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The potential of non-adrenergic, non-cholinergic targets in the treatment of interstitial cystitis/painful bladder syndrome

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Regulation of bladder function involves both divisions of the autonomic nervous system. However, in addition to the classical autonomic transmitters, noradrenaline and acetylcholine, other autonomic transmitters and other signalling components play important roles in physiology and pathophysiology of the lower urinary tract. Several substances of neuronal non-adrenergic, non-cholinergic (NANC) systems have already proven to considerably influence functional responses in the inflamed urinary bladder. Interstitial cystitis (IC) or painful bladder syndrome (PBS) is a chronic inflammatory bladder disease, characterized by urinary frequency, urgency and pelvic pain. IC/PBS is difficult to diagnose, especially because the etiology of the condition is largely unknown. Despite the unclear nature of the cause and manifestation of IC/PBS, it has been shown that the disease involves a significant NANC component. Here, we review the possible roles of ATP, adenosine, nitric oxide, vasoactive intestinal polypeptide, substance P, and pituitary adenylate cyclase-activating peptide in the contribution to IC/PBS development and manifestation of IC/PBS symptoms.

Key words: interstitial cystitis, urinary bladder, pituitary adenylate, cyclase-activating peptide, substance P, vasoactive intestinal polypeptide

INTRODUCTION

Data reported in the literature support a role of non-adrenergic, non-cholinergic (NANC) transmitters in interstitial cystitis (also referred to as painful bladder syndrome) (IC/PBS) development. Consequently, targets for potential treatments of IC/PBS may be found in NANC systems in the lower urinary tract. In view of the lack of knowledge of the etiology and the pathogenesis of the disease, in combination with the currently unsuccessful treatments, new findings of the NANC systems is of utter interest. Therefore, new findings regarding NANC effect are reviewed below in the context of general theories regarding classical neuronal transmitters.

INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

IC/PBS is a chronic inflammation of the submucosa and muscular tissues of the urinary bladder (1). IC/PBS is not caused by any infectious organism, and it is defined by the European Society for the Study of Interstitial Cystitis as pelvic pain associated with other symptoms such as urgency and frequency (2).

A remaining problem concerning IC/PBS is the difficulty of its diagnosis. There is no general agreement of the pathophysiology of the disease. It has been suggested that IC/PBS is best diagnosed from its clinical characteristics (3). Primary symptoms of the disease are urinary frequency (frequent urination) and urgency (sudden feeling of the need to immediately urinate), usually accompanied by suprapubic discomfort or pressure, which fades away when urinating. Because of sharp strong pain related to bladder filling, IC is also referred to as painful bladder syndrome (PBS).

Two forms of IC/PBS exist, the ulcerative and nonulcerative forms. The ulcers are usually found in older persons with low level of bladder capacity, probably caused by fibrosis. Cystoscopy reveals cracks, scars and Hmunner’s ulcers in the bladder wall, and sometimes even bleeding (4). On the other hand, the more common nonulcerative form of IC/PBS lacks all these cystoscopic findings, as described previously; only the clinical symptoms remain. However, even in nonulcerative IC/PBS, small mucosal tears and submucosal haemorrhages may appear, as well as glomerulations (blood vessel abnormalities that may be observed on cystoscopy) in the bladder wall (5).

ABNORMALITIES OF INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

A very common feature of the disease is a sudden attack of the symptoms that can spontaneously disappear for unknown reason, which may make it hard to suggest and also to monitor the effects of treatments. The suggested etiologies have already been reviewed (6-9). An enhanced innervation of the bladder submucosa and detrusor by sympathetic, but not by cholinergic, neurons was suggested by Hohenfellner (10). Furthermore,
IC/PBS has been suggested to be of autoimmune origin, since it shares many features with diseases such as Crohn’s disease and rheumatoid arthritis. Hohenfellner et al. (10) also suggested that the non-adrenergic, non-cholinergic (NANC) system might be involved in IC/PBS development. Even though the effects of the classical autonomic transmitters, *i.e.* acetylcholine and noradrenaline, may be somewhat changed in IC/PBS, the effects of NANC molecules are substantially altered in IC/PBS (11-14). Tentatively, much of the pathology of IC/PBS may originate in alterations of NANC mechanisms (15). Particular interest has been directed to purines and nitric oxide (NO) since it has been shown that both the release of ATP and the expression of nitric oxide synthase (NOS) are enhanced during IC/PBS. However, in view of the functional effects of neuropeptides in the lower urinary tract (16), and further, since they play fundamental roles in neurogenic inflammation (17, 18), they should be considered in IC/PBS as well.

Urothelial NO and ATP are considered to be inhibitory and excitatory mediators of the integration of the afferent bladder control (19). Activation of afferents by ATP seems to be one important factor in the bladder sensation that occurs in IC/PBS and imbalances in inhibitory and excitatory mediators may give rise to a less well-integrated afferent bladder control. Long-term stimulation of TRPA1 receptors has also been discussed in the pathogenesis of IC/PBS and proved to be important for hyperosmolar-induced overactive urinary bladder (20). The transient receptor potential (TRP) channel family consists of important mediators for noxious stimuli. This family consists of six subfamilies of cation channels of which the transient receptor potential V1 (TRPV1; vanilloid) and transient receptor potential A1 (TRPA1; ankyrin) channels are two receptors of particular interest in chronic pain disorders. These receptors do not only act as noci-sensors but may also induce the release of substance P and calcitonine gene-related peptide (21). In chronic cyclophosphamide-induced cystitis, the stimulation of the two receptors may cause bladder overactivity. When blocked, the overactivity is reduced suggesting an involvement of TRPV1 and TRPA1 receptors in disorders in the lower urinary tract (22).

Much interest in the search for key mechanisms in the pathogenesis of IC/PBS has therefore focused on alterations in the afferent signalling systems. However, in view of the direct NO effects on the smooth muscle function in cystitis (11), the mechanisms in IC/PBS may be highly composite. The orthodoxy approach to consider afferent and efferent mechanisms separately may be un-appropriate.

### NON-ADRENERGIC, NON-CHOLINERGIC FEATURES INVOLVED IN BLADDER FUNCTION AND IN INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

Smooth muscle tissue in visceral organs is under the control of the autonomic nervous system. Noradrenaline and acetylcholine were for many years thought to be the only transmitters responsible for physiological function of smooth muscle, in spite of the early observation made by Langley and Anderson (23) that an atropine-resistant parasympathetic response exist in the lower urinary tract. In the 1980's evidence accrued that non-adrenergic, non-cholinergic (NANC) neurotransmitters may be responsible for the "atropine-resistance" and that they may play important roles in peripheral autonomic physiology and pathophysiology, respectively. One reason for the neglecting of NANC mechanisms in the perspective of pharmacotherapies, may be that many of the NANC transmitters are peptides. Today's knowledge in big molecule synthesis may, however, open new strategies.

### PURINES

When first introduced as a neurotransmitter, adenosine 5'-triphosphate (ATP) was highly controversial. The first evidence suggesting that ATP may also be a transmitter, not just a source of energy for cells, was published in 1959 (24), and in 1971 the term "purinergic" was first proposed (25). The idea of ATP being
one of the NANC neurotransmitters was mainly elaborated by Geoffrey Burnstock, who has continued his work over the last decades (25, 26). His work has established purinergic mechanisms by ATP, other nucleotide triphosphates or by their break down products, as important regulatory molecules in health as well as in pathological conditions (25).

In 1978 Burnstock first pointed out that ATP might be a neurotransmitter in the guinea pig urinary bladder (27). In the study, a contractile response to ATP in bladder detrusor was suggested. It was also suggested that the release of ATP is dependent of the concentration of calcium ions in the medium and that prostaglandins E₁, E₂ and F₂α potentiate the effect of ATP. ATP can be released either from effenter nerves, where it excites bladder smooth muscle (28), or from urothelial cells, where it can activate afferent nerves and urothelial cells (29). The ATP molecule activates purinergic receptors, so called purinoceptors, which are plasma membrane receptors involved in physiological as well as in pathological pathways. The family of purinoceptors is divided into two subfamilies; P₁ and P₂ purinoceptors, according to their main agonist; ATP acts preferentially on P₂ purinoceptors and adenosine on P₁ purinoceptors.

In most species, including man, the urothelium expresses P₂X and P₂Y subtypes of purinoceptors. In the rat urothelium, the sub-classification has identified P₂X₁ and P₂X₃ purinoceptors (30). In this matter the rat shows resemblance with humans, since P₂X₁ and P₂X₃ purinoceptors occur in the human urothelium as well (31). The rat urothelium also expresses P₂Y₂ receptors (32). In the feline smooth muscle, the P₂X₁ and P₂X₂ are found, while P₂X₃ and P₂Y₁ purinoceptors are present on nerve terminals (33). In the rat detrusor, P₂X₃ receptors appear in clusters on the smooth muscle cells (34). Of the P₂X purinoceptor phenotypes, the P₂X₁ purinoceptors show the largest expression in the murine smooth muscle and their activation contributes to the contraction of the detrusor (35). The contractile effect by activation of P₂X₃ purinoceptors was observed also in the rat detrusor (36).

ATP causes a contraction of the detrusor muscle. This contraction is followed by a sustained relaxation, which has been suggested to appear due to an ATP breakdown product, adenosine (36-38). Adenosine is generated within the cell by enzymatic breakdown from the hydrolysis of S-adenosyl-L-homocysteine, and is also formed extracellularly and intracellularly from the hydrolysis of ATP, ADP, AMP or cAMP. Extracellular cAMP is converted to adenosine by actions of ectophosphodiesterase and ecto-5′-nucleotidases (38). Adenosine acts through cell surface P₁ purinoceptors in the urothelium in an autocrine and paracrine manner where it modulates exocytic traffic (39). The P₁ purinoceptors consist of four subtypes, P₁A₁, P₁A₂A, P₁A₂B and P₁A₃, all G-protein coupled receptors.

The expression of P₁A₁ purinoceptors is very intense in the rat urothelium, and the purinoceptors seem to be present in the detrusor and suburothelium as well (40). This observation is confirmed by RT-PCR examinations, which show the P₁A₁ purinoceptors to occur moderately frequent. In addition, the RT-PCR shows P₁A₂A and P₁A₂B to be expressed in low and high amounts, respectively (41). The human urothelium also expresses all four subtypes of P₁ receptor family, as established by western blot analysis (39).

In 2004 Birder et al. (33) designed a study where immunohistochemical staining was used for examination of the distribution of purinoceptors in the urothelium, smooth muscle

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Table 1. Summary of non-adrenergic, non-cholinergic transmitters and receptors involved in development of interstitial cystitis/painful bladder syndrome.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Transmitter</th>
<th>Intracellular pathways</th>
<th>Effects on urinary bladder (suggested effect during IC/PBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁A₁</td>
<td>adenosine</td>
<td>↓cAMP; ↑IP₃</td>
<td>• detrusor relaxation, (pro-inflammatory)</td>
</tr>
<tr>
<td>P₂A₂B</td>
<td>adenosine</td>
<td>↑cAMP</td>
<td>• detrusor relaxation, (anti-inflammatory)</td>
</tr>
<tr>
<td>P₁A₃</td>
<td>adenosine</td>
<td>↑cAMP; ↓IP₃</td>
<td>• detrusor contraction, (pro-inflammatory)</td>
</tr>
<tr>
<td>P₂X₁</td>
<td>ATP</td>
<td>↑Ca²⁺, inotropic rs.</td>
<td>• detrusor contraction, reduced expression during IC/PBS</td>
</tr>
<tr>
<td>P₂X₂</td>
<td>ATP</td>
<td>↑Ca²⁺, inotropic rs.</td>
<td>• pain-related effect</td>
</tr>
<tr>
<td>P₂X₃</td>
<td>ATP</td>
<td>↑Ca²⁺, inotropic rs.</td>
<td>• mechanosensory transduction, pain-related effect, responsible for the bladder hyperactivity</td>
</tr>
<tr>
<td>P₂Y₂</td>
<td>ATP</td>
<td>↑Ca²⁺, metabotropic rs.</td>
<td>• modulation of the micturition reflex by release of additional ATP, NO and acetylcholine, (pro-inflammatory)</td>
</tr>
<tr>
<td>P₂Y₄</td>
<td>ATP</td>
<td>↑Ca²⁺, metabotropic rs.</td>
<td>• modulation of the micturition reflex by release of additional ATP, NO and acetylcholine</td>
</tr>
<tr>
<td>NKrs</td>
<td>SP</td>
<td>↑cAMP; ↑Ca²⁺</td>
<td>• detrusor contraction, increased density during IC/PBS, (pro-inflammatory)</td>
</tr>
<tr>
<td>VPAC₁</td>
<td>VIP</td>
<td>↑cAMP; ↑Ca²⁺</td>
<td>• regulation of the micturition reflex, (anti-inflammatory)</td>
</tr>
<tr>
<td>VPAC₂</td>
<td>PACAP</td>
<td>↑cAMP; ↑Ca²⁺</td>
<td>• sensory transduction, detrusor contraction, (pro-inflammatory)</td>
</tr>
</tbody>
</table>

(R₁ receptors, NKrs=neurokinin receptors, ATP=adenosine 5′-triphosphate, SP=substance P, VIP=vasoactive intestinal polypeptide, PACAP=pituitary adenylate cyclase-activating peptide, IP₃=inositol triphosphate, cAMP=cyclic adenosine monophosphate, NO=nitric oxide.)
and nerves in the healthy feline urinary bladder, and their possible change in expression in an animal IC/PBS model. In a number of publications, the increase in ATP release from the urothelium has been reported (42, 43).

P2X₃ purinoceptors are excitatory receptors on nerve terminals and play a role in mechanosensory transduction. This sensory function is mediated by urothelially released ATP acting on these receptors (44). P2X₆ purinoceptors are also involved in peripheral pain responses and afferent pathways controlling the bladder-filling phase (45). Purinergic P2Y₂ receptors, and to a much lesser extent P2Y₁ receptors present in the rat urothelium, have a role in autocrine and paracrine signalling. These receptors, when activated, lead to the release of additional ATP, NO and acetylcholine and hence they directly and indirectly modulate the micturition reflex (32). The activation of cholinergic receptors by for instance carbachol or endogenous acetylcholine, leads to lowering of the purinergic response, showing interplay between cholinergic and NANC systems (46). During IC/PBS, Birders study indicated a reduction of P2X₃ purinoceptors and loss of P2Y₂ purinoceptors in the urothelium. The P2Y purinoceptors were not expressed in the smooth muscle, in contrast to the positive staining for P2X₂ and P2X₆ purinoceptors. In the inflamed detrusor, a significant reduction of P2X₂ purinoceptors occurred. According to previous findings it seems likely that purinoceptors in the smooth muscle and urothelium might be involved in IC/PBS pathophysiology (33). Furthermore, it has been reported that in human IC/PBS, the expression of P2X₂ purinoceptors changes. Tempest et al. (31) suggested that by affecting P2X₂ purinoceptors, pain during the IC/PBS condition might be treated. P2X₂ purinoceptors were also suggested to be involved in the pathophysiology of idiopathic (47) and neurogenic (48) overactive bladder in humans. In the porcine bladder, the decrease of purine breakdown causes smooth muscle hyperactivity. In the condition of up-regulation of purinergic pathway, the P2X₆ purinoceptors showed to be responsible for the condition (49); therefore P2X₂ and P2X₆ purinoceptors might be a promising target for the treatment of various lower urinary tract symptoms (LUTS).

The stimulation of the rat bladder P1A₁ purinoceptors causes bladder relaxation, contrary to bladder contraction caused by P1A₃ purinoceptors (40). The remaining relaxation still appearing while P1A₁ purinoceptors are antagonized seems to be due to P1A₂B receptor activation (40). The specific blockade of P1A₁₅ receptors does not show any change in the relaxatory response, suggesting that this receptor subtype is of low or no importance for the relaxatory or contractile bladder function (36). Thus, purines regulate a number of effects in the lower urinary tract, for which tissue-specific expressions of different purinoceptors are a prerequisite.

The importance of P1 purinoceptors in regulation of inflammation was suggested in various studies (50-52). In the rat IC/PBS model, when cyclophosphamide is injected i.p., a decrease of the total amount of P1A₁ receptors was observed (40). In the same study, the blockade of P1A₁ receptors seemed to have the same influence on detrusor function in healthy and inflamed rat urinary bladders, suggesting that the function of P1A₁ purinoceptors does not change during IC/PBS. The activation of P1A₁ and P1A₃ purinoceptors seems to evoke pro-inflammatory effects, while P1A₂B purinoceptors act anti-inflammatory (40). In the uropathogenic Escherichia coli (UPEC)-infected human urinary tract epithelial cells, the expression of P1A₁ and P1A₃ mRNA showed to be decreased, while the expression of P1A₂B mRNA was increased, suggesting, that adenosine acting on P1A₂B purinoceptors might be a regulator of the inflammatory response in infections of urinary tract (53).

ATP being a key molecule in IC/PBS is even further stressed by its association with mast cells and with cytokines. An interplay between ATP and mast cells has been reported, which may be worth considering in view of increased numbers of activated bladder mast cells that seems to occur in the pathophysiology of IC/PBS (54). The amount of activated mast cells depends on the stage of cyclophosphamide-induced cystitis; in acute IC/PBS, the mast cell infiltration is increased in comparison with that in chronic condition (55). Mast cells may also release factors such as histamine, which may play a role in bladder inflammation (56). The levels of such factors are elevated during IC/PBS and their pro-inflammatory actions seem to contribute to pelvic pain. Substances inhibiting mast cell activation, or down-regulation of the mast cells overproduction, might help to improve the pathological condition. An activation of the inhibitory receptors on mast cells, containing immunoreceptor tyrosine-based inhibitory motifs (ITIMs), seems to be effective in IC/PBS treatment (57, 58). Concerning cytokines, an augmentation of purinergic signalling has been reported to occur in the human urothelial cells and which may be affected in IC/PBS (59). Of particular interest are hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) that may have pivotal roles in the IC/PBS progression. HIF-1 is a transcriptional factor responsible for cellular and tissue adaption to low oxygen levels. VEGF is a factor responsible for new blood vessel formation, which is a feature typical for IC/PBS. An increased expression of HIF-1 in bladder tissue and increased expression of VEGF in umbrella cells were identified in patients with IC/PBS. These alterations were suggested to be responsible for formation of glomerulations in IC/PBS patients (60). VEGF was suggested to contribute also to pain, aside its angioneogenetic effect (61).

All in all this means that purines may interfere at many levels, even though the molecules in the healthy human bladder exert the most important roles in the initiation of the micturition reflex. Apart from alterations at this level, contractile and inflammatory responses may also be affected by IC/PBS.

NITRIC OXIDE

Nitric oxide (NO) is a labile free radical gas, which serves as a neurotransmitter and modulator in the central and peripheral nervous system. It is not stored in synaptic vesicles, as other neurotransmitters are. Instead, it is synthesised by NO synthase (NOS) from its precursor L-arginine. NOS is divided into three different forms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). NO leaves the cell (e.g. neuron or endothelial cell) by simple diffusion, and possesses a relaxant effect in the intestinal tract, not only by direct action on smooth muscle, but also by inhibiting the release of acetylcholine (62).

Apart from numerous other biochemical pathways, NO is also involved in the control of functions in the lower urinary tract. NO can be released from the urothelium as well as from nerves (63). Birder et al. also reported that NO production and its release are influenced by beta-adrenoceptors (64), suggesting the release of NO in rat bladder urothelial cells is activated by beta-adrenoceptor stimulation. Furthermore, it was shown that nerves with the ability to synthetise NO are present in the urinary bladder in animals (65) as well as in humans (66). In studies, the NADPH-dependent enzyme nicotinamide-adenine dinucleotide phosphate-diaphorase, responsible for NO production, was used to indicate the NO production capacity of the neurons. NO released from the urothelium seems to play a role as a chemical messenger in a signalling mechanism between the urothelium and sensory nerves (63). Also, NO may paradoxically cause both relaxation and contraction of the urinary bladder. Namely, the enhancement of spontaneous contractions of guinea pig detrusor is associated
with a cGMP-independent process involving Ca\(^{2+}\) release from intracellular stores, while detrusor relaxation is dependent on a cGMP-dependent mechanism (67).

NO has been shown to contribute to the development of inflammation in the urinary bladder, both in humans and in animals (11, 68). NO is often used to detect inflammation of various organs, and elevated levels were also observed in patients with inflammatory bladder diseases of different origin, including IC/PBS (69). The levels of NO in the bladder could also be used to distinguish between classical, ulcerative IC/PBS, and the nonulcerative form of IC/PBS. High amounts of NO have been observed in patients with classical IC/PBS diagnosis, while other patients do not have any significant increase (68). In the feline IC/PBS, a naturally occurring model of IC/PBS, the levels of NO are altered. It was shown that an iNOS production of NO is increased in feline IC/PBS, probably causing urothelial surface disruptions (70). In a rat model for IC/PBS, in which cystitis was induced by intraperitoneal injection of the cystostatic drug cyclophosphamide, it has been shown that the inflammation is associated with an up-regulation of eNOS, causing decreased contractility to muscarinic receptor activation (71). However, the changes in the NO production cause not only indirect effects via induction of inflammation. The altered NO levels affect direct functional effects evoked by acetylcholine and ATP as well. The ability of NO to cause both relaxatory and contractile effects, and to have different effects in health and in disease, makes its effects obscure and difficult to interpret, but still highly interesting. The complexity of the NO effects in the context of the NOS up-regulation in states of disease in the urinary bladder (72) may be one of the reasons of NO being a molecule of pivotal interest in interstitial cystitis.

**SUBSTANCE P**

Substance P (SP) is a neuropeptide transmitter, first considered as a sensory or pain transmitter; a nociceptive mediator in afferent sensory fibres (73). But SP is involved in other physiologic activities, such as the vomiting reflex, smooth muscle contraction, secretion and vasodilatation (74-77). SP acts on neurokinin (NK) receptors (78), which belong to the G-protein coupled receptor family.

In 1983 Sharkey et al. designed a study where they, by applying capsaicin, known for emptying sensory neurons of their SP content, achieved urine retention, suggesting that SP is responsible for alterations in micturition function (79). SP causes a slow contractile response of the detrusor, as has been shown on cats (80). SP is thus, together with calcitonin gene-related peptide (CGRP), a neuropeptide that mainly transmits signals via the afferent innervation. This has also been confirmed in morphological studies in the rat urinary bladder, in which 14% and 6% of the SP- and CGRP-containing nerves, respectively, may be of non-sensory origin (81).

SP contributes to the pathophysiology of pain and inflammation. The SP-containing nerve fibres are present in the IC/PBS bladders of various species. Their density is increased in IC/PBS in the submucosa, but not in the detrusor. SP might be responsible for the supra-pubic pain appearing during the IC/PBS disorder (82). The involvement of SP in IC/PBS was also suggested by Marchand et al. (83), and an increased expression of NK1 receptors in bladder vascular endothelium was reported. An up-regulation of NK1 receptors was also observed in the feline urinary bladder, where the affinity of SP to its receptors was measured in healthy and inflamed animals (84). The urinary level of SP seems to be related to urinary frequency and urgency. In patients suffering from IC/PBS treated by intravesical administration of DMSO, a reduction of frequency and urgency correlated with reduction of SP in urine was observed (85). However, another study claims that during IC/PBS, the urinary levels of SP are not significantly increased compared to the levels of SP in patients with stress incontinence or normal bladder function (86).

**VASOACTIVE INTESTINAL POLYPEPTIDE**

Vasoactive intestinal polypeptide (VIP) is a 28-amino acid neuropeptide originally isolated from intestinal extracts. VIP is a highly potent vasodilator, and in addition to regulating smooth muscle tension (87), it causes epithelial cell secretion, such as chloride secretion by colonic epithelial cells (88). VIP is widely distributed in the peripheral as well as in central nervous system where it functions as a neurotransmitter and neuromodulator, being released from nerve terminals and acting locally on G-protein coupled VIP receptors (VPAC\(_1\) and VPAC\(_2\)).

The genito-urinary tract contains a rich supply of VIP producing nerves that appear in all species, however the density of the innervation varies (89). In the rat, the VIP-containing fibres have been shown to be non-sensory (81). In the cat, VIP nerves appear in the trigonal part of the bladder, around the urethral openings and in the upper part of the urethra. In the guinea pig urinary tract, VIP nerves occur more frequent in males than in females (90). A fairly high density of VIP fibres occur in the rat urinary bladder and these fibres originate in the pelvic ganglia (91). VIP is present in all regions of the human bladder but is particularly intense beneath the urothelium and in the muscular layer (92). VIP is widely distributed in neuronal pathways regulating the micturition reflex. The exact significance of the relaxatory VIP innervation is largely unknown.

VIP, together with other neuropeptides, was proposed to be an anti-inflammatory modulator of inflammation. In bladder tissues of IC/PBS patients the amount of VIP neurons was higher than in healthy control subjects (10). In VIP knocked out mice, injected intraperitoneally with CYP to induce cystitis, the bladder dysfunction was increased in comparison with CYP-treated mice of wild type (93).

**PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE**

Pituitary adenylate cyclase-activating peptide (PACAP) is a peptide showing large resemblance with VIP. The peptide occurs in two forms, the 37- and 38-amino acid structures. Both are present in the central and in peripheral nervous system, exerting a variety of effects. PACAP and VIP have the ability to modulate innate and adaptive immunity. PACAP can also function as a sensory neurotransmitter, acting on PAC receptors (94). When administrated intrathecally and intra-arterially close to the bladder of the rat, PACAP stimulates micturition and causes detrusor contraction (95). In the rat urinary tract, PACAP is present mainly in the 38-amino acid form. The PACAP positive nerve fibres are localised in the urothelium, blood vessels and detrusor and the peptide is suggested to be a sensory neurotransmitter in the rat urinary tract (96).

PACAP is involved in the regulation of sensory afferents of the micturition pathway. In a rat IC/PBS model, administration of a PACAP receptor antagonist reduced overactivity of the bladder induced by cystitis (97). The same study suggests that the altered expression of PACAP receptors during IC/PBS contribute to bladder dysfunction and is related to the condition. The PACAP expression is regulated by bladder inflammation and the expression of PACAP receptors is significantly increased in the urothelium and in the detrusor in CYP-induced cystitis in the rat. An administration of PACAP to cultured urothelial cells evoked ATP
release that was blocked by the PACAP receptor antagonists (98), showing a possible correlation of PACAP with ATP during IC/PBS.

SUMMARY

Over the years, in studies of lower urinary tract diseases, emphasis has been on examining alterations of signalling by the classical transmitters, acetylcholine and noradrenaline and, in particular regarding sensory mechanisms. However, NANC mechanisms play significant roles in mediating direct functional effects as well as indirect, by affecting inflammation. In this perspective, NANC mechanisms are of particular interest in IC/PBS. In this disease, the main alterations occur regarding the sensory input to the central nervous system, even though changes also appear in the effenter nervous systems. Consequently ATP and SP may be two important players in the disease development, and another key NANC molecule, namely NO, probably affecting inflammation, afferent and efferent signalling as well as muscular spontaneous activity.

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