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Neuropeptides in Lower Urinary Tract (LUT) Function

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Abstract

Numerous neuropeptide/receptor systems including vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating polypeptide, calcitonin gene-related peptide, substance P, neurokinin A, bradykinin, and endothelin-1 are expressed in the lower urinary tract (LUT) in both neural and non-neural (e.g., urothelium) components. LUT neuropeptide immunoreactivity is present in afferent and autonomic efferent neurons innervating the bladder and urethra and in the urothelium of the urinary bladder. Neuropeptides have tissue-specific distributions and functions in the LUT and exhibit neuroplastic changes in expression and function with LUT dysfunction following neural injury, inflammation and disease. LUT dysfunction with abnormal voiding including urinary urgency, increased voiding frequency, nocturia, urinary incontinence and pain may reflect a change in the balance of neuropeptides in bladder reflex pathways. LUT neuropeptide/receptor systems may represent potential targets for therapeutic intervention.

Keywords

substance P; calcitonin gene-related peptide; vasoactive intestinal polypeptide; pituitary adenylate cyclase activating polypeptide; nerve growth factor

19.1 Introduction: Micturition Overview and Anatomy of Lower Urinary Tract

Micturition is regulated by neural circuits in the brain and spinal cord that coordinate the activity of the smooth and striated muscles of the lower urinary tract (LUT) (Fowler et al., 2008). These circuits act as on-off switches to shift the urinary tract between two modes of operation: storage and elimination. Bladder smooth muscle and the urethral outlet must function reciprocally for efficient elimination of urine (Fowler et al., 2008). The bladder smooth muscle must remain relaxed during storage mode to allow for filling, while the urethral outlet is contracted (Fowler et al., 2008). Elimination mode requires contraction of bladder smooth muscle and relaxation of the urethral outlet to allow urine flow (Andersson and Arner, 2004). Precise coordination of the reciprocal functions of the urinary bladder and

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urethra and complex neural organization are required for normal function (Fowler et al., 2008).

The LUT, consisting of the urinary bladder, urethra, internal urethral sphincter and external urethral sphincter, is composed of both striated and smooth muscle, and therefore under both voluntary (somatic) and involuntary (autonomic) influence (Andersson and Arner, 2004). The bladder consists of three layers: a urothelium on the luminal surface, a lamina propria just deep to the urothelium that contains a suburothelial plexus of nerves and vasculature, and an outer muscle layer, named the detrusor, that contains both longitudinal and circular smooth muscle (Andersson and Arner, 2004) (Fig. 19.1). The urothelium in rodents is composed of at least three layers: the basal, intermediate and superficial/umbrella layers. However, in higher mammals including humans, there are additional intermediate layers (Wu et al., 2009). The umbrella cells are connected by tight junctions and are covered on their apical surface by crystalline proteins, which assemble into hexagonal plaques (for review see (Wu et al., 2009)).

19.1.1 Neural control of micturition

The storage and periodic elimination of urine requires a complex neural control system that coordinates the activities of a variety of effector organs including the smooth muscle of the urinary bladder and the smooth and striated muscle of the urethral sphincters (Kuru, 1965; Klück, 1980; de Groat and Steers, 1990; Andersson and Arner, 2004). Three neural pathways regulate the LUT: (1) sacral parasympathetic (pelvic) nerves provide excitatory input to the bladder; (2) thoracolumbar sympathetic (hypogastric) nerves provide an inhibitory input to the bladder and an excitatory input to the bladder neck and urethra; and (3) sacral somatic (pudendal) nerves which innervate the striated muscles of the sphincters and pelvic floor (Kuru, 1965; Klück, 1980; de Groat and Steers, 1990; Middleton and Keast, 2004). Each of these sets of nerves contains afferent (sensory) as well as efferent (motor) axons (Morrison, 1987; Lincoln and Burnstock, 1993).

The central neural pathways controlling the LUT exhibit “all-or-none” or “switch-like” characteristics reflecting the storage and elimination functions of the LUT (de Groat et al., 1993; de Groat and Kruse, 1993; de Groat, 1997). During urine storage, somatic and sympathetic pathways to the sphincters and sympathetic inhibitory inputs to the bladder are tonically active whereas parasympathetic pathways are inactive (Kuru, 1965; de Groat et al., 1993; de Groat and Kruse, 1993; de Groat, 1997). During reflex or voluntary micturition, the activity patterns are reversed such that parasympathetic pathways are excited and somatosympathetic pathways are inhibited thereby promoting urine flow (Middleton and Keast, 2004).

The LUT reflex mechanisms, organized at the level of the lumbosacral spinal cord, are modulated predominantly by supraspinal controls (Kuru, 1965; de Groat, 1975; de Groat et al., 1993; de Groat and Kruse, 1993; de Groat, 1997; Middleton and Keast, 2004). These mechanisms can be summarized as follows: (1) storage reflexes (parasympathetic and somatic) are organized at the spinal level; (2) elimination reflexes (parasympathetic) are organized at a supraspinal site in the pons; and (3) spinal storage reflexes are modulated by inputs from the rostral pons.

19.1.2 Neurochemistry and morphology of afferent and spinal pathways to the urogenital tract

Bladder afferent neurons travel in the hypogastric and pelvic nerves, and their cell bodies are located in dorsal root ganglia (DRG) at spinal segments T11-L2 and S2–S4 in humans and L1-L2 and L6-S1 in rats (Fowler et al., 2008) (Fig. 19.1). Bladder afferent fibers consist of lightly myelinated A δ fibers and unmyelinated C-fibers. Sensation of bladder filling is conveyed by A δ fibers, the most important mechanoreceptors of the bladder. C-fibers are normally “silent”, but they do respond to chemical or noxious stimuli, including extreme bladder pressure (Fowler et al., 2008). A δ and C-fibers terminate in the urothelium, suburothelium, and smooth muscle layers of the bladder (Kullmann et al., 2008). Most bladder afferent fibers project to lumbosacral spinal cord segments, and this is the most important region of the spinal cord relative to signaling the micturition reflex (Holstege, 2005) (Fig. 19.1). Most sensory nerves in the bladder are located in a dense suburothelial plexus just beneath the urothelium (Andersson and Wein, 2004) (Fig. 19.1).

Bladder afferent fibers in the pelvic nerve in rodents pass through the dorsal roots into Lissauer’s tract at the apex of the dorsal horn and then give off collateral branches that extend ventromedially and ventrolaterally along the superficial layers of the dorsal horn to the dorsal commissure and to the area of the sacral parasympathetic nucleus (laminae V–VII) that contains preganglionic parasympathetic neurons that project to the periphery (de Groat et al., 1981; Donovan et al., 1983; de Groat et al., 1986; Steers et al., 1991a; de Groat and Kruse, 1993; de Groat et al., 1994; Nadelhaft and Vera, 1995; de Groat, 1997; Marson, 1997). The most prominent pathway is located in lamina I on the lateral edge of the dorsal horn in a region termed the lateral collateral pathway of Lissauer’s tract. Afferent projections from the pudendal nerve and genital structures follow the medial edge of the dorsal horn into the dorsal commissure region, forming the medial collateral pathway (Kawatani et al., 1990). Bladder afferent fibers contain a variety of neuropeptides, including: calcitonin-gene related peptide (CGRP), substance P (SP), neurokinin A, neurokinin B, vasoactive intestinal polypeptide (VIP), cholecystokinin and enkephalins (Donovan et al., 1983; de Groat et al., 1986; Keast, 1991; de Groat et al., 1996; Vizzard, 2000d; Vizzard, 2001) (Fig. 19.1). We have demonstrated (Vizzard, 2000d) that bladder afferent cells express pituitary adenylate cyclase-activating polypeptide (PACAP) and that expression is increased after cyclophosphamide (CYP)-induced cystitis in rats. With the exception of CGRP, all of these substances are predominantly expressed in small (presumably C-fiber) afferents (Ek et al., 1977; Donovan et al., 1983; de Groat et al., 1986; Su et al., 1986; Keast and de Groat, 1992; Vizzard et al., 1993a; Vizzard et al., 1993b; Vizzard et al., 1994; Vizzard et al., 1995; de Groat et al., 1996; Vizzard and de Groat, 1996). The administration of capsaicin, which acts selectively on small diameter afferent fibers to deplete neurotransmitter stores to induce neuronal degeneration, reduces the levels of substance P, neurokinin A and CGRP within the pelvic viscera but does not affect VIP or enkephalin expression (de Groat, 1987). These findings are consistent with SP, related tachykinins and CGRP expression in afferent pathways to the pelvic viscera (de Groat, 1987).

Many bladder afferent fibers project to the sacral parasympathetic nucleus, synapsing with preganglionic parasympathetic neurons as well as interneurons (Morgan et al., 1981; Fowler

et al., 2008). Primary bladder afferents from the pelvic and hypogastric nerves also project to the dorsal commissure and superficial dorsal horn (Fowler et al., 2008). The lumbosacral dorsal commissure, superficial dorsal horn, and parasympathetic nucleus all contain interneurons important to urinary bladder function (de Groat and Kruse, 1993; Fowler et al., 2008). These interneurons project locally in the spinal cord or to the brain (Fowler et al., 2008). Some bladder afferents synapse with ascending pathways in the spinal cord that project to neuronal populations in the brain involved in micturition control, including the pontine micturition center (de Groat and Kruse, 1993; Fowler et al., 2008).

19.1.3 Neurochemical plasticity in the LUT with bladder inflammation, neural injury or disease

Neuroactive compounds, including neuropeptides, in the afferent pathways to the LUT exhibit either excitatory or inhibitory actions. Non-neural sources of peptides in the LUT include plasma, sites of tissue inflammation or injury, detrusor smooth muscle cells, bladder fibroblasts and the urothelium. Pathology, neural injury and target organ pathology (e.g., bladder inflammation) can alter the known balance of neuropeptides either in the periphery and/or central pathways conceivably shifting the balance to a hyper- or hypo-active reflex state. Changes in micturition reflex function observed with urinary bladder inflammation (Vizzard, 2000d; Vizzard, 2000b; Vizzard, 2001), interstitial cystitis (IC)/painful bladder syndrome (PBS), spinal cord injury (upper motoneuron injury) (Vizzard, 2006; de Groat and Yoshimura, 2009), overactive bladder (OAB) (Yoshimura et al., 2008), and detrusor overactivity secondary to bladder outlet obstruction (BOO) (Andersson, 1999; 2006) may reflect a change in the balance of neuropeptides in LUT reflex pathways. Information presented in this review will address both direct and indirect effects of neuropeptides in the LUT. Due to neuropeptide/receptor expression and diversity of functions in the LUT and subsequent regulation with neural injury, disease, and bladder inflammation, neuropeptide/receptor systems may be potential targets for therapeutic intervention (Fig. 19.1).

The following sections will address the distribution, function and regulation of specific neuropeptide/receptor systems in the LUT under normal and pathological LUT conditions.

19.2 PACAP/VIP and associated receptors signaling in the LUT

A number of peptides have been demonstrated in the LUT and have demonstrated roles in regulating the micturition reflex. PACAP and VIP are members of the glucagon/secretin superfamily of hormones (Dickinson et al., 1999). PACAP and VIP share receptor subtypes coupled to different intracellular effectors; PACAP peptides exhibit high affinity for the PAC1 receptor, whereas VIP and PACAP have similar high affinities for the VPAC1 and VPAC2 receptors (Arimura, 1998; Sherwood et al., 2000). Both PACAP- and VIP-immunoreactivity (IR) have been identified in urinary bladder (Fahrenkrug and Hannibal, 1998; Mohammed et al., 2002). Widespread PACAP-IR exists in nerve fibers in rat LUT with the majority of the PACAP nerve fibers being derived from sensory neurons (Fahrenkrug and Hannibal, 1998; Zvarova et al., 2005). PACAP receptors have been identified in various tissues of the micturition pathway including bladder detrusor smooth muscle, urothelium and major pelvic ganglia (Table 19.1)(Braas et al., 2006; Tompkins et al., 2010).

19.2.1 PACAP/receptors in micturition reflex pathways

Recent studies support roles for PACAP in micturition and suggest that inflammation-induced plasticity in PACAP expression in peripheral and central micturition pathways contribute to bladder dysfunction with cystitis. We have previously demonstrated facilitatory direct effects of PACAP on bladder smooth muscle contractility (Braas et al., 2006). PACAP increased bladder smooth muscle tone and potentiated electric field stimulation (EFS)-induced contractions (Braas et al., 2006). EFS-induced contractions were superimposed on spontaneous muscle contractions and were tetrodotoxin-insensitive suggesting that the responses were direct detrusor smooth muscle effects (Braas et al., 2006). Excitatory effects of PACAP on the micturition reflex pathway are enhanced 2–4 weeks after spinal cord injury in the rat (Yoshiyama and de Groat, 1997).

19.2.2 PACAP expression in bladder afferent pathways and regulation by CYP-induced cystitis

PACAP is expressed in LUT pathways and is regulated by CYP-induced cystitis (Vizzard, 2000d; Braas et al., 2006). In control rats, PACAP-IR was expressed in fibers in the superficial dorsal horn at all segmental levels examined (L1, L2, and L4–S1). Bladder afferent cells (40–45%) in the dorsal root ganglia (DRG; L1, L2, L6, and S1) from control animals also exhibited PACAP-IR (Vizzard, 2000d). After chronic, CYP-induced cystitis, PACAP-IR increased dramatically in spinal segments and DRG (L1, L2, L6, and S1) involved in micturition reflexes (Vizzard, 2000d). The density of PACAP-IR was increased in the superficial laminae (I–II) of the L1, L2, L6, and S1 spinal segments (Vizzard, 2000d). Staining also increased dramatically in a fiber bundle extending ventrally from Lissauer's tract in lamina I along the lateral edge of the dorsal horn to the sacral parasympathetic nucleus in the L6–S1 spinal segments (lateral collateral pathway of Lissauer). After chronic cystitis, PACAP-IR in cells in the L1, L2, L6, and S1 DRG increased significantly, and the percentage of bladder afferent cells expressing PACAP-IR also increased significantly (70–85%) (Vizzard, 2000d).

19.2.3 PACAP/VP receptor expression in LUT and modulation with cystitis

19.2.3.1 Urinary bladder—With acute CYP-induced cystitis, PAC₁ receptor transcript exhibited a significant decrease in expression in both urothelium and detrusor; however, a significant increase in PAC₁ receptor transcript expression in urothelium and detrusor smooth muscle was induced by intermediate and chronic CYP-induced cystitis (Girard et al., 2008).

19.2.3.2 Lumbosacral dorsal root ganglia (DRG)—CYP-induced inflammation of the urinary bladder only affected receptor transcript expression in L6–S1 DRG following acute (4 hr) CYP-induced cystitis. PAC₁ and VPAC₂ receptor transcript expression significantly decreased in both L6 and S1 DRG with acute (4 hr) CYP-induced cystitis. For VPAC₁ receptor transcript expression, a significant increase in expression was demonstrated in the S1 DRG after acute CYP-induced cystitis. No changes in PAC₁, VPAC₁ or VPAC₂ receptor expression in L6 or S1 DRG were demonstrated with intermediate (48 hr) or chronic (8 day) CYP-induced cystitis. PACAP transcript expression significantly increased in the

urothelium with intermediate (48 hr) and chronic (8 days) CYP treatment whereas no changes were observed with acute (4 hr) CYP-induced cystitis in either urothelium or detrusor smooth muscle (Girard et al., 2008). Changes in PACAP transcript with CYP-induced cystitis mirrored those observed in the urinary bladder with acute CYP-induced cystitis decreasing and intermediate (48 hr) and chronic (8 day) treatments significantly increasing PACAP transcript expression in both the L6 and S1 DRG (Girard et al., 2008). In a rat CYP-induced cystitis paradigm, intrathecal or intravesical administration of PAC₁ receptor antagonist, PACAP6-38, reduced cystitis-induced bladder hyperreflexia (Braas et al., 2006). These studies demonstrate that PACAP/receptor are modulated by CYP-induced cystitis in tissue-specific ways and that PACAP/receptor signaling plays a role in urinary bladder afferent pathways after urinary bladder inflammation

19.2.4 PACAP in micturition reflexes and modulation after spinal cord injury (SCI)

SCI rostral to the lumbosacral spinal cord alters the coordination between the urinary bladder and external urethral sphincter in many species and results in detrusor sphincter dyssynergia (DSD) that interferes with efficient voiding and results in urinary retention, bladder hypertrophy, increased voiding pressures, increased bladder capacity, and numerous non-voiding contractions (NVCs) during bladder filling (Vizzard, 2006). PACAP is upregulated in micturition reflex pathways after SCI. These studies (Zvara et al., 2006) demonstrate that intrathecal (L6-S1) administration of the PAC₁ receptor antagonist, PACAP6-38 (10 nM), significantly reduced intermicturition interval, threshold and micturition pressures, and number and amplitude of NVCs after SCI (Zvara et al., 2006). In addition, PACAP6-38 increased voiding frequency (i.e., decreased bladder capacity) after SCI (Zvara et al., 2006). In contrast, intravesical administration of PACAP6-38 was without any effect, possibly due to lack of penetration of the PAC₁ antagonist through the urothelium. The presence of PAC₁ receptor transcript in the lumbosacral spinal cord and DRG has been demonstrated (Girard et al., 2008). Thus, intrathecal administration of PACAP6-38 may act at the lumbosacral spinal cord or DRG. The effects of PACAP6-38 after SCI are consistent with PACAP-27 facilitation of micturition in rats (Ishizuka et al., 1995a). PACAP6-38 reduced the number and amplitude of NVCs after SCI (Zvara et al., 2006). This may be attributed to a reduction in DSD or through an effect on C-fiber bladder afferents (Cheng et al., 1995). An effect of PACAP6-38 on urinary bladder C-fiber afferents is consistent with previous studies that demonstrate that capsaicin depletes PACAP-IR in the LUT (Fahrenkrug and Hannibal, 1998) and that PACAP-IR nerve fibers in the bladder express the vanilloid receptor (Zvarova et al., 2005). PACAP expression is upregulated in micturition pathways after SCI and the present studies demonstrated improved bladder function after intrathecal PACAP antagonist administration. Additional studies addressing the role of PACAP in micturition reflexes suggest that after SCI, PACAP-38 activates spinal circuitry to facilitate the parasympathetic outflow to the urinary bladder and that the elimination of sympathetic pathways enhances this effect (Yoshiyama and de Groat, 2008a; 2008b).

19.2.5 PACAP and VIP expression and effects on major pelvic ganglion (MPG) neurons

Tissue culture experiments modeling neuronal injury have shown PACAP expression regulation in the MPG, the ganglia supplying the majority of autonomic (both sympathetic

and parasympathetic) innervations to the bladder, other urogenital organs and components of the lower bowl. While PACAP expression was devoid in acute (4 hour (hr) cultures (Tompkins et al., 2010), both PACAP-IR and PACAP transcript levels increased significantly by day 3 (Girard et al., 2010a). PACAP was preferentially expressed in parasympathetic neurons (Girard et al., 2010a). Transcripts for VPAC1, VPAC2 and PAC1 were present by 4 hr culture (Tompkins et al., 2010), but only VPAC2 transcript levels increased by day 3 (Girard et al., 2010a). Local application of PACAP or maxadilan, a PAC1-selective agonist, decreased after hyperpolarization and increased neuronal excitability in a subpopulation of neurons (Tompkins et al., 2010). Therefore, PACAP receptor signaling in the MPG may represent another mechanism for neuropeptide-modulated bladder function. Additionally, we have demonstrated VIP expression in the MPG, and local application of VIP results in a decreased after hyperpolarization and increased neuronal excitability in a subpopulation of neurons (Tompkins et al., 2010). However, VIP expression did not increase with prolonged culture (3 days) (Girard et al., 2010a).

19.2.6 PACAP null mice exhibit altered bladder function and somatic sensitivity

PACAP contributions to micturition and somatic sensation were recently studied in PACAP knockout ($PACAP^{-/-}$), littermate heterozygote ($PACAP^{+/-}$) and wildtype (WT) mice using conscious cystometry with continuous intravesical saline or acetic acid (AA; 0.5%) instillation, urination patterns, somatic sensitivity testing of hindpaw and pelvic region with calibrated von Frey filaments and morphological assessments of urinary bladder (May and Vizzard, 2010). $PACAP^{-/-}$ mice exhibit increased bladder mass with fewer but larger urine spots (Fig. 19.2). In $PACAP^{-/-}$ mice, the lamina propria and detrusor smooth muscle are significantly thicker whereas the urothelium is unchanged (Fig. 19.2). $PACAP^{-/-}$ mice exhibit increased bladder capacity, void volume (VV) and longer intercontraction interval (ICI) with significantly increased detrusor contraction duration and large residual volume (May and Vizzard, 2010) (Fig. 19.2). WT mice respond to AA (0.5%) with a reduction in VV and a decreased ICI whereas $PACAP^{+/-}$ and $PACAP^{-/-}$ mice do not respond. $PACAP^{-/-}$ mice are less responsive to somatic stimulation. $PACAP^{+/-}$ also exhibit bladder dysfunction and somatic and visceral sensory abnormalities but to a lesser degree (May and Vizzard, 2010). PACAP gene disruption contributes to changes in bladder morphology, bladder function and somatic and visceral hypoalgesia (May and Vizzard, 2010).

19.2.7 VIP/receptors and micturition reflex pathways

VIP exerts, species-specific, excitatory or inhibitory actions in neural pathways controlling micturition and these functions may be altered with neural injury, disease or inflammation (Erol et al., 1992; Igawa et al., 1993; Uckert et al., 2002; Hernandez et al., 2006). A number of diverse and conflicting roles for VIP have been demonstrated in the urinary bladder from numerous species. VIP has been shown to relax urinary bladder from human (Uckert et al., 2002) or pig (Hernandez et al., 2006), and contract or produce no effects on urinary bladder from the rat (Erol et al., 1992; Igawa et al., 1993). These contradictory findings might be attributable to species differences (Uckert et al., 2002) and differential VIP receptor distribution (Table 19.1). The majority of VIP in the LUT is located in postganglionic efferent neurons of the pelvic ganglia (Chapple et al., 1992; Smet et al., 1997; Wanigasekara

et al., 2003). Surprisingly, VIP had no apparent effects on either bladder tone or EFS-stimulated contractions despite VPAC₂ receptor transcript expression in detrusor (Braas et al., 2006). However, as VIP innervation to the urinary bladder is minimal compared to that for PACAP (Fahrenkrug and Hannibal, 1998), these results may be in keeping with suggestions that PACAP and PAC₁ signaling are more prominent regulators of rat bladder physiology (Braas et al., 2006). These VIP results are consistent with previous studies that demonstrated that VIP application to detrusor smooth muscle had no effect on spontaneous or carbachol-induced bladder contractions despite facilitation of micturition when VIP was administered intrathecally or intraarterially close to the rat bladder (Igawa et al., 1993). Recent studies (Studený et al., 2008) using VIP^{-/-} mice reveal that VIP^{-/-} mice exhibit increased bladder mass and fewer but larger urine spots on filter paper. VIP^{-/-} mice exhibit increased void volumes and shorter intercontraction intervals with continuous intravesical infusion of saline (Studený et al., 2008). No differences in transepithelial resistance or water permeability were demonstrated between VIP^{-/-} and WT mice (Studený et al., 2008). With the induction of bladder inflammation by acute administration of CYP, an exaggerated or prolonged bladder hyperreflexia was demonstrated in VIP^{-/-} mice (Studený et al., 2008). The changes in bladder hyperreflexia may reflect increased expression of neurotrophins and/or of proinflammatory cytokines in the urinary bladder (Studený et al., 2008).

19.2.8 PACAP-mediated ATP release from cultured, rat urothelium

Earlier studies have indicated that the urothelium releases ATP in response to various stimuli (Birder, 2006). In addition, it has been suggested that adenosine triphosphate (ATP) released from the serosal surface of the urothelium during bladder filling stimulates receptors on suburothelial sensory nerve fibers and contributes to bladder filling sensation (Cockayne et al., 2000). ATP in the cell cytoplasm can be released extracellularly by several mechanisms including exocytosis of ATP-containing vesicles (Bodin and Burnstock, 2001; Novak, 2003). PACAP27, PACAP38 and VIP application evoked ATP release from rat urothelial cell cultures; however, ATP release was greatest with PACAP27 treatment and significantly blocked by the PAC₁ receptor selective antagonist, M65 (Girard et al., 2008). Current research supports the suggestion that PACAP and PAC₁ signaling are regulators of bladder physiology at the level of the urinary bladder and specifically, the urothelium (Girard et al., 2008).

19.3 Tachykinins and calcitonin-gene related peptide (CGRP) in micturition reflex pathways

A variety of neuropeptides are present in the somata and processes of urogenital DRG cells including urinary bladder and urethra with CGRP and Substance P (SP) being the most widely distributed (Keast, 1992). Tachykinins (SP, neurokinin A, neurokinin B) and CGRP are present in the LUT and act on neurokinin (NK)₁, NK₂, NK₃ or CGRP receptors, respectively (Andersson, 2002; Canda et al., 2006). NK₁ and NK₂ receptors have been reported in the detrusor smooth muscle whereas NK₂ receptors are present in the urothelium (Ishizuka et al., 1995b; Birder, 2010). In broad terms, the sensory functions of the tachykinins include regulation of micturition threshold, activation of cardiovascular reflexes and perception of pain from the urinary bladder (Maggi et al., 1995; Gu et al., 2000;

Andersson, 2002). Efferent functions of the tachykinins include regulation of local muscle cell activity, nerve excitability, plasma extravasation and blood flow (Andersson, 2002). Involvement of tachykinins located in supraspinal sites on micturition function has also been demonstrated (Gu et al., 2000).

19.3.1 Tachykinins and CGRP in micturition reflex pathways and regulation with inflammation, injury or disease

Expression of tachykinins and associated receptors in the LUT under basal conditions and alterations in expression has been reported in animal models of bladder inflammation and in the clinical syndrome of IC/PBS. Recent studies have demonstrated alterations in SP-immunoreactivity (IR) (Pang et al., 1995) and NK1 mRNA (Marchand et al., 1998) in bladder biopsies from patients with IC/PBS (Johansson and Fall, 1994; Ho et al., 1997; Johansson et al., 1997). In addition, a study involving an acute rat model of urinary bladder inflammation (48 hr following intravesical mustard oil treatment) has demonstrated significant increases in CGRP- and SP-IR in rostral (L1, L2 DRG) and caudal (L6, S1 DRG) bladder afferent neurons (Callsen-Cencic and Mense, 1997). Furthermore, it has been demonstrated (Lecci et al., 1994) that intrathecal injection of SP antagonists reduce CYP-induced bladder hyperreflexia. Although numerous studies have demonstrated changes in CGRP and SP expression in sensory neurons following nerve injury (Hokfelt et al., 1994) or peripheral inflammatory states (Kataeva et al., 1994; Luber-Narod et al., 1997; Traub et al., 1999; Hutchins et al., 2000), there are only a limited number of studies that have examined alterations in CGRP or SP expression following the induction of acute urinary bladder inflammation (Callsen-Cencic and Mense, 1997; Luber-Narod et al., 1997). Increases in CGRP- and SP-IR in bladder afferent neurons in the lumbosacral DRG 48 hr following the induction of cystitis with intravesical mustard-oil have been demonstrated (Callsen-Cencic and Mense, 1997).

19.3.2 SP and CGRP plasticity in LUT pathways with CYP-induced cystitis

Additional plasticity of CGRP and SP expression in LUT pathways has been demonstrated in the CYP model of bladder inflammation in rats (Vizzard, 2001). In control rats, CGRP- or SP-IR was expressed in fibers in the superficial dorsal horn in all segmental levels examined (L4-S1). Bladder afferent cells in the dorsal root ganglia (DRG; L6, S1) from control animals also exhibited CGRP- (41–55%) or SP-IR (2–3%). Following chronic, CYP-induced cystitis, CGRP- and SP-IR were dramatically increased in spinal segments and DRG (L6, S1) involved in micturition reflexes. The density of CGRP- and SP-IR was increased in the superficial laminae (I–II) and lateral collateral pathway of the L6 and S1 spinal segments. Following chronic cystitis, CGRP- and SP-IR in cells in the L6 and S1 DRG significantly increased and the percentage of bladder afferent cells expressing CGRP- (76%) or SP-IR (11–18%) also significantly increased (Vizzard, 2001).

The functional significance of an upregulation of CGRP or SP in bladder pathways following CYP-induced cystitis is not known but changes in neuropeptide expression and presumably release at both central and/or peripheral projections of afferent pathways are possible. Several reports have suggested that neuropeptide-containing, capsaicin-sensitive bladder afferents may mediate urinary bladder hyperreflexia (Maggi, 1991; Giuliani et al.,

1993a; Giuliani et al., 1993b; Ahluwalia et al., 1994; Ahluwalia et al., 1998). It has also been shown that intrathecal SP facilitates normal micturition and SP antagonists delivered intrathecally depress normal micturition indicating that the peptide may be involved as an excitatory transmitter in several types of bladder reflexes in the rat (Mersdorf et al., 1992; Lecci et al., 1993). CYP treatment in the rat induces cystitis that is characterized by increased frequency of voiding in awake rats and urinary bladder hyperreflexia in anesthetized rats (Maggi et al., 1992; Lecci et al., 1994; Lanteri-Minet et al., 1995). Furthermore, it has been demonstrated (Lecci et al., 1994) that intrathecal injection of SP antagonists reduce CYP-induced bladder hyperreflexia. Intrathecal administration of neurokinin receptor (NK)1 antagonists (RP 67580 and CP 96345) increase bladder capacity in normal conscious rats with no changes in voiding pressure whereas NK2 receptor antagonists were ineffective (Lecci et al., 1994). Bladder hyperreflexia induced by capsaicin was reduced by an NK2 antagonist (SR 48965) with no effects on normal micturition (Lecci et al., 1994). Exogenous CGRP application or CRGP release from primary afferent nerves relaxes smooth muscle and produces relaxation with bladder effects being more prominent in guinea pig and dog compared to rat and human (Maggi, 1992; Andersson, 1993). In addition to pharmacological demonstration of involvement of tachykinins/receptors systems in the LUT, studies with the preprotachykinin A (that encodes both SP and neurokinin A) null mouse, have demonstrated involvement of tachykinins in the response to chemical irritation of the LUT as well as to regulation of normal micturition activity (Kiss et al., 2001).

Recent studies with cizolirtine citrate, an inhibitor of CGRP and SP release at the spinal cord level, showed a significant reduction in the total number of voids per 24 hr in patients with urinary incontinence secondary to OAB (Zat'ura et al., 2010). Further, cizolirtine citrate resulted in improvement in urinary incontinence and urgency in symptomatic outpatients with OAB and/or urodynamic diagnosis of detrusor overactivity (Martinez-Garcia et al., 2008). Therefore, it is possible that increased expression of CGRP- or SP-IR in bladder afferent cells and central and peripheral projections could contribute to this hyperreflexia. Changes in neuropeptide expression and release at central terminals could further result in a remodeling of spinal cord circuitry controlling micturition (Lecci et al., 1994). This remodeling may include changes: (1) in the synaptic organization of spinal micturition reflexes; (2) in the neurochemical coding of specific neuronal elements (primary afferent neurons, interneurons) and (3) in the organization of ascending and descending projections to spinal reflexes. Further, recent studies also demonstrate that SP released from nerve fibers or urothelial cells can act on urothelial receptors to release nitric oxide (Birder, 2006; Birder, 2010). In response to stimulation of receptors on urothelial cells, SP and neurokinin A can be released from urothelial cells. Thus, neural as well as non-neural tachykinins may contribute to LUT pathways. Non-neural involvement of neuropeptides in the LUT has been recently addressed (Birder, 2006; Birder, 2010).

The exact role(s) of tachykinins in urethral physiology is not known but tachykinins induce urethral contraction in many species including humans (Maggi et al., 1988; Maggi, 1992; Palea et al., 1996; Parlani et al., 1996; Canda et al., 2006; 2008). In patients with genitourinary prolapse with concomitant urinary incontinence, the density of SP-immunoreactive nerves in the perineal muscles was significantly decreased compared to a

continent group suggestive of a role for tachykinins in urinary continence mechanisms (Busacchi et al., 2004). Additional research focused on the role of tachykinins in urethral physiology is necessary.

19.4 Role of Neurotrophic Factors in Neuropeptide Expression in Micturition Pathways after CYP-induced Cystitis

Altered bladder neurotrophic factor content may underlie neurochemical (Vizzard and de Groat, 1996; Vizzard, 2000d; Vizzard, 2000c; Vizzard, 2000a; Vizzard, 2001) changes in bladder afferent neurons after cystitis. The occurrence of trophic interactions between nerve cells and target tissues is clearly demonstrated during embryonic and postnatal development (Lindsay et al., 1990; Oppenheim et al., 1991; Lapchak et al., 1992; Vantini and Skaper, 1992). Recent experiments have demonstrated the influence of interactions between target organ and neurons in adult animals (Steers and de Groat, 1988; Steers et al., 1991a; Steers et al., 1991b; Tuttle and Steers, 1992; Tuttle et al., 1994; Vizzard, 2000b; Dupont et al., 2001). NGF is expressed under normal conditions in the urinary bladder, and part of its function is likely maintenance of sensory afferent fibers (Chao and Hempstead, 1995; Chuang et al., 2001). We have demonstrated that chronic CYP-induced cystitis alters NGF expression and other neurotrophic factors in the bladder (Vizzard, 2000b). The role(s) of NGF in micturition reflexes and sensation have been evaluated using a variety of addition and subtraction techniques (Dmitrieva and McMahon, 1996; Clemow et al., 1998; Yoshimura et al., 2006). Importantly, exogenous NGF application to the rat bladder detrusor through an osmotic pump reduced bladder capacity and increased expression of CGRP in the lumbosacral spinal cord (Zvara and Vizzard, 2007)(Fig. 19.3).

Recent studies involving a novel NGF overexpressing (OE) mouse model (Cheppudira et al., 2008; Schnegelsberg et al., 2010) are being used to further define NGF-mediated changes in LUT structure and function and effects on neuropeptide/receptor systems (Fig. 19.4). The urinary bladder in NGF OE mice exhibits marked hyperinnervation. To characterize the subpopulations of neurons contributing to the generalized hyperinnervation observed in NGF-OE transgenic mice, we have performed whole-mount immunostaining of bladder urothelium using a panel of neuronal markers. Using the pan-neuronal marker PGP 9.5, a marked increase in total nerve fiber density was seen in the urinary bladders of transgenic mice compared to WT controls (Fig. 19.4), consistent with the histology stains. This dense network was composed of CGRP-positive (Fig. 19.4) and substance P-positive (Fig. 19.4) unmyelinated C-fiber sensory afferents, NF 200-positive myelinated sensory afferents and TH-positive, post-ganglionic sympathetic nerve fibers (Schnegelsberg et al., 2010). The increased nerve fiber density observed in transgenic mouse urinary bladders was evident in both the neck and the dome region of the urinary bladder. However, we observed a higher innervation density within the urinary bladder neck region compared to the dome in both WT and transgenic mice. With CGRP immunostaining, we also observed CGRP-positive ganglia (5–9 CGRP-positive cells per ganglia) interspersed among CGRP-positive nerve fibers within the suburothelial plexus in transgenic mice. These CGRP-positive ganglia were not observed in whole-mount preparations of WT urinary bladder (Schnegelsberg et al., 2010). Studies are currently underway to examine effects of neuropeptide/receptor blockade

on bladder function in NGF OE mice that exhibit bladder hyperreflexia with the presence of NVCs (Schnegelsberg et al., 2010).

Recent studies also demonstrate changes in PACAP/VIP and receptor expression in micturition pathways in NGF-OE mice (Girard et al., 2010b). Results demonstrate upregulation of PAC1 receptor transcript and PAC1-immunoreactivity in urothelium of NGF-OE mice whereas PACAP transcript and PACAP-immunoreactivity were decreased in urothelium of NGF-OE mice (Girard et al., 2010b). In contrast, VPAC1 receptor transcript was decreased in both urothelium and detrusor smooth muscle of NGF-OE mice (Girard et al., 2010b). VIP transcript expression and immunostaining was not altered in urinary bladder of NGF-OE mice (Girard et al., 2010b). Changes in PACAP, VIP and associated receptors transcripts and protein expression in micturition pathways resemble some, but not all, changes observed after induction of urinary bladder inflammation known to involve NGF production.

19.5 Bradykinin/receptors system in micturition reflex pathways

The bradykinin/receptors system plays an important role in normal micturition reflexes as well as in pathology (Lecci et al., 1995; Belichard et al., 1999; Lecci et al., 1999; Meini et al., 2000; Chopra et al., 2005; Fabiyi and Brading, 2006). Kinins are small peptides (8–10 amino acids) produced in plasma or in tissues at the sites of inflammation or tissue damage (Bhoola et al., 1992). Protease activation at the site of inflammation or tissue damage cleaves tissue/plasma kininogen precursors to release the nonapeptide, bradykinin (Dray and Perkins, 1993). The biological effects of bradykinin are mediated by two different receptors, B1 and B2 with the bradykinin B2 receptor being constitutively expressed in various cell types and the B1 receptor being expressed *de novo* after inflammatory stimuli or tissue injury (Marceau et al., 1998; Lecci et al., 1999; Chopra et al., 2005). Recent studies (Chopra et al., 2005) have demonstrated B2 receptor expression in the detrusor smooth muscle and urothelium (apical and basal cells) consistent with reported bradykinin-evoked contractility of detrusor smooth muscle. It has been recently demonstrated that bradykinin-induced facilitation of micturition reflexes may be due to an increase in purinergic (P2) responsiveness (Chopra et al., 2005). Further, stimulation of the B2 receptor in cultured rat urothelial cells resulted in ATP release (Chopra et al., 2005). ATP release was reduced by the selective B2 receptor antagonist Hoe-140 and suggests that bradykinin may elicit bladder hyperreflexia indirectly through the release of ATP from the urothelium (Chopra et al., 2005). Direct effects of bradykinin on pelvic afferent nerves that evoke bladder hyperreflexia have also been demonstrated (Lecci et al., 1995). Thus, direct (neuronal) (Lecci et al., 1995) and indirect (urothelial secretion of ATP) (Chopra et al., 2005) actions of bradykinin on the LUT are likely to affect micturition reflexes.

19.5.1 Bradykinin/receptors in cystitis

The role of the bradykinin/receptor system in the LUT varies under control or pathological states. It is largely agreed that B1 receptor expression is undetectable in control urinary bladder (Ahluwalia and Perretti, 1999; Chopra et al., 2005). Application of the selective B1 receptor agonist, des-Arg⁹-bradykinin exerts little or no effect on $[Ca^{2+}]_i$ or ATP release from cultured rat urothelial cells consistent with no or very low constitutive B1 receptor

expression (Chopra et al., 2005). CYP-induced cystitis significantly upregulates B1 receptor expression in the detrusor smooth muscle and urothelium with acute (24 hr) cystitis (Chopra et al., 2005). In cultured rat urothelial cells obtained from CYP (24 hr)-treated rats, the B1 receptor agonist, des-Arg⁹-bradykinin, evoked release of ATP and elevated calcium levels (Chopra et al., 2005). B1 receptor expression is also upregulated in bladder biopsies from patients with IC/PBS (Ruggieri et al., 1997). Cystometry performed on CYP (24 hr)-treated rats demonstrated that the B1 receptor antagonist, des-Arg¹⁰-Hoe-140, significantly reduced the frequency of non-voiding bladder contractions (Chopra et al., 2005). In contrast, the B2 receptor antagonist, Hoe-140, decreased voiding frequency in CYP (24 hr)-treated rats (Chopra et al., 2005). It has been suggested that one explanation for these findings is the existence of B1 sensitive mechanisms/or afferent pathways underlying the emergence of non-voiding contractions and B2 sensitive mechanisms underlying voiding contractions (Chopra et al., 2005). Additional studies are needed to address this possibility. Interestingly, chronic CYP-induced cystitis (8 days) resulted in a return to baseline of B1 receptor expression in the detrusor smooth muscle whereas expression in the urothelium was similar to that observed with acute (24 hr) CYP-induced cystitis (Chopra et al., 2005). Bradykinin/receptors systems may exert tissue-specific and inflammatory duration-dependent effects on LUT reflexes.

19.6 Endothelin/receptors system in LUT pathways

Endothelin-1 and endothelin receptors, ET_A and ET_B, contribute to LUT function under normal and pathological conditions (Ukai et al., 2006; Ogawa et al., 2008; Ukai et al., 2008). In the LUT, endothelin-1 facilitates detrusor smooth muscle contraction in various species including rabbit, rat, dogs and humans and stimulates proliferation of the prostate and urinary bladder (Maggi et al., 1989; Garcia-Pascual et al., 1990; Persson et al., 1992; Langenstroer et al., 1997; Ogawa et al., 2004). Studies have demonstrated differential endothelin-1 contractile mechanisms depending on species (Persson et al., 1992). Differential endothelin receptor density has been revealed in the LUT with the greatest density of ET_A receptors being present in the ureter (Latifpour et al., 1995; Saenz de Tejada et al., 1992; Afiatpour et al., 2003). In humans and other animals, ET_A expression is more dominant than ET_B in the bladder dome compared to the bladder base or urethra, which exhibits equal ET_A and ET_B receptor expression (Saenz de Tejada et al., 1992; Latifpour et al., 1995; Afiatpour et al., 2003). Endothelin expression has been detected in detrusor smooth muscle, urothelium, and vascular endothelium (Saenz de Tejada et al., 1992; Latifpour et al., 1995; Afiatpour et al., 2003).

19.6.1 Endothelin/receptor system in bladder outlet obstruction and spinal cord transection

In animal models of BOO, modulation of endothelin-1 and ET_A and ET_B expression has been demonstrated in the LUT and studies are researching the endothelin/receptor system as a potential therapeutic target for the treatment of bladder overactivity secondary to BOO (Khan et al., 1999; Ukai et al., 2008). BOO in rabbits is associated with increased expression of ET_A, ET_B and endothelin-1 in detrusor smooth muscle (Khan et al., 1999; Ukai et al., 2008). The ET_A selective receptor antagonist, YM598, dose-dependently reduced the

frequency of premicturition bladder contractions in BOO rats without effects on other voiding parameters (Ukai et al., 2008). Further, inhibition of the endothelin-converting enzyme, which metabolizes big endothelin to endothelin-1, decreased bladder overactivity in BOO rats (Schroder et al., 2004). Additional beneficial effects of repeated administration of endothelin-converting enzyme inhibitor in BOO rats included normalization of: bladder weight, micturition pressures and voiding durations (Schroder et al., 2004). Suppression of ET_A receptors by intravenous infusion of ABT-627 in rats with complete spinal cord transection (thoracic 8–9) significantly decreased the amplitude and number of non-voiding bladder contractions without changing bladder pressures, void volumes or voiding efficiency (Ogawa et al., 2008). No effects on bladder function were observed when rats with spinal cord transection were similarly treated with an ET_B receptor antagonist, A-192621 (Ogawa et al., 2008). Thus, endothelin and ET_A receptors may be a novel therapeutic target to ameliorate the effects of bladder overactivity associated with BOO.

Additional studies are necessary to address the mechanisms by which YM598, an endothelin ETA receptor antagonist, suppresses the bladder overactivity observed in BOO rats; potentiation of postsynaptic norepinephrine effects and endothelin-1 increase of interleukin-(IL) 6 have been suggested (Han et al., 2009). Interestingly, recent studies have demonstrated that endothelin-converting enzyme 1 promotes the recycling and re-sensitization of NK1 receptors and re-sensitization of the pro-inflammatory effects of SP (Cattaruzza et al., 2009). Thus, reductions in bladder overactivity secondary to BOO observed with endothelin-converting enzyme inhibition (described above) may, in part, be due to amelioration of the pro-inflammatory effects of SP (Cattaruzza et al., 2009).

19.6.2 Endothelin/receptor systems in the urethra

There is very limited information concerning the distribution and function of endothelin/receptor systems in the urethra. ET receptors have been demonstrated in the urethral smooth muscle in several species (Latifpour et al., 1995; Afiatpour et al., 2003). ET_A-mediated smooth muscle contractions of the urethra in the rabbit have been demonstrated (Wada et al., 2000).

19.7 Perspectives

A large body of research supports a major role for neuropeptides in LUT function in health and disease thereby identifying neuropeptide/receptor systems as potential novel therapeutic targets for the treatment of LUT disorders (Fig. 19.1). In animal models, pharmacological and/or genetic manipulation of SP, CGRP, VIP, PACAP, bradykinin or endothelin function affects LUT function. Further, in animal models of LUT dysfunction or in clinical assessments, manipulation of neuropeptide/receptor systems improves void function. Furthermore, the field has become increasingly complex, with neuronal plasticity exhibited in pathophysiological situations where changes in neuropeptide expression patterns and receptor density are regularly observed. The study of neuropeptides in animal models has additionally revealed physiological and pathophysiological roles that in turn have led to the ongoing development of new drugs, through utilization predominantly of antagonist activities or blockade of release.

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Abbreviations

AA	acetic acid
ATP	adenosine triphosphate
BOO	bladder outlet obstruction
CGRP	calcitonin gene-related peptide
CYP	cyclophosphamide
DRG	dorsal root ganglia
DSD	detrusor sphincter dyssynergia
EFS	electric field stimulation
hr	hours
IC	interstitial cystitis
ICI	intercontraction interval
IL	interleukin
IR	immunoreactivity
LUT	lower urinary tract
NGF	nerve growth factor
NK	neurokinin
NVCs	non-voiding contractions
OAB	overactive bladder
PACAP	pituitary adenylate cyclase activating polypeptide
PBS	painful bladder syndrome
SCI	spinal cord injury
SP	substance P
VIP	vasoactive intestinal polypeptide
VV	void volume
WT	wildtype

19.8 References

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Figure 19.1. Neuropeptide/receptor systems expression in micturition reflex pathways emphasizing bladder afferent and urothelium participation

A. Diagram of afferent innervation of the urinary bladder. Numerous neuropeptide/receptor systems have been identified in bladder afferent (i.e., sensory) pathways with contributions to normal lower urinary tract function as well as that after neural injury, disease or inflammation. **B.** Potential model of possible reciprocal neuropeptide/receptor interactions among bladder afferent and efferent nerves (not shown), urothelial cells, myofibroblasts located in the suburothelium and detrusor smooth muscle that underlie physiological bladder reflex function as well as pathophysiology in bladder disease. Receptor activation and channel stimulation on urothelial cells can elicit secretion of chemical mediators that can affect adjacent tissues including bladder afferent nerves in the suburothelial plexus, myofibroblasts and detrusor smooth muscle. Urothelial cells can also be responsive to neurotransmitters released from bladder nerves and other cell types including inflammatory cells. Abbreviations: ATP, adenosine triphosphate; TrkA, receptor tyrosine kinase A;

p75^{NTR}, low affinity neurotrophin receptor; PG, prostaglandin; NO, nitric oxide; NGF, nerve growth factor; NP, neuropeptides; Ach, acetylcholine; PAC₁, pituitary adenylate cyclase-activating polypeptide (PACAP) selective receptor; VPAC₂, receptor with equal and high affinity for vasoactive intestinal polypeptide and PACAP; B1, bradykinin receptor 1; B2, bradykinin receptor 2; ET_A, endothelin receptor A; ET_B, endothelin receptor B; NK2, neurokinin receptor 2; NK1, neurokinin receptor 2; NK3, neurokinin receptor 3; CGRP1, calcitonin gene-related receptor. See text for additional details. Figure modified from (Arms et al., 2009).

Figure 19.2. PACAP contributions to bladder morphology and function

A–C. Histological analyses of urinary bladders and bladder function among PACAP^{-/-}, PACAP^{+/-} and WT mice. Representative haematoxylin and eosin stained cryostat bladder sections (15 μm) from WT (A) and PACAP^{-/-} (B) mice that demonstrate significantly (*, p < 0.01) increased thickness of lamina propria (LP) and detrusor smooth muscle (SM) supported by morphometric analyses (C). D–E. Summary figures of the intercontraction interval (ICI, seconds, s; D) and residual volume (RV, μl; E) using conscious cystometry in conscious, unrestrained WT, PACAP^{-/-} and PACAP^{+/-} mice with continuous infusion of saline or AA (0.5%). D. ICI was significantly (p < 0.01) longer in PACAP^{-/-} compared to WT mice with instillation of saline and AA. No changes in ICI were detected with intravesical instillation of AA in PACAP^{-/-} and PACAP^{+/-} mice compared to saline instillation. ICI was significantly (p < 0.01) greater in PACAP^{-/-} and PACAP^{+/-} mice with AA instillation compared to WT. E. RV was significantly (p = 0.01) increased in PACAP^{+/-} and PACAP^{-/-} mice compared to WT. Values represent mean ± S.E.M. for n = 7–10 animals in each group. U, urothelium; WT, wildtype. Calibration bar represents 120 μm in A, B. Figure modified from (May and Vizzard, 2010).

Figure 19.3. CGRP spinal cord expression with exogenous NGF treatment

CGRP-IR increases in lumbosacral spinal cord with exogenous NGF treatment. CGRP-IR in the L6 (A–B) spinal segment in control (A) and NGF-treated (B) rats. A. Fluorescence photographs showing CGRP-IR in the L6 (A) spinal segment of control (saline) + bladder distention. B. Fluorescence photographs showing CGRP-IR in the L6 (B) spinal segment with NGF treatment + bladder distention. Increased density of CGRP-IR was observed in the medial (MDH) to lateral (LDH) extent of the superficial laminae (I–II) of the dorsal horn (DH) with NGF treatment in L6 (C) segments. Summary bar graph of CGRP optical density (O.D.) as measured in specific regions of the L6 spinal cord (C). Calibration bar represents 125 μ m. SPN, sacral parasympathetic nucleus; DCM, dorsal commissure; LCP, lateral collateral pathway. *, $p < 0.01$. Figure modified from (Zvara and Vizzard, 2007).

Figure 19.4. Neuronal hyperinnervation in the urinary bladders of NGF-overexpressing (OE) transgenic mice

Representative fluorescence images of PGP 9.5 (A, B), CGRP (C, D), SP (E, F), immunoreactivity in the bladder neck region in urothelial whole-mount preparations of urinary bladders from 8–10 week-old female WT (A, C, E) and NGF-OE transgenic (B, D, F) mice from F0 line 23 ($n = 3–5$). Scale bars: A–F, 50 μm . Protein Gene Product (PGP 9.5), Calcitonin Gene Related Protein (CGRP), Substance P (SP). Figure modified from (Schnegelsberg et al., 2010).

Table 1

Summary of VIP/PACAP receptor isoforms and tissue distribution in the lower urinary tract

Tissue	Receptor subtypes		
	PAC ₁ receptor	VPAC ₁ receptor	VPAC ₂ receptor
Detrusor smooth muscle	+	-	+
Urothelium	+	-	-
Lumbosacral dorsal root ganglia	+	-	+
Lumbosacral spinal cord	+	-	+
Major pelvic ganglia	+	+	+