<table>
<thead>
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<th>Title</th>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
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</tbody>
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Changes in Angiotensin II type 1 receptor expression in the rat bladder by bladder outlet obstruction.

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Running head: Changes in AT1 in rat bladder by bladder outlet obstruction

Abstract

Purpose
To demonstrate the change in the expression of Angiotensin II type 1 receptor (AT1) in the rat bladder with partial bladder outlet obstruction (P-BOO).

Material and Methods
Bladder specimens were obtained from twelve-week-old Wistar female rats that were divided into two groups, a partial bladder outlet obstruction (P-BOO) group and a control group. The rats of the P-BOO group were divided into six groups: sham-operated control group, 1 day postoperatively, 2 days postoperatively, 4 days postoperatively, 7 days postoperatively and 14 days postoperatively. The cystometrical findings and immunohistochemical staining of the detrusor muscle with AT1 antibody was compared in each group.

Results
AT1 localized on the cell membrane of the detrusor smooth muscle and in cytoplasm of suburothelial myofibroblasts in the control rats. The expression of AT1 disappeared in the detrusor muscle and suburothelial myofibroblasts in P-BOO, but, AT1 was highly expressed...
in urothelial cells 1 day after surgery. The expression of AT1 in urothelial cells gradually
decreased with time after surgery. AT1 completely disappeared in urothelial cells 14 days
after surgery.

Conclusions
The present study demonstrated that the site of AT1 expression changes in response to the
mechanical stress caused by P-BOO, and finally there was no expression of AT1 in rat
bladder tissue following P-BOO. These data suggest the change in AT1 expression may be
play a role in bladder function.

Introduction
Angiotensin II (Ang II) is an important regulatory peptide with multiple physiological
functions (1). The main roles of Ang II are contractility, proliferation and fibrosis. The
local renin-angiotensin II system (RAS) has been demonstrated in various tissues, including
the heart, kidney, adrenal, liver, blood vessels and gonads (2). A few studies investigating
the RAS and bladder function were reported in the 90's. They demonstrated that Ang II
only has a weak contractive potential, and the blockade of the RAS has little influence on
the bladder.

Yamada et al. demonstrated that AT1 decreases in the rat bladder with incomplete
urinary retention using a bladder outlet obstruction model, and stated that the cause might
be down regulation of AT1 induced by the activation of RAS (3).

This study investigated the changes of AT1 expression induced by BOO using
immunohistochemistry.

Materials and Methods

Animals
Adult female Wistar rats weighing 200–225 g and about 3 months of age were used in this
study. The study protocol was approved by the Animals Ethics Committee at Nagasaki
University Graduate School of Biochemical Sciences, Japan. The rats were housed at room
temperature with 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. These rats were
divided into seven groups: a sham-operated control group, 1 day postoperatively, 2 days
postoperatively, 4 days postoperatively, 7 days postoperatively and 14 days postoperatively.

Partial Bladder Outlet Obstruction
A modification of the technique of Mattiasson and Uvelius (4) and Malmgren (5) et al. was
used to obtain a partial obstruction of the urethra. The rats were anesthetized by
intraperitoneal injection of 1 g/kg body weight urethane. 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, and the catheter was removed. The control rats underwent a sham operation.

**Immunohistochemical staining of AT1**

The bladder specimens were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in paraffin. Sections were deparaffinized with xylene and sequentially rehydrated in a graded ethanol series for 5 min each. Endogenous peroxidase activity was blocked by incubation in 3% H2O2 in methanol for 15 min. Sections were then incubated at 4°C for 24 hours with rabbit anti-AT1 receptor polyclonal antibody (Santa Cruz, Santa Cruz, CA) diluted 1:500 in phosphate-buffered saline. The sections were washed and incubated with Histofine Simple Stain Rat Max-PO (Nichirei Co., Tokyo, Japan) for 30 min at room temperature. The sections were stained with diaminobenzidine tetrahydrochloride (Nichirei Co., Tokyo, Japan), counterstained with hematoxylin, and mounted.

**Cystometric investigation**

The rats were anesthetized by intraperitoneal injection of 1 g/kg body weight urethane (6). A PE-50 catheter was inserted through the bladder apex into the lumen and connected to a pressure transducer and a microinjection pump (UD5500, Dantec, Denmark). Warmed saline was then infused into the bladder at a rate of 10 mL/hr for the control and P-BOO rats, respectively.

**Results**

**Cystometric investigation**

The voiding pattern in the control rats was normal. The voiding frequency of the P-BOO rats at 1 day and 2 days were significantly greater than that of the control rats. The P-BOO rats at 14 days showed no detrusor contraction and voiding had the characteristics of overflow (**Figure 3**).

**Immunohistochemistry of AT1**

The localization of AT1 in the rat bladder was examined by immunocytochemistry. **Figure 1** and **2** show the results of the immunohistochemistry. AT1 immunoreactivity was
localized to smooth muscle cells and suburothelial myofibroblasts. AT1 immunostaining in
the detrusor muscle was localized predominantly on the cell membrane and slightly in the
cytoplasm (Figure 1, 2). Replacement of the primary antibody with buffer also yielded
negative staining (Figure 1, 2). The expression of AT1 in the detrusor muscle and
suburothelial myofibroblasts disappeared in P-BOO, but AT1 was highly expressed in
urothelial cells at 1 day after surgery, (Figure 3). The expression of AT1 in urothelial cells
gradually decreased following surgery. There was no AT1 immunostaining in the urothelial
cells at 14 days after the surgery (Figure 3).

Discussion
The present study demonstrated the expression of AT1 on detrusor muscle cells and
suburothelial myofibroblasts in the rat bladder. Previous studies demonstrated that Ang II
have a potential of contraction of rat bladder, these suggested that AT1 exist on rat bladder
tissue. Yamada et al. demonstrated that the expression of AT1 in the rat bladder decreases
with bladder outlet obstruction using a sensitive binding assay (3). The current data
showed the reduction of AT1 expression caused by P-BOO visually and temporally using
immunohistochemical staining. Several investigators have shown that Ang II causes
pronounced contraction of bladder smooth muscles in some species, including humans
(7,8,9,10,11), and the effect is mediated by the AT1. The down-regulation of AT1 in
outlet-obstructed rat was observed by Saito et al. (12), who showed significantly weaker
contractility of human detrusor muscle by Ang II in neurogenic bladders than controls.
This suggests that the down-regulation of bladder AT1 is largely ascribable to the enhanced
activity of Ang II following P-BOO. Dinh et al. reported that dysuria caused by prostatic
hyperplasia induces increased expression of Ang II and down-regulation of AT1 in human
prostatic tissue (13). The same phenomenon might occur in rat bladder tissue.
Pathological conditions of the bladder might reduce expression of AT1, and this mechanism
might be the cause of the reduced response of the human bladder to Ang II that Saito et al.
reported. Although the frequency of urination increased during the period of AT1
expression in urothelial cells, the frequency of urination decreased after AT1 expression
disappeared in urothelial cells. AT1 was not expressed in the urothelial layer without
mechanical stress by P-BOO. The current data demonstrated that AT1 expression in
bladder tissue below the urothelium ceases and new expression of AT1 continues in the
urothelium for a few days after acute incomplete urinary retention, and then AT1 gradually
disappears from the urothelium. The role of this phenomenon in bladder dysfunction is
unknown; however, activated RAS within bladder tissue might induce frequent bladder
contraction via AT1 when AT1 is expressed in the urothelium.
Conclusion

The present study demonstrated that AT1 expression in the rat bladder does not uniformly disappear with mechanical stress by P-BOO. The frequency of urination right after the induction of incomplete urinary retention might therefore be associated with the expression of AT1 in the urothelium for a short period of time.

References


Figure legends

Figure 1. Expression of AT1 on the detrusor muscle in the normal rat bladder. (a) AT1 expressed on the cell membrane of bladder smooth muscle. (b) Negative control   Magnification × 600

Figure 2. Expression of AT1 in the suburothelial layer in the normal rat bladder. (a) AT1 expressed on suburothelial myofibroblasts. (b) Negative control   Magnification × 200

Figure 3. The changes of AT1 expression in bladder in response to P-BOO. The expression of AT1 in urothelial cells gradually decreased.
Figure 1.
Figure 3.
Figure 3.