

## EXPRESSION OF AT1 ON THE INTERSTITIAL CELLS IN SUBUROTHELIAL LAYER

### Hypothesis / aims of study

The local renin-angiotensin II system has been researched in various tissues, including the heart, kidney, adrenal, liver, blood vessels and gonads. Also, in the rat bladder, angiotensin II type 1 receptors (AT1) were considered to mediate the contractile effect of angiotensin II. However, the localization of AT1 in the bladder and the role of AT1 in voiding function have not still been confirmed. The present study aimed to point out the localization of AT1 in the rat bladder.

### Study design, materials and methods

#### Animals

Adult female Wistar rats weighing 240–280 g and about 3 months of age were used in this study. Development humidity of about 65% and a 12:12 h light: dark cycle. They had free access to water and commercial laboratory chow provided ad libitum.

#### Immunohistochemical staining of AT1

The bladder specimens were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in paraffin. Immunohistochemical staining of AT1 was done by using with rabbit AT1 polyclonal antibody (Santa Cruz, California, USA) diluted 1:500 in PBS for the first antibody. Second antibody was used 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 hematoxylin and then mounted.

#### Results

In the rat bladder, AT1 immunoreactivity was detected on the detrusor muscle cells and suburothelial interstitial cells. In the detrusor muscle, AT1 was localized predominantly on the cell membrane and slightly in the cytoplasm. We suspected the AT1 positive suburothelial interstitial cell as to the myofibroblast, we stained the adjacent sections with alpha smooth muscle actin (SMA) and vimentin antibody to identify the characterization of interstitial cell expressed the AT1. Both of SMA and vimentin were expressed predominantly on the AT1 positive interstitial cells. These data confirmed that AT1 expressed interstitial cells in suburothelial layer were myofibroblasts.

#### Interpretation of results

As AT1 was expressed on detrusor muscle, the local renin-angiotensin II system may be associated with the contraction of bladder. Recently, the interstitial cells are considered to have a pacemaker potential, the character of the interstitial cells was revealed in this study. Fry reported that the gap junctions interacted between suburothelial myofibroblasts and nerves. And the activation of gap junction played a key physiological conditions in the over active bladder (OAB) [1]. Recently, myofibroblasts in the bladder were considered to involve in etiology of OAB, Brubaker reported that OAB may be controlled by myofibroblast abnormalities in the bladder [2]. Inoue reported that up-regulation of gap junctions in the rat cultured cardiomyocytes induced atrial fibrillation, and AT1 receptor antagonist prevented the atrial fibrillation induced by up-regulation of gap junction [3]. AT1 expressed myofibroblasts in the suburothelial layer will be up-regulated the interaction with nerves via the gap junction, it may cause to OAB.

#### Concluding message

The present study has demonstrated the expression and the localization of AT1 in the rat bladder. These results indicate that AT1 on suburothelial myofibroblasts may play a role in OAB, and it may lead a new therapeutic strategy to OAB.

[1] Fry CH, et al: Spontaneous activity and electrical coupling in human detrusor smooth muscle: Implications for detrusor overactivity? *Urology* 63: 3-10, 2004

[2] Brubaker L. Urgency: the cornerstone symptom of overactive bladder. *Urology*. 2004 Dec;64(6 Suppl 1):12-6.

[3] Inoue N, et al: Rapid electrical stimulation of contraction modulates gap junction protein in neonatal rat cultured cardiomyocytes: involvement of mitogen-activated protein kinases and effects of angiotensin II receptor antagonist. *J Am Coll Cardiol*. 18; 44 (4): 914-22, 2004

**FUNDING:** None

**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by the Animals Ethics Committee at Nagasaki University Graduate School of Biochemical Sciences, Japan.