent positron emission tomography studies have given information on the brain structures involved in urine storage and voiding (Blok et al., 1997b; Nour et al., 2000; Athwal et al., 2001; Matsuura et al., 2002).

B. Transmitter Systems

The micturition reflexes use several transmitters and transmitter systems that may be targets for drugs aimed at control of micturition (Fig. 3).

1. Glutamic Acid. It is well established that glutamate is a major excitatory transmitter in the mammalian CNS (Mayer and Westbrook, 1987), including pathways controlling the lower urinary tract (de Groat et al., 1999). However, glutamate is involved in many CNS functions, and drugs acting on the different glutamate receptors may affect not only micturition (Downie, 1999; Matsuura et al., 2000). Glutamate is involved in the afferent limb of the micturition reflex at the level of the lumbosacral spinal cord (Birder and de Groat, 1992; Kakizaki et al., 1996, 1998) and in the pathways con-
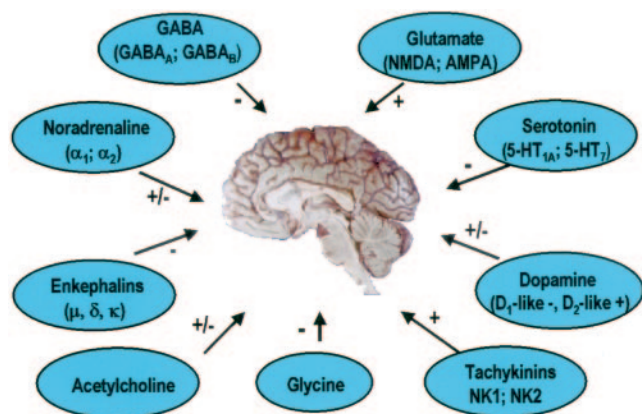
FIG. 3. Transmitter systems in the brain involved in micturition control.

necting the PMC to the preganglionic bladder neurons in the spinal parasympathetic nucleus. L-Glutamate, injected at sites in the brain stem where electrical stimulation evoked bladder contraction, stimulated micturition in rats (Mallory et al., 1991; Matsumoto et al., 1995a,b; Matsuura et al., 2000). For example, coordinated micturition could be evoked by injections of L-glutamate in Barrington's nucleus (Matsuura et al., 2000). However, in cats, injection of L-glutamate into the medullary raphe nuclei, which are known to have an inhibitory function in voiding, elicited only inhibition (Chen et al., 1993), suggesting that glutamate can also activate inhibitory systems.

Glutamate has also been shown to be involved at interneuronal synapses on parasympathetic preganglionic neurons (Araki and De Groat, 1996, 1997). Both N-methyl-D-aspartate (NMDA) and non-NMDA glutamatergic [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)] receptors are involved in micturition control (de Groat et al., 1999), but the receptors may serve different functions in the regulation of bladder and striated urethral sphincter (intramural and extramural or periurethral) activity. Intravenous or i.t. administration of the AMPA receptor antagonist, GYKI52466 [1-(4-amino-phenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzodiazepine hydrochloride], or the NMDA receptor antagonist, MK801 (5H-dibenzo[a,d]cyclohepten-5,10-imine), blocked the bladder contractions evoked by electrostimulation of the PMC, further supporting the view that AMPA and NMDA receptors both mediate excitatory transmission in the descending limb of the spinobulbospinal micturition reflex pathway (Matsumoto et al., 1995a,b).

Striated sphincter activities were also inhibited by the i.v. or i.t. administration of the AMPA receptor antagonist, LY215490 [±6-(2-(1H-tetrazol-5-yl)ethyl) decahydroisoquinoline-3-carboxylic acid], or the NMDA receptor antagonists, LY274614 (6-substituted decahydroisoquinoline-3-carboxylic acid) and MK801 (Yoshiyama et al., 1993, 1997). However, there were differences in the extent of inhibition by the NMDA receptor antagonists between

bladder contraction and striated sphincter activity in the unanesthetized or spinalized rats. In freely moving, awake rats (Vera and Nadelhaft, 1994), unanesthetized decerebrate rats (Yoshiyama et al., 1994, 1997), or spinalized rats (Yoshiyama et al., 1997), NMDA receptor antagonists did not depress bladder reflexes but still depressed striated sphincter activity. These findings suggest that the NMDA receptor regulates bladder contraction through parasympathetic preganglionic neurons (intermediolateral nucleus), mainly at the supraspinal level, and it regulates striated sphincter activity through somatomotor neurons (dorsolateral nucleus neurons) at the spinal level.

Supporting this, Shibata et al. (1999) found that, in rats, sacral parasympathetic preganglionic neurons express high messenger RNA levels of GluR-A and GluR-B AMPA receptor subunits and NR1, but not NR2, NMDA receptor subunits. On the other hand, motoneurons in the urethral sphincter nucleus expressed all four AMPA receptor subunits (GluR-A, -B, -C, and -D) in conjunction with moderate amounts of NR2A and NR2B, as well as high levels of NR1 receptor subunits. The authors concluded that it seems likely that dorsolateral nucleus neurons, but not parasympathetic preganglionic neurons, are provided with functional NMDA receptors, which could induce activity-dependent changes in synaptic transmission in the efferent pathway for the lower urinary tract.

2. Glycine. Glycine can be found in neurons in the sacral dorsal gray commisure, which receives afferent input from the PMC (Sie et al., 2001). In large part, glycine is colocalized with GABA. This is in agreement with the finding that glycine, released from interneurons in the spinal cord in some instances, is co-released with GABA at synapses on parasympathetic preganglionic neurons (Araki, 1994). Relaxation of the striated sphincter during micturition is strongly inhibited by strychnine, which is considered to be a specific glycine receptor antagonist (Shefchyk et al., 1998; Downie, 1999). The interneurons in the sacral DCG are believed to inhibit the striated sphincter motoneurons during micturition (Sie et al., 2001).

Miyazato et al. (2003) studied the influence of lumbosacral glycinergic neurons on the spinobulbospinal and spinal micturition reflexes in different groups of female rats, intact animals, rats with acute injury to the lower thoracic spinal cord, and rats with chronic spinal cord injury. Their results suggested that glycinergic neurons may have an important inhibitory effect on the spinobulbospinal and spinal micturition reflexes at the level of the lumbosacral cord.

3. Enkephalins. Several lines of evidence suggest that enkephalinergic mechanisms in the brain and spinal cord have an important role in the regulation of both the storage and voiding phases of micturition. A rich occurrence of enkephalin-containing nerve terminals has been demonstrated in the region of the PMC and in the sacral parasympathetic and nuclei of Onuf in the

spinal cord. These terminals exert an inhibitory control on the micturition reflex as shown by i.c.v. intrapontine or i.t. administration of enkephalins or opioids (de Groat et al., 1993, 1999; Downie, 1999). Intrathecal administration of morphine in conscious dogs increases the volume threshold for inducing micturition without altering voiding pressure, an effect blocked by naloxone (Bolam et al., 1986). Morphine and its more potent metabolite, morphine-6-glucoronide, also increased volume threshold when given i.t. to conscious rats (Igawa et al., 1993c). This suggests that opioid peptides can suppress the afferent limb of the micturition reflex at a spinal level (Downie, 1999).

Different types of opioid receptors are involved in micturition control. Four types of opioid receptors are recognized (Smith and Moran, 2001). The new designations for opioid-like receptors OP1, OP2, and OP3 correspond to the classic δ-, κ-, and μ-nomenclature, respectively. OP4 was previously known as ORL1, the receptor for the endogenous heptadecapeptide nociceptin/orphanin FQ (see *Section III.A.7.x.*). In the brain, μ-, κ-, and δ-opioid receptors mediate inhibitory effects on micturition, which are blocked by naloxone (Dray and Metsch, 1984a,b,c; Hisamitsu and de Groat, 1984; Dray and Nunan, 1987; Willette et al., 1988; Mallory et al., 1991; Downie, 1999). Naloxone administered alone i.c.v. or injected directly into the PMC facilitated the micturition reflex. In the cat spinal cord, δ-opioid receptors mediate inhibition of bladder activity, and κ receptors mediate inhibition of sphincter activity (Thor et al., 1989). In the rat spinal cord, δ and μ receptors, but not κ receptors, seemed to be involved in the suppression of bladder reflexes (de Groat et al., 1999; Downie, 1999). However, Gotoh et al. (2002) examined the effect of a κ receptor agonist on the bladder motility of anesthetized rats. They found that the κ receptor agonist could inhibit the micturition reflex as effectively as other opioids, and at least part of the inhibition was due to the diminished bladder sensation, based on the activation of the descending monoaminergic systems through the spinal κ-opioid receptors.

The fact that the spinal opioid modulation of the external urethral sphincter is different from that regulating the bladder suggests that this mechanism should be a promising target for selective reduction of sphincter activity (Downie, 1999).

The potent inhibitory effect of opioids on micturition does not seem to have been used therapeutically in voiding disorders, with few exceptions. Tramadol, which is widely used as an analgesic, is by itself a very weak μ receptor agonist, but it is metabolized to several different compounds, some of them almost as effective as morphine at the μ receptor. The drug combines the effects on μ-opioid receptors with inhibition of the uptake of noradrenaline and 5-HT. Pandita et al. (2003) analyzed the combination of these mechanisms by studying the effects of tramadol and its enantiomers on micturition in unanesthetized rats. The most conspicuous effect of i.v. ($\pm$)- and (+)-tramadol (0.1–10 mg/kg) was a dose-dependent increase in threshold pressure and an increase in the bladder contraction interval, eventually resulting in dribbling incontinence. The activity seemed to be produced mainly by the opioid component, which is carried together with the 5-HT uptake inhibition by the (+)-enantiomer, whereas the (−)-enantiomer comprises the noradrenaline inhibitory activity.

($\pm$)-Tramadol, given i.v. at doses below or similar to those shown to be analgesic in rats, abolished apomorphine-induced detrusor overactivity by increasing bladder capacity and abolishing nonvoiding contractions (Pehrson and Andersson, 2003). In rats, tramadol abolished experimentally induced detrusor overactivity caused by cerebral infarction (Pehrson et al., 2003). These data suggest that tramadol may have a clinically useful effect on detrusor overactivity.

4. Serotonin. The major source of 5-HT-containing terminals in the spinal cord is the raphe nuclei. The lumbosacral autonomic and the sphincter motor nuclei receive a dense serotonergic input (de Groat et al., 1993), and the descending bulbospinal pathway to the urinary bladder is essentially an inhibitory circuit, with 5-HT as a key neurotransmitter (de Groat et al., 1993; de Groat, 2002). Electrical stimulation of 5-HT-containing neurons in the caudal raphe and activation of postsynaptic 5-HT receptors in the spinal cord of cats cause marked inhibition of bladder contractions (McMahon and Spillane, 1982; Espey and Downie, 1995).

Multiple 5-HT receptors have been characterized in mammalian species and divided into different families (5-HT$_{1-7}$) based upon structural diversity, transduction mechanism, and pharmacology (Gerhardt and van Heerikhuizen, 1997). Additionally, some 5-HT receptors have been subdivided into subtypes (e.g., the 5-HT$_1$ receptor was further subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, and 5-HT$_{1F}$ subtypes), each of which exhibits a distinct pharmacological profile (Hoyer et al., 2002). Many drugs acting on 5-HT receptors can influence micturition. 5-HT$_{1A}$ Receptors, which have been discussed as an interesting drug target (de Groat, 2002), act as somatodendritic and presynaptic receptors on nerve cells, modulating neural firing, and at the postsynaptic level, where they mediate inhibitory functions. There seem to be multiple sites of serotonergic modulation of micturition in the spinal cord. However, the exact sites of the modulation have not been determined, and the roles of the different 5-HT receptor subtypes have not been established (Downie, 1999; de Groat, 2002).

It has been demonstrated (Lecci et al., 1992) that the selective 5-HT$_{1A}$ agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), injected i.v. or i.c.v., activates the micturition reflex, inducing an increase in the frequency of isovolumic bladder contractions in anesthetized rats. Testa et al. (1999) synthesized several novel *N*-arylpiperazine derivatives and studied their ability to affect the micturi-

tion reflex in anesthetized and conscious rats. Neutral antagonists potently inhibited volume-induced voiding contractions in anesthetized rats. Also, in conscious rats during continuous transvesical cystometry, the neutral antagonists increased bladder capacity, whereas micturition pressure was only slightly, and not dose-dependently, reduced. Based on these and further studies, Testa et al. (1999, 2001) concluded that neutral 5-HT_{1A} receptor antagonists have favorable effects on the bladder, inducing an increase in bladder capacity with no derangement of bladder contractility. This conclusion has been supported by the results of other investigators (Kakizaki et al., 2001; Pehrson et al., 2002b). Kakizaki et al. (2001) showed that rhythmic isovolumetric bladder contractions, evoked by bladder distension, were abolished by i.t. administration of the 5-HT_{1A} antagonist, WAY100635 [[O-methyl-$3H$]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride], in a dose-dependent manner. The drug was effective only if injected at the L6-S1 spinal cord level, but not at the thoracic or cervical cord levels. WAY100635 significantly reduced the amplitude of bladder contractions evoked by electrical stimulation of the PMC. However, the field potentials in the rostral pons evoked by electrical stimulation of pelvic nerve were not affected by i.t. or i.v. injection of WAY100635. These results suggest that the 5-HT_{1A} receptors at the L6-S1 level of the spinal cord have an important role in the tonic control of the descending limb of the micturition reflex pathway in the rat.

Read et al. (2003) injected the selective 5-HT_7 receptor antagonist SB-269970 [(R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol] i.c.v. in urethane-anesthetized female rats. Based on their experiments, Read et al. suggested that 5-HT_7 receptors located supraspinally in the rat are involved in the control of micturition. Whether this receptor can be a useful target for drugs meant for micturition control remains to be established.

Drugs interfering with serotonin or with serotonin receptors (for example, the selective serotonin reuptake inhibitors, or SSRIs) have not been systematically tested as a treatment for voiding disorders in humans. Whether or not imipramine, which blocks the reuptake of serotonin among other effects, depresses detrusor overactivity by this mechanism (Maggi et al., 1989b) has not been established. It has been suggested that there may be a deficiency of serotonin behind both depression and overactive bladder (Zorn et al., 1999; Steers and Lee, 2001; Littlejohn and Kaplan, 2002). If this is the case, the question is if SSRIs are effective for micturition control only in depressed patients, or if selective serotonin uptake inhibition is a general principle that can be used for treatment of the overactive bladder. A model suggesting that serotonin deficiency and urinary incontinence are linked has recently been reported (Na et al., 2003). Clomipramine was given to female rat pups. After

15 weeks, treated rats had developed increased voiding frequency, low bladder capacity, and detrusor overactivity demonstrable at cystometry. If the rats were treated with fluoxetine, micturition was normalized. The authors suggested that this supported the hypothesis that depression was linked to OAB syndrome and idiopathic urge incontinence. This would imply that SSRIs may be useful for treatment of OAB syndrome. On the other hand, there are reports suggesting that the SSRIs in patients without incontinence actually can cause incontinence, particularly in the elderly (Movig et al., 2002). This causes some doubts over the anecdotal reports that SSRIs may be used as a general treatment for OAB syndrome.

Duloxetine, a combined noradrenaline and 5-HT reuptake inhibitor has been shown, in animal experiments, to increase the neural activity to the striated urethral sphincter, and to increase bladder capacity through effects on the CNS (Thor and Katofiasc, 1995; Thor and Donatucci, 2004). Promising clinical experiences with the drug in the treatment of stress incontinence have been reported (Norton et al., 2002; Dmochowski et al., 2003; Millard et al., 2004; van Kerrebroeck et al., 2004). Another dual noradrenaline and 5-HT reuptake inhibitor, venlafaxine, increased intraurethral pressure in rats, and was suggested to be useful for treatment of stress incontinence (Bae et al., 2001). However, no clinical data for this drug are available.

5. Noradrenaline. The role of central nervous noradrenergic pathways in micturition control remains unclear. Noradrenergic neurons originating in the locus coeruleus react to bladder filling (Elam et al., 1986) and project to the autonomic and somatic nuclei in the lumbosacral spinal cord (de Groat et al., 1999). Bladder control through these bulbospinal pathways may involve both α_1- and α_2-adrenoceptors (ARs).

a. α_1-Adrenoceptors. The α_1-ARs seem to be tonically active in both the sympathetic and somatic neural control of the lower urinary tract (Danuser and Thor, 1995; Ramage and Wyllie, 1995). Thus, doxazosin, given i.t., decreased micturition pressure, both in normal rats and in rats with postobstruction bladder hypertrophy (Ishizuka et al., 1996b), the effect being much more pronounced in the animals with hypertrophied/overactive bladders. Doxazosin did not markedly affect the frequency or amplitude of the unstable contractions observed in obstructed rats. On this basis, it was suggested that doxazosin may have an action at the level of the spinal cord and ganglia, thereby reducing activity in the parasympathetic nerves to the bladder and that this effect was more pronounced in rats with bladder hypertrophy than in normal rats.

Urodynamic studies revealed that spontaneously hypertensive rats (SHR) have pronounced detrusor overactivity (Persson et al., 1998b). These animals also have an increased voiding frequency (Steers et al., 1999).

Although the control rats (Wistar-Kyoto rats) have a regular contraction frequency during continuous cystometry, the SHR show both micturition and nonmicturition contractions as well as a decreased bladder capacity. In SHR treated with 6-hydroxydopamine to chemically destroy the peripheral noradrenergic nerves, detrusor overactivity was maintained, as demonstrated by continuous cystometry (Andersson and Pandita, unpublished results). Furthermore, α_1-AR antagonists, injected i.a. near the bladder, did not abolish the detrusor overactivity. On the other hand, when given i.t., the α_1-AR antagonists normalized micturition. This suggested that the effect on detrusor overactivity was exerted within the CNS.

Yoshiyama et al. (2000) studied the role of spinal α_1-AR mechanisms in the control of urinary bladder function using cystometry in anesthetized and decerebrate, unanesthetized female rats. Their results suggested that two types of spinal α_1-AR mechanisms are involved in reflex bladder activity. First, there seems to exist an inhibitory control of the frequency of voiding reflexes, presumably by α_1-ARs regulating afferent processing in the spinal cord. Second, α_1-ARs may mediate a facilitatory modulation of the descending limb of the micturition reflex pathway.

The contribution of different subtypes of α_1-ARs (α_{1A}, α_{1B}, α_{1D}) in the lumbosacral spinal cord to the control of the urinary bladder was examined in urethane-anesthetized rats by Yoshiyama and de Groat (2001). They suggested that different α_1-AR subtypes were involved in the modulation of reflex bladder activity. Via α_{1A}- or α_{1B}-ARs, an inhibitory control of the frequency of voiding reflexes is exerted, presumably by an alteration in the processing of bladder afferent input. α_{1A}-ARs mediate facilitatory modulation of the descending efferent limb of the micturition reflex pathway. Yoshiyama and de Groat (2001) also concluded that spinal α_{1D}-ARs did not appear to have a significant role at either site. Sugaya et al. (2002) investigated the effects of i.t. tamsulosin (selective for $\alpha_{1A/D}$-receptors) and naftopidil (selective for α_{1D}-receptors) on isovolumetric bladder contractions in urethane-anesthetized rats. They found that both tamsulosin and naftopidil transiently abolished isovolumetric rhythmic bladder contractions, and that the effects were reversible. The amplitude of bladder contraction was decreased by naftopidil, but not by tamsulosin. The authors suggested that the noradrenergic projections from the brainstem to the spinal cord promote the afferent limb rather than the efferent limb of the micturition reflex pathway, and that the main α-AR in the afferent limb of this reflex pathway may be α_{1D}-receptors. This finding is of particular interest considering the findings of Smith et al. (1999), who investigated the neuronal localization of α_1-AR subtypes in the human spinal cord. Although all three α_1-AR subtypes were found to be present throughout the human spinal cord, α_{1D}-AR mRNA predominated overall. How-ever, it should be noted that the distribution of α_1-AR mRNA subtypes in the rat spinal cord is not necessarily the same as that in humans (Day et al., 1997).

It has been claimed that although both tamsulosin and naftopidil improve both emptying and storage disorders in benign prostatic hyperplasia patients, tamsulosin is superior for emptying disorders, and naftopidil is superior for collecting disorders (Hayashi et al., 2002). It was speculated that, in addition to an antagonistic action on the α-ARs of the smooth muscle of the lower urinary tract, both drugs (especially naftopidil) may also act on the lumbosacral cord to improve storage disorders.

Clinically, α_1-AR antagonists have been observed occasionally to abolish detrusor overactivity in patients with benign prostatic hyperplasia. α_1-AR antagonists have also been used to treat patients with neurogenic detrusor overactivity, however, with moderate success (Andersson et al., 2002). Whether the site of action is within the CNS or peripherally has not been established.

b. α_2-Adrenoceptors. Several studies have suggested that spinal and/or supraspinal α_2-ARs can modulate lower urinary tract function (de Groat et al., 1993). Smith et al. (1995) found that in the human spinal cord, α_2-AR mRNA was present predominantly in the sacral region. The thoracic, lumbar, and sacral spinal regions showed an increasing predominance of α_{2B}-AR mRNA. This was different from findings in the rat, where α_{2A}-AR and α_{2C}-AR predominated (Stone et al., 1998; Shi et al., 1999).

Ishizuka et al. (1996a) performed continuous cystometry in normal, conscious rats in the presence of α_2-AR stimulation and blockade. Given i.t., the selective α_2-AR agonist, dexmedetomidine, stimulated bladder activity and eventually caused total incontinence. Given i.a., dexmedetomidine decreased micturition pressure, bladder capacity, micturition volume, residual urine, and basal pressure. The selective α_2-AR antagonist, atipamezole, given i.t., increased micturition pressure, bladder capacity, residual urine, and decreased micturition volume. Similar effects were obtained when atipamezole was given i.a.

Kontani et al. (2000) administered the α_2-AR agonists clonidine and oxymetazoline i.t. and i.c.v. to conscious rats and demonstrated that both drugs induced detrusor overactivity, which could be prevented by the selective α_2-AR antagonist, idazoxan. They suggested that this overactivity could be produced via α_{2A}-AR stimulation both at spinal and supraspinal sites.

Collectively, available information suggests that both α_1- and α_2-ARs are involved in central micturition control. The role of α_2-ARs and the possibility that these receptors can be targets for drugs aiming at micturition control remain to be established.

6. Acetylcholine. There is evidence that cholinergic pathways in the cerebral cortex play an important role

in the regulation of the micturition reflex, and studies in animals have indicated that drugs acting on muscarinic receptors may have both excitatory (Sugaya et al., 1987; O'Donnell, 1990; Ishiura et al., 2001; Ishizuka et al., 2002) and inhibitory (Matsuzaki, 1990; Ishiura et al., 2001) effects on these pathways. Yokoyama et al. (2001) suggested, based on their results in rats, that the M_1 receptor is involved in a forebrain inhibitory mechanism controlling the micturition reflex and that muscarinic receptors in the dorsal pontine tegmentum contribute to excitatory control. Since M_1 receptor mRNA does not seem to exist in the pons, it may well be that different muscarinic receptor subtypes mediate inhibition and excitation. In rats, muscarinic receptor mechanisms may mediate a tonic excitatory influence on voiding (Ishiura et al., 2001; Ishizuka et al., 2002), whereas the inhibitory receptors do not appear to be tonically active. Masuda et al. (2001) suggested that excitation of a central muscarinic cholinergic pathway at a supraspinal or spinal site promotes proximal urethral relaxation during the voiding phase through the activation of efferent pathways in the pelvic nerves.

Muscarinic and nicotinic receptors may be involved in the control of voiding function. In the rat, stimulation of nicotinic receptors in the brain increased bladder capacity, suggesting that nicotinic agonists can activate mechanisms that inhibit voiding reflexes (Lee et al., 2003).

The bladder effects of antimuscarinics used for treatment of OAB syndrome are generally considered to be exerted peripherally. However, for those drugs passing into the CNS, effects on central mechanisms controlling lower urinary tract function cannot be excluded. Whether or not such effects are beneficial or not, however, remains to be established.

7. Dopamine. Central dopaminergic pathways can have both facilitatory and inhibitory effects on micturition by actions through D_1-like (D_1 or D_5) and D_2-like (D_2, D_3, or D_4) dopaminergic receptors. Patients with Parkinson's disease often have neurogenic detrusor overactivity and voiding dysfunction (Berger et al., 1987), possibly as a consequence of nigrostriatal dopamine depletion and failure to activate inhibitory D_1-like receptors (Yoshimura et al., 1993). However, through other dopaminergic pathways, micturition can be activated via D_2-like receptors. Facilitation of the micturition reflex mediated via D_2-like receptors may involve actions on brainstem and spinal cord. Thus, microinjection of dopamine into the pontine micturition center reduced bladder capacity and facilitated the micturition reflex in cats (de Groat et al., 1993).

Sillén et al. (1981) showed that apomorphine, which stimulates both D_1- and D_2-like receptors, induced bladder overactivity in anesthetized rats. In female rats, the role of dopamine D_1 and D_2 receptors in the volume-induced micturition reflex, was investigated cystometrically before and after i.v. administration of SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benza-

zepine; a selective D_1 receptor agonist), SCH 23390 (a selective D_1 receptor antagonist), quinpirole (a selective D_2 receptor agonist), and remoxipride (a selective D_2 receptor antagonist) (Seki et al., 2001). The results, which are in agreement with previously obtained data (de Groat and Yoshimura, 2001), suggested that D_1 receptors tonically inhibit the micturition reflex, and that D_2 receptors are involved in its facilitation. Thus, central dopaminergic pathways exhibit different effects on micturition via actions on multiple receptors at different sites in the central nervous system.

Experimental cerebral infarction yields damage to the basal ganglia and motor cortex, which may be areas suppressing D_2-like receptor-mediated actions in the normal rat. Antagonism of D_2-like receptors was without effect on bladder capacity in normal, conscious rats, but it increased bladder capacity in cerebral infarcted rats (Yokoyama et al., 1999). Thus, selective blockade of D_2-like receptors, or a specific D_1-like receptor agonist, might be helpful in stroke patients with detrusor overactivity.

8. GABA. GABA (γ-amino butyric acid) has been identified as an inhibitory transmitter at both spinal and supraspinal synapses in the mammalian CNS. At least in some species, the supraspinal micturition reflex pathway is under a tonic GABAergic inhibitory control (de Groat et al., 1993, 1999). GABA functions appear to be triggered by binding of GABA to its ionotropic receptors, $GABA_A$ and $GABA_C$, which are ligand-gated chloride channels, and its metabotropic receptor, $GABA_B$ (Chebib and Johnston, 1999). Since blockade of $GABA_A$ and $GABA_B$ receptors in the spinal cord (Igawa et al., 1993a; Pehrson et al., 2002a) and brain (Maggi et al., 1987a; Pehrson et al., 2002a) stimulated rat micturition, an endogenous activation of $GABA_{A+B}$ receptors may be responsible for continuous inhibition of the micturition reflex within the CNS. In the spinal cord, $GABA_A$ receptors are more numerous than $GABA_B$ receptors, except for the dorsal horn where $GABA_B$ receptors predominate (Malcangio and Bowery, 1996; Coggeshall and Carlton, 1997).

GABA transporters, present on neuronal and glial cells in the brain, brainstem, and spinal cord (Jursky et al., 1994), are presumed to provide an inactivation mechanism (Malcangio and Bowery, 1996). Four different GABA transporters (GATs) have been described (Bowery, 1993). Tiagabine is a selective inhibitor of one of these GABA transporters, GAT1 (Borden et al., 1994), and is able to increase extracellular levels of GABA (Fink-Jensen et al., 1992). Intravenous administration of tiagabine to rats decreased micturition pressure and decreased voided volume. Given i.t., tiagabine reduced micturition pressure and increased bladder capacity (Pehrson and Andersson, 2002), suggesting that increasing endogenous levels of GABA in the CNS may improve micturition control.

Experiments using conscious and anesthetized rats demonstrated that exogenous GABA, muscimol ($GABA_A$ receptor agonist) and baclofen ($GABA_B$ receptor agonist), given i.v., i.t., or i.c.v., inhibit micturition (Maggi et al., 1987a,c; Pehrson et al., 2002a). Similar effects were obtained in nonanesthetized mice (Zhu et al., 2002).

Baclofen, given i.t., attenuated oxyhemoglobin-induced detrusor overactivity, suggesting that the inhibitory actions of $GABA_B$ receptor agonists in the spinal cord may be useful for controlling micturition disorders caused by C-fiber activation in the urothelium and/or suburothelium (Pehrson et al., 2002a). In mice, where detrusor overactivity was produced by intravesical citric acid, baclofen given subcutaneously had an inhibitory effect which was blocked by the selective $GABA_B$ receptor antagonist CGP55845 [(2S)-3-[[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenyl-methyl)phosphinic acid] (Zhu et al., 2002).

Stimulation of the PMC results in an immediate relaxation of the external striated sphincter and a contraction of the detrusor muscle of the bladder. Blok et al. (1997) demonstrated in cats a direct pathway from the PMC to the dorsal gray commissure of the sacral cord. It was suggested that the pathway produced relaxation of the external striated sphincter during micturition via inhibitory modulation by GABA neurons of the motoneurons in the sphincter of Onuf (Blok et al., 1997a). In rats, i.t. baclofen and muscimol ultimately produced dribbling urinary incontinence (Igawa et al., 1993a; Pehrson et al., 2002a), and this was also found in conscious mice given muscimol and diazepam subcutaneously (Zhu et al., 2002). Thus, normal relaxation of the striated urethral sphincter is probably mediated via $GABA_A$ receptors (Pehrson and Andersson, 2002; Pehrson et al., 2002a), and $GABA_B$ receptors have a minor influence on motoneuron excitability (Rekling et al., 2000).

a. Gabapentin. Gabapentin was originally designed as an anticonvulsant GABA mimetic capable of crossing the blood-brain barrier (Maneuf et al., 2003). However, its effects do not appear to be mediated through interaction with GABA receptors, and its mechanism of action is still controversial (Maneuf et al., 2003). Gabapentin is also widely used not only for seizures and neuropathic pain, but also for many other indications such as anxiety and sleep disorders, due to its apparent lack of toxicity.

In a pilot study, Carbone et al. (2003) reported on the effect of gabapentin on neurogenic detrusor activity. They found a positive effect on symptoms and a significant improvement of urodynamic parameters after treatment and suggested that the effects of the drug should be explored in further controlled studies in both neurogenic and non-neurogenic detrusor overactivity.

9. Tachykinins. The main endogenous tachykinins, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), and their preferred receptors, NK1, NK2, and NK3, respectively, have been demonstrated in various CNS regions, including those involved in micturition control (Lecci and Maggi, 2001; Saffroy et al., 2001, 2003; Covenas et al., 2003). NK1 receptor expressing neurons in the dorsal horn of the spinal cord may play an important role in detrusor overactivity. Thus, Ishizuka et al. (1994) found that at the spinal level, there was a tachykinin involvement via NK1 receptors in the micturition reflex induced by bladder filling. This was demonstrated in normal rats, and more clearly, in rats with bladder hypertrophy secondary to bladder outflow obstruction. Seki et al. (2002) demonstrated that NK1 receptor-expressing neurons in the spinal cord could be eliminated by using i.t. substance P-saponin conjugate (SSP-SAP). They found that SSP-SAP capsaicin-induced detrusor overactivity was reduced, and they suggested that SSP-SAP could be effective to treat overactivity induced by bladder irritation without affecting normal bladder function.

Spinal NK1 receptor blockade could suppress detrusor activity induced by dopamine receptor (L-DOPA) stimulation (Ishizuka et al., 1995a).

Intracerebroventricular injection of SP, a selective NK1 receptor agonist, but not selective NK2 and NK3 receptor agonists, was found to inhibit isovolumetric contractions in urethane-anesthetized rats (Palea et al., 1993b). In conscious rats, however, i.c.v. SP stimulated micturition (Dib et al., 1998). Furthermore, in conscious rats undergoing continuous cystometry, antagonists of both NK1 and NK2 receptors inhibited micturition, decreasing micturition pressure and increasing bladder capacity at low doses, and inducing dribbling incontinence at high doses. This was most conspicuous in animals with outflow obstruction (Gu et al., 2000). Intracerebroventricular administration of NK1 and NK2 receptor antagonists to awake rats suppressed detrusor activity induced by L-DOPA stimulation (Ishizuka et al., 2000). The differences between the results of Palea et al. (1993b) and later investigators probably can be attributed to differences in experimental conditions (including anesthesia).

Taken together, available information suggests that spinal and supraspinal NK1 and NK2 receptors may be involved in micturition control. Involvement of NK3 receptors does not seem to have been demonstrated (Lecci and Maggi, 2001). Whether or not spinal and/or supraspinal NK receptors can be useful targets for drugs aiming at control of micturition disturbances remains to be established.

III. Peripheral Targets

A. Bladder

1. Urothelium and Interstitial Cells. The uroepithelium (urothelium) was long regarded as a protecting barrier that allowed for urine storage. However, recent investigations indicate that urothelial cells sense and respond to mechanical stimuli such as pressure, and

that they may communicate mechanical stimuli to the nervous system (Apodaca, 2004). Thus, the urothelium may serve as a mechanosensor which, by producing nitric oxide, ATP, acetylcholine, and other mediators, can control the activity in afferent nerves, and thereby the initiation of the micturition reflex (Andersson, 2002b). Low pH, high K^+, increased osmolality, and low temperatures can all influence afferent nerves, possibly via effects on the vanilloid receptor (capsaicin-gated ion channel TRPV1), which is expressed both in afferent nerve terminals and in the urothelial cells (Birder et al., 2001, 2002) A network of interstitial cells, extensively linked by Cx43-containing gap junctions, was found to be located beneath the urothelium in human bladder by Sui et al. (2002, 2004) This interstitial cellular network was suggested to operate as a functional syncytium, integrating signals and responses in the bladder wall, or as an electrical network acting as a control step in bladder sensory function (Sui et al., 2004).

Interstitial cells can also be found within the detrusor (McCloskey and Gurney, 2002; Hashitani et al., 2004). Hashitani et al. (2004) suggested that these cells modulated signal transmission within the bladder. Interestingly, these cells reacted to muscarinic receptor stimulation by initiating calcium transients.

If the bladder interstitial cells are important for the generation of OAB syndrome, they may be an interesting target for drugs meant for treatment of this disorder.

2. Afferent Nerves. From the dorsal root ganglia, afferent nerve cell bodies project both to the bladder, where information is received, and to the spinal cord, where connections with other neurons are established. Retrograde tracing studies have shown that most of the afferent innervation of the bladder and urethra originates in the dorsal root ganglia of the sacral region and travels via the pelvic nerve (Morrison et al., 2002). In addition, some afferents originating in ganglia at the level of the sympathetic outflow project via the hypogastric nerve. The afferent nerves of the striated muscle in the external striated sphincter (rhabdosphincter) travel in the pudendal nerve to the sacral region of the spinal cord. Sacral afferent nerve terminals are uniformly distributed to all areas of the detrusor and urethra, whereas lumbar afferent nerve endings are most frequently found in the trigone and are scarce in the bladder body (Lincoln and Burnstock, 1993). The afferent hypogastric and pelvic pathways mediate the sensations associated with normal bladder filling and with bladder pain. The pelvic and pudendal pathways are concerned also with the sensation that micturition is imminent and with thermal sensations of the urethra.

In both humans and animals, afferent nerves have been identified suburothelially as well as in the detrusor muscle (Gosling and Dixon, 1974; Maggi, 1993, 1995; Wakabayashi et al., 1993; Gabella and Davis, 1998). Suburothelially, they form a nerve plexus, which lies immediately beneath the epithelial lining. Some terminals may even be located within the basal parts of the urothelium. This suburothelial plexus is relatively sparse in the dome of the bladder, but becomes progressively denser near the bladder neck, and it is particularly prominent in the trigone (Gabella and Davis, 1998). Gabella and Davis (1998) studied in detail the distribution of afferent axons in the bladder of rats, using immunohistochemistry for calcitonin gene-related peptide (CGRP). They found that the afferent axons were distributed over four distinct targets: at the base of the epithelium, inside the epithelium, on blood vessels (both arteries and veins), and along muscle bundles. The afferent innervation of the musculature was diffuse, and appeared uniform throughout the bladder. There was a bilateral innervation of many regions of the lamina propria and the musculature, including individual muscle bundles. However, the epithelium and lamina propria of the dome of the bladder had no afferent axons.

Wakabayashi et al. (1993) studied the ultrastructure of SP-containing axon terminals in the lamina propria of the human urinary bladder. Numerous SP-immunoreactive varicose nerve fibers were observed, and most of them ran freely in the connective tissue. Many SP-immunoreactive nerve fibers were found beneath the epithelium, and perivascular SP-immunoreactive nerves were also seen in the lamina propria. The density of suburothelial, presumptive sensory nerves in the bladder wall was assessed in women with idiopathic detrusor overactivity and compared with the density of these nerves in asymptomatic women (Moore et al., 1992; Smet et al., 1997). The results suggested that a relative abundance of suburothelial sensory nerves may serve to increase the appreciation of bladder filling, giving rise to the frequency and urgency of micturition, which are characteristic of patients with detrusor overactivity.

The most important afferents for the micturition process are myelinated Aδ-fibers and unmyelinated C-fibers traveling in the pelvic nerve, conveying information from receptors in the bladder wall to the spinal cord. The Aδ-fibers respond to passive distension and active contraction, thus conveying information about bladder filling (Janig and Morrison, 1986). The activation threshold for Aδ-fibers is 5 to 15 mm H_2O, which is the intravesical pressure at which humans report the first sensation of bladder filling (de Groat et al., 1993). Afferents, which respond only to bladder filling, have been identified in the rat bladder and appear to be volume receptors, possibly sensitive to stretching of the mucosa. Experiments in rats suggested that ATP may play a significant role in driving volume-evoked micturition reflexes mediated by Aδ-fibers (Smith et al., 2002a).

C-fibers have a high mechanical threshold and respond primarily to chemical irritation of the bladder mucosa (Habler et al., 1990) or cold (Fall et al., 1990). Following chemical irritation, the C-fiber afferents exhibit spontaneous firing when the bladder is empty and increased firing during bladder distension (Habler et al.,

1990). These fibers are normally inactive and are therefore termed "silent fibers." Some of these C-fibers may be nociceptive, and glutamate may be the predominant transmitter (Smith et al., 2002a). They are sensitive to chemical stimulation and may then become mechanosensitive. Both high-threshold fibers (>15 mm Hg; known to be associated with nociception) and low-threshold fibers (<15 mm Hg; probably associated with non-nociceptive events) could be induced to discharge by intravesical α,β-methylene ATP, suggesting that purinergic mechanisms contribute to both nociceptive and non-nociceptive (physiological) mechanosensory transduction in the urinary bladder (Rong et al., 2002). There is evidence that epithelial and suburothelial afferents may respond to changes in the chemical composition of the urine or to chemicals [e.g., nitric oxide (NO), prostaglandins, and ATP] released from the urothelial cells (Andersson, 2002b).

3. Efferent Nerves. Bladder emptying and urine storage involve a complex pattern of efferent and afferent signaling in parasympathetic, sympathetic, and somatic nerves. These nerves are parts of reflex pathways that either maintain the bladder in a relaxed state, enabling urine storage at low intravesical pressure, or facilitate micturition by relaxing the outflow region and mediating a coordinated contraction of the bladder smooth muscle. Contraction of the detrusor smooth muscle and relaxation of the outflow region result from activation of parasympathetic neurons located in the sacral parasympathetic nucleus in the spinal cord at the level of S2-S4 (de Groat et al., 1993). 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) (Lincoln and Burnstock, 1993). The preganglionic neurotransmission is predominantly mediated by acetylcholine acting on nicotinic receptors, although the transmission can be modulated by adrenergic, muscarinic, purinergic, and peptidergic presynaptic receptors (de Groat et al., 1993). The postganglionic neurons in the pelvic nerve mediate the excitatory input to the human detrusor smooth muscle by releasing acetylcholine, acting on muscarinic receptors. However, an atropine-resistant component has been demonstrated, particularly in functionally and morphologically altered human bladder tissue (see *Section III.A.7.*). The pelvic nerve also conveys parasympathetic fibers to the outflow region and the urethra. These fibers exert an inhibitory effect and therefore relax the outflow region. This is mediated partly by NO (Andersson and Persson, 1993), although other transmitters might be involved (Bridgewater and Brading, 1993; Hashimoto et al., 1993; Werkström et al., 1995).

Most of the sympathetic innervation of the bladder and urethra originates from the intermediolateral nuclei in the thoraco-lumbar region (T10-L2) of the spinal cord. The axons travel either through the inferior mesenteric ganglia and the hypogastric nerve, or they pass through the paravertebral chain and enter the pelvic nerve. Thus, sympathetic signals are conveyed in both the hypogastric and pelvic nerves (Lincoln and Burnstock, 1993).

The predominant effects of the sympathetic innervation of the lower urinary tract in humans 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 contribute to relaxation of the detrusor by releasing noradrenaline (Andersson, 1993). The normal response of isolated bladder body tissue to noradrenaline, released by electrical stimulation of nerves, or added exogenously, is relaxation (Andersson, 1993; Nomiya and Yamaguchi, 2003).

4. Neurotransmission: Cholinergic Mechanisms.
a. Cholinergic Nerves. Although histochemical methods that stain for acetylcholine esterase (AChE) are not specific for acetylcholine-containing nerves (Lincoln and Burnstock, 1993), they have been used as an indirect indicator of cholinergic nerves. The vesicular acetylcholine transporter (VAChT) is considered a specific marker for cholinergic nerve terminals (Arvidsson et al., 1997). For example, in rats, bladder smooth muscle bundles were supplied with a very rich number of VAChT-positive terminals also containing neuropeptide Y (NPY), NOS, and vasoactive intestinal polypeptide (VIP) (Persson et al., 1997a). Similar findings have been reported in human bladders of neonates and children (Dixon et al., 2000). 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 an afferent function or a neurotrophic role with respect to the urothelium cannot be excluded (Dixon et al., 2000).

b. Muscarinic Receptors. As described in detail elsewhere (Caulfield and Birdsall, 1998), muscarinic receptors comprise five subtypes, encoded by five distinct genes. The five gene products correspond to pharmacologically defined receptors, and M_1 through M_5 are used to describe both the molecular and pharmacological subtypes. Muscarinic receptors are coupled to G-proteins, but the signal transduction systems vary. M_1, M_3, and M_5 receptors couple preferentially to $G_{q/11}$, activating phosphoinositide hydrolysis, in turn leading to mobilization of intracellular calcium. M_2 and M_4 receptors couple to pertussis toxin-sensitive $G_{i/o}$, resulting in inhibition of adenylyl cyclase activity.

In the human bladder, the mRNAs for all muscarinic receptor subtypes have been demonstrated (Sigala et al., 2002), with a predominance of mRNAs encoding M_2 and M_3 receptors (Yamaguchi et al., 1996; Sigala et al., 2002). These receptors are also functionally coupled (Eg-

len et al., 1996; Hegde and Eglen, 1999; Chess-Williams, 2002). Also, in most animal species, detrusor smooth muscle contains muscarinic receptors of the M_2 and M_3 subtypes (Eglen et al., 1996; Hegde and Eglen, 1999; Chess-Williams, 2002).

The M_3 receptors in the human bladder are believed to be the most important for detrusor contraction (Fig. 4) and to cause contraction through phosphoinositide hydrolysis (Andersson et al., 1991b; Harriss et al., 1995). In cat detrusor muscle, contraction induced by acetylcholine was found to be mediated via M_3 receptor-dependent activation of $G_{q/11}$ and phospholipase C-β_1 and inositol tris phosphate (IP_3)-dependent Ca^{2+} release (An et al., 2002). Jezior et al. (2001) found that bethanechol-induced contractions in the rabbit detrusor were practically abolished by inhibitors of Rho-kinase [Y27632 ((+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl)cyclohexane carboxamide), HA 1077 (fasudil)] in combination with a nonselective cation channel inhibitor, LOE-908 [(R,S)-(3,4-dihydro-6,7-dimethoxy-isochinolin-1-yl)-2-phenyl-N,N-di[2-(2,3,4-trimethoxyphenyl)ethyl]acetamid mesylate]. They suggested that muscarinic receptor activation of detrusor muscle includes both nonselective cation channels and activation of Rho-kinase. Supporting a role of Rho-kinase in the regulation of rat detrusor contraction and tone, Wibberley et al. (2003) found that Rho-kinase inhibitors (Y27632, HA 1077) inhibited contractions evoked by carbachol without affecting the contraction response to KCl. They also demonstrated high levels of Rho-kinase isoforms (I and II) in the bladder. Supporting a role for Rho-kinase in detrusor contraction, Fleichman et al. (2004) demonstrated in the rat bladder that carbachol-induced contraction did not involve protein kinase C, phosphatidylinositol-3-kinase, tyrosine kinases, or extracellular signal-regulated kinases, but that Rho-associated kinases were involved. In the human detrusor,

FIG. 4. Transmitter signal pathways (1–4) involved in activation of detrusor contraction via muscarinic M_3 receptors. Ach, acetylcholine; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; MLC, myosin light chain; SR, sarcoplasmic reticulum; CIC, calcium-induced calcium release. There seem to be differences between species in the contribution of the different pathways in contractile activation. In human detrusor, Ca^{2+} influx (3) is of major importance.

Schneider et al. (2004) confirmed that the muscarinic receptor subtype mediating carbachol-induced contraction was the M_3 receptor: They also demonstrated that the phospholipase C inhibitor U-73122 [1-[6-[[17β-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione] did not significantly affect carbachol-stimulated bladder contraction, despite blocking IP_3 generation. A phospholipase D inhibitor caused only a small inhibition of the contraction. However, the L-type calcium channel blocker, nifedipine, almost completely inhibited carbachol-induced detrusor contraction, whereas an inhibitor of store-operated Ca^{2+} channels, caused little inhibition. Protein kinase C inhibition did not significantly affect carbachol-induced contraction, but in contrast, the Rho-kinase inhibitor, Y27632, concentration-dependently and effectively attenuated the carbachol-induced responses. Schneider et al. (2004) concluded that carbachol-induced contraction of human urinary bladder is mediated via M_3 receptors and largely depends on Ca^{2+} entry through nifedipine-sensitive channels and activation of the Rho-kinase pathway.

Previous studies have indicated an important role for extracellular calcium in muscarinic receptor activation of the human bladder (Fovaeus et al., 1987), but the sources of calcium used for contractile activation have been a matter of debate (Andersson 1993). As pointed out by Hashitani et al. (2000), in most studies of the contribution of IP_3 production to muscarinic receptor-mediated contractions in the detrusor, relatively high concentrations of muscarinic receptor agonists have been used. Hashitani et al. (2000) speculated that the concentration of neurally released acetylcholine, which acts on the muscarinic receptors of the detrusor, is not always sufficiently high to stimulate IP_3 production. They suggested that M_3 receptors, which on stimulation produce IP_3, operate at high concentrations of muscarinic agonists, whereas M_2 receptors, which do not trigger the formation of IP_3, may be activated by lower concentrations.

Thus, the main pathway for muscarinic receptor activation of the detrusor via M_3 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 (Fig. 4).

The functional role for the M_2 receptors has not been clarified, but it has been suggested that M_2 receptors may oppose sympathetically mediated smooth muscle relaxation, mediated by β-ARs (Hegde et al., 1997). M_2 receptor stimulation may also activate nonspecific cation channels (Kotlikoff et al., 1999) and inhibit K_{ATP} channels through activation of protein kinase C (Bonev and Nelson, 1993b; Nakamura et al., 2002). Even in the obstructed rat bladder, M_3 receptors were found to play a predominant role in mediating detrusor contraction (Krichevsky et al., 1999). On the other hand, in certain
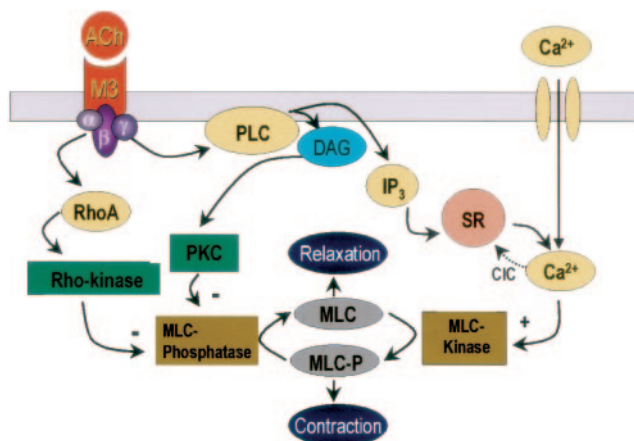
disease states, M_2 receptors may contribute to contraction of the bladder. Thus, in the denervated rat bladder, M_2 receptors or a combination of M_2 and M_3 mediated contractile responses, and the two types of receptor seemed to act in a facilitatory manner to mediate contraction (Braverman et al., 1998, 1999, 2002). In obstructed, hypertrophied rat bladders, there was an increase in total and M_2 receptor density, whereas there was a reduction in M_3 receptor density (Braverman and Ruggieri, 2003). The functional significance of this change for voiding function has not been established. Pontari et al. (2004) analyzed bladder muscle specimens from patients with neurogenic bladder dysfunction to determine whether the muscarinic receptor subtype mediating contraction shifts from M_3 to the M_2 receptor subtype, as found in the denervated, hypertrophied rat bladder. They concluded that whereas normal detrusor contractions are mediated by the M_3 receptor subtype, in patients with neurogenic bladder dysfunction, contractions can be mediated by the M_2 receptors.

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 M_2 in the rabbit (Tobin and Sjögren, 1995; Inadome et al., 1998) and rat (Somogyi and de Groat, 1992), and M_4 in the guinea pig (Alberts, 1995), rat (D'Agostino et al., 1997), and human (D'Agostino et al., 2000) bladder. Prejunctional facilitatory muscarinic receptors appear to be of the M_1 subtype in the rat and rabbit urinary bladder (Somogyi and de Groat, 1992; Tobin and Sjögren, 1995; Inadome et al., 1998). Prejunctional muscarinic facilitation has also been detected in human bladders (Somogyi and de Groat, 1999). The muscarinic facilitatory mechanism seems to be up-regulated in hyperactive bladders from chronic spinal cord-transected rats. The facilitation in these preparations is primarily mediated by M_3 muscarinic receptors (Somogyi and de Groat, 1999; Somogyi et al., 2003).

Muscarinic receptors have also been demonstrated on the urothelium, but their functional importance has not been clarified. It has been suggested that they may be involved in the release of an unknown inhibitory factor (Hawthorn et al., 2000; Chess-Williams, 2002).

The muscarinic receptor functions may be changed in different urological disorders, such as outflow obstruction, neurogenic bladders, idiopathic detrusor overactivity, and diabetes. However, it is not always clear what the changes mean in terms of changes in detrusor function.

There is good evidence that outflow obstruction may change the cholinergic functions of the bladder. Thus, detrusor denervation as a consequence of outflow obstruction has been demonstrated in several species, including humans (Gosling et al., 1986; Sibley, 1987; Speakman et al., 1987; Pandita et al., 2000a). In pigs with experimental outflow obstruction, detrusor response to intramural nerve stimulation was decreased, but there was a supersensitivity to acetylcholine (Sibley, 1984). Similar changes were found in bladders of obstructed patients with detrusor overactivity (Harrison et al., 1987). It was suggested that the supersensitivity was due to partial denervation of the bladder, and that one consequence of this may be detrusor overactivity (Sibley, 1987). On the other hand, Yokoyama et al. (1991) found that the responses to acetylcholine of detrusor strips from patients with detrusor overactivity were not significantly different from those without.

The reasons for these conflicting results are unclear, but they may be partly explained by the occurrence of "patchy denervation". Immunohistological investigations of the obstructed mouse bladder (also exhibiting detrusor overactivity) revealed that the nerve distribution patterns were markedly changed. In large parts of the detrusor, the smooth muscle bundles were completely devoid of accompanying VAChT-immunoreactive varicose terminals. In other areas, the densities of nerve structures were nearly normal (Pandita et al., 2000a).

The obstructed human bladder often shows an increased (up to ≈50%) atropine-resistant contractile component (Sjögren et al., 1982; Sibley, 1984; Bayliss et al., 1999). This may be taken as indirect evidence of changes in the cholinergic functions of the bladder, since normally the atropine-resistant component is almost negligible (Andersson, 1993; Tagliani et al., 1997; Bayliss et al., 1999). Alternatively, there may also be an up-regulation of the nonadrenergic, noncholinergic (NANC) component of contraction (see *Section III.A.7.*).

Turner and Brading (1997) suggested that in the "unstable bladder" (exhibiting detrusor overactivity), alterations of the smooth muscle are seen, which may be a consequence of patchy denervation of the detrusor (Fig. 5). This was supported by studies on unstable human bladders (Charlton et al., 1999; Mills et al., 2000). However, other investigators have arrived at other conclusions. Kinder and Mundy (1987) compared detrusor muscle from human normal bladders to that from patients with idiopathic or neurogenic detrusor overactivity. They found no significant differences in the degree of inhibition of electrically induced contractions produced by tetrodotoxin or atropine in detrusor strips from any of these bladders and no significant differences in the concentration-response curves for acetylcholine. In such bladders, a decreased number of muscarinic receptors was demonstrated (Restorick and Mundy, 1987), but the relation to overactivity was unclear.

Conflicting results concerning changes in the cholinergic functions in neurogenic bladders have been reported. In patients with myelomeningocele and detrusor dysfunction, Gup et al. (1989) found no supersensitivity to carbachol and no changes in the binding properties of the muscarinic receptors. However, German et al. (1995) found that isolated detrusor strips from patients with detrusor hyperreflexia were supersensitive to both car-
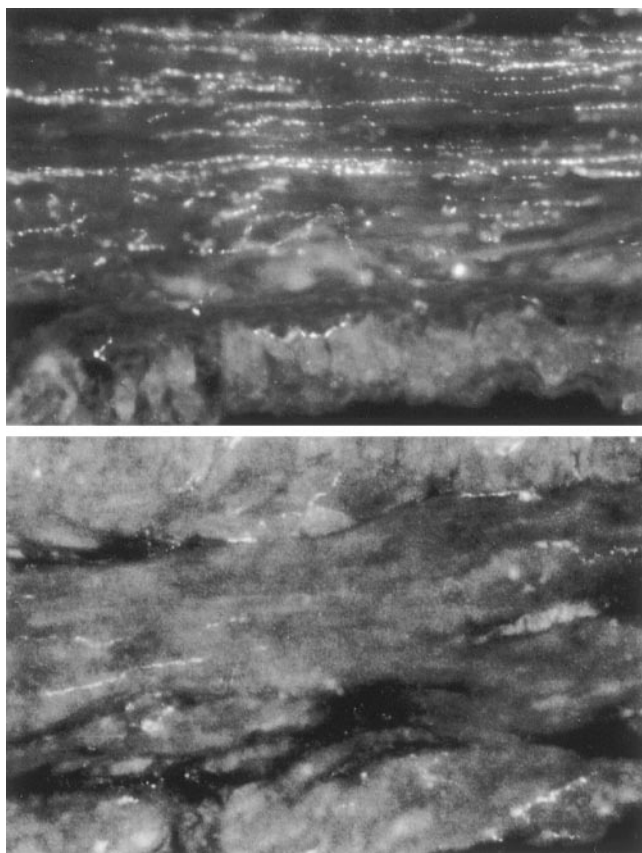
FIG. 5. Cholinergic innervation (VAChT immunohistochemistry) after partial bladder outflow obstruction in a mouse. Upper panel, normal unobstructed bladder. Lower panel, patchy denervation. Courtesy of Dr. Petter Hedlund.

bachol and KCl, but responded like normal controls to intramural nerve stimulation. The results were interpreted to suggest a state of postjunctional supersensitivity of the detrusor secondary to a partial parasympathetic denervation (German et al., 1995). An atropine-resistant component of the contraction of the human neurogenic bladder has been reported by some investigators (Saito et al., 1993a), but not by others (Bayliss et al., 1999).

Patients with diabetes mellitus and voiding dysfunction show a variety of urodynamic abnormalities, including detrusor overactivity and impaired detrusor contractility (Kaplan et al., 1995). In bladders from diabetic animals, an increased density of muscarinic receptors accompanied by an enhanced muscarinic receptor-mediated phosphoinositide hydrolysis was found (Latifpour et al., 1989; Mimata et al., 1995). An up-regulation of M_2 receptor mRNA has been demonstrated in rats with streptozotocin-induced diabetes (Tong et al., 1999), and also a supersensitivity of postjunctional muscarinic receptors may develop (Hashitani and Suzuki, 1996). However, it is unclear what these receptor changes mean for the functional bladder disorders seen in diabetic patients.

5. Neurotransmission: Adrenergic Mechanisms.

a. Adrenergic Nerves. The body of the bladder receives a relatively sparse innervation by noradrenergic nerves. The density of noradrenergic nerves increases markedly toward the bladder neck, where the smooth muscle receives a dense noradrenergic nerve supply, particularly in the male (Gosling et al., 1999). 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 established (Gosling et al., 1999).

b. α-Adrenoceptors. In the human detrusor, β-ARs dominate over α-ARs, and the normal response to noradrenaline is relaxation (Andersson, 1993). Goepel et al. (1997) found that the number of α-ARs in the human detrusor was low, the order of abundance being $\beta > \alpha_2 \gg \alpha_1$. The amount of α_1-ARs was too small for a reliable quantification. Walden et al. (1997) reported a predominance of α_{1A}-AR mRNA in the human bladder dome, trigone, and bladder base. This contrasts with the findings of Malloy et al. (1998), who found that among the high-affinity receptors for prazosin, only α_{1A} and α_{1D}-mRNAs were expressed in the human bladder. The relation between the different subtypes was $\alpha_{1D} = 66\%$ and $\alpha_{1A} = 34\%$ with no expression of α_{1B}. The total α_1-AR expression was low, 6.3 ± 1.0 fmol/mg, but very reproducible. A low α_1-AR expression was confirmed by Nomiya and Yamaguchi (2003).

Even if the α-ARs have no significant role in normal bladder contraction, there is evidence that this may change after, for example, bladder outlet obstruction, parasympathetic decentralization, and in overactive bladders.

i. Outflow Obstruction. Whether or not outflow obstruction changes the sensitivity of the bladder α-ARs to agonists has been discussed for decades. Dog detrusor muscle, which is normally relaxed by noradrenaline, responded with contraction in 7 of 12 dogs with bladder outlet obstruction (Rohner et al., 1978). However, this was suggested to be dependent on a decrease in β-AR function rather than to an increased α-AR function. In rats with outflow obstruction, Mattiasson et al. (1987) found that the number of α-ARs in the detrusor, as determined by [³H]dihydroergocryptine binding was decreased, and that α-AR-mediated contraction was impaired. In contrast, Saito et al. (1993) found that, in mildly obstructed rats, there was an increased detrusor response to phenylephrine, suggesting an enhanced α-AR function. A significant subtype-selective α_{1D}-AR mRNA up-regulation was found in rats with outflow obstruction (Hampel et al., 2002), but functional correlates were not reported. Contributing to these conflicting results, factors such as the degree and duration of obstruction may have had an important influence on the α-ARs in the detrusor.

Results obtained in bladders from patients are also conflicting. Perlberg and Caine (1982) showed that noradrenaline caused contraction instead of the normal relaxant response in bladder strips from patients with benign prostatic obstruction. On the other hand, a

change in α-AR function was not confirmed by Smith and Chapple (1994), who found that only 5 of 72 human bladder strips responded to phenylephrine, results which are not in agreement with the view of a change in α-AR function. Based on the α-AR subtype expression pattern (Malloy et al., 1998), Schwinn (2000) suggested that in the human detrusor, the α_{1D} receptors may be responsible for the storage (irritative) symptoms often associated with bladder outflow obstruction. However, no functional data were presented. Recently, Nomiya and Yamaguchi (2003) confirmed the low expression of α-AR mRNA in normal human bladder, and further demonstrated that there was no up-regulation of any of the adrenergic receptors (α- or β-AR mRNA) with obstruction. In addition, in functional experiments they confirmed previous functional results (Andersson, 1993), showing that the human detrusor has a low sensitivity to α-AR stimulation; phenylephrine at high drug concentrations produced a weak contraction with no difference between normal and obstructed bladders. Thus, in the obstructed human bladder, there seems to be no evidence for α-AR up-regulation or change in subtype. This would mean that it is unlikely that the α_{1D}-ARs in the detrusor muscle are responsible for detrusor overactivity or OAB syndrome. This does not exclude that the α-ARs on bladder vascular smooth muscle, for example, may have important roles in the changes in bladder function found after outflow obstruction (Das et al., 2002; Pinggera et al., 2003).

ii. Neurogenic Bladders. In parasympathetically decentralized cat bladder, a change in AR-mediated function was reported, with a shift from a β-AR-dominated relaxant influence in the normal bladder to an α-AR-dominated response (Norlén et al., 1976). However, other investigators were unable to confirm this finding (Malkowicz et al., 1985; Andersson et al., 1991a). The discrepancy in results is difficult to explain but can probably be attributed to differences in experimental approaches.

iii. Detrusor Overactivity. Detrusor tissue from patients with detrusor overactivity (without neurological disorders) had an almost 4-fold increase in the density of α-ARs compared with the density in normal subjects (Restorick and Mundy, 1989). The importance of this finding for detrusor overactivity is, however, unclear.

c. β-Adrenoceptors and the cAMP Pathway. The relaxant effect of noradrenaline on the detrusor can be exerted both pre- and postjunctionally (Åmark, 1986). Stimulation of α_2-ARs on cholinergic neurons may lead to a decreased release of acetylcholine. Since β-ARs dominate over α-ARs postjunctionally in the bladder (Andersson, 1993; Nomiya and Yamaguchi, 2003), noradrenaline relaxes the detrusor through stimulation of β-ARs. β-AR agonists are considered to stimulate adenylyl cyclase to increase cAMP. In turn, cAMP activates protein kinase A to mediate the biological effects. Isoprenaline prevented spontaneous action potential dis-

charges and associated calcium transients through the activation of protein kinase A. Isoprenaline hyperpolarized the cell membrane, probably by stimulating sodium pump activity. It was also found that there was no effect of different K^+ channel blockers on the hyperpolarization, and it was concluded that activation of K^+ channels was not involved in this effect (Nakahira et al. (2001). This is in contrast to the findings of other investigators, who reported that the isoprenaline-induced relaxation of guinea pig bladder smooth muscle was mediated mainly by facilitation of large-conductance calcium-activated potassium (BK_{Ca}) channels subsequent to the activation of the cAMP/protein kinase A pathway (Kobayashi et al., 2000).

There seems to be a decrease with age in density of β-ARs (Nishimoto et al., 1995), in adenylyl cyclase activity, and in content and activity of pertussis-sensitive G-proteins (Derweesh et al., 2000), and it has been suggested that different β-AR subtypes may be differently affected by age (Nishimoto et al., 1995).

The existence of subtypes of β_1- and β_2-ARs in the bladder is well established (Andersson, 1993). Early studies showed that in most species, β_2-ARs predominated (Andersson, 1993), but in guinea pig detrusor, for example, which contains both β_1- and β_2-ARs, the relaxant effect was mediated mainly by β_1-ARs (Li et al., 1992; Yamamoto et al., 1998). However, evidence has accumulated that suggests the occurrence of additional β-ARs. The relaxant response of rat bladder to isoproterenol and other nonselective β-AR agonists seems to be mediated by both β_2- and β_3-ARs (Oshita et al., 1997; Iizuka al., 1998; Yamazaki et al., 1998; Longhurst and Levendusky, 1999; Takeda et al., 2000; Woods et al., 2001). Furthermore, mRNA for β_1-, β_2-, and β_3-ARs has been detected in rat bladder using reverse transcription-polymerase chain reaction (RT-PCR) (Seguchi et al., 1998; Fujimura et al., 1999).

Both normal and 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 (Igawa et al., 1999, 2001; Takeda et al., 1999; Morita et al., 2000). Thus, it seems that the β-AR-mediated response in the human bladder is mediated by β_3-ARs. These findings would suggest β-AR, and particularly β_3-AR, stimulation as a way of keeping the bladder relaxed during filling. However, this does not necessarily mean that this mechanism is active during normal filling. The importance of the sympathetic input for human bladder function is controversial. Sympathectomy has no distinct effect on bladder filling, and neither has blockade of β-ARs (Andersson, 1986). Furthermore, since patients with deficiency of dopamine β-hydroxylase, the enzyme that converts dopamine to noradrenaline, void normally (Gary and Robertson, 1994), the sympathetic nervous system may not be essential for urine storage in humans. However, if released noradrenaline

contributes to bladder relaxation during filling, it may be through stimulation of both β_2- and β_3-adrenoceptors.

It may be speculated that in detrusor overactivity, there is a lack of an inhibitory β-AR-mediated noradrenaline response. However, detrusor muscle from patients with detrusor overactivity was reported to show a similar degree of inhibition in response to isoprenaline as normal detrusor (Eaton and Bates, 1982), even if the inhibitory effect of isoprenaline on the response to electrical stimulation was less in normal detrusor muscle.

Woods et al. (2001) found in rats that the selective β_3-AR agonist, CL-316243 [5-[(2R)-2-[[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylic acid disodium salt], increased the voiding interval and bladder compliance and decreased the number of spontaneous contractions during the filling phase in models of neurogenic and obstruction-induced detrusor overactivity. They suggested that activation of the β_3-ARs in rat bladder can directly inhibit smooth muscle contractility and detrusor overactivity, induced experimentally or associated with outflow obstruction and neurologic lesions.

Whether or not this is valid also for humans and if β_3-AR stimulation is an effective way of treating detrusor overactivity remain to be established

i. Forskolin. Forskolin, a naturally occurring diterpene and plant alkaloid, is believed to stimulate directly the catalytic subunit of the adenylyl cyclase enzyme and thus increase the intracellular concentration of cAMP. However, the drug may also have effects that are not mediated via cAMP (Laurenza et al., 1989). Forskolin caused a concentration-dependent relaxation of rabbit (Morita et al., 1986) and guinea pig (Longhurst et al., 1997) detrusor muscle strips and was also shown to effectively relax both porcine and human detrusor muscle (Truss et al., 1995, 1996a,b,c; Uckert et al., 2002). Forskolin and isoproterenol were equipotent in relaxing rabbit detrusor strips, and produced the same amount of relaxation. However, forskolin caused a much higher increase in cAMP levels than isoproterenol, suggesting compartmentalization of cAMP in the detrusor (Morita et al., 1992a). Forskolin has been used mostly as a tool for stimulation of adenylyl cyclase in various tissues. Whether or not forskolin or any of its analogs (Laurenza et al., 1992; Sutkowski et al., 1994) may have a clinically useful effect in the treatment of bladder disorders remains to be demonstrated.

ii. Phosphodiesterases. Bladder and urethral smooth muscle can be relaxed by drugs, which increase the intracellular concentrations of cAMP or cGMP (Andersson, 1999a). Multiple isoforms of adenylyl cyclases exist that catalyze the formation of cAMP (Soderling and Beavo, 2000; Fawcett et al., 2000). In general, increases in cAMP seem to have a main role in bladder relaxation, whereas cGMP is important for urethral relaxation (Morita et al., 1992b; Dokita et al., 1994; Persson and Andersson, 1994; Persson et al., 2001). A decrease of cAMP concentration may increase bladder tone. As mentioned above, stimulation of M_2 muscarinic receptors, which are negatively coupled to adenylyl cyclase, was found to contribute to muscarinic receptor-mediated rat bladder contraction both in vitro and in vivo (Hegde et al., 1997).

Cyclic nucleotide phosphodiesterases (PDEs) catalyze the degradation of cAMP and cGMP, and the cellular levels and effects of these nucleotides are determined in part by the rate of degradation. Presently, 11 different families of PDE are known which differ in their specificity for cAMP and cGMP, cofactor requirements, and kinetic properties (Essayan, 2001). Inhibitors of PDE can influence the intracellular cyclic nucleotide metabolism and therefore contraction and relaxation of detrusor smooth muscle. In both porcine (Truss et al., 1995, 1996c) and human (Truss et al., 1996a,b) detrusor, several PDE isoenzyme families have been demonstrated. Longhurst et al. (1997) found that inhibition of cAMP-specific PDE4 isozymes by rolipram significantly reduced the contractile response of guinea pig bladder strips to field stimulation, but that inhibition of cGMP-specific PDE5 isozymes by zaprinast had no effect. Kinetic characteristics together with functional in vitro studies suggest that PDE1 may be of importance in the intracellular regulation of human detrusor tone. The PDE1 inhibitor vinpocetine was more effective than other selective PDE inhibitors in relaxing carbachol-contracted detrusor strips (Truss et al., 1996b).

Truss et al. (2000) reported preliminary, promising experiences with vinpocetine in patients with urge incontinence and low-compliance bladder. Whether this means that vinpocetine or any other selective inhibitor of PDE1 will be useful in the treatment of detrusor overactivity remains to be established in controlled clinical trials.

6. Neurotransmission: Other Amines.

a. 5-Hydroxytryptamine (Serotonin). 5-HT has been shown to contract the bladder or isolated bladder smooth muscle from several species, although its potency seems to vary with the species investigated. 5-HT also potentiates the contractions induced by electrical field stimulation of isolated urinary bladder (Andersson, 1993). The response to the amine may be caused by direct actions on the smooth muscle cells or by indirect effects on the autonomic innervation of these organs. Saxena et al. (1985) suggested that both actions were implicated in the effects of 5-HT on the cat bladder, but, in general, there is evidence in favor of predominating indirect effects.

Contractions due to direct stimulation of 5-HT_2 receptors of smooth muscle cells of cat, rat (Cohen, 1990; Saxena et al., 1985; Kodama and Takimoto, 2000; Kim et al., 2002), and human (Klarskov and Hørby-Petersen, 1986) bladders, have been reported. Activation of 5-HT_3 receptors causes contractions mediated by stimulation of the receptor located on excitatory neurons in the bladder

of cats (Saxena et al., 1985), mice (Holt et al., 1986), guinea pigs (Messori et al., 1995), rabbits (Chen, 1990; Barras et al., 1996), pigs (Sellers et al., 2000), and humans (Corsi et al., 1991; Tonini et al., 1994). The 5-HT$_4$ receptor has been suggested to be located on the excitatory bladder neurons, and its activation facilitates cholinergic transmission in pigs (Sellers et al., 2000) and humans (Tonini et al., 1994; Candura et al., 1996). However, in the monkey bladder, the 5-HT$_4$ receptor is located postjunctionally, and its activation causes inhibition of neurogenic contractions (Waiker et al., 1994). In the isolated guinea pig bladder, it has been reported that 5-HT$_{2A}$ and 5-HT$_4$ receptors are involved in enhancing purinergic transmission and that the 5-HT$_3$ receptor is involved in enhancing cholinergic transmission (Messori et al., 1995).

Yoshida et al. (2002) studied the 5-HT receptors in guinea pig urinary bladder preparations by in vitro receptor autoradiography and determinations of mechanical activity and acetylcholine release. Specific binding sites were detected evenly throughout the urinary bladder. They concluded that the contractile response to 5-HT was mediated by activations of 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors. The 5-HT$_2$ receptor seemed to have high affinity to 5-HT and was located on the smooth muscle cells. The 5-HT$_4$ and 5-HT$_3$ receptor had low affinity to 5-HT and was located on the cholinergic neurons.

5-HT-immunoreactive cells can be demonstrated in human urinary tract tissue (Fetissof et al., 1983), but the importance of 5-HT for bladder function in humans is not known. In humans, the 5-HT$_2$ receptor antagonist ketanserin was found to have little effect on bladder function, as judged by urodynamic investigation, but it did reduce intraurethral pressure (Hørby-Petersen et al., 1985; Andersson et al., 1987a; Delaere et al., 1987). This was, however, attributed mainly to ketanserin's blocking effect on urethral α-adrenoceptors.

i. Detrusor Overactivity. Takimoto et al. (1999) observed in an open study that patients with diabetes and increased frequency, resistant to antimuscarinic treatment, got symptom relief with a 5-HT$_2$ receptor antagonist. In patients with neurogenic bladder dysfunction, administration of clomipramine, which inhibits 5-HT uptake, decreased the intravesical volume where the first detrusor reflex activity was observed and reduced residual urine (Vaidyanathan et al., 1981). Whether this observation reflects effects exerted by 5-HT on the bladder can only be speculated upon.

Animal data suggest that the effects of 5-HT may be changed in disorders known to be associated with detrusor overactivity. In rabbits with partial bladder outlet obstruction, autoradiography demonstrated a time-dependent up-regulation of 5-HT binding sites in the detrusor muscle. By immunohistochemistry, it was confirmed that the binding sites were neuronal (Khan et al., 1999c). It was speculated that such a change could in-

fluence the release of acetylcholine, which in turn could play a role in development of detrusor overactivity.

ii. Diabetes. In rats with experimental diabetes, an increased contractile response to 5-HT was found compared with nondiabetic control. This response was inhibited by a selective 5-HT$_{2A}$ antagonist. However, when this response was related to the muscle mass (which was increased in diabetic animals), no difference was found (Kodama and Takimoto, 2000).

In rabbits with alloxan-induced diabetes, neurogenic bladder contractions were significantly potentiated by exogenously applied 5-HT compared with normoglycemic controls, and also direct contractile effects of the amine were enhanced. The potentiation was ascribed to facilitated cholinergic transmission, and the enhanced direct contractility, at least in part, was linked to the associated hyperglycemia (Ichiyanagi et al., 2002).

b. Histamine. Patients suffering from the inflammatory condition of interstitial cystitis frequently exhibit an increased number of mast cells in the bladder. Histamine is a known mediator of allergic and acute inflammatory reactions and a major inflammatory mediator of mast cell origin. Histamine contracts bladder smooth muscle (Andersson, 1993) and can possibly contribute to the symptoms of interstitial cystitis. The effects of the amine on isolated urinary tract smooth muscle have been studied by several investigators. In the rabbit and guinea pig urinary bladder, histamine was suggested to produce contraction through H$_1$-receptors located on the smooth muscle (Fredericks, 1975; Khanna et al., 1977; Kondo et al., 1985; Poli et al., 1988). However, in the rabbit bladder, part of the contraction seemed to be mediated by release of acetylcholine, since the histamine-induced contraction was effectively inhibited by atropine or propantheline (Fredericks, 1975). Poli et al. (1988) found that histamine enhanced the atropine-resistant response to field stimulation and also suggested that H$_1$ receptors were involved in both types of response, but that the receptors populations pre- and postjunctionally were heterogeneous. Patra and Westfall (1994) investigated the nerve- and agonist-induced contractions in strips of guinea pig urinary bladder. Their findings suggested that histamine potentiated the neurogenic response of the bladder by influencing the purinergic component of contraction, apparently at postjunctional sites.

Histamine is known to release Ca^{2+} from internal stores by activating H$_1$ receptors in smooth muscle (Hill, 1990), including urinary bladder (Chambers et al., 1996). It has been shown that histamine increases phosphoinositide metabolism (Hill, 1990) and IP$_3$ levels (Chilvers et al., 1994) via activation of phospholipase C by a pertussis toxin-insensitive G protein (Leurs et al., 1994).

Rueda et al. (2002) studied the Ca^{2+}-dependence and wortmannin-sensitivity of the initial IP$_3$ response induced by activation of H$_1$ receptors in smooth muscle

from guinea pig urinary bladder. They suggested that the histamine-induced increase in IP_3 was more the consequence of Ca^{2+} release from internal stores than a direct activation of phospholipase C by H_1 receptors. They also found evidence for the existence of a Ca^{2+}-dependent amplification loop for the histamine-induced IP_3 response.

In detrusor specimens from patients with interstitial cystitis, an important portion of the electrically induced contraction was shown to be atropine-resistant (Palea et al., 1993a). H_1 and H_2 receptor antagonists did not affect this noncholinergic contractile response, which was probably caused by ATP. The sensitivity of interstitial cystitis detrusor muscle to histamine was much lower than that of control detrusor, suggesting that there was a desensitization of histamine receptors present in the bladder wall.

Data from open-label studies initially suggested that the H_1 receptor antagonist, hydroxyzine, reduces interstitial cystitis symptoms (Theoharides and Sant, 1997). Hydroxyzine could inhibit carbachol-induced bladder mast cell activation, but this was not found with other H_1 receptor antagonists (diphenhydramine or azatadine), which suggested that the effect was mediated through a mechanism which was unrelated to H_1 receptor antagonism (Minogiannis et al., 1998). A recent National Institutes of Health clinical trial, however, reported that this type of clinical beneficial effect was minor (Sant et al., 2003).

7. Neurotransmission: Nonadrenergic, Noncholinergic Mechanisms.

a. Atropine Resistance and Nonadrenergic, Noncholinergic Activation. In most mammalian species, part of the bladder contraction induced by electrical stimulation of nerves is resistant to atropine (Andersson, 1993). The proportion of NANC-mediated response to the total contraction varies with species and the frequency of stimulation. Thus, in bladder strips from rats and guinea pigs, atropine had little effect on the response to single nerve stimuli, but at 20 Hz, 75% of the response was resistant to atropine. In bladders from rabbits, mice, and pigs, 60, 70, and 25%, respectively, was resistant to atropine (Brading and Inoue, 1991; Wust et al., 2002). In female mice lacking both M_3 and M_2 receptors, the atropine-resistant response to electrical nerve stimulation was increased compared with controls (116% versus 84% of K^+-induced contraction), whereas in males, it was decreased (40% versus 102% of K^+-induced contraction) (Matsui et al., 2002).

i. Normal Bladder. The role of a NANC mechanism in the contractile activation of the human bladder is still disputed (Andersson, 1993; de Groat and Yoshimura, 2001). Cowan and Daniel (1983) found that acetylcholine was responsible for approximately 50% of the electrically induced contraction in strips of the normal human detrusor. In contrast, Sjögren et al. (1982), who investigated morphologically normal detrusor samples from

patients undergoing bladder surgery for various reasons, found that atropine caused more than 95% inhibition of electrically evoked contractions. Sibley (1984) compared the effects of atropine on contractions induced by electrical field stimulation in isolated detrusor preparations from rabbits, pigs, and humans. He found that in human preparations, obtained from patients undergoing lower urinary tract surgery for different disorders, or donor nephrectomy, atropine abolished nerve-mediated contractions. He concluded that nerve-mediated contractile activity in human detrusor is exclusively cholinergic. This was supported by the results of Kinder and Mundy (1985a), who found that atropine caused an almost total inhibition of the electrically induced contraction in human detrusor tissue taken from patients with urodynamically normal bladders. Luheshi and Zar (1990) investigated whether the reported full atropine sensitivity of the human detrusor was due to a genuine absence of a noncholinergic element in its motor transmission or was dependent on the experimental protocols, which in most investigations involve prolonged electrical stimulation. They used an experimental protocol where field stimulation was limited to the minimum required to elicit consistent and reproducible contractions and found that part of the electrically induced response (about 30%) was resistant to atropine. With a more conventional stimulation protocol involving long trains of pulses, however, the responses were enhanced by physostigmine and fully blocked by atropine. Atropine-resistant, tetrodotoxin (TTX)-sensitive contractions evoked by electrical stimulation in normal human detrusor tissue also have been reported by other investigators (Hoyle et al., 1989; Ruggieri et al., 1990; Bayliss et al., 1999; O'Reilly et al., 2002). However, the contribution of NANC neurotransmission to bladder excitation in humans, and also in pigs, seems to be small.

These apparently conflicting data may partly be explained by differences in the tissue materials investigated and by varying experimental approaches. Most probably, normal human detrusor muscle exhibits little "atropine resistance." This does not exclude that atropine resistance may exist in morphologically and/or functionally changed bladders. Thus, the NANC component of the nerve-induced response may be responsible for up to 40 to 50% of the total bladder contraction in different conditions associated with detrusor overactivity (Sjögren et al., 1982; Sibley, 1984; Kinder and Mundy, 1985b; Palea et al., 1993a; Bayliss et al., 1999; Moore et al., 2002; O'Reilly et al., 2002).

ii. Outflow Obstruction. In detrusor strips from male patients with a diagnosis of benign prostatic hyperplasia and detrusor overactivity, and in particular from those with bladder hypertrophy, an atropine-resistant component of up to 50% of the electrically induced contraction could be demonstrated (Sjögren et al., 1982). These results were confirmed by Smith and Chapple (1994), who compared the responses to electrical stimulation of de-

trusor strips from normal individuals and from patients with bladder outflow obstruction, with or without detrusor overactivity. They found a NANC response only in strips from patients with detrusor overactivity (15 of 21), amounting to 25% of the original response. Nergårdh and Kinn (1983) found a varying degree of atropine resistance (0–65%) in isolated detrusor preparations from male patients, most of them having outflow obstruction. Sibley (1984) verified the occurrence of atropine resistance in hypertrophic bladder muscle, but also showed that the atropine-resistant response was resistant to TTX, suggesting that it was not nerve mediated, but that it was caused by direct muscle stimulation. Tagliani et al. (1997) concluded, based on their results, that the atropine-resistant component may reflect direct smooth muscle excitation, since the human detrusor has a very short chronaxie. In contrast, Smith and Chapple (1994) showed that the NANC response from overactive, obstructed bladders was eliminated by TTX.

iii. Detrusor Overactivity. O'Reilly et al. (2002) were unable to detect a purinergic component of nerve-mediated contractions in control (normal) bladder preparations, but they found a significant component in overactive bladder specimens, in which the purinergic component was approximately 50%. They concluded that this abnormal purinergic transmission in the bladder might explain symptoms in these patients and the lack of response to conventional antimuscarinic agents in some patients.

iv. Aging. Yoshida et al. (2001) found a significant positive correlation between age and the NANC response and a significant negative correlation between age and the cholinergic neurotransmissions in human isolated detrusor preparations.

Taken together, these results suggest that there is a NANC component contributing to motor transmission in the isolated human detrusor, and also that no obvious qualitative difference exists between humans and other mammalian species. In normal detrusor, this component of contraction seems to be small. However, the importance of the NANC component for detrusor contraction in vivo, normally, and in different micturition disorders, remains to be established.

b. ATP. Evidence has been presented (Hoyle et al., 1989; Ruggieri et al., 1990; Palea et al., 1993; O'Reilly et al., 2002) 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. (1983) showed that ATP produced a concentration-dependent contraction in isolated human detrusor muscle, and 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 P2X receptors. Hardy et al. (2000) demonstrated the presence of the $P2X_1$ receptor subtype in the human

bladder, using RT-PCR, and confirmed that activation of purinergic P2X receptors, putatively $P2X_1$, may be important in the initiation of contraction in human detrusor. Purinergic transmission seemed to be more important in muscle taken from patients with detrusor overactivity. Their results also indicated the possibility that human bladder expresses multiple isoforms of the $P2X_1$ receptor, which may be potential sites for modifying or regulating putative purinergic activation of the human bladder. Supporting such a concept, mice deficient in $P2X_3$ exhibited a marked urinary bladder hyporeflexia, characterized by decreased voiding frequency and increased bladder capacity, but normal bladder pressures (Cockayne et al., 2001). Immunohistochemical studies localized $P2X_3$ to nerve fibers innervating the urinary bladder of wild-type mice and showed that loss of $P2X_3$ did not alter sensory neuron innervation density. $P2X_3$ thus seemed to be critical for peripheral afferent pathways 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.

c. Nitric Oxide. Not only constitutive NO synthase (neuronal NOS, nNOS; endothelial NOS, eNOS) but also inducible NO synthase (iNOS) can be demonstrated in lower urinary tract smooth muscle from animals and humans (Dokita et al., 1994; Ehren et al., 1994a,b; Olsson et al., 1998; Lemack et al., 1999, 2000; Johansson et al., 2002b; Masuda et al., 2002a,b; Felsen et al., 2003). There is so far no evidence that nNOS is produced by detrusor smooth muscle cells, and in unstimulated detrusor cells, iNOS was not detected. However, the cells expressed the enzyme when exposed to lipopolysaccharide or cytokines (Fig. 6), known to be produced during urinary tract infections (Weiss et al., 1994; Olsson et al., 1998; Johansson et al., 2002b). In biopsies taken from the lateral wall and trigone regions of the human bladder, a plexus of NADPH-diaphorase-containing nerve fibers was found (Smet et al., 1994). 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 NOS immunoreactivity was higher in trigonal and urethral tissue than in the detrusor (Persson et al., 1993). Most NOS-containing (nitrergic) nerves also contained VAChT and can thus be considered cholinergic.

L-Arginine-derived NO seems to be responsible for the main part of the inhibitory NANC responses in the lower urinary tract. However, the functional role of NO in the detrusor has not been established (Andersson, 1993; Andersson and Persson, 1995; Mumtaz et al., 2000,
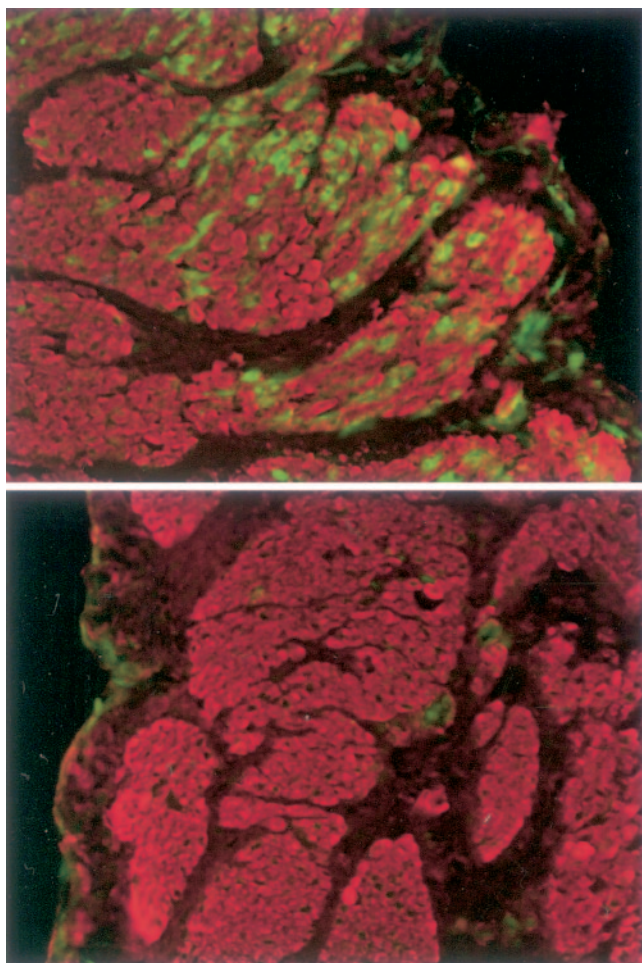
Fig. 6. Inducible iNOS expression in rat bladder. Upper panel, normal control. Lower panel, after incubation with a cytokine mixture. Courtesy of Dr. Rebecka Johansson.

Mamas et al., 2003). It has been suggested that NO might be generated from the detrusor muscle and may be an important factor for bladder relaxation during the filling phase (James et al., 1993; Theobald, 1996). The normal bladder responds to filling at a physiological rate with relaxation and can accommodate large volumes of urine, with a minimal increase in intravesical pressure (Coolsaet, 1985). The phenomenon has been attributed not only to the physical properties of the bladder, but also to the existence of an inhibitory neural mechanism operative during filling and storage. If NO has an important role in detrusor relaxation, it may be expected that the detrusor muscle has a high sensitivity to agents acting by increasing the intracellular concentrations of cyclic GMP. In the pig detrusor, the NO-donor SIN-1 (morpholinosydnonimine) and NO relaxed carbachol, and ET-1 (endothelin 1) contracted preparations by approximately 60%. However, 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 with their effects on the urethral muscle (Persson and Andersson, 1992;

Persson et al., 1993). These results agree well with those of Morita et al. (1992b), who found that in rabbits, cyclic GMP is mainly related to urethral relaxation and cyclic AMP to urinary bladder relaxation. They also agree with the findings of Masuda et al. (2002a,b), demonstrating that in the detrusor, in contrast to the urethra, NOS and soluble guanylyl cyclase activities were mainly detected in the mucosa and not in the smooth muscle. In human detrusor, NO donors and dibutyl-cGMP evoked a complex response in precontracted tissue (relaxant, contractile, or biphasic), and a clear role for a NO/cGMP signaling system could not be defined (Moon, 2002).

i. Outflow Obstruction. In male nNOS-deficient mice, Burnett et al. (1997) found markedly dilated bladders with muscular hypertrophy, findings compatible with deficient urethral relaxation and increased outflow resistance. Supporting the hypothesis that the bladder changes were caused by disturbances in outflow relaxation, the decrease in tension produced by low-frequency stimulation of nerves of isolated urethral preparations from wild-type controls was absent in preparations from nNOS-deficient mice. In contrast to these findings, Sutherland et al. (1997), investigating female nNOS-deficient mice with voiding, urodynamic, and muscle strip testing, as well as histological examination, found no marked differences between these animals and normal controls. NO-mediated smooth muscle relaxation is mediated by cGMP through activation of cGMP-dependent protein kinase I (PKG; cGKI). cGKI-deficient mice, male or female, showed no sign of bladder hypertrophy (Persson et al., 2000). The role of NO (or lack of it) in the development of bladder hypertrophy associated with outflow obstruction in humans has not been established.

Bladder outflow obstruction can cause an increase in iNOS activity and a decrease in nNOS activity (Lemack et al., 2000; Johansson et al., 2002a; Felsen et al., 2003). Thus, expression of iNOS was enhanced, both at the cDNA and protein level, 1 and 3 weeks after experimental outflow obstruction in mice, suggesting that iNOS-derived NO is involved in the early response to bladder outlet obstruction (Lemack et al., 1999). It was speculated that the enhanced iNOS expression was a response to overcome the effects of obstruction-induced ischemia. Felsen et al. (2003) presented evidence that iNOS-derived NO, forming reactive nitrogen species, promoted the spontaneous bladder contractions and fibrosis induced by outlet obstruction. They also suggested that iNOS inhibitors have a therapeutic potential.

ii. Detrusor Overactivity. Evidence that baseline NOS activity prevents detrusor overactivity exists from urodynamic studies performed in a fetal lamb model (Mevorach et al., 1994). If the nitrergic nerves observed in the detrusor, and particularly within and beneath the urothelium, are afferent terminals, NO may be involved in the regulation of the threshold for bladder afferent firing. Supporting such a view, intravesical oxyhemoglobin, a nitric oxide scavenger, induced detrusor overac-

tivity in normal rats (Pandita et al., 2000b). It was suggested that intravesical oxyhemoglobin induced detrusor overactivity by interfering with NO generated in the urothelium or suburothelially, and that NO may be involved in the regulation of the threshold for afferent firing in the bladder. Inhibition of the L-arginine/NO pathway by NOS inhibitors also resulted in detrusor overactivity and decreased bladder capacity (Persson et al., 1992b). Furthermore, cGKI-deficient mice showed detrusor overactivity characterized by decreased intercontraction intervals and nonvoiding bladder contractions (Persson et al., 2000). This further supports the view that the L-arginine/NO pathway is involved in the control of bladder activity.

d. Neuropeptides. The functional roles of many of the neuropeptides that have been demonstrated to be synthetized, stored, and released in the human lower urinary tract (Maggi, 1993, 1995; Lecci and Maggi, 2001) have not been established. For example, bombesin-like immunoreactivity has been found in the rat urinary bladder, and bombesin has a potent contractile effect on the bladder of several species, but a physiological role for the peptide in bladder function has not been established (Andersson, 1993). Galanin has also been demonstrated in nerves of the rat and human urinary tract, and the peptide concentration-dependently caused a pronounced reduction of the responses to electrical stimulation in human detrusor strips. However, its functional role, if any, in the lower urinary tract remains to be established (Andersson, 1993). In detrusor from rabbits and humans, higher levels of arginine vasopressin-like immunoreactivity were detected than those normally found in plasma, but arginine vasopressin had little effect on the isolated human bladder, and a V_1 receptor selective antagonist had no effect on contractions induced by electrical field stimulation (Holmquist et al., 1991). However, it cannot be excluded that these and other peptides (e.g., pituitary adenylate cyclase-activating polypeptide) can be involved as neurotransmitters/modulators of both afferent and efferent signaling, or that they serve other functions in the lower urinary tract.

i. Tachykinins and Capsaicin-Sensitive Primary Afferents. Capsaicin-sensitive primary afferents in the bladder and urethra have been shown to contain tachykinins, including SP, NKA, and NKB. These peptides may have not only a sensory function, but also a local effector or efferent function (Maggi, 1993, 1995; Lecci and Maggi, 2001). In addition, they may play a role as neurotransmitters and/or neuromodulators in the bladder ganglia and at the neuromuscular junctions. As a result, these 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 ("neurogenic inflammation"). 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 (Maggi, 1993). However, cystometric evidence that capsaicin-sensitive nerves may modulate the afferent branch of the micturition reflex in humans has been presented (Maggi et al., 1989a). In a small number of patients suffering from bladder hypersensitivity disorders, intravesical capsaicin produced a long-lasting, symptomatic improvement after an initial period with increased symptoms. The site of action may include the urothelium (Birder et al., 2002).

The tachykinins act on different NK receptors, and SP, NKA, and NKB possess the highest affinity for NK1, NK2, and NK3 receptors, respectively. All receptor subtypes have been identified in urinary bladders of various mammals, both in vitro and in vivo (Maggi, 1995; Lecci and Maggi, 2001). In the rat detrusor, NK1, NK2, and NK3 receptors have been demonstrated, as evidenced by radioligand binding and autoradiographic and functional experiments, whereas in hamster, mouse, dog, and human detrusor, NK2 receptors predominate (Lecci and Maggi, 2001).

In the human detrusor, the presence of tachykinins, their receptors, and their contractile effects have been well documented (Erspamer et al., 1981; Dion et al., 1988; Maggi et al., 1989c; Giuliani et al., 1993; Zeng et al., 1995; Palea et al., 1996; Smet et al., 1997; Burcher et al., 2000; Werkström et al., 2000; Warner et al., 2002). The potencies of the peptides were shown to be NKA > NKB ≫ SP (Dion et al., 1988; Maggi et al., 1989c). As mentioned previously, the predominant tachykinin receptor-mediating contraction of the human bladder seems to be of the NK2 type.

Whether or not any of the tachykinins is responsible for part of the atropine-resistant component of the detrusor contraction induced by electrical stimulation of nerves is unclear. The potential involvement of SP has been studied by several investigators. With few exceptions, these studies did not favor the view that SP, released from postganglionic nerve terminals, has an excitatory transmitter role. On the other hand, evidence has been presented that SP may play a role in the afferent, sensory branch of the micturition reflex (Andersson, 1993). At the peripheral level, neither NK1 nor NK2 receptors seem to have any importance for normal initiation of the micturition reflex in the rat (Lecci et al., 1993). This does not exclude that they have such a role in, for example, detrusor overactivity induced by irritants.

The NK2 receptor-mediated contraction in the human detrusor is largely dependent on the activation of L-type Ca^{2+} channels and is sensitive to nifedipine (Maggi et al., 1989c). However, a role of intracellular Ca^{2+} cannot be excluded, since NK2-receptor stimulation also activates phospholipase C (Suman-Chauhan et al., 1990; Martin et al., 1997). Prostanoids generated following NK2 receptor activation may amplify the direct contrac-

tile effect of NK2 receptor stimulation (Tramontana et al., 2000). Additional mechanisms may be involved, Thus, Wibberley et al. (2003) found that NKA-induced contractions of the rat detrusor could be attenuated by the Rho-kinase inhibitor, Y27632, suggesting involvement of the Rho-kinase pathway.

Detrusor Overactivity. Bladder outflow obstruction, which is often associated with detrusor overactivity, was shown to reduce the density of neuropeptide-containing nerves in the bladder (Chapple et al., 1992). On the other hand, in bladder tissue from women with idiopathic detrusor overactivity, a significant increase in the density of subepithelial, presumptive sensory nerves compared with stable controls was reported (Moore et al., 1992; Smet et al., 1997). Capsaicin-sensitive afferents may be a part of a spinal, vesico-vesical excitatory (short-loop) reflex providing a neurogenic mechanism for overactive detrusor contractions, both idiopathic and neurogenic (Maggi, 1993). In detrusor tissue from patients with idiopathic detrusor overactivity, blockade of NK receptors did not influence the atropine-resistant response (67%) to nerve stimulation (Moore et al., 2002), thus favoring an effect on the afferent loop of the micturition reflex.

Taken together, the available information suggests that tachykinins may be involved in pathophysiological afferent signaling associated with detrusor overactivity.

Vanilloids and Vanilloid Receptors. Both capsaicin and resiniferatoxin have been used successfully to treat bladder function disturbances (Andersson et al., 2002). They are known to bind to the VR1 receptor, a nonselective cation channel, on the peripheral terminals of nociceptive neurons, but the role of VR1 receptors in normal bladder function and in the pathogenesis in detrusor overactivity has not been established.

There is experimental and clinical evidence that capsaicin-sensitive afferents are involved in neurogenic and in other forms of detrusor overactivity (Birder et al., 2002; Cruz, 2002; Silva et al., 2002). Birder et al. (2002) investigated bladder function in conscious mice lacking the VR1 receptor. VR1 receptor-knockout mice had an increased voiding frequency compared with wild-type mice. The VR1-knockouts also had an increased frequency of nonvoiding bladder contractions. In vitro, stretch-induced ATP release was decreased in bladders from VR1-knockout mice, and also hypo-osmolality-induced ATP release from cultured urothelial cells was reduced (Birder et al., 2002). The authors suggested that VR1 receptors participate in normal bladder function, are involved in ATP release, and are essential for normal mechanically evoked purinergic signaling by the urothelium.

Clinical trials of intravesical resiniferatoxin in patients with idiopathic detrusor overactivity, refractory to antimuscarinic therapy, revealed that a single instillation of resiniferatoxin caused little initial discomfort. When evaluated at 3 months, 11 of 12 patients reported marked improvement of their urinary symptoms, particularly urge incontinence (Cruz, 2002).

ii. Calcitonin Gene-Related Peptide. Two discrete isoforms of CGRP have been described: αCGRP, which is produced by alternative splicing of a calcitonin gene transcript, and βCGRP, the product of a separate gene (Poyner et al., 2002). In the rat bladder, CGRP-immunoreactive nerves were found in smooth muscle and around blood vessels, and they formed a dense suburothelial plexus with some nerve fibers penetrating within the urothelium (Gabella and Davis, 1998). Binding sites for the peptide were demonstrated in the mucosa in the rat bladder (Nimmo et al., 1988; Banasiak and Burcher, 1994). In human bladders, CGRP receptors were expressed densely over the smooth muscle layer of arteries and arterioles, and weakly over collecting venules. They were not observed over the detrusor muscle (Burcher et al., 2000).

The family of CGRP receptors that has been characterized molecularly consists of the seven-transmembrane G-protein-coupled calcitonin receptor-like receptor linked to one of three single membrane-spanning receptor activity-modifying proteins. Receptor activity modifying proteins are required for both receptor trafficking and ligand binding (Poyner et al., 2002). At least two receptor types, CGRP-1 and CGRP-2, have been proposed from functional pharmacological experiments.

Even if the CGRP content of the bladder is of primary afferent origin, CGRP may have an efferent function when released, including relaxation of smooth muscle and vasodilatation. The action of CGRP is of different intensity in different regions of the urinary tract of the same species (Maggi et al., 1992). Thus, in the guinea pig, its relaxant action effect was much more intense in the bladder neck than in the bladder dome (Maggi et al., 1988b). In guinea pig and canine urinary bladder, CGRP is a potent relaxant agent. However, in the rat bladder, CGRP had little or no effect on either bladder motility or vascular permeability (Maggi et al., 1987b), and it had no effect on contractile activity in the human bladder (Maggi et al., 1989c). In agreement with these findings, Persson et al. (1991) found no functionally important mechanical effects in isolated pig detrusor strips, but the peptide was a potent dilator of vesical arteries through an endothelium-independent mechanism. It has been reported that CGRP increases blood flow in the cat bladder (Andersson, 1989), but whether or not CGRP has any importance for bladder function through this action remains to be established. In the hamster urinary bladder, CGRP and NKA are co-released by electrical nerve stimulation, and CGRP exerts an inhibitory effect on the detrusor (Giuliani et al., 2001). This confirms previous findings that CGRP and tachykinins have opposite motor effects on the bladder (Maggi et al., 1988a).

It has been suggested that CGRP is a neurotransmitter regulating motility in the mammalian urinary tract (Maggi et al., 1992). There are hardly enough data to

support that this is the case in, for example, the normal human bladder. However, it cannot be excluded that CGRP and tachykinins, released by different noxious stimuli from capsaicin-sensitive afferents, may contribute to symptoms in different disorders of the lower urinary tract, particularly those with damage of the urothelium due to inflammation, trauma, or cancer (Maggi et al., 1992). Supporting this, Smet et al. (1997) found that the density of CGRP and SP-immunoreactive nerves within the subepithelium was increased by 82% in women with urodynamically proven detrusor overactivity compared with control without any lower urinary tract symptoms. In patients with spinal cord injury, the subepithelial nerve plexus and its density of CGRP-containing nerves were reduced (Drake et al., 2000).

iii. Vasoactive Intestinal Polypeptide. VIP-containing nerves were found to form a dense subepithelial plexus which also projected to the detrusor muscle bundles (Smet et al., 1997). VIP is known to bind to bind to two types of receptor, $VPAC_1$ and VPC_2, in several types of smooth muscle (Harmar et al., 1998), including the human urinary bladder (Reubi, 2000). Both receptor subtypes are G-protein coupled. The effects of VIP on isolated urinary detrusor vary from species to species. In cats, VIP was released in response to pelvic nerve stimulation in vivo (Andersson et al., 1987b). In isolated detrusor muscle from humans, the peptide inhibited spontaneous contractile activity, but was found to have little effect on contractions induced by muscarinic receptor stimulation or by electrical stimulation of nerves (Kinder et al., 1985; Sjögren et al., 1985). Uckert et al. (2002), on the other hand, demonstrated a concentration-dependent relaxation of carbachol-induced contractions in human detrusor strips. This effect was paralleled by a 3- to 4-fold elevation in tissue cGMP and a 2-fold rise in cAMP, suggesting stimulation of both guanylyl and adenylyl cyclase. In detrusor strips from rat and guinea pig, no effect or contraction, was demonstrated, whereas relaxation and inhibition of spontaneous contractile activity was found in preparations of the rabbit detrusor (Andersson, 1993). If the low-amplitude myogenic contractile activity demonstrated in human, as well as animal bladders, is of primary importance for the genesis of reflex bladder contraction, direct inhibitory effects of VIP on bladder smooth muscle may be physiologically important.

Outflow Obstruction. Chapple et al. (1992) studied the levels of several neuropeptides in the bladder from normal patients and from patients with outflow obstruction. A reduction in the density of VIP, CGRP, and SP, but not NPY-immunoreactive nerve fibers, was shown in the obstructed bladder. They suggested that there may be an afferent nerve dysfunction resulting from bladder outflow obstruction.

Detrusor Overactivity. VIP levels were markedly reduced in patients suffering from idiopathic detrusor overactivity (Gu et al., 1983; Chapple et al., 1992) or neurogenic detrusor overactivity (Kinder et al., 1985). These results were interpreted to suggest that VIP (or rather lack of it) can be involved in some forms of detrusor overactivity. In rats with outflow obstruction, bladder hypertrophy, and detrusor overactivity, the concentrations of VIP in the middle and lower parts of obstructed bladders were *higher* than in controls (Andersson et al., 1988). Neither in the hypertrophic nor in the normal isolated rat bladder did VIP have relaxant or contractant effects, and the peptide did not influence contractions induced by electrical stimulation (Andersson et al., 1988; Igawa et al., 1993b).

iv. Neuropeptide Y. The human bladder is richly endowed with NPY-containing nerves (Gu et al., 1984; Iwasa, 1993; Crowe et al., 1995; Dixon et al., 1997). It seems that NPY can be found in adrenergic as well as cholinergic nerves. In neonates and children, Dixon et al. (2000) found small ganglia were scattered throughout the detrusor muscle of the 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 subepithelial location in all the organs examined, the majority containing NPY, whereas a small proportion contained NOS. The number of NPY-containing nerves did not change in the obstructed human bladder, where a general loss of sensory peptides was demonstrated (Chapple et al., 1992).

The presence of functional NPY receptors in human bladder was investigated by Davis et al. (2000) using peptide YY (PYY) as the agonist and $[^{125}I]$PYY as the radioligand. No quantifiable specific $[^{125}I]$PYY binding was detected in human bladder, and PYY caused no contraction of bladder preparations; furthermore, field stimulation-induced contraction was not affected by PYY. The authors concluded that human bladder expresses only very few, if any, functional NPY receptors. This is in contrast to findings in animal bladders. NPY-containing nerves were shown to be present in abundance in the rat detrusor (Andersson, 1993). Iravani and Zar (1994) found that exogenously added NPY contracted strips of rat detrusor and potentiated the noncholinergic motor transmission. The effects were blocked by nifedipine, and it was concluded that the abundant presence of NPY-like immunoreactive nerve fibers in the detrusor muscle was consistent with a motor transmitter function of the peptide in the rat bladder. These results are not in agreement with those of Zoubek et al. (1993), who found that porcine NPY did not induce any direct contractile effect in isolated strips of rat detrusor. On the other hand, porcine NPY had a marked inhibitory effect on the cholinergic component of electrically induced contractions in rat bladder strips, particularly at low (<10 Hz) frequencies of stimulation. In the isolated guinea pig detrusor, NPY had no effects per se, but

it decreased the electrically induced NANC response (Lundberg et al., 1984).

Thus, available information on the effects of NPY on detrusors from different species is conflicting. 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 (Tran et al., 1994), there is no convincing information that this is the case in humans.

v. Endothelins. The presence of ETs in animal and human detrusor has been well established (Sullivan et al., 2000). Garcia-Pascual et al. (1990) found ^{125}I-ET-1 binding sites mainly in the outer longitudinal muscle layer, in vessels, and in the submucosa of the rabbit bladder. The highest density of binding sites appeared to be in vessels and the outer muscle layer. Saenz de Tejada et al. (1992) demonstrated ET-like immunoreactivity in the transitional epithelium, serosal mesothelium, vascular endothelium, smooth muscles of the detrusor and vessels, and in fibroblasts in both human and rabbit bladder. This cellular distribution was confirmed in in situ hybridization experiments. Traish et al. (1992) characterized the ET receptor subtypes in the rabbit bladder using radioligand binding and suggested that at least two subtypes exist in rabbit bladder tissue, ET-1 and ET-2, binding to one subpopulation (ET_A) and ET-3 to the other (ET_B). Autoradiographic and radioligand binding studies have further demonstrated the presence of both ET_A and ET_B receptors in the epithelium and smooth muscle in human as well as rabbit and guinea pig bladders (Kondo et al., 1992; Mumtaz et al., 1999; Yoshida et al., 2003). The density of both ET receptors varied among the different parts of the bladder, the density in the dome being greater that in the base and the urethra (Latifpour et al., 1995; Mumtaz et al., 1999).

ET-1 is known to induce slowly developing, concentration-dependent contractions in animal as well as human detrusor muscle with a marked tachyphylaxis to the effects of the peptide (Andersson, 1993). The ET-1-induced contractions were not significantly affected by scopolamine or indomethacin, but could be abolished by incubation in a Ca^{2+}-free solution, and nifedipine had a marked inhibitory action. On the other hand, in human detrusor, the ET-1-induced contractions were resistant to nifedipine (Maggi et al., 1989d), illustrating species variation in the activation mechanisms. In rabbit bladder, Traish et al. (1992) found that ET-1, ET-2, and ET-3 all caused concentration-dependent contractions. The threshold concentrations of ET-3 to initiate contraction were higher than for ET-1 and ET-2.

The main role of ET_A receptors in the contractile effects of ETs in the detrusor has been confirmed by several other investigators in animal as well as human bladders (Donoso et al., 1994; Latifpour et al., 1995; Okamoto-Koizumi et al., 1999; Calvert et al., 2002; Yoshida et al., 2003). In human detrusor, Okamoto-Koizumi et al. (1999), using isometric contraction experiments and RT-PCR, demonstrated that the ET-1-

induced contractions were mediated mainly by the ET_A receptor and not by the ET_B receptor. RT-PCR revealed positive amplification of the ET_A, but not ET_B, receptor mRNA fragments (Okamoto-Koizumi et al., 1999). This is in contrast to findings in guinea pig bladder, where both ET_A and ET_B receptors contributed to ET-induced contraction (Yoshida et al., 2003).

The contractile effects of the ETs in the detrusor seem to involve both activation of L-type Ca^{2+} channels and activation of phospholipase C (Maggi et al., 1989e; Garcia-Pascual et al., 1990, 1993; Persson et al., 1992a). Thus, in the pig detrusor, contractile effects were associated with an increase in IP_3 concentrations and were blocked by the protein kinase C inhibitor, H-7, and by nifedipine (Persson et al., 1992a).

The functional role of ETs in the detrusor has not been established. The slow onset of the contractile effects seems to preclude direct participation in bladder emptying. Still, Yoshida et al. (2003) suggested that ETs may be involved in regulation of detrusor muscle tone by a direct effect. However, Donoso et al. (1994) found in the rat bladder that ET-1 potentiated the contractions evoked by both transmural nerve stimulation and applications of ATP at peptide concentrations 10-fold below those needed to produce an increase in bladder tone. This suggests a modulatory effect on detrusor neurotransmission.

Outflow Obstruction and Detrusor Overactivity. In a model of partial outflow obstruction in rabbits, Khan et al. (1999a,b, 2000) found a significant increase in the binding sites for both ET_A and ET_B receptors in the detrusor. In cells from obstructed rabbit bladders, proliferation could be inhibited by ET receptor antagonists (Khan et al., 2000). Since a mitogenic effect of ET-1 is well established, this would suggest that ET receptors can be involved in detrusor hyperplasia and hypertrophy associated with outlet obstruction, and that ET receptor antagonist could prevent this process. On the other hand, in the bladder of patients with benign prostatic obstruction, the density of ET receptors was decreased (Kondo et al., 1995).

Westfall et al. (2003) showed that i.v. ET-1 decreased micturition volume, an effect that could be blocked by both selective and nonselective ET-receptor antagonists. Whether or not this means that ET-receptor antagonist may have an effect on detrusor overactivity remains to be established.

Diabetes. ET_B receptors were shown to be up-regulated in the bladder dome, bladder neck, and urothelium of rabbits with experimental (alloxan) diabetes (Mumtaz et al., 1999). However, ET-1-induced contractions were impaired in the bladder neck of these diabetic animals. This could be related to the release of NO by activated ET_B receptors. In diabetic rabbit bladder cells, the proliferative effect could be inhibited by ET-receptor antagonists (Mumtaz et al., 2000). If this is

the case also in humans, ET-receptor antagonists may be useful for treatment of diabetic bladder dysfunction.

vi. Angiotensins. An autocrine/paracrine renin-angiotensin system has been identified in the urinary bladder (Weaver-Osterholtz et al., 1996), and angiotensin II (ATII) formation was demonstrated in human isolated detrusor smooth muscle (Andersson et al., 1992; Lindberg et al., 1994; Waldeck et al., 1997). ATII receptors have been demonstrated by different methods in animal as well as human detrusor (Anderson et al., 1984; Lam et al., 2000). ATII was reported to contract the urinary bladder of several species, but with a wide range of relative potencies (Erspamer et al., 1973; Anderson et al., 1984). Several investigators (Erspamer et al., 1981; Andersson et al., 1992, Saito et al., 1993; Lindberg et al., 1994; Waldeck et al., 1997), but not all (Lam et al., 2000), have shown that in human detrusor muscle, ATII was a potent and effective contractile agent. In dog bladder, the responses to both ATII and ATI were minor or lacking (Steidle et al., 1990), illustrating the wide variation in response to the peptide between species.

The responses in human detrusor were antagonized by the AT1 receptor antagonist losartan, but not by the AT2 receptor antagonist PD123319 [S-(+)-1-([4-(dimethylamino)-3-methylphenyl]methyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo(4,5-c)pyridine-6-carboxylic acid], indicating interaction with the AT1 receptor (Lam et al., 2000). Also, in the rat bladder, AT1 receptors mediated the contractile effect of ATII (Tanabe et al., 1993). ATI caused concentration-dependent contractions in the human detrusor, which, like those evoked by ATII, could be blocked by saralasin. This suggests that the actions of both ATI and ATII were mediated through stimulation of ATII receptors (Andersson et al., 1992). The contractile effect of ATII was very sensitive to removal of extracellular calcium, but less so to calcium antagonists, suggesting that calcium influx may occur through pathways other than L-type Ca^{2+} channels (Andersson et al., 1992; Saito et al., 1993c).

The effects of ATI could not be blocked by the angiotensin-converting enzyme (ACE) inhibitors captopril and enalaprilate (Andersson et al., 1992). Further studies revealed that a serine protease was responsible for ATII formation in the human bladder in vitro, probably human chymase or an enzyme similar to human chymase (Lindberg et al., 1994; Waldeck et al., 1997).

The functional importance of ATII in the detrusor has not been established. The delayed onset of action of the contractile effect of exogenous ATII, and the fact that saralasin was not able to block completely the atropine-resistant component of electrically induced contractions, compelled Anderson et al. (1984) to suggest that if ATII is involved in neurotransmission, it may be as a neuromodulator. Based on experiments in rabbits (Cheng et al., 1996, 1997), Cheng et al. (1999) suggested that 1) outlet obstruction of the bladder can cause increased cell stretch/strain, which in turn induces the local production of ATII. ATII may also influence cell stretch/strain via its direct effects on bladder tone. 2) ATII then acts as a trophic factor in the bladder wall to cause smooth muscle cell hypertrophy/hyperplasia and increased collagen production via an autocrine and/or paracrine pathway. 3) The cellular effect(s) of ATII may be mediated by secondary growth factors such as basic fibroblast growth factor and transforming growth factor-β. Stretch-stimulated growth of rat bladder smooth muscle cells was shown to involve the ATII receptor system (Nguyen et al., 2000). On the other hand, inhibition of ACE or blockade of ATII receptors had no effect on the development of bladder hypertrophy in rats (Persson et al., 1996; Palmer et al., 1997).

vii. Bradykinin. Bradykinin can contract the detrusor of several animal species with a wide range of relative potencies (Erspamer et al., 1973; Andersson, 1993). The physiological role of bradykinin as a contractile agonist in the bladder and its role in voiding are, however, largely unknown. In guinea pig urinary bladder, in which bradykinin was found to have a rather low relative potency (Erspamer et al., 1973), binding of [³H]bradykinin was located to the subepithelial lamina propria; it was absent over the muscle layers (Manning and Snyder, 1989). It was therefore suggested that bradykinin had an indirect action on urinary tract smooth muscle. Of the two bradykinin receptors identified, B1 and B2 (Regoli and Barabe, 1980; Hall, 1997), the B2 receptor seems to be the predominant receptor mediating the contractile response in the bladder under normal conditions. In isolated dog bladder muscle, bradykinin was a potent contractile agonist (Steidle et al., 1990); the effect was not enhanced by ACE inhibition. Bradykinin was also found to contract isolated human detrusor muscle (Andersson et al., 1992). However, it was considerably less potent and effective than ATI and ATII. The contractile effect was significantly increased after pretreatment with captopril or enalaprilate, which suggests that ACE inhibitors may reduce the degradation of bradykinin in the human detrusor.

Maggi et al. (1989e) found that multiple mechanisms were involved in the motor responses of the guinea pig isolated bladder to bradykinin. They found that bradykinin-induced contraction involved activation of both B2 receptors and prostanoid synthesis. In addition, they found that in carbachol-contracted detrusor strips, bradykinin produced relaxation. This response involved activation of B2 receptors and opening of apamin-sensitive K^+-channels. Maggi et al. (1989f) also found that bradykinin stimulated sensory nerves in the bladder, mainly by prostanoid production.

The cellular action of bradykinin seems to include a direct action on a G protein resulting in increased inositol 1,4,5-triphosphate turnover (Hall, 1992) and in part

a secondary release of prostanoids (Downie and Rouffignac, 1981; Nakahata et al., 1987; Andersson et al., 1992).

B1-dependent contractile responses are mainly absent under normal conditions, but they can be up-regulated by prolonged incubation in vitro. This has been demonstrated in isolated rabbit bladder (Butt et al., 1995). Lecci et al. (1999) reported an increased B1 response in inflamed bladders. Interleukin-1 has been proposed as a possible mediator in the increased response to bradykinin.

Sjuve et al. (2000) investigated bradykinin effects in vitro in isolated control and hypertrophic smooth muscle strips from rat bladder and from normal human bladders. The peptide caused contractions of small amplitude in normal and hypertrophic rat tissues. After a 4-h equilibratory period, contractile responses to bradykinin and the B1 specific bradykinin receptor agonist, desArg9 bradykinin, were slightly increased in the controls, but there was approximately a 6-fold increase in the hypertrophic muscle strips. After 4 h of equilibration, the human bladder strips showed a smaller but still significant increase in contractile response to bradykinin. Indomethacin almost abolished the increased response, which suggests that prostanoids are involved in the up-regulated response. The protein synthesis inhibitor, cycloheximide, inhibited up-regulation by approximately 50% in hypertrophic and control muscle strips from rat bladder and normal muscle from human bladder.

Available information suggests that bradykinin receptor responses are present in animal and human detrusor muscle, and they can be up-regulated in vitro. Even if the role for bradykinin in normal detrusor function is unclear, it cannot be excluded that it may contribute significantly to lower urinary tract symptoms in inflammatory conditions, for example. In hypertrophic rat bladder, bradykinin receptor seems to be up-regulated.

viii. GABA. GABA, glutamate decarboxylase, and the GABA transporter, GAT1, have all been shown to be present in the urinary bladder of guinea pigs and rats (Tanaka, 1985; Erdö et al., 1989; Pehrson and Andersson, 2002), and GABA may influence bladder motility by acting not only in the CNS, but also at the pelvic ganglionic (de Groat, 1970; Maggi et al., 1985a; Kataoka et al., 1994) and postganglionic levels, by reducing neurotransmitter release from neurons innervating the detrusor (Andersson, 1993; Pehrson and Andersson, 2002; Sanger et al., 2002). Pehrson and Andersson (2002) showed in the rat bladder that the GABA reuptake inhibitor tiagabine, by blocking GAT1 and increasing local levels of GABA, attenuated bladder contractions induced by electrical field stimulation, but did not affect contractions induced by carbachol. It was also found that tiagabine inhibited electrically induced acetylcholine release.

Both $GABA_A$ and $GABA_B$ receptors have been demonstrated to influence bladder activity peripherally. Both isoforms of the $GABA_B$ receptor were expressed in many peripheral organs, including the urinary bladder (Castelli et al., 1999). In the bladder from several species, prejunctional $GABA_B$ receptors were found to reduce the cholinergic component of the postganglionic excitatory neurotransmission (Andersson, 1993). However, in the mouse urinary bladder, GABA inhibited cholinergic as well as NANC, components of electrically induced contractions through stimulation of prejunctional $GABA_B$ receptors (Santicioli et al., 1986). In the rabbit bladder, GABA, again acting via $GABA_B$ receptors, inhibited carbachol-induced detrusor contraction by a direct effect on the muscle (Chen et al., 1992). This was not the case in human detrusor muscle strips, in which no inhibition of carbachol-mediated contractions by GABA could be demonstrated (Chen et al., 1994). In human detrusor muscle, Chen et al. (1994) therefore concluded that GABA, via the $GABA_B$ receptor, has an inhibitory effect on excitatory neurotransmission, and that the site of action was on the postganglionic nerves.

$GABA_A$ receptors were also found to be involved in the regulation of motility in the guinea pig isolated bladder through a modulatory effect on both cholinergic and NANC components of the postganglionic excitatory innervation (Taniyama et al., 1983; Kusunoki et al., 1984; Maggi et al., 1985b).

Activation of $GABA_A$ and $GABA_B$ receptors by muscimol and baclofen, respectively, reduced release of CGRP-like immunoreactivity from capsaicin-sensitive afferents in the guinea pig urinary bladder. This suggested that GABA receptors may be able to modulate the efferent function of afferent nerves (Santicioli et al., 1991).

In the normal, unanesthetized rat, muscimol and baclofen, injected i.a. near the bladder, had little effect on micturition. Only in high doses, where central nervous effects of the drug could not be excluded, were inhibitory effects recorded. It was therefore concluded that in vivo, GABA receptor agonists (muscimol and baclofen) have insignificant peripheral effects on the lower urinary tract, they but depress micturition by actions on the central nervous system (Igawa et al., 1993a).

ix. Enkephalins. By immunohistochemistry, enkephalins have been demonstrated in parasympathetic ganglia on the surface of the urinary bladder (Andersson, 1993), where they were suggested to be involved in δ-receptor-mediated inhibitory mechanisms (de Groat and Kawatani, 1989). Both methionine and leucine enkephalin were found to inhibit electrically evoked contractions in human detrusor and pig lower urinary tract smooth muscle, probably by a prejunctional effect (Klarskov, 1987a). In guinea pig urinary bladder, neither methionine nor leucine enkephalin had any prominent direct action on the smooth muscle, nor did they significantly modify the cholinergic or noncholinergic components of the contractile response to electrical stimulation of nerves (Mackenzie and Burnstock, 1984).

An involvement of opioid mechanisms in the peripheral control of detrusor muscle was suggested by Berg-

gren et al. (1992). They found in isolated rat and human detrusor muscle that naloxone caused a significant facilitation of the response to electrical field stimulation, which was counteracted by morphine and the synthetic δ-receptor agonist, DADLE [[d-Ala$_2$,d-Leu$_5$]enkephalin]. Morphine and DADLE had by themselves no effect on the electrically induced contractions, which is in agreement with previous findings in mouse urinary bladder (Acevedo et al., 1986). The authors suggested that there was a tonic, inhibitory action on the detrusor contraction elicited by electrical field stimulation exerted via μ- and possibly δ-receptors. Whether or not a peripheral action complements to the central depressant effects on micturition exerted by morphine is an open question. There is a possibility that opioid receptors in the urothelium may influence afferent signaling, not only for pain, but also for initiation of bladder activity. In a rat model of visceral pain, Craft et al. (1995) showed that intravesical instillation of a δ-receptor agonist (but not μ- and κ-receptor agonists) was effective, and they suggested that δ-receptors may be present on bladder nociceptive afferents. Intravesical administration of morphine has been investigated in patients with varying results. In children undergoing vesico-ureteral surgery, Duckett et al. (1997) demonstrated a pain-relieving effect in an open study, and an effect on bladder spasm of intravesical opioid has also been observed (McCoubrie and Jeffrey, 2003). However, in a controlled randomized study of postoperative pain in children undergoing vesico-ureteral surgery, no advantages of a continuous bladder infusion of morphine compared with placebo could be demonstrated (El-Ghoneimi et al., 2002). Whether or not opioid receptors in the bladder (urothelium, nerves, and ganglia) can be targets for drugs influencing micturition remains to be established.

x. Nociceptin.   Nociceptin or orphanin FQ, is a 17-amino acid neuropeptide, which has been identified as the endogenous ligand of opioid-like receptor-4 (OP4, previously known as ORL1), a G-protein-coupled receptor sharing sequence analogies with μ, δ, and κ opioid receptors, but displaying very low affinity for classic opioid ligands (Lecci et al., 2000a). Animal studies have demonstrated that nociceptin/orphanin FQ exerts an inhibitory effect on the micturition reflex in the rat (Giuliani et al., 1998, 1999; Lecci et al., 2000b,c). Nociceptin (10–100 nmol/kg), given i.v., inhibited the micturition reflex in a naloxone-resistant manner. These effects were not observed in capsaicin-pretreated animals, indicating that i.v. nociceptin inhibits the micturition reflex by inhibiting afferent discharge from capsaicin-sensitive nerves. Supporting this interpretation, nociceptin also inhibited the reflex, but not the local bladder contraction induced by topical capsaicin. Intrathecal nociceptin produced urodynamic modifications similar to those induced by i.v. administration. Intracerebroventricular nociceptin also inhibited the micturition reflex in a naloxone-resistant manner, suggesting a direct ef-

fect on supraspinal sites controlling micturition. A peripheral excitatory effect mediated by capsaicin-sensitive fibers was also detected in rats. Application of nociceptin onto the bladder serosa when the intravesical volume was subthreshold for triggering of the micturition reflex activated the reflex in a dose-dependent manner. In addition to the triggering of micturition reflex, topical nociceptin evoked a local tonic-type contraction that was abolished by the coadministration of tachykinin NK1 and NK2 receptor antagonists. Altogether these results indicate that ORL1 receptors are present at several sites for the integration of the micturition reflex, and that their activation may produce both excitatory and inhibitory effects, depending on the route of administration and the experimental conditions.

Lazzeri et al. (2001) investigated the urodynamic and clinical effects of intravesical instillation of nociceptin/orphanin FQ (1 μM) in five normal subjects and nine patients with neurogenic detrusor overactivity refractory to standard therapy. Intravesical instillation of nociceptin/orphanin FQ produced no significant functional changes in the controls. In the patients, there was a statistically significant increase in mean bladder capacity and volume threshold for the appearance of detrusor overactivity. A randomized, placebo-controlled, double-blind study was then performed in a selected group of 14 patients who had neurogenic detrusor overactivity due to spinal cord injury (Lazzeri et al., 2003). Intravesical infusion of a solution containing nociceptin/orphanin FQ, but not placebo, increased significantly bladder capacity and volume threshold for the appearance of detrusor overactivity. The authors suggested nociceptin/orphanin FQ receptor agonists as potential novel drugs for the treatment of neurogenic urinary incontinence.

xi. Parathyroid Hormone Releasing Protein.   Distension of the bladder wall can cause the synthesis of agents that may increase bladder compliance. One such factor is parathyroid hormone-related protein (PTHrP). Yamamoto et al. (1992) demonstrated that PTHrP mRNA levels changed in response to stretching of the rat bladder wall. PTHrP mRNA increased substantially with distension of the bladder, and in vitro, PTHrP-1(1–34)-NH$_2$ relaxed carbachol-induced contractions in strips from bladders kept empty in vivo. To test whether PTHrP could be increased solely by stretch rather than other possible in vivo variables, Steers et al. (1998) stretched cultured bladder smooth muscle cells and analyzed the culture medium for the protein. In response to mechanical stretch, PTHrP was increased in the smooth muscle cell cultures. PTHrP (1–100 nM) relaxed carbachol-contracted bladder body, but it did not affect bladder contractions induced by KCl (124 mM) or α,β-methylene ATP (10 μM). Steers et al. (1998) suggested the possibility that increased PTHrP secretion in response to stretching of smooth muscle can be an autocrine action to relax the bladder during filling. However, PTHrP may also exert a paracrine action on vessels

regulating blood flow during bladder filling or it may modulate neural activity. The mechanism for PTHrP-induced bladder relaxation does not seem to have been clarified, nor whether or not PTHrP has a role in the normal micturition cycle, increasing compliance during bladder filling.

8. Prostanoids and Leukotrienes. It is well established that prostanoids (prostaglandins and thromboxanes) are synthesized by cyclo-oxygenase (COX) in the bladder (Maggi, 1992; Andersson, 1993; Khan et al., 1998; Azadzoi et al., 2003). This enzyme exists in two isoforms, one constitutive (COX-1) and one inducible (COX-2). It has been suggested that, in the bladder, the constitutive form is responsible for the normal physiological biosynthesis, whereas the inducible COX-2 is activated during inflammation (Tramontana et al., 2000). Prostanoids are generated locally in both detrusor muscle and mucosa (Brown et al., 1980; Downie and Karmazyn, 1984; Jeremy et al., 1987; Khan et al., 1998), and their synthesis is initiated by various physiological stimuli such as stretching of the detrusor muscle (Gilmore and Vane, 1971), but also by injuries of the vesical mucosa, nerve stimulation, and by agents such as ATP and mediators of inflammation, e.g., bradykinin and the chemotactic peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (Maggi, 1992; Andersson, 1993; Khan et al., 1998).

There seem to be species variations in the spectrum of prostanoids and the relative amounts synthesized and released by the urinary bladder. Biopsies from the human bladder were shown to release prostaglandins (PG) and thromboxane A_2 (TXA$_2$) in the following quantitative order: $PGI_2 > PGE_2 > PGF_{2\alpha} > TXA_2$ (Jeremy et al., 1987). Chronic ischemia increased bladder 5-lipoxygenase and COX-1 and COX-2 protein expression, and it altered leukotriene and prostaglandin production (Azadzoi et al., 2003). Prostanoid actions are mediated by specific receptors on cell membranes. The receptors include the DP, EP, FP, IP, and TP receptors that preferentially respond to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA$_2$, respectively. Furthermore, EP is subdivided into four subtypes: EP_1, EP_2, EP_3 and EP_4 (Narumiya et al., 1999; Breyer et al., 2001). The signaling pathways vary. For example, TP receptors are known to signal via the G_q G protein, activating Ca^{2+}/diacylglycerol pathways, but also other G-proteins may be involved. EP_1 receptors signal via IP_3 generation and increased cell Ca^{2+}, activation of EP_2 and EP_4 leads to an increase in cAMP, and EP_3 activation seems to inhibit cAMP generation via a pertussis toxin-sensitive G_i-coupled mechanism and may also signal via the small G-protein Rho (Breyer et al., 2001).

The leukotrienes (LT) LTC_4, LTD_4, and LTE_4 were found to be synthesized in the guinea pig bladder (Bjorling et al., 1994), and LTD_4 and LTE_4 had contractile effects (Viggiano et al., 1985; Bjorling et al., 1994). No contractions were produced in rat bladder (Viggiano

et al., 1985). LTD_4 receptors have been demonstrated on human detrusor myocytes (Bouchelouche et al., 2001). LTD_4 was found to induce contraction in human detrusor smooth muscle cells, and the induced force and increased $[Ca^{2+}]_i$ were entirely dependent on Ca^{2+} release from intracellular stores (Bouchelouche et al., 2003).

Several investigators have shown that $PGF_{2\alpha}$, PGE_1, and PGE_2 contract isolated human, as well as animal, detrusor muscle (Andersson, 1993), which has led to the suggestion that they contribute to the maintenance of detrusor tone. Under ischemic conditions leukotrienes dominate bladder tone and appear to have a leading role in increased smooth muscle contraction and bladder overactivity (Azadzoi et al., 2003).

Prostanoids may affect the excitation-contraction coupling in the bladder smooth muscle in two ways, directly by effects on the smooth muscle, and/or indirectly via effects on neurotransmission. The membrane potential of guinea pig and rabbit smooth muscle cells was unchanged by low concentrations of PGE_2 (up to 10^{-6} M), but at higher concentrations the cells depolarized and the frequency of spontaneous action potential increased. It was concluded that prostanoids are not normally released by the nerves to the guinea pig urinary bladder. They are able to facilitate excitation-contraction coupling, possibly by mobilizing Ca^{2+} (Creed and Callahan, 1989). The contractile response of detrusor muscle to prostanoids is slow, and it is unlikely that these agents are directly involved in the evacuation of the bladder by exerting direct effects on the detrusor smooth muscle (Andersson and Sjögren, 1982).

The prostanoid receptor most important for detrusor function has not been established. Mice lacking EP_1 receptors had normal cystometry, but did not react to intravesical PGE_2 instillation, which caused detrusor overactivity in wild-type controls (Schröder et al., 2004). Prostanoids probably do not act as true effector messengers along the efferent arm of the micturition reflex, but rather as neuromodulators of the efferent and afferent neurotransmission; they may also have other functions in the bladder (Maggi, 1992; Andersson, 1993; Khan et al., 1998). It has been demonstrated that the expression of COX-2 is increased during bladder obstruction (Park et al., 1997), and that this can be a response to mechanical stress (Park et al. (1999). Obstruction of EP_1 receptor-knockout mice did not prevent the resulting increase in bladder weight but prevented the increase in spontaneous contractile activity (nonvoiding contractions) seen in the wild-type controls (Schröder et al., 2004).

9. Ion Channels.

a. Ca^{2+} Channels. Activation of detrusor muscle, both through muscarinic receptor and NANC pathways, seems to require both influx of extracellular Ca^{2+} through Ca^{2+} channels and mobilization of intracellular Ca^{2+} (Andersson, 1993; Fry et al., 2002). The importance of each mechanism may vary between species and also with respect to the transmitter studied. As men-

tioned previously, neuronal stimulation releases both acetylcholine and ATP in most animal bladders, whereas in the normal human detrusor, neurally mediated contractions are thought to occur predominantly through the actions of acetylcholine (Andersson, 1993). In rabbit and rat rabbit detrusor, ATP and acetylcholine have been proposed to cause either or both extracellular Ca^{2+} influx and release from stores (Andersson, 1993; Munro and Wendt, 1994; Yu et al., 1996; Mimata et al., 1997). In human detrusor smooth muscle, the contribution of intracellular store release and extracellular influx to cholinergic responses often differs between studies (Andersson, 1993; Harriss et al., 1995; King et al., 1998; Masters et al., 1999; Wu et al., 1999; Visser and van Mastrigt, 2000). However, a role of extracellular Ca^{2+} entry through dihydropyridine-sensitive Ca^{2+} channels in the contraction induced by muscarinic and/or purinergic stimulation is generally accepted. Imaizumi et al. (1998) studied $[Ca^{2+}]_i$ transients and electrical events in guinea pig detrusor and concluded from their experiments that the entry of Ca^{2+} in the early stages of an action potential evokes Ca^{2+}-induced Ca^{2+} release from discrete subplasmalemma storage sites and generates "hot spots" close to the cell membrane that spread to initiate a contraction. Calcium entry through L-type Ca^{2+} channels may result in potentials and in spontaneous contractions of detrusor smooth muscle cells (Hashitani and Brading, 2003a).

When contracted by high K^+ solutions or prolonged application of agonists, the detrusor muscle has an inability to sustain the contraction, and the response fades rapidly. Brading (1992) suggested that this is due to a transient increase in the membrane permeability to Ca^{2+}, the Ca^{2+} channels closing even at persistent depolarization. A well developed Ca^{2+}-induced inactivation of the channels may account for the phasic nature of the detrusor contraction and its inability to sustain tone.

The role of other Ca^{2+} channels than the L-type in detrusor activation has been debated. In cultured detrusor cells, the action potential was found to be completely abolished by L-type Ca^{2+} channel blockers, but incompletely so in freshly isolated cells (Sui et al., 2001). It was concluded that in freshly isolated guinea pig cells, both T- and L-type Ca^{2+} currents are present. Based on their experiments, Chow et al. (2003) concluded that Ca^{2+} influx through both T-type and L-type Ca^{2+} channels determines the contractile status of guinea pig detrusor smooth muscle, and that T-type channel activity is more important at membrane potentials near the resting level. The presence of both T- and L-type calcium channels also in human detrusor muscle has been demonstrated (Sui et al., 2003), and a role for these channels in spontaneous contractile activity was suggested.

i. Ca^{2+} Antagonists. L-type calcium antagonists, have a potent inhibitory effect on isolated human detrusor muscle (Andersson, 1993). Inhibitory effects have also been demonstrated clinically in patients with detru-

sor overactivity (Andersson, 1988). However, even though experimental data provide a theoretical basis for the use of Ca^{2+} antagonists in the treatment of detrusor overactivity, there have been few clinical studies of the effects of Ca^{2+} antagonists in patients with detrusor overactivity (Naglie et al., 2002), and available information does not indicate that oral therapy with these drugs is effective, possibly owing to the use of low doses to limit cardiovascular side effects (Andersson et al., 2002).

b. K^+ Channels.

i. K_{ATP} Channels. The presence of mRNA for sulfonylurea receptors has been demonstrated in both pig and human detrusor (Buckner et al., 2000) and the ATP-sensitive K^+ (K_{ATP}) channels in the detrusor of e.g., guinea pigs have been characterized (Gopalakrishnan et al., 1999). These channels have been shown to be involved in the regulation of bladder contractility (Andersson, 1992; Bonev and Nelson, 1993a).

Studies on isolated human detrusor muscle and on bladder tissue from several animal species have shown that K_{ATP} channel openers reduce not only spontaneous contractions (Buckner et al., 2002), but also contractions induced by electrical stimulation, carbachol, and low, but not high, external K^+ concentrations (Andersson, 1993). The drugs also increase the outflow of ^{86}Rb or ^{42}K in preloaded tissues, further supporting the view that they relax bladder tissue by K_{ATP} channel opening, subsequent hyperpolarization, and reduction in Ca^{2+} influx (Andersson, 1993; Trivedi et al., 1995; Wojdan et al., 1999).

ii. K_{Ca} Channels. The BK_{Ca} channels were shown to play an important role in controlling membrane potential and contractility of urinary bladder smooth muscle (Heppner et al., 1997; Christ et al., 2001). Measurements of BK_{Ca} currents and intracellular Ca^{2+} have revealed that BK_{Ca} currents are activated by Ca^{2+} release events (Ca^{2+} sparks) from ryanodine receptors in the sarcoplasmic reticulum (Herrera et al., 2000, 2001). Herrera et al. (2001) proposed that the membrane potential or any other factor that modulates the Ca^{2+} sensitivity of BK_{Ca} channels profoundly alters the coupling strength of Ca^{2+} sparks to BK_{Ca} channels.

Not only BK_{Ca} channels, but also small-conductance (SK_{Ca}) channels, are regulators of excitability in detrusor smooth muscle. Herrera and Nelson (2002) and Herrera et al. (2003) examined the role of SK_{Ca} channels in guinea pig detrusor, using the SK_{Ca} channel blocker apamin. Their results indicated that Ca^{2+} entry through voltage-dependent calcium channels activates both BK_{Ca} and SK_{Ca} channels, but Ca^{2+} release (Ca^{2+} sparks) through ryanodine receptors activates only BK_{Ca} channels.

Christ et al. (2001) demonstrated the importance of the BK_{Ca} channel, finding that local hSlo cDNA (i.e., the BK_{Ca} channel) injection ameliorated detrusor overactivity in a rat model of partial urinary outlet obstruction. They suggested that expression of hSlo in rat bladder

functionally antagonized the increased contractility normally observed in obstructed animals and thereby improved detrusor overactivity. They also thought that their observations could indicate a potential utility of gene therapy for urinary incontinence.

iii. K_v Channels. In addition to BK and SK channels, voltage-gated K^+ channels (K_V) may play an important role in the regulation of electrical activity of detrusor smooth muscle (Hashitani and Brading, 2003b). Davies et al. (2002) determined whether K_V channels are expressed and functional in human detrusor, and concluded, based on their data, that $K_{V\alpha1}$ subunits are expressed and are functionally important. How important these channels are for bladder function remains to be established, and also whether they can be targets for future treatment of detrusor overactivity.

iv. K^+ Channel Openers. It has been well established that the first generation of K_{ATP} channel openers (cromakalim, pinacidil, and nicorandil) has relaxant effects on the detrusor both in vitro and in vivo (Andersson, 1993), and these properties also characterize newer compounds. K_{ATP} channel openers have been shown to be particularly effective in hypertrophic rat bladder muscle in vitro (Malmgren et al., 1990; Wojdan et al., 1999), and they effectively suppressed detrusor overactivity in rats with bladder outflow obstruction (Malmgren et al., 1989; Wojdan et al., 1999; Fabiyi et al., 2003). In rats with spinal cord injury, the K_{ATP} channel openers, ZD6169 and ZD0947, very effectively inhibited detrusor overactivity (Abdel-Karim et al., 2002).

Newer K_{ATP} channel openers, such as ZD6169 (Howe et al., 1995; Trivedi et al., 1995) have been claimed to be more selective for the bladder than first-generation K_{ATP} openers (cromakalim, pinacidil). Oral administration of ZD6169 reduced voiding frequency in rats and dogs without lowering blood pressure (Howe et al., 1995; Wojdan et al., 1999; Lynch III et al., 2003). However, bladder selectivity was not confirmed by all investigators (Fabiyi et al., 2003). The reasons for the discrepancy in results have not been clarified.

Intravesical administration in rats increased the bladder volume for inducing a micturition reflex, decreased the frequency and amplitude of spontaneous bladder contractions, and decreased voiding pressure in both normal and outlet-obstructed animals (Pandita et al., 1997; Pandita and Andersson, 1999). It has been suggested that the drug acts not only on bladder smooth muscle but also on capsaicin-sensitive bladder afferents to reduce afferent firing induced by bladder distension or chemical irritation of the mucosa (Yu and de Groat, 1999).

BK_{Ca} channel openers may offer an alternative approach, but little information on their bladder effects is available. Tanaka et al. (2003) studied the effects of a 2-amino-3-cyano-5-(2-fluorophenyl)-4-methylpyrrole (NS-8), on the micturition reflex in rats. They found in a cystometry study that the drug increased bladder capacity without affecting the maximal bladder contraction pressure. Intravesical as well as intravenous injection of NS-8 inhibited isovolumetric bladder contractions in a dose-dependent manner without affecting their amplitude, whereas i.c.v. injection of NS-8 had no effect. During filling of the bladder, NS-8 decreased the discharge rate of the afferent pelvic nerve. The authors concluded the NS-8 might have the potential for treating patients with urinary frequency and incontinence. Future clinical studies will decide whether or not this is the case.

In clinical trials performed with the first generation of K^+ channel openers, no bladder effects have been found at oral doses already lowering blood pressure (Hedlund et al., 1991; Komersova et al., 1995). No other K^+ channel opener seems to have passed the proof of concept stage, and there is at present no convincing evidence showing that K^+ channel opening is a useful principle for treatment of detrusor overactivity (Andersson et al., 2002).

10. Unknown Factors. The mechanism by which the bladder maintains a low pressure during filling has not yet been established. It has been suggested that factors released from the urothelium should be able to modify the bladder response to distension (Andersson, 2002b), similar to what is found in the endothelium and vascular smooth muscle (Burnstock, 1999). Unidentified smooth muscle-relaxing factors, released from the bladder wall and/or the urothelium and causing bladder relaxation, have been described (Levin et al., 1995; Fovaeus et al., 1998, 1999; Hawthorn et al., 2000; Templeman et al., 2002). Fovaeus et al. (1999), using a coaxial bioassay system with endothelium-free, noradrenaline-contracted rat aortic preparations mounted within urothelium-intact urinary bladder, showed that carbachol caused a concentration-dependent relaxation of the vessel preparation. Carbachol had no relaxant effect if the urinary bladder segment was removed. However, the relaxation was affected neither by removal of the urothelium nor by bladder segment inversion. It was resistant to inhibition of the L-arginine/nitric oxide and COX pathways and remained unaffected by propranolol. The effect was interpreted as being caused by an unidentified relaxant factor released from bladder tissue (Fovaeus et al., 1999). The presence of a relaxant factor, released from the rat bladder by acetylcholine, was confirmed by Inci et al. (2003), who also showed that bladder inflammation did not alter the synthesis and/or release of this bladder-derived relaxant factor.

Hawthorn et al. (2000) reported the presence of a diffusable, urothelium-derived inhibitory factor in the pig bladder, which could not be identified, but did not appear to be NO, a COX product, a catecholamine, adenosine, GABA, or an endothelium-derived relaxing factor sensitive to apamin. A similar factor was found in the pig trigone, where cross-talk with the α-AR system was suggested (Templeman et al., 2002).

11. Botulinum Toxin. Seven immunologically distinct antigenic subtypes of botulinum toxin have been identified: A, B, C1, D, E, F, and G. Types A and B are in clinical use in urology, but most studies have been performed with botulinum toxin A type. Botulinum toxin acts by inhibiting acetylcholine release from cholinergic nerve terminals interacting with the protein complex necessary for docking acetylcholine vesicles (Smith et al., 2002b; Yokoyama et al., 2002). This results in decreased muscle contractility and muscle atrophy at the injection site. The produced chemical denervation is a reversible process, and axons are regenerated in about 3 to 6 months. Given in adequate amounts botulinum toxin inhibits release not only of acetylcholine, but also of several other transmitters. The botulinum toxin molecule cannot cross the blood-brain barrier and therefore has no CNS effects.

This type of chemical denervation of the bladder implies an effective treatment approach. In urology, botulinum toxin injected into the external urethral sphincter was initially used to treat spinal cord-injured patients with detrusor-external sphincter dyssynergia (Smith et al., 2002a; Yokoyama et al., 2002). The use of botulinum toxin has increased rapidly, and successful treatment of neurogenic detrusor overactivity by intravesical botulinum toxin injections has now been reported by several groups (Reitz et al., 2003). However, toxin injections may also be effective in refractory idiopathic detrusor overactivity (Radziszewski and Borkowski, 2002; Chancellor et al., 2003; Leippold et al., 2003; Rapp et al., 2004).

B. Urethra

The bladder and the urethra work as a functional unit, and under normal conditions, there is a reciprocal relationship between the activity in the detrusor and the activity in the outlet region. During voiding, contraction of the detrusor muscle is preceded by a relaxation of the outlet region, thereby facilitating bladder emptying (Tanagho and Miller, 1970). On the contrary, during the storage phase, the detrusor muscle is relaxed, and the outlet region is contracted to maintain continence.

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. NO has emerged as an important mediator of urethral smooth muscle relaxation, but a role of other transmitters cannot be excluded (Bridgewater and Brading, 1993; Hashimoto et al., 1993; Werkström et al., 1995). The relative contribution to intraurethral pressure of various factors is still a matter of discussion (Rud et al., 1980; Torrens, 1987; DeLancey, 1997). However, there is good pharmacological evidence supporting the view that noradrenaline-mediated contraction of the smooth muscle component has an important role.

Deficient urethral closure function may result in stress urinary incontinence. The pharmacological treatment of this condition, mainly based on α-AR agonists, has so far been disappointing (Andersson et al., 2002).

1. Innervation of the Urethra. The urethra of both males and females receives sympathetic (adrenergic), parasympathetic (cholinergic), and sensory innervation. The pelvic nerve conveys parasympathetic fibers to the urethra, and activity in these fibers results in an inhibitory effect on urethral smooth muscle and therefore in relaxation of the outflow region. Most of the sympathetic innervation of the bladder and urethra originates from the intermediolateral nuclei in the thoraco-lumbar 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 the pelvic nerves (Lincoln and Burnstock, 1993).

Both adrenergic and cholinergic nerves contain transmitters/transmitter-generating enzymes other than noradrenaline and acetylcholine. These agents, some of which are yet to be identified, are responsible for the NANC efferent neurotransmission, which can be demonstrated in urethral smooth muscle (see *Section III.B.6. and 7.*).

Most of the sensory innervation of the 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 (Lincoln and Burnstock, 1993). These sensory nerves are usually identified by their content of peptides, such as CGRP and tachykinins.

2. Adrenergic 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 preprostatic part of the urethra is richly innervated by both cholinergic and adrenergic nerves (Gosling et al., 1977). 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 (Ek et al., 1977a; Gosling et al., 1977). 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 hydroxy-lase) also contain NPY (Alm et al., 1995).

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 (Persson et al., 1997b). This suggests that NOS is not contained within adrenergic nerves.

3. Cholinergic Nerves. Urethral smooth muscle receives a rich cholinergic innervation. Most probably, the cholinergic nerves cause relaxation of the outflow region at the start of micturition by releasing NO and other relaxant transmitters (see *Section III.B.6. and 7.*), but otherwise their functional role is largely unknown. The cholinergic nerves of the bladder produce detrusor contraction, and disturbances in the co-ordination of the contractant effects on the bladder and the relaxation of the outflow region, may lead to the detrusor-sphincter dyssynergia, often seen in suprasacral spinal cord injuries.

In the pig urethra, colocalization studies revealed that ACh-positive and some NOS-IR nerves had profiles that were similar. These nerves also contained NPY and VIP immunoreactivity. NO-containing nerves were present in a density lower than that of the AChE-positive nerves, but higher than the density of any peptidergic nerves (Persson et al., 1995). Coexistence of acetylcholine and NOS in the rat major pelvic ganglion was demonstrated by double immunohistochemistry using antisera raised against NOS and choline acetyltransferase (Persson et al., 1998a). In the rat urethra, colocalization studies using antibodies to VAChT confirmed that NOS and VIP are contained within a population of cholinergic nerves. The distribution of immunoreactivities to nNOS, heme oxygenases (HO), and VIP was assessed by Werkström et al. (1998). HO-2 immunoreactivity was found in all nerve cell bodies of intramural ganglia, localized among smooth muscle bundles in the detrusor, bladder base, and proximal urethra. About 70% of the ganglionic cell bodies were also NOS immunoreactive, whereas a minor part was VIP immunoreactive.

4. Autonomic Receptor Functions in the Urethra.

a. α-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 (Appell et al., 1980; Furuya et al., 1982). 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 (Andersson, 1993; Brading et al., 1999). 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 that 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 rabbits) or in the distribution

of α_1-AR subtypes, do not seem to occur (Nasu et al., 1998). Taki et al. (1999) separated the entire length of the isolated female human urethra into seven parts, from the external meatus to the bladder neck, and examined for regional differences in contractile responses to various agents, including noradrenaline (α_1 and α_2) and clonidine (α_2). Noradrenaline, but not clonidine, produced concentration-dependent contractions in all parts, with a peak in middle to proximal urethra. They found a similarity in patterns between noradrenaline-induced contraction and the urethral pressure profile in the human urethra. Such a similarity has previously been shown in the female guinea pig (Ulmsten et al., 1977).

Among the three high-affinity α_1-AR subtypes identified in molecular cloning and functional studies ($\alpha_{1a/A}$, $\alpha_{1b/B}$, $\alpha_{1d/D}$), $\alpha_{1a/A}$ seems to predominate in the human lower urinary tract. However, the receptor with low affinity for prazosin (the α_{1L}-AR), which has not been cloned and may represent a functional phenotype of the α_{1A}-AR (Daniels et al., 1999), was found to be prominent in the human male urethra (Fukasawa et al., 1998). In the human female urethra, the expression and distribution of α_1-AR subtypes were determined by in situ hybridization and quantitative autoradiography. mRNA for the α_{1A} subtype was predominant, and autoradiography confirmed the predominance of the α_{1A}-AR.

The studies cited above suggest that the sympathetic innervation helps to maintain urethral smooth muscle tone through α_1-AR receptor stimulation. If urethral α_1-ARs are contributing to the lower urinary tract symptoms, which can also occur in women (Chai et al., 1993; Jollys et al., 1993; Lepor and Machi, 1993), an effect of α_1-AR antagonists should be expected in women with these symptoms. This was found to be the case in some studies but was not confirmed in a randomized, placebo-controlled pilot study (Lepor and Theune, 1995), which showed that terazosin was not effective for the treatment of "prostatism-like" symptoms in aging women.

Urethral α_2-ARs are able to control the release of noradrenaline from adrenergic nerves as shown in in vitro studies (Andersson, 1993). In the rabbit urethra, incubated with [³H]noradrenaline, and electrical stimulation of nerves caused a release of [³H], which was decreased by noradrenaline and clonidine and increased by the α_2-AR antagonist rauwolscine. Clonidine was shown to reduce intraurethral pressure in humans (Nordling et al., 1979), an effect that may be attributed partly to a peripheral effect on adrenergic nerve terminals. More likely, 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 [³H]noradrenaline secretion in the isolated guinea pig urethra was suggested to be of the α_{2A} subtype (Alberts, 1992).

Prejunctional α_2-ARs regulating transmitter release are not confined to adrenergic nerves (Andersson, 1993). Werkström et al. (1997) showed that elec-

trical field stimulation (EFS; frequencies > 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 N^G-nitro-L-arginine (L-NOARG), suggesting the release of an unknown relaxation-producing mediator. Treatment with the selective α_2-AR agonist UK-14,304 [5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine] 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.

b. β-Adrenoceptors. Both α- and β-ARs can be demonstrated in isolated urethral smooth muscle from animals (Persson and Andersson, 1976; Levin and Wein, 1979; Latifpour et al., 1990) and humans (Ek et al., 1977b). In humans, the β-ARs in the bladder neck were suggested to be of the β_2 subtype, as shown by receptor binding studies using subtype selective antagonists (Levin et al., 1988). However, the predominating β-AR in the human bladder seems to be of the β_3 subtype (Fujimura et al., 1999; Igawa et al., 1999; Takeda et al., 1999), and β_3-ARs can be found in the striated urethral sphincter also (Morita et al., 2000). The role of this subtype in urethral function does not seem to have been explored.

Even if 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 (Laval et al., 1978; Rao et al., 1980; Vaidyanathan et al., 1980; Thind et al., 1993). However, β-AR antagonists have not been shown to influence intraurethral pressure acutely (Thind et al., 1993).

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 noradrenaline 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 (Andersson et al., 2002).

Since 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 (Yasuda et al., 1993). The positive effects were suggested to be a result of an action on urethral striated muscle and/or the pelvic floor muscles (Morita et al., 1995, 2000).

c. Muscarinic Receptors. The number of muscarinic receptor binding sites in the rabbit urethra was lower than in the bladder (Johns, 1983). Muscarinic receptor agonists contract isolated urethral smooth muscle from several species, including human, but these responses seem to be mediated mainly by the longitudinal muscle layer (Andersson, 1993). Taki et al. (1999) investigated the whole length of the female human urethra and 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 (Persson and Andersson, 1976; Andersson et al., 1978b). In humans, tolerable doses of bethanechol (Ek et al., 1978) and emeprone (Ulmsten and Andersson, 1977) had little effect on intraurethral pressure.

Prejunctional muscarinic receptors may influence the release of both noradrenaline and acetylcholine 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 (Mattiasson et al., 1984). This would mean that released acetylcholine could inhibit noradrenaline release, thereby decreasing urethral tone and intraurethral pressure. The muscarinic receptor subtypes involved in contractile effects on smooth muscle or controlling transmitter release in the urethra have not been established. This may have clinical interest since subtype selective antimuscarinic drugs (M_3) are being introduced as a treatment of bladder overactivity.

5. Nonadrenergic, Noncholinergic Relaxant Mechanisms. The normal pattern of voiding in humans is characterized by an initial drop in urethral pressure followed by an increase in intravesical pressure (Tanagho and Miller, 1970; Andersson, 1993). The mechanism of this relaxant effect has not been definitely established, but several factors may contribute. One possibility is that the fall in intraurethral pressure is caused by stimulation of muscarinic receptors on noradrenergic nerves diminishing noradrenaline release and, therefore, tone in the proximal urethra. Another is that contraction of longitudinal urethral smooth muscle in the proximal urethral, produced by released acetylcholine, causes shortening and widening of the urethra, with a concomitant decrease in intraurethral pressure. A third possibility is that a NANC mechanism mediates this response (Andersson, 1993).

The mechanical responses of the cat urethra to autonomic nerve stimulation and to intraarterial acetylcholine injection were analyzed by Slack and Downie (1983). 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 acetylcholine 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 now reasons to believe that this transmitter is NO.

a. Nitric Oxide. NO has been demonstrated to be an important inhibitory neurotransmitter in the lower urinary tract (Andersson and Persson, 1993; Burnett, 1995). NO-mediated responses in smooth muscle preparations are most often linked to an increase in cGMP formation. This has also been demonstrated in the rabbit urethra (Morita et al., 1992b; Dokita et al., 1994; Persson and Andersson, 1994). Subsequent activation of a cGMP-dependent protein kinase 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 (Robertson et al., 1993; Peng et al., 1996). There have also been reports suggesting that NO in some smooth muscles might act directly on the K^+ channels (Bolotina et al., 1994; Koh et al., 1995). 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+} (Warner et al., 1994), 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 and Kimoto (1985) reported a hyperpolarization after NANC stimulation in some preparations of urethral smooth muscle from male rabbits. Furthermore, KRN 2391 (*N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboximidamide methanesulfonate), a combined NO donor and K^+-channel opener, had a pronounced relaxant effect accompanied by a hyperpolarization in the female rabbit urethra (Waldeck et al., 1995). These effects were suggested to be 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, which was not mediated by NO.

It seems reasonable to believe that the relaxant effect of NO in the rabbit urethra is mediated by increased levels of cyclic GMP. Evidence for this has been demonstrated by several investigators (Morita et al., 1992b; Dokita et al., 1994; Persson and Andersson, 1994). Accordingly, the cGMP-analog 8-Br-cGMP (8-bromo-cyclic guanosine monophosphate) was able to induce relaxation of rabbit urethra, further supporting the view of cGMP as a mediator of relaxation also in this tissue. The cGK phosphorylates K_{Ca} channels and thus increases their open probability, leading to hyperpolarization (Robertson et al., 1993). Furthermore, cGMP might affect sequestering of intracellular Ca^{2+}, affect Ca^{2+} extrusion pumps, and/or decrease the sensitivity for Ca^{2+}

(Warner et al., 1994). The latter may occur without changing the membrane potential. Thus, cGMP 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; Persson et al., 2000). 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 resting membrane potential of the smooth muscle of the guinea pig urethra was found to be -42.2 ± 4.0 mV (Callahan and Creed, 1981), and similar values (-40.1 ± 3.1 mV) were found in the rabbit (Ito and Kimoto, 1985). Spontaneous electrical activity was recorded from all regions of the organ (Callahan and Creed, 1981). Excitatory and inhibitory junction potentials have been recorded in rabbit urethra (Ito and Kimoto, 1985). The excitatory junction potentials were found to have a fast and a slow component. Only the slow component was blocked by atropine; the fast component was partially, but not completely, blocked by guanethidine and phentolamine. The inhibitory junction potentials were not affected by adrenergic or cholinergic antagonists, suggesting that they were generated by activation of a NANC mechanism.

Spontaneous electrical activity was found to occur in most preparations in the rabbit urethra, and the depolarizations were of two types: slow waves with high amplitude and short irregular spikes with low amplitude (Hashitani et al., 1996; Waldeck et al., 1998). Hashitani et al. (1996) suggested that these changes have a myogenic origin, caused by the release of Ca^{2+} from intracellular stores, acting on Ca^{2+}-activated chloride channels. In contrast to several other smooth muscle tissues in which NO act as an inhibitory NANC transmitter (Dalziel et al., 1991; Thornbury et al., 1991; Ward et al., 1992), the nitrergic inhibitory neurotransmission in the rabbit urethral smooth muscle seemed to be mediated through mechanisms, which do not involve membrane hyperpolarization (Waldeck et al., 1998). This contradicts results reported by Ito and Kimoto (1985), who observed inhibitory junction potentials in some registrations from rabbit urethral smooth muscle. However, these experiments were performed on male rabbits, and a difference in neurotransmission between the male and female urethra cannot be excluded.

The rich occurrence of NOS-immunoreactive nerve fibers also supports the present view of NO as the main inhibitory NANC mediator in rabbit urethra (Persson and Andersson, 1994). Waldeck et al. (1998) demon-

strated spindle-shaped cyclic GMP-IR cells, distinct from the smooth muscle cells, forming a network around and between the smooth muscle bundles. These results confirmed the findings of Smet et al. (1996) who found similar cyclic GMP-IR cells in the guinea pig and human bladder and urethra. The function of these interstitial cells has not been established, but they have been suggested to be pacemaker cells involved in the regulation of urethral tone (Sergeant et al., 2000, 2001, 2002). Thus, based on results obtained in freshly dispersed rabbit urethral interstitial cells, Sergeant et al. (2002) suggested that stimulation of α_1-ARs releases Ca^{2+} from an IP_3-sensitive store in the urethra. In turn, this activates a Ca^{2+}-activated Cl^- current, which elevates slow wave frequency in the cells. This may underlie the mechanism responsible for increased urethral tone during nerve stimulation.

b. ATP. ATP is believed to cause smooth muscle relaxation via G-protein-coupled P2Y receptors (Dalziel and Westfall, 1994). ATP may also induce relaxation via breakdown to adenosine. In strips of precontracted guinea pig urethra, Callahan and Creed (1981) showed that ATP caused relaxation, and ATP also inhibited spontaneous electrical activity. Ohnishi et al. (1997) studied the effects of ATP on isolated male rabbit circular urethral smooth muscle and also measured the outflow of ATP elicited by EFS, using the luciferase technique. In precontracted preparations, ATP had almost no effect on EFS-induced relaxation; however, suramin, a nonselective P2Y-purinoceptor antagonist, and L-NOARG both concentration-dependently attenuated the relaxation. ATP and related purine compounds (adenosine, AMP, and ADP) each reduced induced tonic contractions in a concentration-dependent manner. The outflow of ATP was markedly increased by EFS. The findings suggested that P2Y-purinoceptors exist in the male rabbit urethra, and that ATP and related purine compounds may play a role in NANC neurotransmission. This conclusion was further supported by Pinna et al. (1998), who studied the effect of EFS on circular strips of hamster proximal urethra precontracted with arginine vasopressin. EFS caused frequency-dependent relaxations, which were attenuated by suramin and reactive blue. Exogenous ATP produced concentration-related relaxations, which also were attenuated by suramin and reactive blue. The results were consistent with the view that ATP released from nerves is producing the NANC relaxation of hamster proximal urethra through stimulation of P2Y receptors.

In circular smooth muscle strips from the female pig urethra, which develop a spontaneous contractile tone, ATP caused a concentration-dependent relaxation (Werkström and Andersson, unpublished results). This relaxation was slowly developing and long-lasting. The P2Y-receptor agonist 2-methylthioATP relaxed the preparations with potency and efficacy similar to that of ATP. The ATP-induced relaxation was not significantly affected by treatment with a G-protein activator [guanosine 5′-*O*-(3-thiotriphosphate)], a G-protein inhibitor [guanosine 5′-*O*-(2-thio-diphosphate)], suramin, or reactive blue. Also, adenosine induced a concentration-dependent relaxation of the smooth muscle tone, which was not affected by treatment with the adenosine (P1) receptor antagonist, 8-(*p*-sulfophenyl) theophylline. Electrical field stimulation caused slowly developing and long-lasting relaxations in the presence of phentolamine, scopolamine, and L-NOARG. Guanosine 5′-*O*-(3-thiotriphosphate), guanosine 5′-*O*-(2-thio-diphosphate), suramin, and reactive blue did not affect these relaxations. It was suggested that exogenous ATP and adenosine relax the smooth muscle of the pig urethra in a manner similar to that evoked by EFS, but no evidence for involvement of a definable P2Y receptor subtype was found.

c. Carbon Monoxide. The distribution of the carbon monoxide (CO) producing enzymes HO-1 and -2 was studied by immunohistochemistry in the pig lower urinary tract (Werkström et al., 1997). CO has been suggested to mediate relaxation in the pig urethra (Werkström et al., 1997). Like NO, the relaxant effect of CO, was thought to be mediated by increased levels of cyclic GMP. However, no relaxant effect was observed in the rabbit urethra, and accordingly, no HO-1 or HO-2 immunoreactivity was observed. Thus, CO is less likely to be involved in the inhibitory neurotransmission in this tissue. HO-2 immunoreactivity was observed in coarse nerve trunks within the smooth muscle of the urethra, and HO-1 immunoreactivity was seen in nerve cells, coarse nerve trunks, and varicose nerve fibers in the smooth muscle. In urethral smooth muscle preparations, exogenously applied CO evoked a marked relaxation associated with an 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 (Werkström et al., 1998). 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 urethral strip preparations, electrical field stimulation evoked long-lasting, frequency-dependent relaxations. The relaxations were not inhibited by the NO-synthesis inhibitor, L-NOARG, or the inhibitor of guanylate cyclase, ODQ. The heme precursor, 5-aminolevulinic acid (5-ALA), did not affect the relaxations. Exogenously applied NO, SIN-1, and VIP relaxed the preparations by approximately 50%, whereas the relaxation evoked by exogenous CO was minor. These results suggested that CO is probably not involved in NANC inhibitory control

of the guinea pig urethra, where non-NO/cGMP-mediated relaxation seems to be predominant. However, in female pig urethral smooth muscle, 3-(5′-hydroxy-methyl-2′-furyl)-1-benzylindazole (YC-1), which increases the catalytic rate of soluble guanylyl cyclase by binding to an allosteric site, was found to increase the potency and maximal relaxant effect of CO to levels similar to those obtained with NO in the absence of YC-1 (Schröder et al., 2002). The finding that the response to CO was greatly increased after sensitizing soluble guanylyl cyclase suggests a potential for CO as a relaxant mediator in urethral smooth muscle.

6. Other Nonadrenergic, Noncholinergic Transmitters/Modulators. Additional systems with as yet unknown mediators have been observed in the urethra from pig (Bridgewater and Brading, 1993; Werkström et al., 1995) and dog (Hashimoto et al., 1993). Other agents shown to influence urethral function include neuropeptides, prostanoids, and serotonin.

a. Neuropeptides. Neuropeptides have been suggested to be involved in both contraction and relaxation of urethral smooth muscle, and similar to the bladder in afferent signaling. However, to a large extent, their functional roles remain to be established.

i. Vasoactive Intestinal Polypeptide. In various species, VIP-containing urethral ganglion cells have been demonstrated, and numerous VIP-IR nerve fibers 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 (Lincoln and Burnstock, 1993). Several investigators have shown that VIP is able to relax urethral smooth muscle from various species, including human, and the peptide was suggested to be responsible for the NANC-mediated urethral relaxation that could be demonstrated (Andersson, 1993). Sjögren et al. (1985) showed that VIP had a marked inhibitory effect on isolated female rabbit urethra contracted by noradrenaline or electrical stimulation of nerves. No effect was found on noradrenaline release. In human urethral smooth muscle, relaxant responses were less consistent, but a modulatory role in neurotransmission could not be excluded (Sjögren et al., 1985). Infusion of VIP in humans in amounts that caused circulatory side effects had no effect on urethral resistance (Klarskov et al., 1987). Plasma concentrations of VIP were obtained that, in other clinical investigations, had been sufficient to cause relaxation of the lower esophageal sphincter and to depress uterine contractions (Klarskov et al., 1987). Therefore, the physiological importance of VIP for the lower urinary tract function in humans was questioned (Klarskov et al., 1987).

In the pig urethra, VIP and NOS seem to be partly colocalized within nerve fibers (Persson et al., 1995). Waldeck et al. (1998) showed that VIP-IR nerve fibers 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 (Waldeck et al., 1998), and the relaxant mechanism for VIP seemed to be independent of changes of the membrane potential. This is in contrast to observations in the gastrointestinal tract, e.g., the feline esophageal smooth muscle (Ny et al., 1995), where exogenously applied VIP resulted in a relaxation and a distinct hyperpolarization. The ability of VIP to relax K$^+$-contracted preparations strengthens the hypothesis that the relaxant mechanism is independent of hyperpolarization (Waldeck et al., 1998).

Both pelvic and hypogastric nerve stimulation in dogs increased the bladder venous effluent VIP concentration (Andersson et al., 1990), which supports the view that VIP can be released also from urethral nerves. However, it is still unclear whether or not VIP contributes to NANC-mediated relaxation of the urethra.

ii. Neuropeptide Y. In the isolated female rabbit urethra, NPY reduced the electrically induced contraction of the longitudinal muscle, probably by selectively inhibiting the release of acetylcholine. It had no effect on circular muscle preparations. In the rabbit urethra, the peptide did not appear to have any significant postjunctional effects or to interfere with the release or effects of noradrenaline or NANC transmitters (Sjögren et al., 1988). However, in the spirally cut rat urethra, Zoubek et al. (1993) found that NPY exhibited a nearly maximal inhibition (90–100%) of electrically induced contractions over a broad range of stimulus frequencies (1–20 Hz). This finding suggests that NPY, at least in some species, may also affect the release of noradrenaline in the urethra.

iii. Tachykinins. Along the whole rat urethra, SP-immunoreactive terminals ran closely beneath the urothelium and sometimes gave off branches that penetrated into it. The smooth muscle supply of SP-containing fibers was sparse (Persson et al., 1997b). This localization would suggest a mainly afferent function for SP-containing nerves. However, this does not exclude an efferent function. Tachykinins produced powerful contractions of isolated rat, guinea pig, and human urethral smooth muscle (Maggi et al., 1988a; Parlani et al., 1990; Maggi and Patacchini, 1992; Palea et al., 1996). The contractile effects were not influenced by TTX, favoring the view that the receptors for the peptides are localized on the urethral smooth muscle cells. In rat proximal urethra, both NK1 and NK2 receptors seem to be present (Maggi et al., 1988a). In human urethral tissue the rank order of potency was found to be NKA > NKB ≫ SP, suggesting NK2 receptors are the dominating species. This was supported by results obtained with receptor-selective synthetic peptide agonists (Parlani et al., 1990; Palea et al., 1996). On the other hand, in guinea pig, isolated proximal urethra NK1 receptors were found to be the main, if not the only, mediator of tachykinin-mediated responses (Maggi and Patacchini, 1992), illustrating differences between species.

In the rat, intravesically administered tachykinins increase bladder activity by stimulating NK2 receptors, probably located to the urothelium or on suburothelial nerves (Maggi et al., 1991; Ishizuka et al., 1995b). Since the density of afferent nerves of the suburothelial plexus is highest in the bladder base and proximal urethra, it may be speculated that tachykinins, locally released by chemical or mechanical irritation of the primary sensory afferent nerves, may have a role in the genesis of abnormal urethral contractions (urethral instability) and are seen in humans (Kulseng-Hanssen, 1983), which may lead to detrusor overactivity.

iv. Endothelins. In the rabbit urethra, ^{125}I-ET-1 binding sites were found mainly in the outer longitudinal muscle layer, in vessels, and in the submucosa. The highest density of binding sites appeared to be in vessels and the outer muscle layer (Garcia-Pascual et al., 1990). Binding sites for both ET_A and ET_B receptors were demonstrated (Latifpour et al., 1995), but ET_A receptors were located only in the smooth muscle, whereas ET_B receptors were found both in the urothelium and in the smooth muscle layers (Wada et al., 2000; Afiatpour et al., 2003). Garcia-Pascual et al. (1990) showed that in isolated urethral smooth muscle, ET-1 caused concentration-related, slowly developing contractions. These contractile effects on urethral smooth muscle seem to be mediated by ET_A receptors (Sullivan et al., 2000). There was a marked tachyphylaxis in the effects of the peptide. The ET-1-induced contractions were not significantly affected by phentolamine or indomethacin, suggesting that they were produced by a direct effect on the smooth muscle cells. Incubation for 30 min in a nominally Ca^{2+}-free solution abolished the ET-1-induced contractions, but nifedipine had no effect. In the presence of ET-1, Ca^{2+}-induced contractions were not significantly blocked by nifedipine. ET-1 stimulated phosphoinositide hydrolysis in the rabbit urethra. The formation of inositol phosphates was dependent on extracellular Ca^{2+}. The Ca^{2+} entry pathway used was Ni^+sensitive and nifedipine resistant (Garcia-Pascual et al., 1993).

Pretreatment with ET-1 produced a significant increase in the contractions induced by electrical stimulation, but it had no significant effect on contractions induced by exogenous noradrenaline. ET-1 did not affect spontaneous or stimulation-induced efflux of [^{3}H]noradrenaline (Garcia-Pascual et al., 1990).

The role of ETs for urethral function has not been established. Considering the slowly developing and long-lasting contractile effect and the ability to enhance the contractile effects of noradrenaline, it may be speculated that ETs contribute to long-term regulation of urethral smooth muscle tone and therefore to continence.

7. Prostanoids. It is well established that prostanoids have a dual effect on lower urinary tract smooth muscle. PGE_1, PGE_2, and $PGF_{2\alpha}$ all contract detrusor muscle, but in the urethra, PGE_1 and PGE_2 cause relaxation, whereas $PGF_{2\alpha}$ produces contraction (Andersson, 1993). This pattern of effects has been demonstrated in animal (Persson and Andersson, 1976; Khanna et al., 1978; Gotoh et al., 1986; Morita et al., 1994; Pinna et al., 1996), as well as human urethral smooth muscle (Andersson et al., 1978a). In women receiving PGE_2 intravesically or intraurethrally, a decrease in the maximum urethral pressure and a reduction of the closure pressure were found (Andersson et al., 1978a; Schussler, 1990).

Morita et al. (1994) showed that PGE_1 and PGE_2 significantly relaxed the male rabbit urethral smooth muscle strips, whereas $PGF_{2\alpha}$ caused contraction. In the strips, cAMP, but not cGMP, increased significantly after administration of PGE_1 or PGE_2.

The dual direct effects of PGE_2, for example, on bladder and urethral smooth muscle may seem appropriate for bladder emptying. However, the time course of the mechanical effect is slow, and it is unlikely that they play a role for bladder emptying. As has been suggested previously (Andersson, 1993), an effect involving local modulation of afferent nerve activity seems more plausible.

8. 5-Hydroxytryptamine (Serotonin). Hills et al. (1984) found that in the isolated pig bladder neck preparation, 5-HT produced concentration-dependent relaxations. The effect was antagonized by methysergide, but the relaxant response to electrical nerve stimulation was not. In rabbit trigonal and urethral muscle strips, 5-HT produced a concentration-dependent contraction (Chen, 1990). Although no detailed data for the urethra were presented, it was concluded that, like in the bladder, the $5\text{-}HT_3$ receptor was involved in the mediation of the response. In humans, the $5\text{-}HT_2$ receptor antagonist ketanserin reduced intraurethral pressure (Hørby-Petersen et al., 1985, Andersson et al., 1987a; Delaere et al., 1987). This was, however, attributed mainly to ketanserin's blocking effect on urethral α-adrenoceptors.

IV. Effects of Sexual Hormones

A. Estrogen and Progesterone

It is well established that lower urinary tract innervation, receptor density and distribution, and contractile function may change significantly during periods of marked changes in female hormone levels, such as puberty, pregnancy, and menopause (Andersson, 1993). The lower urinary tract in animals and humans has been shown to express estrogen receptors (Blakeman et al., 2000; Nilsson et al., 2001). However, in human bladders, estrogen receptors were found only in squamous epithelia (trigone, proximal and distal urethra). No estrogen receptors were found in transitional urothelium or detrusor muscle, and there was no variation with estrogen status (Blakeman et al., 2000). In the urethra, the expression is greater but still inconsistent (Blakeman et al., 2000). In animals, both estrogen-α and estrogen-β receptors have been demonstrated in epithelium, detrusor muscle, and rhabdosphincter, however, many of the rapid estrogen-induced changes have been

postulated to be nonreceptor mediated (Nilsson et al., 2001).

The effects of estrogen on lower urinary tract structure and function and on the autonomic nervous control have been examined in many animal studies (Andersson, 1993; Longhurst and Levendusky, 2000; Yono et al., 2000, Fleischmann et al., 2002; Liang et al., 2002; Longhurst, 2002; Aikawa et al., 2003), often with conflicting results. In particular, there has been disagreement on how estrogens affect muscarinic receptor functions. Levin et al. (1980) found that estrogen treatment (estradiol) to immature female rabbits induced a marked increase in the detrusor response to stimulation of α-adrenoceptors, muscarinic receptors, and ATP, and that in the bladder body and midsection, there was a significant increase in the number of α-adrenoceptors and muscarinic receptors. Shapiro (1986) reported that treatment of mature female rabbits with estradiol led to a significant decrease in muscarinic receptor density. This observation was confirmed by Batra and Andersson (1989), who also found that the decrease in muscarinic receptor number had little effect on the responses to carbachol and electrical stimulation. Elliott et al. (1992) found a decreased sensitivity to acetylcholine, carbachol, and electrical stimulation, but not to K^+ in isolated detrusor tissue from female rats treated with estradiol. Addition of diethylstilbestrol further reduced these responses, an effect previously investigated by the same group and attributed to a reduction of calcium influx into the detrusor cells (Elliott et al., 1992). Selective β_3-AR agonists relaxed the detrusor muscle of female rats with low estrogen levels (Matsubara et al., 2002), which was suggested to have potential clinical implications.

In nonanesthetized mice lacking estrogen-α, estrogen-β, or both receptor subtypes, in vitro contractility and cystometry were no different from those in wild-type controls. However, mice lacking estrogen-α receptors did not respond to intravesical capsaicin instillation, suggesting a role of estrogen receptors in afferent signaling in the bladder (Schröder et al., 2003).

In the urethra, estrogens may increase the smooth muscle sensitivity to α-AR stimulation, and also improve the function of the vasculature and connective tissue of the lamina propria (Andersson, 1993).

Taken together, the above results suggest that estrogens, at least in animals, can influence lower urinary tract structure and function and modify the responses of the detrusor to autonomic nervous influences. The results are not always consistent, and some of the discrepancies may be attributed to differences in experimental approach. Even if an estrogen effect on afferent signaling in humans seems possible, available data do not allow conclusions to be drawn that consistently are applicable to treatment of urine storage disorders, for example.

Little is known about the effects of progesterone on bladder function, and the functional importance of the progesterone receptors that have been demonstrated in the lower urinary tract (Batra and Iosif, 1987; Wolf et al., 1991) has not been established. In vitro administration of progesterone reduced the response of bladder strips to electrical field stimulation and KCl (Elliott and Castleden, 1994). In contrast, long-term, in vivo treatment with progesterone increased the maximal response of rabbit bladder preparations to electrical field stimulation and increased the sensitivity to muscarinic receptor stimulation compared with ovariectomized controls (Ekström et al., 1993). In contrast, Tong et al. (1995) found that in vivo treatment with progesterone decreased the maximal response of rat bladder strips to acetylcholine and that there was a decrease in muscarinic receptor density.

As for estrogens, available information does not allow conclusions to be drawn that are applicable to treatment of lower urinary tract disorders.

B. Androgens

The effects of androgens on lower urinary tract functions have not been widely investigated. In rabbits, androgen receptors were found in the highest concentrations in urethral and bladder epithelium, and the concentrations in smooth muscle were low or moderate (Rosenzweig et al., 1995). Androgen receptors were also identified on neurons in the medial preoptic area projecting to the pontine micturition center in male cats (Blok and Holstege, 1998).

Based on observations in castrated rats, testosterone seems to be essential for maintaining some of the neural pathways involved in urine storage (Keast, 1999). A significant increase in the density of muscarinic receptors in bladder tissue was demonstrated in rabbits treated with testosterone. There was, however, no change in the maximal response to carbachol, and no change in the EC_{50} value, and the treatment did not affect the density of α- or β-adrenoceptors (Anderson and Navarro, 1988). In the rabbit, acute application of testosterone had no effect on isolated detrusor preparations (Ratz et al., 1999). However, Hall et al. (2002) concluded from their experiments on isolated rat bladder that testosterone may act on a postjunctional nongenomic receptor to inhibit detrusor muscle contraction.

Thus, under certain experimental conditions, androgens may be able to influence lower urinary tract function in animals. However, available information does not permit any extrapolations to either normal or castrated humans.

C. Pregnancy

Urinary incontinence (particularly stress incontinence) is common during pregnancy and has been attributed, at least in part, to changes in bladder and urethral function (Toozs-Hobson and Cutner, 2001).

In rats, pregnancy was reported to increase bladder weight and capacity, decrease the responses to muscarinic and α-AR stimulation, and increase the response to ATP (Tong et al., 1995). Thus, in the presence of bethanechol, bladder strips from pregnant rabbits generated 50% less tension in response to calcium than those from nonpregnant rabbits (Zderic et al., 1990). The isolated whole bladder from pregnant animals responded to low-frequency stimulation and to ATP with a greater increase in intravesical pressure than did preparations from virgin rabbits, whereas the response to bethanechol was greater in the virgin rabbits (Levin et al., 1991). Receptor binding studies in bladder tissue from pregnant animals revealed a significantly reduced muscarinic receptor density (50%), corresponding to the decrease in response to bethanechol of the whole bladder. The results were interpreted to mean that pregnancy induced an increase in the purinergic and a decrease in the cholinergic components of the urinary bladder response to field stimulation (Levin et al., 1991). A reduction of the muscarinic receptor density in the pregnant rabbit bladder was confirmed by other investigators (Baselli et al., 1999).

To what extent the receptor and functional changes demonstrated in the pregnant bladder from different species can explain the voiding disturbances found in pregnant women remains to be established.

V. Summary and Conclusions

In the last decade, research in the field of the physiology and pharmacology of the lower urinary tract has provided much new information and the emergence of several new concepts regarding both the central nervous and peripheral control of voiding and the etiology of voiding dysfunction. The search for new therapies to treat voiding disorders has been intensive, and new targets for drugs aiming at micturition control have been defined, such as CNS transmitter/modulator systems, urothelial signaling mechanisms, and afferent nerves. Despite the many potential targets, few drugs with modes of action other than antimuscarinic have passed the proof of concept stage. A major problem has been to find drugs exhibiting "clinical uroselectivity" (Andersson 1998). There are multiple mechanisms, some proven in concept, but more theoretical, by which a pharmacological agent could facilitate lower urinary tract filling/urine storage and emptying. These include peripheral and central efferent and afferent sites of action. Drugs, however, do not affect only the smooth or striated musculature of the bladder and bladder outlet or the reflexes/pathways controlling their activities. This lack of selectivity is responsible for a given agent's side effects, which limit its ability to be used in dosages above a certain level. Improved uroselectivity can be approached in a number of ways: 1) receptor selectivity; 2) alterations in drug delivery, metabolism, and distribution;

and 3) organ selectivity. Receptor selectivity may be of little use unless the receptor under consideration is not expressed or operative in other organs or pathways or unless a receptor subtype exists which is specific for the organ being treated or its neurological connections. Organ specificity remains the holy grail of drug therapy. Finally, useful drugs should not interfere with the ability to empty the bladder in a normal manner. Micturition control is complex, and most probably, several classes of drugs will eventually be used to treat voiding problems. However, drug development is a time-consuming process, and since the potential of the treatment targets defined so far is promising, there are reasons to believe that new drugs effective for treatment of voiding disorders will be introduced in the coming decade.

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