

The expression of Angiotensin II type 1 receptor in rat bladder smooth muscle cells in respond to streptozotocin induced diabete mellitus model.

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Abstract

Introduction ; The aim of this study is to investigate angiotensin II type 1 (AT1) receptors on rat bladder smooth muscle cells and the alterations of AT1 receptors by diabetes mellitus and diuretic states.

Materials and Methods ; Diabetes and diuresis were induced in adult female rats by a single intraperitoneal injection of streptozotocin (STZ) and feeding 5% sucrose in water.

Cystometry was performed on control, diuretic, and diabetic rats at 2 and 8 weeks after treatment. To detect the expression of AT1 receptors on the bladder smooth muscle cell membrane, immunohistochemical staining was performed.

Results and Conclusions ;

In diabetic rats, the expression of AT1 receptors on the bladder smooth muscle cell membrane was increased at 2 weeks, and was further increased at 8 weeks. The local Renin-Angiotensin System (RAS) in the rat bladder might be activated by the continuous hyperglycemia caused by STZ administration.

Introduction

Diabetes mellitus results as a long-term complication of the lower urinary tract is referred

to as diabetic cystopathy (1). Many factors probably contribute to the large capacity, atonic bladder that characterizes diabetic cystopathy, including changes in the bladder smooth muscle (2). Diuresis originating from diabetes mellitus causes increases in the bladder mass and changes the bladder strip contractility (1, 2).

Angiotensin II (AngII) is a potent vasoconstrictor, and an integral part of the systemic renin-angiotensin system, which regulates the vascular tone, blood pressure, and sodium homeostasis. In addition to the systemic effects of this hormone, AngII may regulate cellular growth and function in several organ systems through a local renin-angiotensin system (RAS). In fact, there is increasing evidence suggesting that AngII is a local trophic factor in the wall of bladder that is responsible for regulating smooth muscle cell growth under both normal and pathological conditions (3). Ang II may also play a role in the control of collagen production (4).

Previous studies demonstrated that a local renin-angiotensin system plays a role in the functional disorder and fibrosis of many organs, including the kidneys (5), heart (6), lungs (7), liver (8), and so on. Moreover, the functional disorder and fibrosis caused by diabetes mellitus are reported to be associated with a local renin-angiotensin system.

However, to our knowledge, whether the RAS is associated with bladder dysfunction caused by diabetes mellitus has not been investigated. Therefore, in the present study, we investigated angiotensin II type 1 (AT1) receptors on rat detrusor muscle and the alterations of AT1 receptors by diabetes mellitus and diuretic states.

Materials and Methods

Induction of Diabetes and Diuresis

Diabetes was induced in adult, female Sprague-Dawley rats (weight 230-280 g; Hilltop Laboratory, Pittsburgh, PA) by a single intraperitoneal injection (65 mg/kg) of streptozotocin (STZ) dissolved in an ice-cold 0.1-M citrate buffer. The blood glucose level of these rats was checked 1 week after STZ injection and rechecked before death to confirm the presence of diabetes (blood glucose >300 mg/dL). Chronic diuresis was induced by feeding 5% sucrose in water. Normal rats were used as controls. The study protocol was approved by the Animal Ethics Committee at Nagasaki University Graduate School of Biochemical Sciences, Japan.

Cystometry

Cystometry was performed on control, diuretic, and diabetic rats at 2 and 8 weeks after treatment. The rats were anesthetized by intraperitoneal injection of 1 g/kg body weight urethane. A PE-50 catheter was inserted through the bladder apex into the lumen and then was connected to a pressure transducer and a microinjection pump (UD5500, Dantec,

Denmark). Warmed saline was infused into the bladder at a rate of 10 mL/hr for each the control, diuretic, and diabetic rats.

Saline voided from the urethral meatus was collected and measured to determine the voided volume. After constant voided volumes were collected, the infusion was stopped temporarily; the postvoid residual urine volume was measured by withdrawing intravesical fluid through the catheter, first by gravity and then by manually expressing the bladder. The bladder capacity was calculated as the sum of the voided volume and the volume of the postvoid residual urine. The voiding efficiency was estimated as follows: voiding efficiency (%) = [(voided volume/bladder capacity) × 100]. The maximal voiding pressure was also measured.

Histological evaluation

The bladder specimens of the control, diuretic and DM rats 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 (100%, 95%, 90%, 80%, and 70%) for 5 min each. The endogenous peroxidase activity was blocked by incubation in 3% H₂O₂ in methanol for 15 min. The sections were blocked with 1% goat serum and 5% skim milk, and incubated at 4°C for 24 hours with 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. The sections were stained with diaminobenzidine tetrahydrochloride (Nichirei Co., Tokyo, Japan), counterstained with hematoxylin and then were mounted. Moreover, sections were stained by Masson's trichrome staining to visualize the fibrosis of the bladder wall tissues.

Statistical Analysis

All data were expressed as the means ± standard error. The data are expressed as the means ± standard error. Student's *t*-test was used for comparisons between the control, diuretic and diabetic rat groups. Statistical significance was accepted at a value of *P* < 0.05.

Results

General Characteristics

The body weight of the diuretic rats was significantly higher in comparison to that of control rats, and that of the diabetic rats was significantly decreased in comparison to that of control rats (Table 1). The blood glucose levels of the diabetic rats were about four times

higher than those of the control and diuretic rats. The bladder weights of the diuretic and diabetic rats were approximately twice those of the control rats.

Cystometry

Cystometry revealed that the mean bladder capacity and compliance increased in all treated rats compared with controls (Table 1). Although diabetic rats had a significantly decreased voiding efficiency, the mean maximal voiding pressure was similar in all groups.

Histological findings

Masson's trichrome staining revealed that the color of bladder smooth muscle cell changed red to blue. This suggests that continuous hyperglycemia induced the fibrotic change in the bladder wall.

To detect the expression of AT1 receptors on the bladder smooth muscle cell membrane, immunohistochemical staining was performed. AT1 receptors were expressed sparsely on the bladder smooth muscle cell membrane in control and diuretic rats. However, in diabetic rats, the expression of AT1 receptors on the bladder smooth muscle cell membrane was increased at 2 weeks, and was further increased at 8 weeks.

Discussion

Ang II within the rat bladder tissue has been rarely studied, and to the best of our knowledge, Ang II within the bladder of a diabetic rat model has not been investigated. A previous report indicated that the hyperglycemia caused by STZ strongly induced the expression of AT receptors on the rat kidney (9). Our results suggest that the same phenomenon occurs in rat bladder tissue.

Harrison et al reported that the expression of plasma Ang II is increased in STZ-induced diabetic rats in comparison to control rats, and that the RAS is activated in rat kidney tissue by continuous hyperglycemia (9). Our results suggested that the RAS could also be activated in rat bladder tissue by continuous hyperglycemia. The bladder weight was increased by diuresis compared to that of control rats, so that the STZ-induced hyperglycemia increased the rat bladder weight more than that of diuretic rats. These findings suggest that an increase in bladder weight could be induced by chronic mechanical stress to the rat bladder wall by diuresis, and that continuous hyperglycemia could further increase the rats' bladder weight.

Interestingly, the AT1 receptor was highly expressed in STZ-induced diabetic rat bladders, although the expression of AT1 receptors of diuretic rats did not change in comparison to the control rats. This suggests that the activation of the RAS might occur in

STZ-induced diabetic rat bladder tissue.

It is now accepted that blockade of the RAS can contribute to improving the pathogenesis of diabetic nephropathy, and angiotensin receptor blockers (ARBs) are now commonly used in clinical practice. It is unknown whether ARBs can improve the pathogenesis of diabetic cystopathy. However, the present findings indicate that an investigation of the potential of ARBs to prevent or treat diabetic cystopathy is warranted.

Conclusion

In this study, we demonstrated that the expression of AT1 receptors was enhanced by severe hyperglycemia in a rat diabetic model. The local RAS in the rat bladder might be activated by the continuous hyperglycemia caused by STZ administration.

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Tabale 1. General characteristics and cystometry in control, diuretic and diabetic rats.

	Rat s (n)	Rat weight (g)	Bladder Weight (g)	Blood Glucose (mg/dl)	Bladder Capacity (ml)	Voiding Efficiency (%)	Max voiding pressure (ml/cm H2O)
Contr ol	7	256±6	0.11±0.0 2	93±5	0.53±0.07	91.5±1.4	31.6±3.7
Diure tic (wk)							
2	7	291±8 *	0.20±0.0 2*	94±10	1.29±0.15*	84.1±1.7	31.8±4.7
8	7	342±6 *	0.25±0.0 3*	97±3	2.30±0.20* ‡	83.8±4.8	33.3±5.1
Diabe tic (wk)							
2	7	219±9 *†	0.24±0.0 1*†	401±23*†	1.21±0.09*	57.6±4.8*†	37±5.6
8	7	228±4 *†	0.41±0.0 2*†	411±27*†	2.58±0.15* 	39.6±5.2*†	39.5±5.9

* Significantly different from controls.

† Significantly different from diuretic rats of same week.

‡ Significantly different from 2-wk diuretic rats.

|| Significantly different from 2-wk diabetic rats.

Figure legends

Figure1. Histological analysis of detrusor muscle cells in 2wk rats.

Masson's trichrome staining (A-C). The color of the bladder smooth muscle of 2-wks diabetic rats change into blue. AT1 receptors sparsely expressed on detrusor smooth muscle

membrane of control (D) and diuretic (E) rats. And, in STZ-induced diabetic model, the expression of AT1 receptors was increased in comparison to the control and diuretic rats (F).

Magnification of Masson's trichrome staining $\times 40$

Magnifications of immunohistochemical staining , $\times 600$

Figure 2. Histological analysis of detrusor muscle cells in 8wk rats.

Masson's trichrome staining (A-C). The color of the bladder smooth muscle of 8-wks diabetic rats change into blue too. In diabetic rats, the expression of AT1 receptors on the bladder smooth muscle cell membrane was further increased at 8 weeks (F).

D: control rats E: diuretic rats

Magnification of Masson's trichrome staining $\times 40$

Magnifications of immunohistochemical staining , $\times 600$

Figure 1.

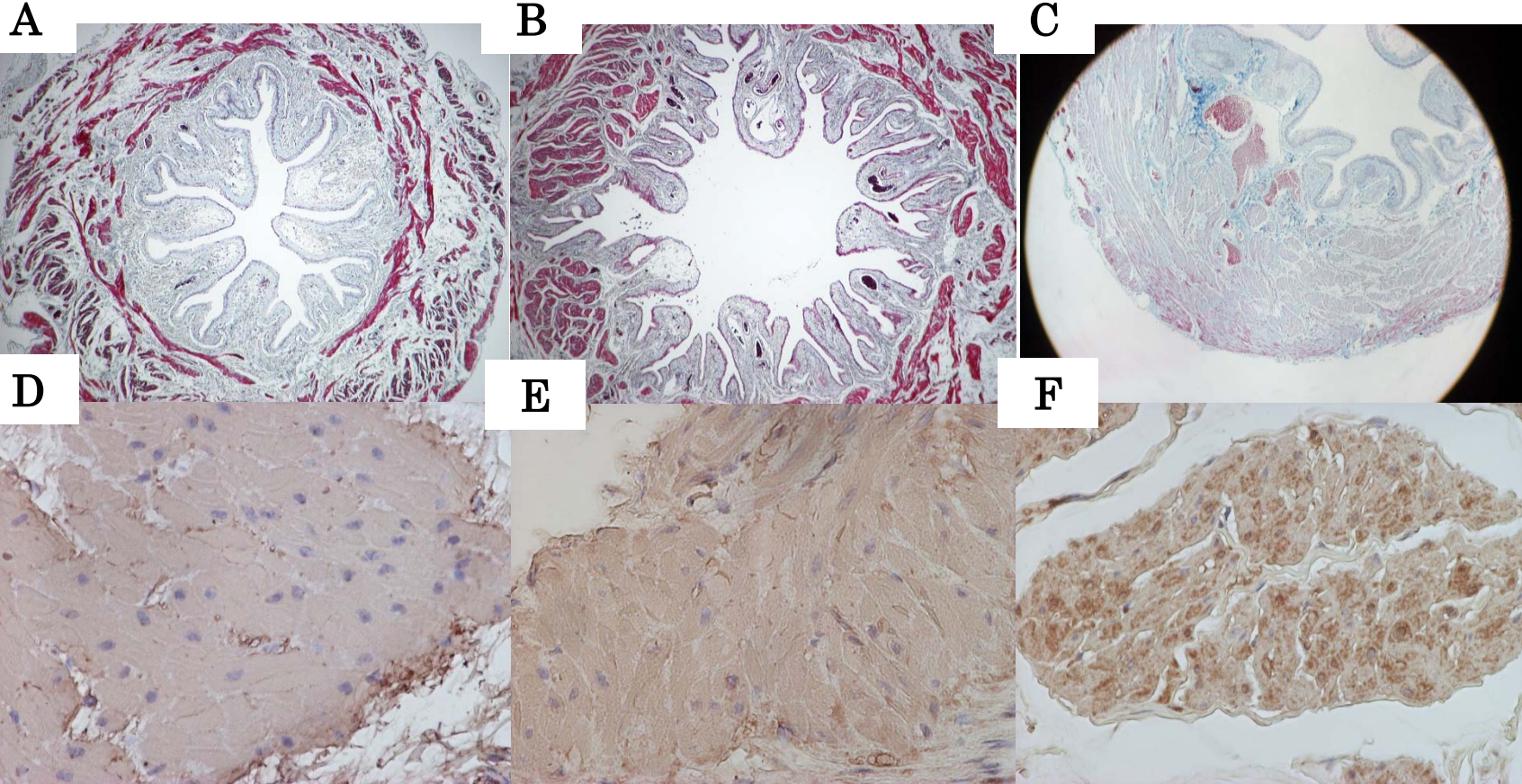


Figure 2.

