Published in final edited form as:

J Urol. 2010 February ; 183(2): 772–779. doi:10.1016/j.juro.2009.09.077.

Urinary Bladder Dysfunction and Altered Somatic Sensitivity in Pituitary Adenylate Cyclase Activating Polypeptide Knockout (PACAP^{-/-}) Mice

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Abstract

Purpose—Pituitary adenylate cyclase activating polypeptide (PACAP) and receptors are expressed in micturition pathways. Studies demonstrated roles for PACAP in detrusor smooth muscle contraction, facilitating ATP release from urothelium and PACAP antagonism reduced cyclophosphamide-induced bladder hyperreflexia.

Materials and Methods—PACAP contributions to micturition and somatic sensation were 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.

Results—PACAP^{-/-} mice exhibit increased bladder mass with fewer but larger urine spots. In PACAP^{-/-} mice, the lamina propria and detrusor smooth muscle are significantly thicker whereas the urothelium is unchanged. PACAP^{-/-} mice exhibit increased bladder capacity, void volume (VV) and longer intercontraction interval (ICI) with significantly increased detrusor contraction duration and large residual volume. 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.

Conclusions—PACAP gene disruption contributes to changes in bladder morphology, bladder function and somatic and visceral hypoalgesia.

Keywords

cystometry; pelvic sensitivity; hindpaw sensitivity; nociceptive reflexes

Introduction

The neuropeptide, pituitary adenylate cyclase activating polypeptide (PACAP), is involved in lower urinary tract (LUT) function^{1–3}. PACAP is a member of the vasoactive intestinal polypeptide (VIP), secretin, glucagon family of hormones and exhibits ~68% homology to

VIP⁴. PACAP can act on three types of G protein-coupled receptors including PAC₁, VPAC₁ and VPAC₂ receptors⁴.

Dense PACAP-immunoreactive (IR) sensory fibers are present in bladder including suburothelial plexus^{2, 5}. PACAP/receptors expression in dorsal root ganglia (DRG) and afferent projections in the spinal cord are upregulated with cyclophosphamide (CYP)-induced bladder inflammation¹. We have demonstrated specific PACAP receptor expression in bladder smooth muscle as well as in urothelium, lumbosacral DRG and spinal cord suggesting that PACAP and PAC₁ receptor signaling participate at multiple levels in the LUT². Functionally, PACAP peptides increase contractility in rat and mouse detrusor smooth muscle^{2, 3}. Urinary bladder hyperreflexia induced by CYP is reduced by a PAC₁ antagonist, PACAP6-38². PACAP application evoked ATP release from rat cultured urothelium that is blocked by the PAC₁ receptor selective antagonist, M65⁶. In addition to roles for PACAP/receptor signaling in LUT function, differential effects of PACAP have been demonstrated in peripheral nociception¹¹

This study determined the effects of PACAP gene disruption in mice (PACAP^{-/-}, PACAP^{+/-} and wildtype (WT) littermates) on urinary bladder function, somatic sensitivity, urinary bladder morphology and somatic (pelvic, hindpaw) sensitivity. These studies demonstrate that PACAP gene disruption contributes to bladder morphology changes, bladder function changes and somatic and visceral hypoalgesia.

Methods

Animals

The PACAP^{-/-} mouse model was generated in a pathogen-free facility at UCLA and backcrossed for at least eight generations with C57Bl/6^{7, 8}. PACAP^{-/-}, littermate PACAP^{+/-} and WT mice^{7, 8} were bred locally at The University of Vermont (UVM) and lack of the PACAP gene was confirmed by PCR genotyping⁸. The UVM IACUC approved animal use. Animal care was under the supervision of the UVM Office of Animal Care Management following AAALAC and NIH guidelines.

Urination Patterns

PACAP^{-/-}, PACAP^{+/-} and WT mice of either gender were placed individually in standard cages for 1 h with the bedding replaced with Whatman Grade 3 filter paper. Food and water were provided ad libitum. Urine spots were photographed under UV light⁹, area (cm²) of spots determined and large (0.2 – 10 cm²)¹⁰ and small (< 0.2 cm²)¹⁰ spots counted. Mice were placed into cages and data analyzed by an individual blinded to mouse strain; groups were decoded after data analysis.

Mechanical sensitivity testing

Due to divergent roles for PACAP in somatic sensation¹¹ and PACAP expression in sensory neurons^{1, 14}, tactile allodynia was tested using calibrated von Frey hairs with forces of 0.04, 0.16, 0.4, 1, and 4 g to the abdomen^{9, 12} and hindpaw^{9, 12} among genotypes. Mice were tested in individual Plexiglas chambers with a stainless steel wire grid floor. Mice were acclimated to the chambers for 2 h. Due to limited supply, the same mice were evaluated for somatic sensitivity 1–2 weeks prior to intravesical catheter implantation and 1–2 weeks prior to both catheter implant and somatic testing for determination of urination patterns. The von Frey hairs were applied in an up-down method for 1–3 sec with an interstimulus interval of 15 sec. Pelvic region stimulation was confined to the lower abdominal area overlying the urinary bladder. Testing of the plantar region of the hindpaw and lower abdominal area was performed by perpendicular application of the von Frey hairs until the hair bent slightly. We

used established criteria for determining positive responses to hindpaw or pelvic region stimulation^{9, 12}. Pelvic and hindpaw testing was performed in the same mice but somatic regions were evaluated in randomly assigned mice at different times. All somatic testing was performed in a blinded manner; groups were decoded after data analysis.

Bladder Catheter Implant

A lower midline abdominal incision was performed under general anesthesia (isoflurane 2.5–3.5%) and PE-10 tubing with the end flared by heat was inserted into the dome of the bladder and secured in place with a 6-0 nylon purse string suture⁹. The distal end of the tubing was sealed, tunneled subcutaneously and externalized. Animals were maintained for 72 h to ensure complete recovery. Postoperative analgesics were given for 48 h.

Bladder Function: Cystometry

Animals were placed conscious and unrestrained in recording cages with a balance and pan for urine collection and measurement placed below^{2, 9}. Intravesical pressure changes were recorded using a Small Animal Cystometry System (Med Associates, Inc.). Saline at room temperature was infused (25 μ l/min) to elicit repetitive bladder contractions. At least six reproducible micturition cycles were recorded after an initial stabilization period (25 – 30 minutes). Bladder capacity (BC) was measured as the amount of saline infused into the bladder at the time when micturition commenced^{10, 13}. Voided saline was collected to determine voided volume (VV). After collection, the infusion was stopped and residual volume (RV) was determined by withdrawing from the bladder catheter with a syringe. Intercontraction interval (ICI), maximal voiding pressure, pressure threshold for voiding and baseline resting pressure were measured¹⁴. Detrusor contraction duration (DCD) was determined by the width of the micturition contraction at its base. Non-voiding bladder contractions (NVC; defined as greater than 5 cm H₂O from baseline pressure without a release of fluid from the urethra) during the filling phase were quantified. Acetic acid (AA; 0.5%) was infused into the urinary bladder in some littermates after saline infusions to evaluate nociceptive bladder responses. Mice were removed from study when adverse events occurred that included: $\geq 20\%$ reduction in body weight post surgery, a significant postoperative event, lethargy, pain or distress not relieved by our IACUC-approved regimen of post-operative analgesics or hematuria in control rodents⁹. No mice were excluded according to established criteria; however, three mice were removed because they chewed the exteriorized tubing. Behavioral movements (e.g., grooming, defecation) rendered bladder pressure recordings unusable during these events. Experiments were conducted at similar times of the day⁹. Mice were euthanized (isoflurane (4%) and thoracotomy) at the conclusion of study and urinary bladder dissected and weighed.

Urinary Bladder Histological Analysis

Histological analysis of the urinary bladder sections (15 μ m), from intravesical saline studies only, was performed using haematoxylin and eosin (H&E) staining. Digital images of H&E-stained sections were captured using a MagnaFire camera and an Olympus microscope using a 20x objective. Measurements of bladder layer thickness were performed using MetaMorph image analysis software (version 4.5r4; Universal Imaging, Downingtown, PA). Measurements consisted of urothelium, lamina propria and muscular layer thickness; measurements on sections were performed in triplicate and averaged. We matched the urinary bladder region being compared (i.e., bladder dome or trigone) among littermates. Parameters were measured as distances along a line drawn normal to the long axis of the specimen from the luminal edge to the serosal edge of urothelium, lamina propria and detrusor¹³.

Statistical Analyses

All values represent mean $\pm$ S.E.M. Data were compared using analysis of variance using SAS® statistical software. When F ratios exceed the critical value ($p \leq 0.05$), the Newman-Keul's post-hoc test was used to compare means.

Results

General properties of WT, PACAP^{+/-} and PACAP^{-/-} mice

There were no differences in body mass among WT, PACAP^{+/-} and PACAP^{-/-} littermates but urinary bladder/body weight ratios were significantly increased in PACAP^{-/-} mice due to increased bladder mass (Fig. 1). Fluid intake measured over a 24 h period was similar among littermates (Table 1). Urine spots from PACAP^{-/-} mice, as quantified on filter paper over a 1 h period, were significantly ($p \leq 0.01$) fewer in number but greater in area compared to those made by WT animals ($8.2 \pm 3.5 \text{ cm}^2$ vs. $3.3 \pm 2.5 \text{ cm}^2$, respectively; Table 1). PACAP^{+/-} mice also produced significantly ($p \leq 0.01$) fewer urine spots compared to WT. Both large ($0.2 - 10 \text{ cm}^2$) and small ($< 0.2 \text{ cm}^2$) diameter urine spots were observed among all genotypes but differences in numbers of only large diameter urine spots (likely representing actual voiding events) were observed in PACAP^{-/-} and PACAP^{+/-} mice. No differences between PACAP^{-/-} and PACAP^{+/-} mice were observed (Table 1).

Urinary bladder histology—Histological analyses of urinary bladder at the bladder dome and trigone regions demonstrated significantly ($p \leq 0.01$) increased thickness of lamina propria and detrusor in PACAP^{-/-} mice (Fig. 1A–C). There were no differences in bladder layer thickness between bladder dome and trigone regions. No differences in urothelial thickness were observed between WT and PACAP^{-/-} mice.

Bladder function—In response to continuous intravesical instillation of saline, PACAP^{-/-} mice exhibited significantly ($p \leq 0.01$) larger bladder capacity (BC; Figs. 2, 4B), void volumes (VV; Figs. 2, 4B) and longer intercontraction intervals (ICI)(Fig. 4A) compared to WT and PACAP^{+/-} mice (Figs. 2–4). RV in the PACAP^{-/-} and PACAP^{+/-} mice was also significantly ($p \leq 0.01$) greater compared to WT (Fig. 4D). The DCD associated with voiding was significantly lengthened (1.9– to 4.1-fold longer) in PACAP^{-/-} mice compared to WT and PACAP^{+/-} mice (Figs. 2, 3, 4C). In WT mice, AA (0.5%) instillation into the urinary bladder decreased the ICI and VV (Fig. 3–4A, B). Intravesical AA instillation in PACAP^{-/-} or PACAP^{+/-} mice produced no significant change in VV or ICI (Fig. 2, 4A, B). We observed NVCs during bladder filling with intravesical saline or AA instillation infrequently in any littermate and these were not considered further. No significant differences in maximum voiding, micturition threshold or baseline filling pressure with intravesical saline instillation were observed among WT, PACAP^{-/-} and PACAP^{+/-} mice (Table 1).

Somatic sensitivity—We evaluated baseline mechanical somatic sensitivity using a calibrated series of von Frey hairs on the plantar surface of the hindpaw and pelvic region overlying the urinary bladder in WT, PACAP^{-/-} and PACAP^{+/-} 9, 12. Somatic sensitivity in the pelvic region was significantly ($p \leq 0.01$) decreased in PACAP^{-/-} mice with von Frey hairs among filament forces tested (0.1 – 4 g) compared to WT and PACAP^{+/-} mice (Fig. 5A). Pelvic sensitivity was also significantly ($p \leq 0.01$) reduced in PACAP^{+/-} mice although the magnitude of change was less than that observed in PACAP^{-/-} mice (Fig. 5A). Somatic sensitivity was significantly ($p \leq 0.01$) reduced in the hindpaw in both PACAP^{-/-} and PACAP^{+/-} compared to WT (Fig. 5B). PACAP^{-/-} mice exhibited significantly ($p \leq 0.01$) less hindpaw sensitivity than PACAP^{+/-} mice (Fig 5B).

Discussion

This study demonstrates several novel roles of pituitary adenylate cyclase activating polypeptide (PACAP) in micturition reflexes and somatic and visceral sensation. PACAP^{-/-} mice produce fewer but larger urine spots. The urinary bladders of PACAP^{-/-} mice have increased mass, correlating with significantly thicker lamina propria and detrusor smooth muscle layers, whereas the urothelium appears unchanged. PACAP^{-/-} mice exhibit greater BC, VV and longer ICI. In addition, PACAP^{-/-} mice have significantly increased DCD and larger RV. WT mice respond to AA (0.5%) intravesical instillation with a reduction in VV and a decreased ICI whereas PACAP^{+/-} and PACAP^{-/-} mice do not respond. PACAP^{-/-} mice are less responsive (i.e., hypoalgesic) to somatic stimulation of the hindpaw or pelvic region with calibrated von Frey filaments. PACAP^{+/-} also exhibit bladder dysfunction and somatic and visceral sensory abnormalities but to a lesser degree. These studies suggest that PACAP gene disruption contributes to changes in bladder morphology, bladder function and somatic and visceral hypoalgesia.

PACAP and PAC₁ signaling are prominent regulators of bladder physiology through actions on urothelium, detrusor smooth muscle and/or bladder afferent nerves ¹⁻³. PACAP and VIP peptides regulate smooth muscle function in a tissue- and species-specific manner ^{15, 16}. We have demonstrated direct effects of PACAP on bladder smooth muscle contractility ². PACAP increased bladder smooth muscle tone and potentiated electric field stimulation-induced contractions ² that were tetrodotoxin-insensitive suggesting direct smooth muscle effects ². PACAP evoked ATP release from rat urothelial cell cultures that was significantly blocked by the PAC₁ receptor selective antagonist, M65 ⁶. PACAP/receptor expression is regulated by CYP-induced cystitis¹ and administration of the PAC₁ receptor antagonist, PACAP6-38, significantly reduced CYP-induced bladder hyperreflexia ².

Mice with PACAP gene deletion (PACAP^{-/-}) or disruption (PACAP^{+/-}) exhibited bladder dysfunction consistent with outlet obstruction. Conscious cystometry in PACAP^{-/-} mice demonstrated larger VV, longer ICI. PACAP^{-/-} mice do not achieve complete bladder emptying, have large RV and exhibit prolonged bladder emptying (increased DCD). One interpretation of these data is that PACAP gene deletion results in incomplete compensation by the detrusor muscle to overcome outlet obstruction. Despite evidence of detrusor smooth muscle hypertrophy, increases in peak micturition pressure in PACAP^{-/-} mice were not observed suggestive of incomplete compensation. Given previous studies demonstrating PACAP stimulatory effects on detrusor function ^{2, 3}, PACAP^{-/-} mice may have reduced detrusor contractility. Among genotypes, more detailed analyses of urethral outlet morphology and detrusor contractility *in vitro* may help clarify some of these issues.

The current studies in PACAP^{-/-} mice revealed some distinctions in urinary bladder structure and function compared with the recent work in VIP^{-/-} mice ⁹. Although VIP^{-/-} and PACAP^{-/-} mice both exhibit increased detrusor smooth muscle thickness, increased VV and increased ICI, VIP^{-/-} mice, in contrast to PACAP^{-/-} animals, have low RV, no differences in baseline hindpaw sensitivity and respond as expected to intravesical instillation of AA ⁹. Further, only PACAP^{-/-} mice exhibit cystometric signs consistent with outlet obstruction (i.e., large RV and increased DCD). Previous studies have demonstrated that PACAP is found in capsaicin-sensitive DRG populations ¹⁷, co-expressed with TRPV-1 immunoreactivity in bladder nerves of the suburothelial plexus (Vizzard et al., unpublished) and released from the spinal cord in response to capsaicin ¹⁸. Given that PACAP^{-/-} and PACAP^{+/-} mice exhibit reduced somatic (hindpaw, pelvic) sensitivity in response to calibrated filament probing and do not respond to intravesical instillation of AA (0.5%), the changes in sensory function may also be a significant contributor to the differences in bladder/urethral defects between the PACAP and VIP null animals. The sensory dysfunction

demonstrated with somatic testing techniques may also extend to sensory dysfunction of mechanosensitive bladder afferents. PACAP^{-/-} mice showed increased bladder capacity and increased VV and RV with a tendency of increased pressure threshold (Table 1) suggesting that bladder mechanosensitive afferents may be less sensitive to bladder distention after PACAP depletion. This is supported by reduced somatic mechanosensitivity in the pelvic area. Further, increases in RV and DCD, apart from sensory dysfunction, could indicate detrusor dysfunction. Thus, sensory and detrusor dysfunction in PACAP null mice may both contribute to the observed bladder dysfunction.

Partial urethral obstruction, and CYP-induced cystitis are associated with increases in bladder mass and altered expression of urinary bladder NGF^{19, 20}. Studies of intestinal²¹ and bladder morphology⁹ in VIP^{-/-} mice demonstrated significant increases in smooth muscle thickness. Given the ability of VIP to inhibit airway smooth muscle proliferation²², a regulatory role for VIP in smooth muscle proliferation has been suggested²¹. The present results suggest a similar regulatory role for PACAP in detrusor smooth muscle. The increased bladder mass of the PACAP^{-/-} mice was consistent with cystometry and urine spot data that demonstrated increased VV and fewer but significantly larger urine spots. PACAP^{-/-} mice also exhibit increased lamina propria thickness as previously shown in animal models of outlet obstruction²³. Reasons underlying increased lamina propria thickness in PACAP^{-/-} mice may include tissue remodeling of the afferent and efferent nerves²⁴ present in the suburothelial plexus and detrusor²⁵, myofibroblasts and/or vasculature²⁶ as previously described in animal models of outlet obstruction and potentially influenced by changes in urinary bladder NGF content^{24, 27}. Future studies will explore the relationship between urinary bladder neurotrophic factor expression and bladder morphological changes in PACAP^{-/-} mice.

Conclusions

These data suggest that PACAP gene disruption results in (1) broad effects on LUT function that may reflect underlying sensory and bladder dysfunction and (2) novel effects on somatic sensation.

Acknowledgments

The authors gratefully acknowledge the technical expertise and support provided Ms. Susan Malley and Ms. Abbey Dattilio. The authors thank Dr. Bopaiah Cheppudira, Department of Pharmacology, University of Illinois at Chicago, for instruction in somatic testing techniques. The authors thank Dr. Peter Zvara for helpful discussions regarding the manuscript.

Grants

This work was funded by NIH grants DK051369, DK060481, and DK065989. NIH Grant Number P20 RR16435 from the COBRE Program of the National Center also supported the project for Research Resources.

References

1. Vizzard MA. Up-regulation of pituitary adenylate cyclase-activating polypeptide in urinary bladder pathways after chronic cystitis. *J Comp Neurol* 2000;420:335. [PubMed: 10754506]
2. Braas KM, May V, Zvara P, et al. Role for pituitary adenylate cyclase activating polypeptide in cystitis-induced plasticity of micturition reflexes. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R951. [PubMed: 16322346]
3. Herrera GM, Braas KM, May V, et al. PACAP enhances mouse urinary bladder contractility and is upregulated in micturition reflex pathways after cystitis. *Ann N Y Acad Sci* 2006;1070:330. [PubMed: 16888187]

4. Arimura A. Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Regul Pept* 1992;37:287. [PubMed: 1313597]
5. Fahrenkrug J, Hannibal J. Pituitary adenylate cyclase activating polypeptide immunoreactivity in capsaicin-sensitive nerve fibres supplying the rat urinary tract. *Neuroscience* 1998;83:1261. [PubMed: 9502264]
6. Girard BM, Wolf-Johnston A, Braas KM, et al. PACAP-mediated ATP release from rat urothelium and regulation of PACAP/VIP and receptor mRNA in micturition pathways after cyclophosphamide (CYP)-induced cystitis. *J Mol Neurosci* 2008;36:310. [PubMed: 18563302]
7. Colwell CS, Michel S, Itri J, et al. Selective deficits in the circadian light response in mice lacking PACAP. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1194. [PubMed: 15217792]
8. Girard BA, Lelievre V, Braas KM, et al. Noncompensation in peptide/receptor gene expression and distinct behavioral phenotypes in VIP- and PACAP-deficient mice. *J Neurochem* 2006;99:499. [PubMed: 17029602]
9. Studeny S, Cheppudira BP, Meyers S, et al. Urinary bladder function and somatic sensitivity in vasoactive intestinal polypeptide (VIP)-/- mice. *J Mol Neurosci* 2008;36:175. [PubMed: 18561033]
10. Birder LA, Nakamura Y, Kiss S, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nat Neurosci* 2002;5:856. [PubMed: 12161756]
11. Sandor K, Bolcskei K, McDougall JJ, et al. Divergent peripheral effects of pituitary adenylate cyclase-activating polypeptide-38 on nociception in rats and mice. *Pain* 2009;141:143. [PubMed: 19091468]
12. Rudick CN, Chen MC, Mongiu AK, et al. Organ cross talk modulates pelvic pain. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R1191. [PubMed: 17626130]
13. Herrera GM, Pozo MJ, Zvara P, et al. Urinary bladder instability induced by selective suppression of the murine small conductance calcium-activated potassium (SK3) channel. *J Physiol* 2003;551:893. [PubMed: 12813145]
14. Maggi CA, Santicioli P, Meli A. The nonstop transvesical cystometrogram in urethane-anesthetized rats: a simple procedure for quantitative studies on the various phases of urinary bladder voiding cycle. *J Pharmacol Methods* 1986;15:157. [PubMed: 3702468]
15. Mizumoto A, Fujimura M, Ohtawa M, et al. Pituitary adenylate cyclase activating polypeptide stimulates gallbladder motility in conscious dogs. *Regul Pept* 1992;42:39. [PubMed: 1362001]
16. Seebeck J, Lowe M, Kruse ML, et al. The vasorelaxant effect of pituitary adenylate cyclase activating polypeptide and vasoactive intestinal polypeptide in isolated rat basilar arteries is partially mediated by activation of nitrergic neurons. *Regul Pept* 2002;107:115. [PubMed: 12137973]
17. Zhang YZ, Hannibal J, Zhao Q, et al. Pituitary adenylate cyclase activating peptide expression in the rat dorsal root ganglia: up-regulation after peripheral nerve injury. *Neuroscience* 1996;74:1099. [PubMed: 8895878]
18. Zhang Y, Malmberg AB, Yaksh TL, et al. Capsaicin-evoked release of pituitary adenylate cyclase activating peptide (PACAP) and calcitonin gene-related peptide (CGRP) from rat spinal cord in vivo. *Regul Pept* 1997;69:83. [PubMed: 9178350]
19. Steers WD, Kolbeck S, Creedon D, et al. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. *Journal of Clinical Investigations* 1991;88:1709.
20. Vizzard MA. Alterations in spinal cord Fos protein expression induced by bladder stimulation following cystitis. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R1027. [PubMed: 10749792]
21. Lelievre V, Favrais G, Abad C, et al. Gastrointestinal dysfunction in mice with a targeted mutation in the gene encoding vasoactive intestinal polypeptide: a model for the study of intestinal ileus and Hirschsprung's disease. *Peptides* 2007;28:1688. [PubMed: 17606312]
22. Maruno K, Absood A, Said SI. VIP inhibits basal and histamine-stimulated proliferation of human airway smooth muscle cells. *Am J Physiol* 1995;268:L1047. [PubMed: 7541947]
23. Pampinella F, Roelofs M, Castellucci E, et al. Proliferation of submesothelial mesenchymal cells during early phase of serosal thickening in the rabbit bladder is accompanied by transient keratin 18 expression. *Exp Cell Res* 1996;223:327. [PubMed: 8601410]

24. Steers WD, Ciambotti J, Etzel B, et al. Alterations in afferent pathways from the urinary bladder of the rat in response to partial urethral obstruction. *Journal of Comparative Neurology* 1991;310:1. [PubMed: 1658087]
25. Andersson KE, Hedlund P. Pharmacologic perspective on the physiology of the lower urinary tract. *Urology* 2002;60:13. [PubMed: 12493344]
26. Matsumoto S, Hanai T, Ohnishi N, et al. Bladder smooth muscle cell phenotypic changes and implication of expression of contractile proteins (especially caldesmon) in rats after partial outlet obstruction. *Int J Urol* 2003;10:339. [PubMed: 12757606]
27. Steers WD, Creedon DJ, Tuttle JB. Immunity to nerve growth factor prevents afferent plasticity following urinary bladder hypertrophy. *Journal of Urology* 1996;155:379. [PubMed: 7490891]

Figure 1.

Histological analyses of urinary bladders 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 (*, $p \leq 0.01$) increased thickness of lamina propria (LP) and detrusor smooth muscle (SM) supported by morphometric analyses (C). Body weights (g) among mice were not different (D) but bladder/body weight (mg/g) ratio was significantly (*, $p \leq 0.01$) increased in PACAP^{-/-} mice due to increased bladder mass (E). U, urothelium. Values represent mean $\pm$ S.E.M. for $n = 7-10$ animals in each group. Calibration bar represents 120 μm in A, B.

Figure 2.

Representative cystometrograms from conscious, unrestrained $PACAP^{-/-}$ mice with continuous intravesical infusion (25 μ l/min) of room temperature saline (A–C) and acetic acid (AA, 0.5%)(D–F). Infused volume (IF, μ l), bladder pressure (BP, cm H_2O) and voided volume (VV, μ l) are shown. $PACAP^{-/-}$ mice exhibit large bladder capacity before micturition (A), long detrusor contraction duration (B) and large VV (C) with incomplete emptying compared to WT (Fig. 3). $PACAP^{-/-}$ mice do not respond to AA compared to WT (Fig. 3).

Figure 3.

Representative cystometrograms from conscious, unrestrained wildtype (WT) mice with continuous intravesical infusion (25 μ l/min) of room temperature saline (A–C) and acetic acid (AA, 0.5%)(D–F). Infused volume (IF, μ l), bladder pressure (BP, cm H₂O) and voided volume (VV, μ l) are shown. WT mice responded to intravesical instillation of AA with an increase in voiding frequency (E) and decreased VV (F).

Figure 4.

Summary figures of the intercontraction interval (ICI, seconds, s; A), bladder capacity with saline or AA (volume, μl; B), detrusor contraction duration (DCD, s; C) and residual volume (RV, μl; D) using conscious cystometry in conscious, unrestrained WT, PACAP^{-/-} and PACAP^{+/-} mice with continuous infusion of saline or AA (0.5%). A. ICI was significantly ($p \leq 0.01$) 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 ($p \leq 0.01$) greater in PACAP^{-/-} and PACAP^{+/-} mice with AA instillation compared to WT. B. Bladder capacity with saline or AA was significantly ($p \leq 0.01$) greater in PACAP^{-/-} and PACAP^{+/-} mice compared to WT. Saline and AA VV were also significantly ($p \leq 0.01$) greater in PACAP^{-/-} and PACAP^{+/-} mice. Significant ($p \leq 0.01$) differences between bladder capacity and VV for saline and AA instillation were apparent in PACAP^{-/-} and PACAP^{+/-} mice. AA instillation in PACAP^{-/-} and PACAP^{+/-} mice did not affect bladder capacity compared to WT mice. C. DCD in PACAP^{-/-} mice was significantly ($p \leq 0.01$) longer compared to WT and PACAP^{+/-} mice. D. RV was significantly ($p \leq 0.01$) increased in PACAP^{+/-} and PACAP^{-/-} mice compared to WT. Values represent mean $\pm$ S.E.M. for $n = 7-10$ animals in each group.

Figure 5.

Baseline pelvic (A) and hindpaw (B) somatic sensitivity testing in WT, PACAP^{-/-} and PACAP^{+/-} mice. A. Pelvic region testing with calibrated von Frey hairs applied in an up-down method for 1–3 sec with an interstimulus interval of 15 sec. For pelvic region stimulation, stimulation was confined to the lower abdominal area overlying the urinary bladder. The following behaviors were considered positive responses to pelvic region stimulation: sharp retraction of the abdomen, jumping, or immediate licking or scratching of the pelvic area^{9, 10}. PACAP^{-/-} mice exhibited significantly (*, $p \leq 0.01$) decreased pelvic response frequency with all von Frey hairs (0.1 – 4 g) tested compared to WT (*) or PACAP^{+/-} (#) mice. PACAP^{+/-} mice also exhibited significantly (*, $p \leq 0.01$) decreased pelvic response frequency with some filaments tested compared to WT. B. Hindpaw sensitivity testing with calibrated von Frey hairs in WT, PACAP^{-/-} and PACAP^{+/-} mice. PACAP^{-/-} mice exhibited significantly (*, $p \leq 0.01$) decreased hindpaw response frequency with some von Frey hairs tested compared to WT (*) or PACAP^{+/-} (#) mice. PACAP^{+/-} mice also exhibited significantly (*, $p \leq 0.01$) decreased hindpaw response frequency with some filaments tested compared to WT. The following behaviors were considered positive responses to hindpaw stimulation: sharp withdrawal of the paw or licking of the tested hindpaw^{9, 10}. Values represent mean $\pm$ S.E.M. for $n = 8$ –10 animals in each group. All somatic testing was performed in a blinded manner.

Table 1
Comparison of urine spots and fluid intake among wildtype (WT), PACAP^{-/-} and PACAP^{+/-} littermates

Comparison of water intake (ml/24 hour, h) and urine spots (number/h) on filter paper and urinary bladder pressure (cm H₂O) among PACAP^{-/-}, PACAP^{+/-} and WT mice during continuous intravesical instillation of saline. Values represent mean $\pm$ S.E.M. for $n = 10$ animals in each group.

	WT ($n=10$)	PACAP^{-/-} ($n=10$)	PACAP^{+/-} ($n=10$)
Fluid intake (ml/24 h)	7.2 $\pm$ 0.8	7.4 $\pm$ 1.0	7.0 $\pm$ 0.5
Urine spots (number/h)	12.4 $\pm$ 2.5	3.2 $\pm$ 1.2*	6.2 $\pm$ 1.5*

* , $p \leq 0.01$ compared to WT.

Table 2
Comparison of bladder pressure with intravesical saline instillation among wildtype (WT), PACAP^{-/-} and PACAP^{+/-} littermates

	<u>WT (n=10)</u>	<u>PACAP^{-/-} (n=10)</u>	<u>PACAP^{+/-} (n=10)</u>
Max voiding pressure (cm H ₂ O)	67.2 ± 1.5	68.5 ± 1.0	66.5 ± 1.5
Baseline pressure (cm H ₂ O)	12.4 ± 2.5	14.2 ± 2.2	13.2 ± 1.5
Threshold pressure (cm H ₂ O)	14.5 ± 2.5	21.5 ± 4.5	16.8 ± 2.0