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ANALYSIS OF CELL TYPE-SPECIFIC GENE EXPRESSION IN THE RAT BLADDER USING LASER-CAPTURE MICRODISSECTION TECHNIQUES

Hypothesis / aims of study

There is increasing evidence that alterations in sensor molecule expression in the urothelium and/or the detrusor could be important for the pathogenesis of bladder overactivity/pain conditions. However, it is often difficult to identify cell type-specific changes in expression of receptors or ion channels in urothelial vs. derusor muscle cells or in different urothelial cell layers (i.e., apical vs. underlying intermediate/basal cells) as previous studies usually used the macroscopically-separated bladder mucosa, which contains the suburothelial layer, to examine the changes of molecular expression in the bladder. Thus, this study examined whether the laser-capture microdissection (LCM) method, which allows us to perform cell type-specific tissue dissection under a microscope, can achieve separation of urothelial cells from detrusor cells, and of superficial cells from intermediate/basal cells within the urothelium using a smooth muscle marker, alpha-smooth muscle actin (SMA) and a differentiated apical urothelial cell marker, cytokeratin 20 (CK20) (1), respectively. In addition, it has been reported that urothelially expressed muscarinic receptors (MR) play a significant role in modulation of bladder afferent activity (2, 3). Therefore, we further investigated the changes in expression of MR in urothelial and detrusor cells using LCM and real-time PCR in rats with chronic cystitis.

Study design, materials and methods

Female SD rats were used. For the production of a chronic cystitis model, rats were injected with cyclophosphamide (75mg/kg) intraperitoneally at day 1, 4, 7 and 10 to induce chronic cystitis (CC: N=3), and sacrificed at day 11. Saline was injected in the same protocol for controls (CTR: N=3). Bladder specimens were embedded in O.C.T. compound and cut at 8 μm thickness. Tissue sections were then fixed in 70% ethanol for 1 min and rinsed with double distilled H_2O. The sections were stained lightly with Hematoxylin & Eosin followed by laser-capture microdissection (LCM) under a microscope using Leica LMD6000. Superficial (apical) urothelium, intermediate/basal urothelium and detrusor muscles were laser-captured separately and each specimen included as few as 500-600 cells. Captured individual tissue specimens were then lysed and RNA isolation, reverse transcription, and real-time PCR were performed to examine expression of alpha-SMA (smooth muscle marker), CK20 (differentiated apical urothelial cell marker) (1), muscarinic M2 receptor (M2R) and 3 (M3R). The reactions were analyzed in triplicate and normalized to GAPDH or HPRT.

Results

In comparison of laser-captured urothelial and detrusor cells, expression of alpha-SMA mRNA in detrusor muscle cells was 200 times higher than that in urothelial cells in control bladders. In the comparison of laser-captured detrusor muscle, and apical and intermediate/basal urothelial cells, CK20 mRNA expression in apical urothelial cells was 55 times more than that in detrusor muscle and four times more than that in intermediate/basal urothelial cells. When MR mRNA expression in laser-captured urothelial cells and detrusor muscles was compared in control and cystitis rats, expressions of M2R and M3R mRNA were increased in urothelial cells (M2R: CC/CTR=5.55 and M3R: CC/CTR=6.62) and decreased in detrusor muscles (M2R: CC/CTR=0.20 and M3R: CC/CTR=0.16) following chronic cystitis.

Interpretation of results

These results indicate that cell-type specific dissection of different layers of urothelium and detrusor muscle with minimal contamination can be achieved using the LCM method as evidenced by high expression of smooth muscle (alpha-SMA) or differentiated apical urothelial cell marker (CK20) in laser captured detrusor muscle or apical urothelial cells, respectively. Moreover, LCM followed by RT-PCR detected the changes in expression of M2R and M3R after chronic cystitis, which were increased and decreased in urothelium and detrusor muscle, respectively.

Concluding message

The LCM could be useful for tissue collection of detrusor muscle and different layers of urothelial cells with minimal contamination of other cell types and, when combined with real-time PCR, cell type-specific changes in molecular expression could accurately be analyzed. Since muscarinic cholinergic receptors such as M2R and M3R expressed in the urothelium are reportedly involved in the modulation of bladder afferent activity (2, 3), increased expression of urothelial MR might enhance urothelial-afferent interactions to induce bladder overactivity/pain conditions associated with bladder inflammation.

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