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DIFFERENTIAL EXPRESSION OF ANGIOTENSIN II TYPE-1 RECEPTOR IN THE RAT DETRUSOR MUSCLE WITH PARTIAL BLADDER OUTLET OBSTRUCTION AND DIABETES MELLITUS MODEL

Hypothesis / aims of study

Angiotensin II (Ang II) has been known to be an important regulatory peptide with multiple physiological functions. Also, it is reported that Angiotensin II type 1 receptors (AT1) mediated the contractile effect of Ang II in the rat bladder [1]. The main roles of Ang II are the following three effects; contractility, proliferation and fibrosis. It is considered that renin-angiotensin system (RAS) may be related to bladder hypertrophy or fibrosis caused to the voiding dysfunction. Some reports demonstrated RAS blockage improves the morbidity and prevent the fibrosis and dysfunction in several organs [2, 3]. In this study, we demonstrated weather the expression and alteration of AT1 receptors in detrusor muscle with partial bladder outlet obstruction (BOO) and diabetes mellitus (DM) rat model.

Study design, materials and methods

Animals

Adult female Wistar rats (240–280 g) were used in this study. These rats were divided into three groups as follows, a partial bladder outlet obstruction (P-BOO) group, diabetes mellitus (DM) group and a sham-operated control group. Cystometrical findings and analysis of detrusor muscle by using the immunohistochemical staining with AT1 antibody in each group.

Partial Bladder Outlet Obstruction

To obtain a partial obstruction of the urethra, a modified technique of Mattiasson and Uvelius was used. The rats were anesthetized by intraperitoneal injection of urethane at 1 g/kg body weight. The urethra was intubated with a PE-50 polyethylene catheter and a double 4-0 silk ligature was placed loosely around the proximal urethra producing a standardized degree of obstruction.

Induction of diabetes mellitus

Diabetes mellitus was induced by a single intra-peritoneal injection of 60 mg/kg body weight of streptozotosin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) dissolved in a citrate buffer (0.1 M, pH 4.5).

Immunohistochemistry of AT1

The detrusor muscles were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in paraffin. After blocking with 1% goat serum and 5% skim milk, the sections were incubated at 4^{o}C overnight with primary rabbit AT1 polyclonal antibody (Santa Cruz, California, USA) diluted 1:500 in PBS. After washing, the sections were incubated with Histofine Simple Stain Rat Max-PO (Nichirei Co., Tokyo, Japan) for 30 minutes at room temperature respectively. The sections were stained with diaminobenzidine tetrahydrochloride (Nichirei Co., Tokyo, Japan), counterstained with hematoxilin and then mounted.

Results

The vesical pressure of the P-BOO rats at 2 weeks was significantly greater than that of the control rats. However, the voiding pressure of the P-BOO groups at 4 weeks was lower than that of the P-BOO rats at 2 weeks. Finally, in the P-BOO rats at 8 weeks, no detrusor contraction was observed and the voiding had the characteristics of overflow. On the other hand, the vesical pressure of the DM rats at 2 weeks was kept as same as the control rats. At 4 and 8 weeks, the vesical pressure was gradually decreased and the residual urine was increasing. However, the vesical pressure was not significantly different between 2, 4 and 8 week in DM rats. Immunohistochemical staining revealed that AT1 receptor was expressed on the detrusor cells membrane in control rat. In P-BOO, the expressions of AT1 receptors in detrusor muscle were gradually decreased. At 8 weeks after P-BOO, the expression of AT1 receptor on the detrusor muscle was disappeared. However, the expression of AT1 receptor in detrusor muscle in DM rats was gradually increased. At 8 weeks later, AT1 receptors were highly expressed on the detrusor cell membrane than 2, 4 weeks after induction of DM.

Interpretation of results

AT1 receptor was expressed on the detrusor muscle, it is considered that renin-angiotensin II system via AT1 receptor may involved to the detrusor contraction and fibrosis. Our data suggested that AT1 may be down-regulated by mechanical stress and up-regulated by high glucose concentration. Contractile potential of bladder was remained in DM rats than P-BOO rats; it was considered that the expression of AT1 in rats' bladder may be related to keep the detrusor contraction and bladder compliance.

Concluding message

Our results suggested that AT1 may contribute to maintain the contraction of the detrusor muscle. Renin-angiotensin system may be related to voiding function.

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