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GENE EXPESSION AND FUNCTIONAL ROLE OF PROSTAGLANDIN E2 RECEPTOR SUBTYPES IN THE RAT URINARY BLADDER MUCOSA AND DETRUSOR: HOW DO THESE CHANGES IN ASSOCIATION WITH BLADDER OUTLET OBSTRUCTION?

Hypothesis / aims of study

Prostanoids synthesis occurs locally in bladder muscle and mucosa, and it is initiated by various physiological stimuli, such as detrusor muscle stretch and nerve stimulation as well as by noxious stimulations. Prostanoids, especially prostaglandin (PG) E2, has been implicated as endogenous modulator of bladder function in the normal physiological state and under pathological conditions. Intravesical administration of PGE2 produces detrusor overactivity and stimulates reflex micturition. The release of PGE2 from the bladder has been reported to be increased as a consequence of bladder outlet obstruction (BOO), suggesting a possible role of PGE2 in detrusor overactivity associated with BOO.

The endogenous activity of PGE2 is mediated thorough the EP receptor family of G protein coupled receptors, of which 4 subtypes are identified to date. However, the receptor subtypes that mediate the actions of PGE2 on bladder function in normal and pathological conditions have not been characterized. Therefore, we investigated the expression and functional role of EP receptor subtypes and how these change in association with bladder outlet obstruction in rats.

Study design, materials and methods

Quantitative real-time RT-PCR: Seven weeks old female Sprague-Dawley rats were used for this study. Under pentbarbital anesthesia (50 mg/Kg i.p.), partial urethral obstruction was created by a standard method (n = 10). Sham operation was made similarly without tying the urethra (n = 5). Seven weeks later, the whole bladder were removed, weighed and immediately separated into mucosa and smooth muscle layers in RNA stabilization solution under stereoscopic microscope. Total RNA was isolated from mucosa and detrusor. The extracted RNA was reverse-transcribed with random primer. Quantitative real-time PCR was performed with a Smart Cycler System (Cephied) using SYBR Green I as the fluorescent dye. The product of RT-PCR, cDNA, was subjected to 40 PCR cycles with each pair of gene-specific primers. The expression of EP receptors was normalized as the ratio to YWHAZ expression in each sample.

Cystometric investigations: Cystometric investigations were performed under urethane anesthesia (1.2 g/Kg s.c.) 3 weeks after the operation. The abdomen was opened through a midline incision, and 27 gauge needle was put into the bladder through the dome. The bladder needle was connected via a T-tube to a transducer and microinjection pump. Saline with or without 10 μM ONO-AE1-329 (EP4 selective agonist) at room temperature was continuously infused into the bladder at a rate of 2 ml per hour. Intercontraction interval (ICI), maximal micturition pressure (MP), basal intravesical pressure (BP) and pressure threshold for micturition (PT) were recorded on a Power Lab Chart5. These cystometric parameters were examined in control saline, during ONO-AE1-329 instillation and after washout of the drug (n = 5). The same experiment was performed in sham operated rats (n = 5).

Results

The weight of obstructed bladders (221.1±17.9mg, n=10) was significantly ($p<0.01$) greater than that of control bladders (86.6±3.4mg, n=5).

Quantitative analysis of PGE2 receptor subtype gene expression

	mucosa		smooth muscle layer	
	BOO bladder	control bladder	BOO bladder	control bladder
EP1/YWHAZ	0.1867±0.0223	0.2686±0.0339	0.2040±0.0189	0.2476±0.0332
EP2/YWHAZ	0.1088±0.0106	0.1065±0.0153	0.0674±0.0290	0.0363±0.0019
EP3/YWHAZ	0.0332±0.0031 *	0.0554±0.01	0.1138±0.0100 *	0.2020±0.018
EP4/YWHAZ	0.1554±0.0247 *	0.0000±0	0.0518±0.0065 *	0.0000±0

There was no difference in EP1 or EP2 receptor gene expression between control and obstructed bladders. However, EP3 receptor gene expression was significantly decreased in obstructed bladders ($p=0.017$ in mucosa and $p<0.001$ in muscle layer). EP4 receptor gene was virtually absent in controls, but its gene was clearly expressed in obstructed bladders ($p<0.001$ in both mucosa and muscle layer).

In controls, no significant differences were found in any cystometric parameters. In obstructed bladders, however, ICI was significantly increased by intravesical infusion of ONO-AE1-329 (paired Student's t test, $p = 0.01$). The effect of ONO-AE1-329 on ICI was reversible by washing out of the drug (vs ONO-AE1-329, $p = 0.002$; vs control saline, $p = 0.34$). There were no significant differences in other parameters.

Interpretation of results

Quantitative PCR analysis revealed that the expression level of EP4 and EP3 receptor mRNA was significantly increased and decreased in hypertrophic bladder due to BOO, respectively. Activation of EP3 and EP4 receptor has been suggested to produce the detrusor relaxation. In addition, cystometric experiment showed that ICI in BOO rats increased by EP4 agonist, which suggests the afferent inhibition by EP4 receptor activation. Thus, changes of EP3 and EP4 expression in obstructed bladder might play some roles in the induction process of overactive bladder due to BOO.

Concluding message

This study showed that the expression of EP3 and EP4 gene and functional role of EP4 change in obstructed bladders of the rat. EP4 receptor may play a significant role in the induction process of overactive bladder due to BOO with modulating detrusor relaxation status and/or afferent activity.