PACAP/PAC1 Regulation in Cystitis Rats: Induction of Bladder Inflammation Cascade Leading to Bladder Dysfunction

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Hypothesis / aims of study

Interstitial cystitis/bladder pain syndrome (IC/BPS) presents itself as a perplexing chronic inflammatory disorder of the bladder, characterized by bladder pain, nocturia, urgency, sterile urine, and frequent urination(1,2).

Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide, is involved in regulating lower urinary tract (LUT) functions(3–5).

The hypothesis of this study is that Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and its receptor PAC1 play a crucial role in the pathophysiology of Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS), a chronic and painful bladder disorder. By investigating the PACAP/PAC1 pathway in both human bladder tissue and a rat model of cystitis, the study aims to:

- Understand the involvement of PACAP/PAC1 in bladder inflammation and dysfunction.
- Determine whether modulating this pathway with the PACAP6-38 antagonist can alleviate symptoms of bladder inflammation and dysfunction.
- Identify potential therapeutic targets for the treatment of IC/BPS through PACAP/PAC1 pathway inhibition.

Study design, materials and methods

Study Design:
This study used both clinical and experimental approaches. Bladder tissue samples from IC/BPS patients were analyzed for gene expression, and a rat model of chronic cystitis was induced using cyclophosphamide. (Figure 1A)

Materials and Methods:

Human Samples: Bladder biopsies were collected from IC/BPS patients, and RNA sequencing was performed to analyze inflammation-related gene expression. Controls were from adjacent normal tissue.

Animal Model: Female rats were injected with CYP (25 mg/kg) every third day to induce cystitis. PACAP6-38 was administered intravesically (300 nM) or intrathecally (50 nM). Rats were divided into six groups for comparison.

Bladder Function Assessment:

Von Frey Test: Measured bladder sensitivity using von Frey filaments.

Urination Patterns: Urine spots were analyzed using UV light and ImageJ.

Filling Cystometry: Urodynamic testing assessed maximum bladder capacity (MBC) and bladder compliance (BC).

Immunohistochemistry: Inflammatory markers (IL-6, IL-8, TNF-α, VEGF, NGF) and PACAP expression were analyzed in bladder tissues.

Transcriptomic Analysis: DESeq2 was used to identify differentially expressed genes (DEGs), with GO and KEGGanalyses highlighting pathways related to inflammation and sensory function.

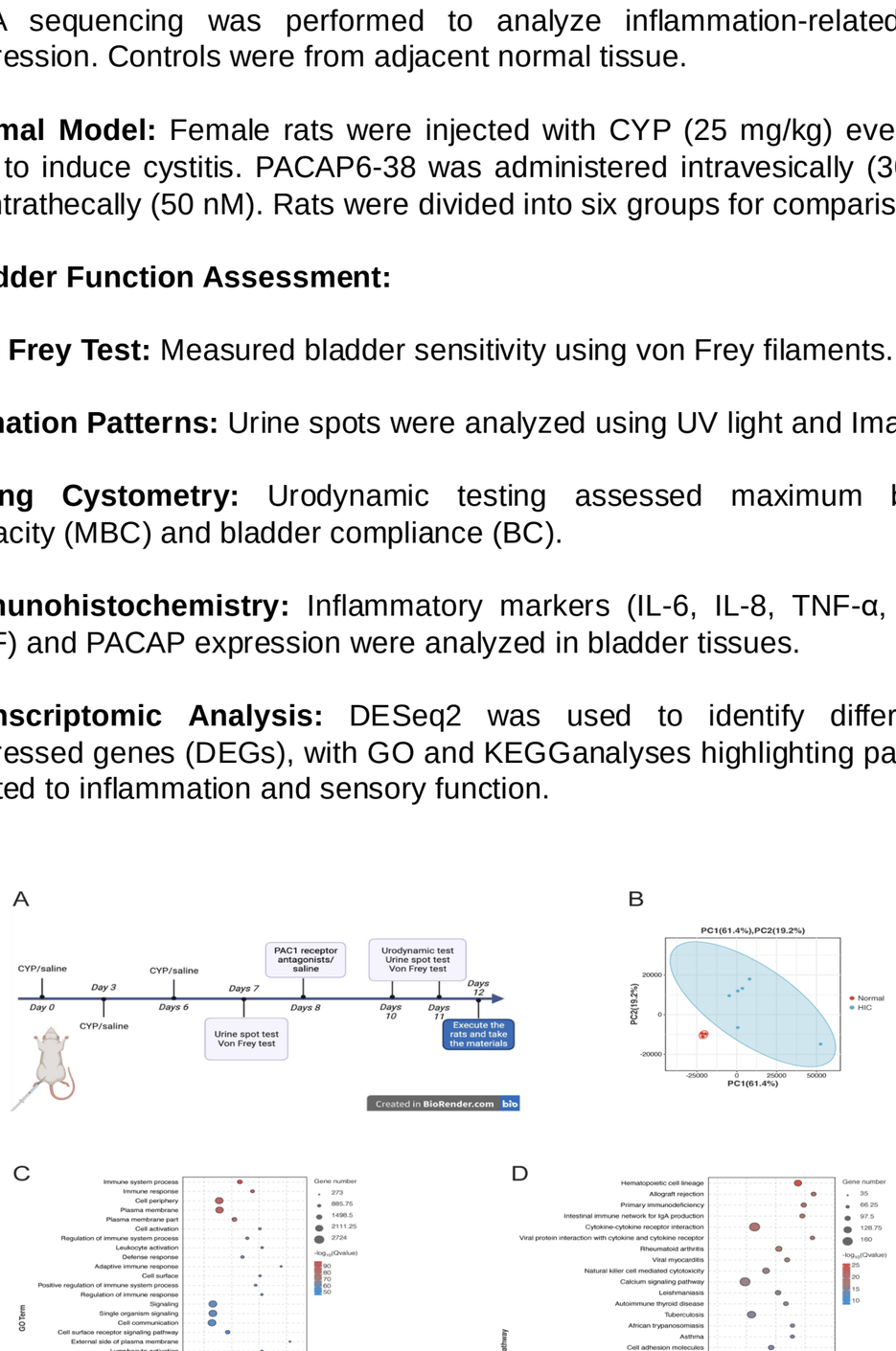

Figure 1

Results and interpretation

PACAP Expression in IC/BPS:
Transcriptomic analysis showed significant upregulation of PACAP in IC/BPS patients, highlighting it as a key biomarker and potential therapeutic target. (Figure 1F–G) Immunohistochemistry confirmed elevated PACAP levels in bladder tissues. (Figure 2A)

Bladder Function and Pain in Rats:
Urination Patterns: PACAP6-38 treatment increased urine spot frequency in CYP-PAC rats, restoring bladder function without affecting urine volume.
Urodynamics: PACAP6-38 improved bladder capacity and compliance in treated rats .(Figure 2B,C)
Inflammatory Markers:
PACAP6-38 reduced elevated cytokines (IL-6, IL-8, TNF-α, VEGF) and NGF levels in cystitis rats, correlating with improved bladder function and pain reduction .(Figure 2D-K)
Pain Reduction: PACAP6-38 reduced pain sensitivity in CYP-treated rats, as shown by the von Frey test. (Figure 2M,N)

Histopathology:
PACAP6-38 reduced bladder edema and improved tissue structure in cystitis rats, supporting its anti-inflammatory effects. (Figure 1 L)

Transcriptomic Pathway Analysis:
GO, KEGG revealed immune dysregulation as a major factor in IC/BPS, with downregulation of energy metabolism pathways, indicating metabolic changes in IC/BPS patients. (Figure 1C,D)

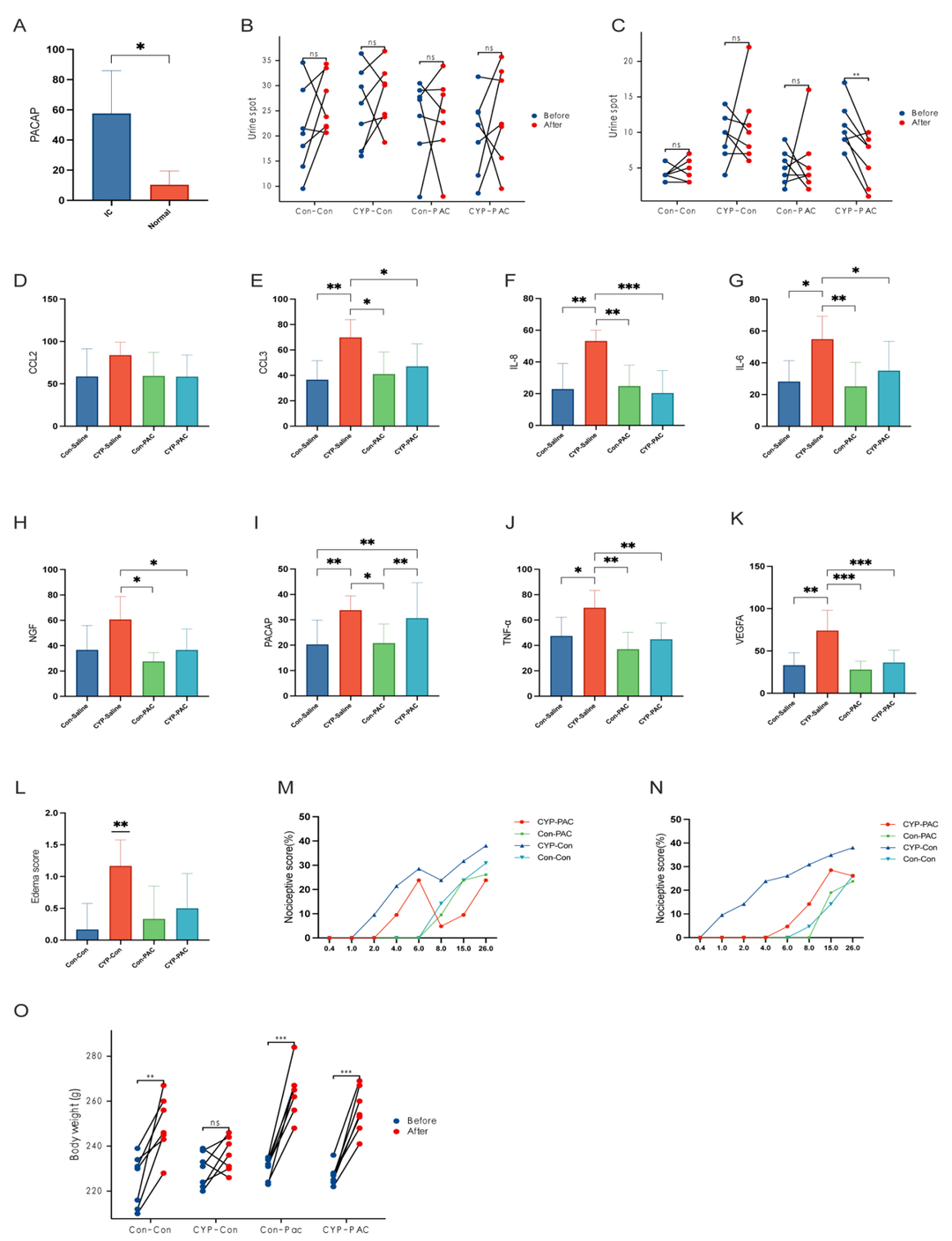

Figure 2

Conclusions

This study highlights the critical role of the PACAP/PAC1 pathway in mediating bladder inflammation and dysfunction in IC/BPS. The following key findings support the therapeutic potential of targeting this pathway:

- PACAP expression is significantly upregulated in both human IC/BPS patients and rat models of cystitis.
- Treatment with PACAP6-38, a PAC1 receptor antagonist, effectively reduces bladder inflammation, improves bladder function, and alleviates chronic visceral pain in cystitis models.

These findings open new avenues for developing targeted treatments aimed at modulating neuroinflammatory and immune responses in IC/BPS, with PACAP/PAC1 as a promising therapeutic target.

References

1. Hanno PM, Erickson D, Moldwin R, Faraday MM. Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome: AUA Guideline Amendment. Journal of Urology. 2015 May;193(5):1545–53.

2. LEPPILAHTI M, SAIRANEN J, TAMMELA TLJ, AALTOMAA S, LEHTORANTA K, AUVINEN A, et al. PREVALENCE OF CLINICALLY CONFIRMED INTERSTITIAL CYSTITIS IN WOMEN: A POPULATION BASED STUDY IN FINLAND. Journal of Urology. 2005 Aug;174(2):581–3.

3. Vizzard MA. Up-regulation of pituitary adenylate cyclase-activating polypeptide in urinary bladder pathways after chronic cystitis. J Comp Neurol. 2000 May 8;420(3):335–48.

4. HERRERA GM, BRAAS KM, MAY V, VIZZARD MA. PACAP Enhances Mouse Urinary Bladder Contractility and Is Upregulated in Micturition Reflex Pathways after Cystitis. Annals of the New York Academy of Sciences. 2006 Jul;1070(1):330–6.

5. Braas KM, May V, Zvara P, Nausch B, Kliment J, Dunleavy JD, et al. Role for pituitary adenylate cyclase activating polypeptide in cystitis-induced plasticity of micturition reflexes. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2006 Apr;290(4):R951–62.