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Nishikawa N[1], Kanematsu A[2], Okinami T[1], Negoro H[1], Sugino Y[1], Imamura M[1], Yoshimura K[1], Ogawa O[1]
1. *Department of Urology, Kyoto University Graduate school of medicine,* ***2.*** *Department of Urology, Hyogo medical college*

PTHRP SIGNALLING SYSTEM IS ACTIVATED BY ACUTE BLADDER DISTENSION

Hypothesis / aims of study
Acute bladder retention is a frequently encountered urological phenomenon, but transcriptional changes in the bladder have not been fully explored. In the present study, we used in vivo rat bladder acute distension model to investigate comprehensive gene expression profile by microarray, and identified parathyroid hormone-related peptide (PTHrP) as a novel signalling candidate. Thus we investigated function of PTHrP-related signalling system in this model.

Study design, materials and methods
1) Identification of the genes associated with acute distension: 8weeks Female Sprague-Dawley rats were used. Rats were divided into 3 groups (empty group: bladder kept empty for 6hrs with transurethral catheterization; cycling group: bladder kept in urine storing-voiding cycle for 3 hours; distension group: after the cycling with group 2, bladder kept distended for 3 hours). Total RNA was extracted from bladder using RNeasy mini kit ® (Qiagen), and cDNA was synthesized using First-Strand cDNA synthesis kit® (GE healthcare Lifescience). First, expression pattern of the 3 genes (Heparin-binding epidermal growth factor(HB-EGF), Thrombomodulin(THBD), and Cyclooxygenase 2 (Cox2)), reported to be upregulated with bladder smooth muscle cell (BSMC) stretch(1), was examined by quantitative real-time PCR(qPCR). Next, a comprehensive transcriptome analysis for differences between two groups (empty group vs. distension group. n=3) was performed by Affymetrix Genechip rat Gene 1.0 ST Array®. Further, transcriptional upregulation of PTHrP indicated by the Affymetrix array was confirmed by qPCR.
2) Localization of PTH1R in the bladder: The bladder wall was manually separated to urothelial tissue (UT) and smooth muscle tissue (SMT). PTH1R localization was evaluated by qPCR and immunoblotting.
3) The downstream pathway of PTH1R in smooth muscle: PTH1R expression of BSMC was confirmed by qPCR. BSMC was cultured with serum-free DMEM for 24 hours, and treated with PTHrP (1-34) amide. Intracellular cAMP concentrations were assayed with cAMP-EIA kit (RPN225: GE healthcare Lifescience) in quadruplicate.

Results
1) This animal model was validated by upregulation of all 3 stretch-related genes tested. In a total of 27,342 genes on microarray, 353 genes showed >2 fold upregulation, PTHrP (25th highest upregulation, fold change = 6.17, p= 0.013) was identified as a novel gene related with distension, and significant upregulation of PTHrP was confirmed by qPCR (Fig.1).
2) Three smooth muscle markers(Calponin, Smooth muscle-myosin heavy chain(SM-MHC), and alpha smooth muscle actin(aSMA) , and two urothelial markers(uroplakin 3a (UP3a) and cytokeratin 20(CK20)) showed a clear difference in expression between UT and SMT. SMT showed significantly higher PTH1R expression than UT in mRNA level (Fig2) and protein level .
3) PTH1R protein was expressed in primary cultured BSMC at a higher level than in primary cultured urothelial cell (UC). Temporal increase of intracellular cAMP was observed with PTHrP (1-34) amide treatment of BSMC (Fig3).

Interpretation of results
PTHrP may function as an acute response signal upregulated in bladder distension, dominantly through PTH1R in smooth muscle layer.

Concluding message
PTHrP/PTH1R signalling could be a novel target for bladder remodelling associated with bladder distension/stretch injury.

Figure 1.

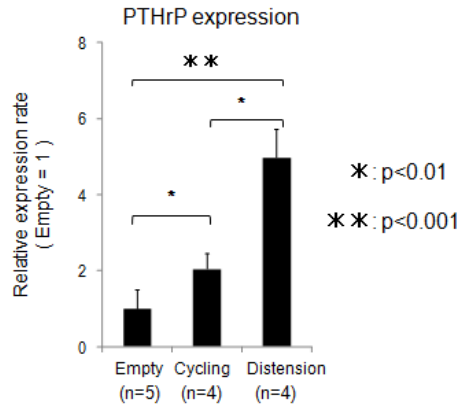


Figure 2.

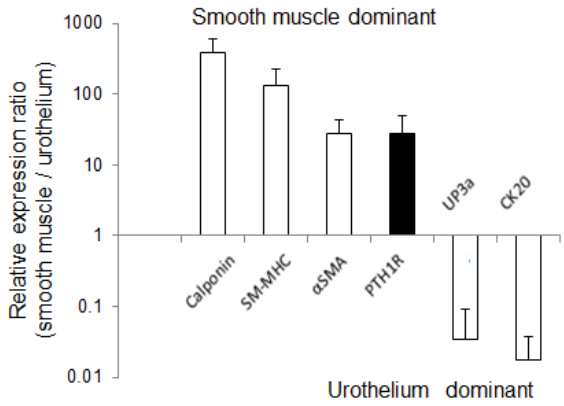
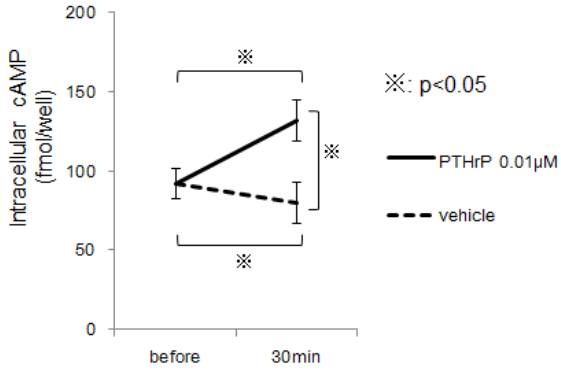


Figure 3. Intracellular cAMP change with PTHrP(1-34)treatment



References

1. Adams RM et al. Mechanical stretch is a highly selective regulator of gene expression in human bladder smooth muscle cells. Physiol Genomics 20:36-44, 2004.

Disclosures

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