Herpes simplex virus vector-mediated gene delivery for the treatment of lower urinary tract pain

WF Goins[1], **JR Goss**[1], **MB Chancellor**[2,4], **WC de Groat**[3], **JC Glorioso**[1], and **N Yoshimura**[2]

[1] Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

[2] Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

[3] Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Abstract

Interstitial cystitis (IC)/painful bladder syndrome (PBS) is a painful debilitating chronic visceral pain disorder of unknown etiology that affects an estimated 1 million people in the, United States alone. It is characterized by inflammation of the bladder that results in chronic pelvic pain associated with bladder symptoms of urinary frequency and urgency. Regardless of the etiology, IC/PBS involves either increased and/or abnormal activity in afferent nociceptive sensory neurons. Pain-related symptoms in patients with IC/PBS are often very difficult to treat. Both medical and surgical therapies have had limited clinical utility in this debilitating disease and numerous drug treatments, such as heparin, dimethylsulfoxide and amitriptyline, have proven to be palliative at best, and in some IC/PBS patients provide no relief whatsoever. Although opiate narcotics have been employed to help alleviate IC/PBS pain, this strategy is fraught with problems as systemic narcotic administration causes multiple unwanted side effects including mental status change and constipation. Moreover, chronic systemic narcotic use leads to dependency and need for dose escalation due to tolerance: therefore, new therapies are desperately needed to treat refractory IC/PBS. This has led our group to develop a gene therapy strategy that could potentially alleviate chronic pelvic pain using the herpes simplex virus-directed delivery of analgesic proteins to the bladder.

Keywords

interstitial cystitis; painful bladder syndrome; visceral pain; dorsal root ganglia; herpes simplex virus

Interstitial cystitis/painful bladder syndrome

Interstitial cystitis (1C) or painful bladder syndrome (PBS) is a non-malignant chronic inflammatory pain condition of the visceral organs of the lower urinary tract (LUT), characterized by a constellation of symptoms including bladder/pelvic pain most commonly associated with urinary urgency and frequency that alters urinary output due to pain upon voiding.[1–4] In many instances, the pain and discomfort felt by patients with IC/PBS is substantial, with their quality of life being similar to patients with end-stage renal disease. Although IC/PBS affects both men and women of all ages, it predominantly affects women, with up to 12% of women showing some symptoms of the disease during their lifetime. Diagnosis is usually based on the presence of the prior listed symptoms, which unfortunately

Correspondence: Dr WF Goins, Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, W1157 Biomedical Science Tower, 200 Lothrop St., Pittsburgh, PA 15261, USA. goins@pitt.edu.
[4]Current address: Department of Urology, William Beaumont, Hospital, Royal Oak, MI 48073, USA

are characteristic of numerous other bladder conditions, such as cancer and infectious disease, making definitive diagnosis of IC/PBS difficult. Once these other disorders have been ruled out, IC/PBS is then diagnosed as painful bladder symptoms in the absence of infection or other identifiable conditions. Urinalysis including urine culture can be employed to rule out infection, whereas tests such as the prostate-specific antigen can be employed in men to rule out cancer as the cause. In some instances, other clinical tests, such as urodynamic studies, cytoscopy with hydrodistension and the potassium chloride sensitivity test, can be employed in the diagnosis. However, the variability of the potassium chloride sensitivity and specificity of this test for IC/PBS and the fact that hydrodistension testing generates a painful response in patients and can generate lesions similar to those found in IC/PBS patients make these tests less attractive for use in the diagnosis of IC.

Epidemiology of IC/PBS

Oravisto (1975)[5] reported an incidence of 18.1 per 100 000 female individuals and an overall incidence of 10.6 per 100 000 in Finland. In 1987, Held *et al.*[6] performing population-based studies in the United States reported a prevalence of 20 000–90 000 documented cases of IC/PBS where the duration of patient symptoms was for 30–50 months before diagnosis. Curhan *et al.* (1999)[7] also reported that the incidence of IC/PBS is much more prevalent than was initially thought. IC/PBS affects over 700 000 people in the United States, an overall prevalence 50% higher than earlier estimates and more than three times that reported in Europe. More than 90% of IC/PBS patients are women, but IC/PBS occurs in people of both sexes and all ages. More recently, Payne *et al.*[8] documented that the prevalence of IC-like symptoms is much higher at approximately 5000/100 000 although the prevalence of a formal physician diagnosis of IC is relatively low at approximately 200/100 000 population. Thus, it is likely that the prevalence of IC/PBS or chronic pelvic pain is greater than was first anticipated and that IC/PBS has now become more readily recognized as an important diagnosis with greater awareness of this disorder.

Pathophysiology of IC/PBS

Although the etiology of IC/PBS remains unknown it is likely to be a multifactorial and not a single-source disease that can be initiated by various triggers such as autoimmune response to host antigens, allergic reaction to substances that have leaked into the bladder, neurogenic inflammation, sensitization of the afferent pathways, dysfunction of the bladder epithelium, inherited susceptibility[9] and also bladder mucosal ischemia as potential mediators of the observed disease pathology. In the past, bladder infection was proposed as a primary mechanism for the creation of the IC/PBS pathophysiologic state; it has been difficult to ascribe support for this hypothesis compared with the other possible triggering mechanisms.

It has been proposed that a defect in the protective glycosaminoglycan (GAG) layer that lines the epithelium of the bladder, resulting from a wide range of stimuli, may lead to increased permeability across the uroepithelium (Figure 1). This change in membrane permeability then can result in irritating substances present within urine breaching this layer leaking into the surrounding tissues, triggering the painful symptoms observed in IC/PBS.[10] This initial injury to the bladder epithelium[9] thereby induces other events, such as irritation of the afferent pathways responsible for neurogenic control of bladder function as well as the release of soluble mediators at the site of injury, which in turn results in an increase in and further activation of mast cells, all of which are signs of immune involvement in the disease process and in some instances point to an autoimmune or allergic function that may contribute to IC/PBS pathophysiology.[9,11] The role of the host immune response in IC/PBS pathogenesis is further supported by the fact that numerous patients with IC/PBS also suffer from allergies, various autoimmune disorders such as inflammatory/irritable bowel syndrome (IBS), asthma, sensitive skin and lupus as well as fibromyalgia, vulvodynia and migraine headaches,[11,12] which may

further lead to mast cell activation and overall inflammation in the region of the initial insult and the afferents innervating this site.

The stimulation of mast cell infiltrate to the bladder urothelium site of initial injury and the subsequent release of soluble mediators by these cells, such as cytokines like tumor necrosis factor, histamines and prostaglandins, contributes to further local inflammation, resulting in additional damage to the bladder mucosal lining. This in turn results in an additional immune cell infiltration primarily consisting of mast cells, the further activation of the release of soluble mediators as well as the release of tachykinins such as substance P (SP), which ultimately leads to the sensitization of sensory nerves.[9,12] Thus, the combination of SP stimulation of afferent processes with a cycle of increasing local inflammation surrounding these processes, further contributes to the role of the inflammatory process in the disease pathogenesis.

Standard IC/PBS treatment regimens

Treatment strategies for IC/PBS have been just as puzzling as the diagnosis of this syndrome. Although a variety of treatment regimens have been used to manage other chronic painful symptoms, none uniformly eradicate the symptoms of severe suprapubic pain, urinary frequency, and urgency experienced by patients with IC/PBS.[3,4,13] Thus, the treatment regimen of IC/PBS is often specific to the individual patient and frequently involves a combination of therapies. However, some patients are not helped by any of the current treatment regimens.

Although surgery has proven an effective therapy for some forms of chronic lower back pain, it is rarely used to treat IC/PBS pain only as a last resort should other standard therapies fail. However, even following surgery, many patients still experience urinary frequency, urgency and pain even phantom bladder pain after cystectomy. Neuromodulation strategies are also usually employed only when other standard therapies have failed. These approaches use either the external transcutaneous electrical nerve stimulator (TENS) units or InterStim implants to send varying modulatory impulses to the nerves controlling bladder function in the hope of regulating urinary frequency and urgency. In addition, these devices have shown some relief in patients with mild-to-moderate IC/PBS pain, but controlled studies to assess the number of patients with improved pain scores have not been initiated and some adverse side effects have been observed with these devices.

As the initial injury leading to IC/PBS occurs through damage to the urothelium, agents like GAGs, such as heparin,[14] Uracyst (chondroitin sulfate)[15] and Cystistat,[16,17] have been administered by intravesical instillation over a 20-min period weekly for 1–2 months, then monthly thereafter in an effort to help restore or maintain the lining of the bladder. These agents also appear to have some anti-inflammatory action in addition to being potent protective factors for repairing the bladder surface. Another drug that acts at the same level is Elmiron (pentosan polysulfate sodium), the only oral medication specifically approved by the Food and Drug Administration to treat IC/PBS. In addition to the use of Elmiron as an oral agent, it can also be given by direct instillation into the bladder. Clinical trials using Elmiron have shown promise with ~50% of patients displaying some form of improvement in their bladder symptoms; however, it may take more than 6 months for these results to occur.[18–21] Bladder instillation with RIMSO-50, a 50% solution of dimethylsulfoxide, the only other Food, and Drug Administration-approved treatment for IC/PBS or other agents,[3,4,22] also has helped relieve the symptoms of IC/PBS because these agents may not only possess anti-inflammatory and analgesic properties but also may act by relaxing bladder detrusor muscle activity.[23] Some unwanted side effects have been observed in patients treated with the 50% dimethylsulfoxide solution that seem to lessen over time; however, the use of a 25% solution fails to induce these harmful effects yet maintains some activity toward managing the IC/PBS symptoms.[24] Dimethylsulfoxide has been used in many combination therapies in which it increases the

uptake of other drugs in the cocktail across the bladder lining following intravesicular instillation.[25,26]

Histamines, leukotrienes and prostaglandins are generated upon mast cell infiltration of the site of bladder epithelium injury; thus, drugs that block their inflammatory activities have been employed to treat IC/PBS. The histamine H1R blocker hydroxyzine (Atarax) has been employed alone or in combination with other drugs, such as Elmiron,[27] with limited success while possessing mild sedation as an unwanted side effect. Even H2R-specific histamine blockers, such as Tagamet, have resulted in a reduction in pain in ~50% of IC/PBS patients tested.[28] The leukotriene inhibitor Montelukast (Singulair), which is taken for allergy relief, has shown some reduction in IC/PBS patient bladder-related symptoms[29] as well as the prostaglandin analog Misoprostol.[30] Other agents that may offer relief include tricyclic antidepressants that have analgesic properties with both anti-cholinergic and anti-histamine effects such as amitriptyline (elavil).[31,32]

Non-opioid pain medications employed in the treatment of other chronic pain syndromes, such as the drugs gabapentin (Neurontin), aspirin (Bufferin), acetaminophen (Tylenol) and non-steroidal anti-inflammatory drugs (Advil, Motrin, Aleve), have also been prescribed for IC/PBS pain, with gabapentin showing the greatest efficacy (~50% pain reduction) due to its action against neuropathic pain.[33] Opioid analgesics, such as tramadol (Ultram), codeine (+/− aspirin, acetaminophen) and hydrocodone (Vicodin), have been used to treat moderate bladder pain with a modicum of success. Although all possess narcotic and dependency issues, tramadol seems to possess a superior safety profile compared with the other opioids and has just recently been proven to be effective in the treatment of IC/PBS-related bladder pain.[34,35] Patients with frequent or unremitting pain may require more aggressive pain management, such as long-acting opioids, for example morphine (MS Contin and Oramorph) and oxycodone (OxyContin).[3,36] However, the use of systemic opioid therapy has been limited because of its untoward side effects and dependency.[37,38]

On account of the multiple factors that have been shown to play a role in the initiation of IC/PBS, it has been suggested that multimodal therapeutic intervention may hold the best promise in treating patients with IC/PBS. Today, several such approaches have shown varying degrees of efficacy, including the combination of Elmiron with other meds such as heparin,[31] hydroxyzine[27] or steroids such as cyclosporin A (CSA).[39] Other treatments have combined a membrane-protective agent like heparin with lidocaine[14] or heparin and hydrocortisone,[40] which has higher efficacies than using either agent alone.

C-fiber activation in IC/PBS pathology and pain management

The activation of C-fiber afferents that innervate the bladder by a variety of potential mechanisms represents crucial initial steps to the development of pain associated with IC/PBS. This process may involve components of an inflammatory nature or may also involve the activation of chemo- and mechanoreceptors.

Afferent pathways innervating bladder and urethral function

Sensory information including the feeling of bladder fullness or bladder pain is conveyed to the spinal cord through afferent axons in the pelvic and hypogastric nerves.[41,42] Neuronal somata of these afferent nerves are located in the dorsal root ganglia (DRG) at S2–S4 and T11–L2 spinal segmental levels in humans. These afferent fibers carry impulses from tension receptors and nociceptors in the bladder wall to neurons in the dorsal horn of the spinal cord (Figure 2). Afferent axons in the pelvic, hypogastric and pudendal nerves transmit information from the LUT to the spinal cord. The primary afferent neurons of the pelvic and pudendal nerves are present within the sacral DRG, whereas afferent innervation in the hypogastric

nerves arises in the rostral-lumbar DRG.[42–44] The central axons of the DRG neurons carry the sensory information from the LUT to second-order neurons in the spinal cord[43,45–47] (Figure 2). Visceral afferent fibers of the pelvic[46] and pudendal[47] nerves enter the cord and travel rostrocaudally within Lissauer's tract.

Afferent fibers passing in the pelvic nerve to the sacral cord are responsible for initiating the micturition reflex. These bladder afferents are composed of myelinated Aδ-fiber or unmyelinated C-fiber axons.[45,48] In rats, there is now evidence that many C-fiber bladder afferents are volume receptors that do not respond to bladder contractions, a property that distinguishes them from 'in series tension receptors'.[49] In cats, Aδ-bladder afferents appear to be low-threshold mechanoreceptors,[50] whereas C-fiber bladder afferents[41] are generally mechanoinsensitive ('silent C-fibers'). Some of the latter may be nociceptive, and are sensitized by intravesical administration of chemicals such as high potassium, low pH, high osmolality and irritants such as capsaicin and turpentine.[41,51–53] Following exposure to these substances, the sensitivity of bladder mechanoreceptors to distension, increases and some 'silent' afferents become mechanoceptive. Immunohistochemical studies indicate that bladder afferent neurons contain various neuropeptides, such as SP, calcitonin gene-related peptide and vasoactive intestinal peptide.[51,54] The distribution of these peptidergic afferent terminals in the spinal cord is similar to that of central projections of bladder afferent neurons.[43,55] The release of these neuropeptides in the bladder wall is known to trigger inflammatory responses similar to those characteristic of IC/PBS, including plasma extravasation or vasodilation.[56] However, the release of these neuropeptides in central afferent nerve terminals activates second-order neurons in the spinal cord to transmit pain sensation to the brain. Thus, during inflammation and possibly other pathological conditions including IC/PBS or chronic pelvic pain, there is recruitment of mechanosensitive C-fibers that form a new functional afferent pathway.[57]

Hyperexcitability of C-fiber afferent pathways as a mechanism for LUT pain

Pain is a defining characteristic of IC/PBS or chronic pelvic pain syndrome. One mechanism by which pain is induced is postulated to involve chronic tissue inflammation that can lead to functional changes in C-fiber afferents.[12,58–60] These normally silent fibers appear to have a specific function in signaling noxious events in the bladder. Hyperactivity of C-fiber afferents may, therefore, lead to increased pain sensation. Chronic conditions that involve tissue inflammation or irritation can induce changes in sensory pathways that lead to hyperalgesia (heightened response to painful stimuli) and allodynia (pain in response to normally non-painful stimuli). For example, tissue inflammation in visceral organs such as urinary bladder can increase afferent nerve excitability in response to both noxious and non-noxious stimuli. [41,61] Therefore, changes in afferent nerves might contribute to painful symptoms in patients with IC/PBS or chronic pelvic pain syndrome, a chronic pain syndrome of unknown etiology that appears to have an inflammatory component. It has also been reported that C-fiber desensitization induced by intravesical application of high-dose capsaicin and resiniferatoxin is effective for treating painful symptoms in IC/PBS patients,[62] although a more recent prospective, randomized clinical trial using intravesical resiniferatoxin application was not effective in patients with IC.[63]

Indirect evidence for this postulate comes from histologic analysis of bladders from patients with IC/PBS, which is marked by edema, vasodilation, proliferation of nerve fibers and infiltration of mast cells,[64,65] and from chemically induced cystitis in animals, in which increased urinary frequency is initiated by sensitizing mechanosensitive afferents and/or recruitment of afferents normally unresponsive to mechanical stimulation.[41,61,66] In addition, proinflammatory agents, such as prostaglandin E_2, serotonin, histamine and adenosine, as well as neurotrophic factors such as nerve growth factor (NGF), can induce functional changes in

C-fiber afferents that can lead to these relatively unexcitable afferents becoming hyperactive or hyperexcitable.[66–70]

A more direct evidence linking chronic inflammation with functional changes in C-fiber afferents has been derived from a rat model of chronic cystitis induced by systemic application of cyclophosphamide, which undergoes hepatic metabolism to acrolein, an irritant excreted in the urine[71] or intravesical application of hydrochloric acid (HCI).[72] In the cyclophosphamide model, it has been documented that the majority of bladder afferent neurons from both control and chronic cystitis rats are capsaicin sensitive and exhibit tetrodotoxin-resistant action potentials, whereas those from treated rats exhibit significantly lower thresholds for spike activation and show high-frequency firing characteristics.[73] Other significant changes in bladder afferents from cyclophosphamide-treated rats include increased somal diameter, increased input capacitance and decreased density of slowly inactivating A-type K^+ (K_A) currents. Together, these data suggested that chronic inflammation induces both cell hypertrophy and hyperexcitability of C-fiber visceral afferent neurons.[73] More recently, hyperexcitability of C-fiber afferent neurons due to decreased K^+ currents was also reported in cats with IC/PBS.[74] C-fiber-mediated nociceptive responses, such as urinary frequency and C-fiber afferent hyperexcitability, were also identified in rats with urethral inflammation or pudendal nerve injury.[60] Thus, targeting hyperexcitable C-fiber activity represents a mechanism to treat LUT pain.

Opioid mechanisms in the peripheral nervous system and central nervous system

Multiple opioid peptides and receptors

Opioid peptides are derived from three different precursor proteins: pro-opiomelanocortin, prodynorphin and proenkephalin.[75–77] The main groups of opioid peptides, enkephalins, dynorphins and β-endorphin, derive from proenkephalin, prodynorphin and pro-opiomelanocortin, respectively. Proenkephalin is the source of [Met^5]- and [Leu^5]-enkephalins and several longer peptides. Three members of the receptor family were cloned. The cloned μ-opioid receptor is a morphine-like receptor[78–82] and endomorphins may be its endogenous ligands. The enkephalins bind to the δ-opioid receptor[80,83–86] with great affinity and, therefore, are considered to be endogenous δ-opioid receptor agonists. The affinity of β-endorphin binding to μ- and δ-opioid receptors was found to be similar. Dynorphins bind to κ-opioid receptors[83,87–91] and therefore appear to function as its endogenous ligands. However, opioid peptides do not bind exclusively to one specific receptor type but have some affinity for other opioid receptors as well, similar to that of the neurotrophins for their cognate receptors.

High expression levels of opioid receptors (μ-, κ- and δ-binding) were found in the substantia gelatinosa where sensory afferents terminate as well as on the terminals of primary afferents,[92] explaining the significant binding of opioid peptides to the dorsal sensory roots in the DRG.[93,94] Opioid receptors have also been shown on peripheral terminals of nociceptive sensory nerves in animals and humans.[95,96] Earlier studies have shown that all three opioid receptor types (μ, κ and δ) can be functionally active in peripheral tissues[97,98] although some other studies suggested that κ-opioid receptor mechanisms, rather than μ- or δ-receptors, are more likely to be involved at the peripheral site in visceral pain induced in the bladder or colon.[99,100]

Central afferent terminals, opioids and presynaptic inhibition

It has been well documented that opioids have a direct inhibitory effect upon the excitability of the afferent terminals, leading to a net decrease in the release of neurotransmitter into the synapse. Changes in central terminal excitability could reflect three general mechanisms: (1)

a hyperpolarization of the terminal by the activation of terminal receptors that alter ion permeability, leading to a shunting of membrane currents and thus making the terminal more difficult to excite; (2) depolarization of the terminal such that voltage-sensitive Ca^{2+} channels may become inactivated, negating the ability of any subsequent terminal depolarization to evoke neurotransmitter release; or (3) direct inhibitory coupling to voltage-sensitive Ca^{2+} channels, blocking Ca^{2+}-dependent exocytosis,[92,101−103] Consequence of these events could then reduce the amount of neurotransmitter that gets released secondary to depolarization.

Populations of unmyelinated C-fiber afferents are known to contain a variety of neuropeptides such as SP and calcitonin gene-related peptide, which are released in a Ca^{2+}-dependent fashion following the physiological or pharmacological activation of nociceptive afferents.[104,105] The presence of opioid receptors on the terminals and the coupling of opiate receptors with voltage-sensitive Ca^{2+} channels suggest that opioids might act to block directly the release of the neurotransmitters contained in the respective terminals. Thus, it appears that opioids acting through μ- and δ-receptors act presynaptically to inhibit the release of peptides from nociceptive C-fibers that release neuropeptides such as SP.

Role of opioids in dorsal horn neuron postsynaptic inhibition

Various studies have shown that opioids at or near the terminals of the C-fibers in the dorsal horn of the spinal cord reduced both spontaneous activity and the firing of spinothalamic projection dorsal horn neurons evoked by noxious and innocuous stimuli.[92] Both μ- and δ-agonists exerted a suppressive effect upon nociceptive-specific neurons in lamina I of the spinal cord. Opioid peptides also produce a hyperpolarization and a decrease in cell firing of the dorsal horn neurons in the substantia gelatinosa.[106]

Opioid peptides in the control of LUT function

Various neurotransmitters at the spinal and supraspinal level are involved in the regulation of LUT activities.[107,108] Among them, opioid peptides and GABA have been shown to be important inhibitory transmitters suppressing LUT activity. Enkephalinergic pathways in the central nervous system exert an inhibitory control on the micturition reflex.[107,108] Enkephalinergic varicosities have been shown by immunocytochemistry at various sites including the primary motor cortex (PMC), the sacral parasympathetic nucleus and urethral sphincter motor nucleus in the spinal cord. Administration of opioid drugs or enkephalins to the brain or spinal cord suppresses micturition and sphincter reflexes.[109−112] In the brain, both μ- and δ-opioid receptors mediate inhibitory effects, which are blocked by naloxone.[110,113,114] In the cat spinal cord, δ-opioid receptors mediate the inhibition of bladder activity and κ-receptors mediate the inhibition of sphincter activity.[112] In the rat spinal cord, δ- and μ-receptors but not κ-receptors are involved in the suppression of bladder reflexes.[108,110,111] In conscious dogs, intrathecal administration of morphine increases the volume threshold for inducing micturition without altering voiding pressure that can be blocked by naloxone. These observations indicate that spinal opioid mechanisms can control the afferent limb of the micturition reflex[110] but not C-fiber bladder afferents, which are reportedly not involved in normal voiding in rats.[115]

It has been shown that visceral pain induced by colon or bladder noxious distention was suppressed by the activation of spinal μ- and δ-opioid receptors and peripheral κ-receptors in rats.[99,100] Craft *et al.*[116] also reported that behavioral pain responses induced by the intravesical application of resiniferatoxin, a potent analog of capsaicin, were suppressed by the activation of systemic μ-, δ- and κ-opioid receptors and peripheral δ-receptors in rats. Meen *et al*[117] have also shown that bladder nociceptive responses induced by cyclophosphamide in rats were suppressed by the intrathecal application of opioids.

Gene therapy: an alternative therapy for LUT pain

Gene therapy offers considerable promise for treating otherwise intractable diseases of the human nervous system, including the treatment of chronic pain, compared with standard drug-related therapies.[118–121] In many instances in which drug therapies have been employed, the therapeutic factor delivered systematically cannot pass the blood–brain barrier in sufficient quantities to be therapeutic or systemic delivery of the agent results in side effects associated with expression of the agent in cells or tissues in which its expression is unwanted or deleterious.

Both non-viral and viral delivery vehicles have been employed to deliver genes to bladder and other tissues. Each delivery system has its own advantages and disadvantages for delivery to different target cells and tissues. Overall, a major advantage of the non-viral delivery systems has been the low immunogenicity of this approach compared with viral vectors that all show some level of host response to the vector itself, which may only amplify any host response to the therapeutic gene. In addition, the non-viral systems have not been plagued by the short-term nature of vector-delivered transgene expression due to the limited maintenance of the delivery vehicle in the transduced cells. On the other hand, viral vectors are exceedingly more efficient at delivering their genetic payload to the target cell compared with non-viral systems because over millennia viruses have acquired efficient methods to deliver their own genetic material to cells to replicate their genomes and further propagate themselves. Another advantage is that most, viral vectors can be readily produced and purified for *in vivo* gene transfer, whereas the non-viral methods are limited by the production of sufficient quantities of DNA for transduction.

The majority of gene transfer studies for the bladder have revolved around gene delivery to bladder tumors, in which the goal is to achieve high-level transduction of the tumor in the absence of gene delivery to normal bladder epithelium; both non-viral and viral vectors have been used for gene transfer to urothelium. Non-viral approaches to transfer to the bladder have used direct injection of naked, liposome-mediated transfer of DNA as well as electroporation of DNA into the bladder wall and gene gun-mediated delivery of DNA linked to gold particles, although most of these approaches have met with limited success in achieving high-level transduction of either the urothelium or the smooth muscle layer. The GAG layer present on the uroplakin-covered umbrella cells has limited access of agents to the urothelium in undamaged bladder, and thus simple administration of genes by cationic liposomes[122,123] led to poor transduction of the bladder suggesting that other means of abrogating this physical barrier are needed for effective gene transfer. To bypass this barrier, various treatments have been employed; however, their damage to the urothelium would be detrimental to the already damaged urothelium present in IC/PBS patients, and thus would not be of clinical use to improve transduction. Other groups have exposed the bladder by a lower midline incision followed by the direct injection of plasmid DNA expressing a luciferase, eGFP or lacZ reporter gene combined with electroporation to achieve gene delivery, where the voltage, number, duration and frequency of the electric pulses were optimized to provide efficient delivery primarily to cells of the smooth muscle layer.[124–126] Moreover, the groups did not detect any difference in KCl-induced detrusor muscle activity following electroporation, suggesting that this approach did not damage the urothelium. Otani *et al*.[125] showed that the transduction of bladder by this method with a plasmid encoding the muscarinic M3 receptor did result in detrusor muscle changes that may potentially be therapeutic, whereas Iwashita *et al*.[124] showed that nNOS gene transfer led to an increase in NO production within the bladder smooth muscle layer. Finally, gene gun delivery of gold particle-linked DNA (Figure 2) to the bladders of animals in which the bladder has been exposed by a midline incision enabled delivery of molecules that alter the painful responses to bladder nociceptive stimuli in animal models of cystitis[127,128] discussed in detail below.

Viral vectors (adenoviruses, pox viruses and herpes simplex virus (HSV)) have been employed in gene transfer studies to the bladder and have met with greater success than the various non-viral methods, although the main goal of many of these studies was to efficiently transduce urogenic tumors over that of the normal bladder urothelium. The same GAG barrier that blocked transduction using non-viral methods also proved troublesome for initial studies using intravesical delivery of adenoviral vectors,[129,130] so continued studies to improve transduction employed agents to disrupt this barrier such as ethanol,[130] polyamines such as CHAP and Big-CHAP,[131] HCI[32] and dodecyl-β-D-maltoside or SDS.[133] Engler *et al.*[130] found that while 40% ethanol provided enhanced gene transfer, it also resulted in hemorrhagic cystitis, but its reduction to 22% yielded a high-level expression (84% of surface expression of lacZ), either as a pretreatment or when co-administered with the vector. It is interesting to note that the alcohol had no effect on vector-mediated gene transfer *in vitro,* supporting the role of the agent in altering the GAG barrier *in vivo.* Various cationic, anionic, zwitterionic and non-ionic agents were employed to break down the GAG layer hydrophilic polyanionic barrier restricting adenovirus transduction; however, most either gave little improvement or led to cystitis.[131] One agent, Big CHAP, yielded high transduction in the absence of toxicity, but more-purified Big CHAP preparations of the agent failed to enhance vector-mediated transduction, whereas the impurities Syn3-Lac and Syn3-Mel were found to possess the ability to enhance the transduction of adenovirus. Another study employed 60 mM HCl to alter the GAG barrier, [132] which did improve adenovirus-mediated gene transfer, which could be blocked using Elmiron, yet the authors did not examine the effects of this short (10 min) acid treatment on bladder integrity/toxicity as many groups actually employ acid treatment to create an animal model of cystitis. Ramesh *et al.*[133] used a variety of agents including alcohols, chlorpactin, tween, oxychlorosene, SDS and even dodecyl-β-D-maltoside. Most of these did not significantly improve transduction but rather acted as bladder irritants. Even treatment with 0.1–0.2% oxychlorosene, which gave a modest enhancement to vector-mediated transgene expression, resulted in edema, neutrophil infiltration, ulceration and mucosal erosion. However, pretreatment of with 0.1% dodecyl-β-D-maltoside, an alkyl disaccharide, yielded increased adenoviral transduction without measurable signs of bladder irritation. Initial studies using Pox virus vectors delivered intravesically failed to transduce the urothelium,[134] which is likely to be the result of a block in vaccinia infection by the GAG barrier. The use of similar agents employed to increase adenovirus transduction to make that barrier more penetrable to the Pox vector, such as HCl, NH_4Cl, chlorpactin and oxychlorosene,[135] yielded similar results, with oxychlorosene providing the greatest enhancement with the least toxicity, whereas agents such as acids tended to produce severe changes to the urothelium that in turn result in cystitis.

Herpes simplex virus vectors have been employed for delivery of genes to the bladder[136] and bladder afferents.[137–139] Unlike studies using Adeno and Pox viral vectors, the main goal of the HSV studies were the delivery of therapeutic genes to the bladder and the DRG afferents that innervate the bladder either for treatment of diabetic neuropathy[138,139] or for cystitis/PBS. [137] Delivery of vector in these studies was through the injection of the vector directly into the bladder wall following a lower midline incision to expose the bladder (Figure 2). Brooks *et al.*[136] showed high-level expression of either the lacZ reporter gene or the tumor necrosis factor therapeutic gene within the bladder and not any other tissue, demonstrating the specificity achieved using HSV, yet they did not examine whether vector injection led to transgene expression within bladder afferents. The studies examining the effects of NGF delivery to the bladder and its afferents by replication-defective HSV vectors showed both short-term and long-term NGF expression in both bladder smooth muscle layer and L6 and S1 DRG small-medium neurons, with the length of expression determined by the promoter used to drive transgene expression.[138,139] In functional studies using the streptozotocin (STZ)-treated diabetic animal rat model, NGF vector-treated animals displayed reduced bladder capacity and postvoid volumes in metabolic cage studies and also cystometry compared with STZ-treated diabetic animals that received the control vector expressing the lacZ reporter gene,[138]

suggesting a partial restoration of function that correlated with the expression of the NGF transgene within the bladder and afferents. Using similar delivery approaches, replication-defective HSV vectors expressing opioid gene products have also been recently employed in a cystitis model of bladder pain[137] described in detail below.

Non-viral delivery methods for treating LUT pain

On account of the clinical observation that opiate drugs are effective in suppressing painful symptoms in patients with IC/PBS,[3] and that opioids were able to block pain signaling in animal models,[95,98,109] it seems reasonable to assume that opiate gene therapy could reduce LUT pain induced by chronic inflammation if the therapy is able to provide enough of the transgene products in the target organ-specific afferent pathways following the local inoculation of the vectors into target organs in the LUT such as the bladder, urethra or pelvic floor. Toward this goal, non-viral vectors using the gene gun technology to transfer either the pro-opiomelanocortin[127] or preproenkephalin (PPE)[128] expression cassette plasmid DNAs linked to gold particles into the bladder of rats where the bladder is exposed by a low midline incision were employed in cystitis models. Cystometric measurements in rats receiving pro-opiomelanocortin gold particles[127] showed an increased ICI compared with control animals following the infusion of 0.3% acetic acid irritant into the bladder at 3 days after the introduction of three gold particle-DNA bullets into three sites within the bladder wall (Figure 3a), which correlated with β-endorphin expression within the bladder. A lesser effect was seen at 7 days, which disappeared by 2 weeks, attesting to the rather short-term nature of using non-viral methodologies. Moreover, the specificity of the response could be reversed by treatment of these animals with opioid receptor antagonist naloxone hydrochloride. In an attempt to block δ-rather than μ-opioid receptor pain signaling, PPE plasmid DNA linked to gold particles were employed in a similar cystitis model except that 15 μM capsaicin was used as a bladder irritant instead of acetic acid.[128] As seen earlier, opioid gene expression at 4–7 days postinjection resulted in a lessening of the effects of capsaicin of δ-opioid receptor activation/signaling that again was specifically blocked by naloxone HCI (Figure 3b). The use of non-viral vectors for opioid gene delivery in these two approaches was limited by the short-term nature of transgene expression and moreover by the expression of the opioid gene only within the bladder itself with none of the product being released within the synaptic cleft of the spinal cord.

HSV vector-mediated delivery of enkephalin to inhibit LUT pain

HSV represents an alternative viral vector system that has many biological features that make it attractive for gene delivery to the peripheral nervous system as the virus naturally establishes latency in DRG sensory neurons, in which viral genomes persist for the life of the host in a non-integrated state without altering host cell metabolism. Completely replication-defective genomic viruses can be constructed that retain the ability to establish latency without the threat of viral growth *in vivo* or the potential of reactivation from the latent state. As part of the virus's natural life cycle, it possesses the ability to spread from cells of the periphery such as epithelial or mucosal cells to the sensory nerves that innervate the site of primary infection by retrograde axonal transport, thus these vectors can be applied peripherally. Moreover, by selecting the correct dermatome for direct vector inoculation, one can specifically target the sensory neurons in which one wants to express the therapeutic product. For example, inoculation of the footpad with an HSV vector expressing PPE leads to transgene expression in the lumbar DRG and subsequent transport and release of enkephalin from both central (spinal cord) and peripheral (foot) processes of sensory bipolar neurons. Such an approach has been used to treat forms of peripheral nerve pain including formalin-induced pain,[140] pain associated with bone cancer[141] and neuropathic pain due to spinal nerve ligation.[142] In this last example, the effective dose (ED50) of morphine was reduced 10-fold in animals treated with the HSV vector.[142]

On account of the previous experience using HSV vectors to enable efficient gene transfer in the bladder injection model and the ability of the HSV vectors expressing PPE to block sensory nerve pain in other peripheral models, we employed the identical vectors in a model of capsaicin-induced LUT visceral pain. In this study,[137] animals received injection of enkephalin-expressing vector (HSV-PPE) or a control vector expressing the β-galactosidase reporter gene (HSV-lacZ) and cystometry was performed 7–14 days post-transduction before and following infusion of 15 μM capsaicin. Similar to previous non-viral vector studies, we observed a lessening of capsaicin-induced pain response in the HSV-PPE-injected rats compared with the control vector (HSV-lacZ)-injected animals (Figure 3c) that was once again antagonized by naloxone HCI. It is interesting to note that behavioral studies showed a decrease in motionless freezing events but no effect on lower abdominal licking events as we previously showed that freezing and licking events are predominantly correlated with bladder and urethral pain, respectively.[143] Both viral genomes and transgene expression were found in both bladder smooth muscle tissue and in L6 and S1 small-medium DRG neurons, mostly corresponding to C-fiber afferent neurons, which correlated with the observed changes in function and behavior.

Summary

Considerable progress has been made in better defining IC/PBS and the components involved in establishing the complex series of symptoms/behaviors associated with this disorder. With the development of new and improved drug therapies for inflammatory and neuropathic pain, the number of patients who receive some relief of their IC/PBS symptoms is increasing. However, a significant number of patients either briefly respond, display numerous unwanted side effects or fail to respond even to the new drug treatments. Gene therapy, using either non-viral or viral vectors represents a new and potentially promising way to deliver anti-nociceptive products directly to the bladder or more importantly the bladder afferents involved in pain signaling, with the viral vector system holding more promise than non-viral methodologies. In particular, HSV vectors represent the ideal delivery vehicle based on their effective transduction of the DRG afferents as a part of that viruses' natural biology. Replication-defective vectors expressing the PPE opioid gene are currently in use in a phase-I human clinical trial for chronic cancer-induced pain, and their success in such patient trials will have a direct impact on their future use to treat cystitis pain.

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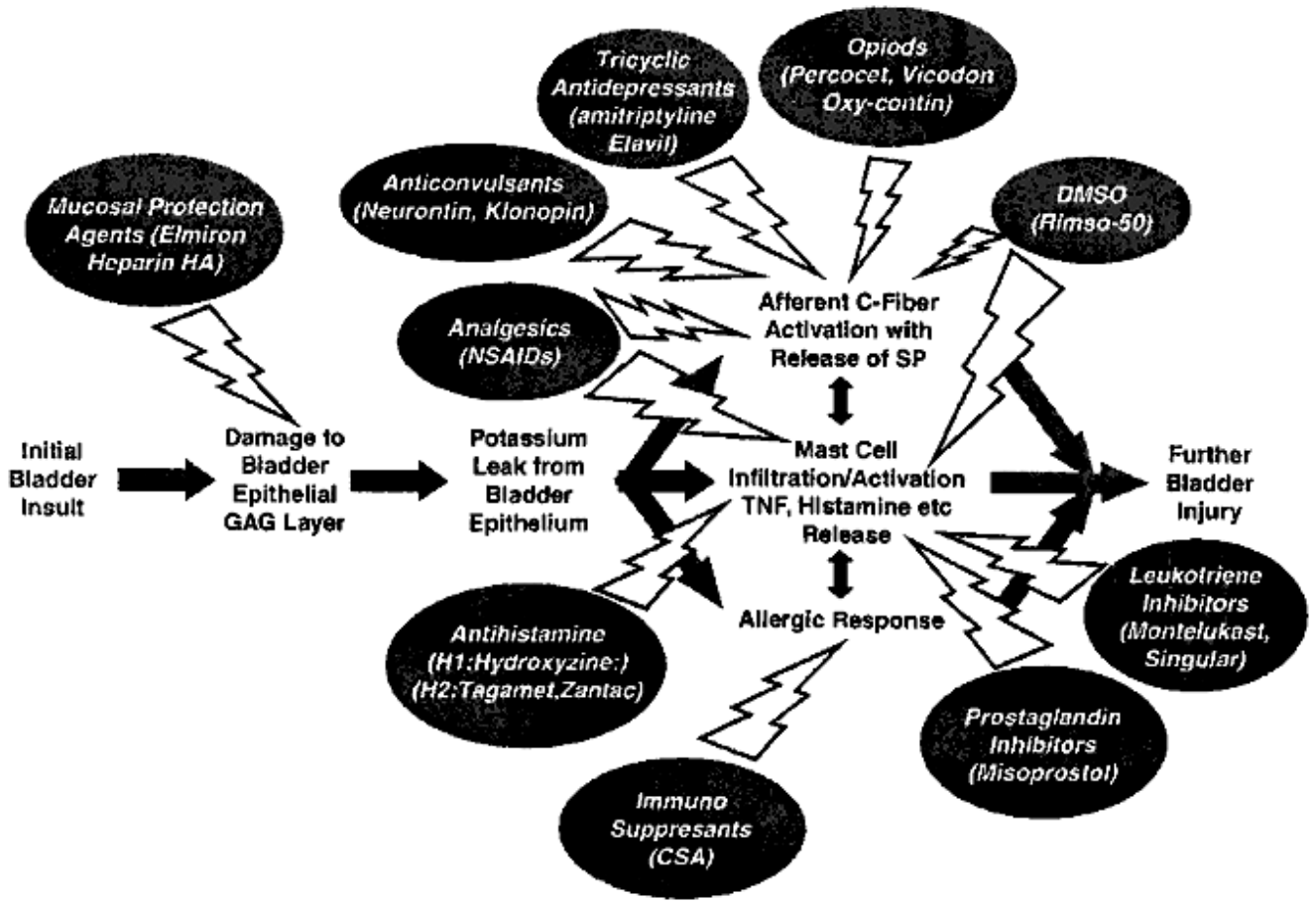
Figure 1.
The proposed pathogonesis of IC/PBS and standard therapies for IC/PBS. An initial insult to the bladder results in the damage of the bladder epithelial layer that, for example, allows potassium to leak in and to prompt a cascade of events, each contributing to tissue inflammation and sensory fiber activation thereby inducing pain sensation in the lower urinary tract (LUT). Various standard drug treatments that have been employed for IC/PBS are depicted showing the level(s) at which these factors are involved in blocking the inflammatory or neurogenic processes that lead to IC/PBS pain. IC/PBS, interstitial cystitis/painful bladder syndrome.
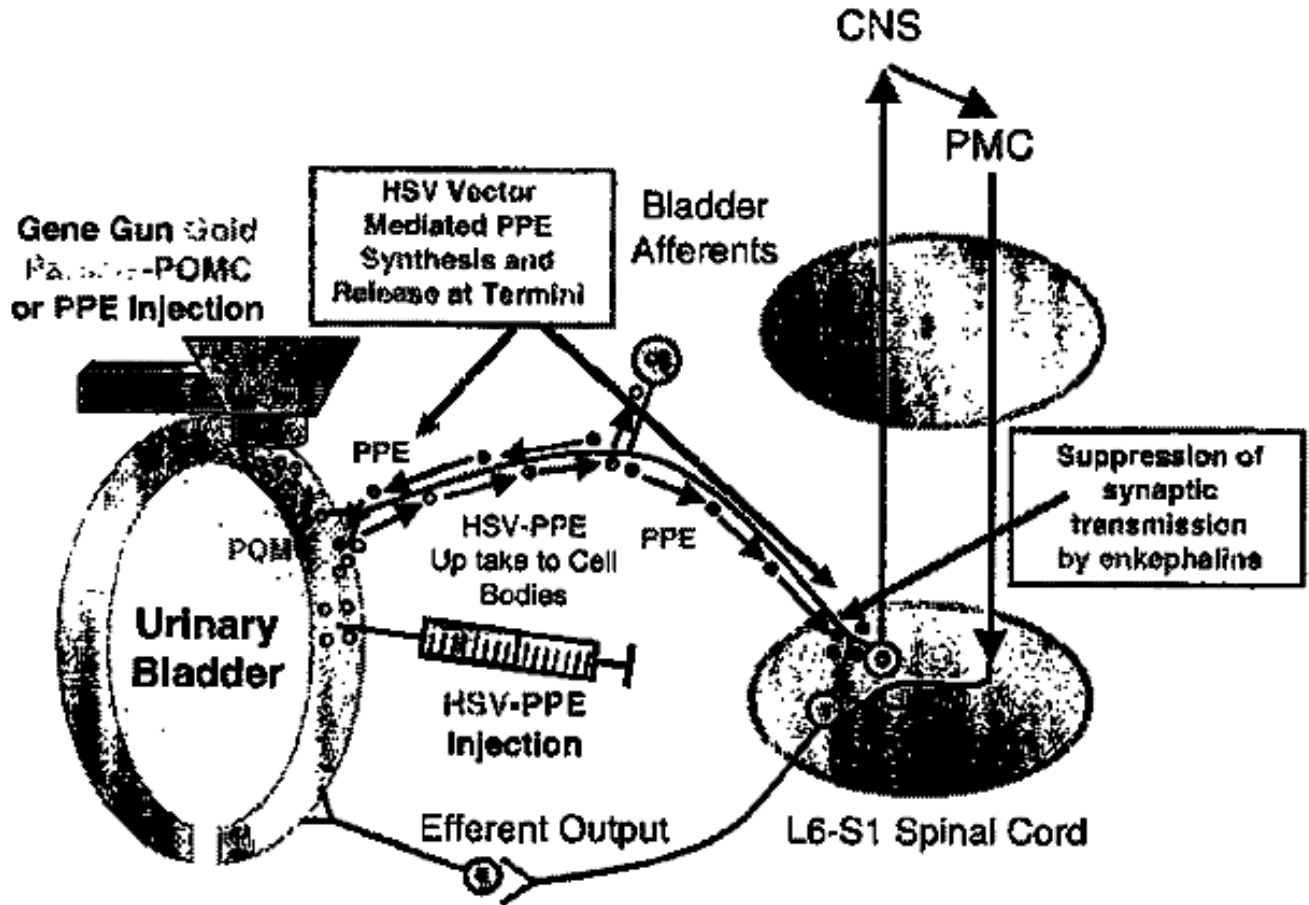
Figure 2.
Schematic diagram of gene therapy strategies for IC/PBS. Gold particles coated with POMC or PPE DNA were used to bombard the bladder wall. These plasmid DNAs encode the peptides (POMC and PPE) that can only locally block pain by suppressing further neuropeptide release. HSV vectors expressing either the PPE gene (HSV-PPE) or the lacZ control (HSV-lacZ) are injected into bladder wall where viral genomes can encode enkephalins locally and virus can be transported to bladder afferent pathways. In contrast to using gold particles with the gene gun technology, HSV-PPE vector genomes present within L6-S1 DRG bladder afferents synthesize and release enkephalins within the dorsal horn of spinal cord, and binding of met- and leu-enk to opioid receptors present on postsynaptic second-order spinal tract neurons and presynaptic bladder afferents may allow better suppression of synaptic transmission of the bladder pain responses. DRG, dorsal root ganglia; HSV, herpes simplex virus; IC/PBS, interstitial cystitis/painful bladder syndrome; POMC, pro-opiomelanocortin; PPE, preproenkephalin.
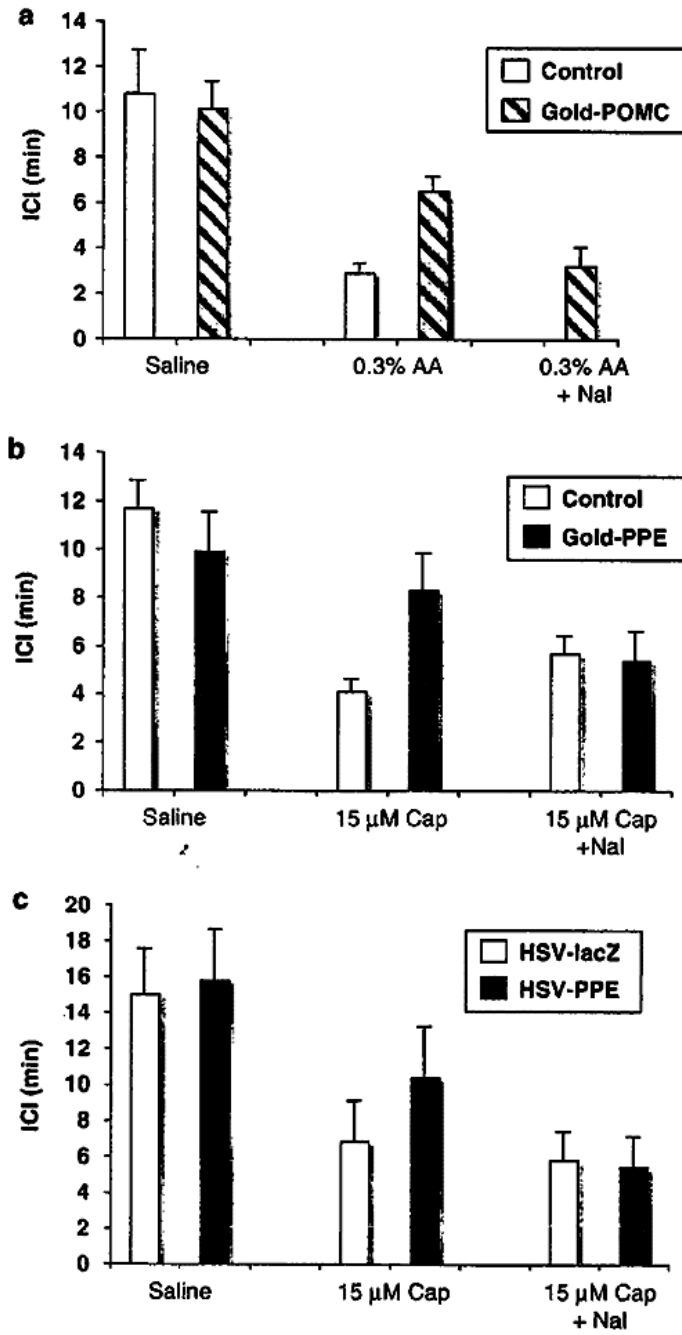
Figure 3.
Effects of non-viral and viral vector-mediated opioid gene expression on bladder hyperactivity induced by nociceptive stimuli in rats. Gene gun-mediated delivery of gold particles linked either to the (**a**) POMC or (**b**) PPE gene expression cassettes was used to deliver three particles to three different sites within the bladder wall of rats with a lower midline incision to expose the bladder. In similar studies, (**c**) HSV-PPE or HSV-lacZ control vector were injected into the rat bladder wall Intercontraction intervals (ICIs) during cystometry were decreased by intravesical application of acetic acid (AA) or capsaicin (Cap). However, gene gun (GOLD-PPE or GOLD-POMC) or HSV-PPE-treated animals exhibited a smaller reduction in ICI

compared with controls, which was reversed by naloxon (Nal). HSV, herpes simplex virus; POMC, pro-opiomelanocortin; PPE, preproenkephalin.