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Effect of an Angiotensin-Converting Enzyme Inhibitor on Smooth Muscle Myosin Isoforms in Afferent Arterioles from Spontaneously Hypertensive Rats

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Abstract I used immunohistochemical analysis to examine the expression of smooth muscle myosin heavy chain (MHC) isoforms (SM1 and SM2) in renal arterioles from 20-week-old Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). I also examined the effect of an angiotensin-converting enzyme inhibitor, enalapril, on the expression of MHC isoforms in renal afferent arterioles from SHR. SM1 was expressed in both afferent and efferent arterioles from WKY and SHR, but much less expressed in efferent arterioles compared with afferent arterioles. On the other hand, SM2 was only expressed in afferent arterioles, and was less expressed in vehicle SHR than WKY. The expression of SM1 and SM2 in afferent arterioles from enalapril-treated SHR for 6 weeks and WKY were more increased than those from vehicle SHR (WKY vs vehicle SHR; $p < 0.01$, vehicle SHR vs enalapril-treated SHR; $p < 0.05$, WKY vs enalapril-treated SHR; not significant). Thus, pressure load and the antihypertensive treatment resulted in the changes of the expression of smooth muscle MHC isoforms in renal afferent arterioles, suggesting that smooth muscle MHC isoforms in renal afferent arterioles may play an important role in the regulation of glomerular function in hypertension.

Key words: Smooth muscle myosin heavy chain isoforms, Spontaneously hypertensive rat, Afferent arteriole, ACE inhibitor

Introduction

Spontaneously hypertensive rats (SHR) show a distinctive pattern of proteinuria and glomerular sclerotic changes with a drop in creatinine clearance during the first year of life and these animals are used as models of human essential hypertension and benign nephrosclerosis [1, 2] . The mechanism of glomerular damage has been thought to be associated with glomerular capillary hypertension and glomerular ischemia, mainly due to the dysfunction of afferent arterioles, which are important in regulating glomerular capillary pressure [3] . Micropuncture studies have revealed functional changes in afferent arterioles and abnormal glomerular hemodynamics in 20-week-old SHR [3-6] .

Vascular smooth muscle cells contain at least two isoforms of smooth muscle myosin heavy chain (MHC), SM1 (204 kDa) and SM2 (200 kDa) [7-10] . Isoforms of smooth muscle MHC have been reported to be markers of smooth muscle cell differentiation and have been used to detect structural changes in vascular walls that occur during physiologi-

cal and pathological remodeling [7, 11, 12] . Kimura et al found that smooth muscles in pre - and postglomerular arterioles differ in phenotype, which suggests that the different contractile properties of afferent arterioles are critical to the regulation of glomerular filtration [13] . In addition, recent studies suggested that enalapril, an angiotensin-converting enzyme inhibitor (ACEI), not only decreases blood pressure but can also correct the deteriorated glomerular hemodynamics in SHR [14] . In the present study, I made the immunohistochemical evaluation of the expression of SM1 and of SM2 in afferent arterioles from Wistar-Kyoto rats (WKY) and SHR. I also examined the effect of enalapril on the expression of smooth muscle MHC isoforms in renal afferent arterioles from SHR.

Methods

Animals

Male SHR and WKY, 10 to 14 weeks old, were obtained from Charles River Japan, Inc., Kanagawa, Japan. All rats were allowed free access to water and standard laboratory rat chow (Oriental Kobo Co., Chiba, Japan) in cages maintained at a temperature of 22 ± 1 ℃ and 45 ± 5% humidity.

Study protocol

Male SHR (14 weeks old, n = 13) were divided into two groups: the enalapril-treated (10 mg/kg body weight/day, n = 7) one, and vehicle-treated (vehicle, n = 6) one. WKY were used as a control (n = 6). Enalapril was administered orally.

After 6-week-treatment, body weight was measured. Heart rate and systolic blood pressure (BP) of unanesthetized rats were measured by a tail-cuff plethysmography (Programmed Electro Sphygmomanometer PE-300, NARCO BIO-SYSTEMS, Houston, TX, USA) at a constant room temperature (26 ℃).

Immunohistochemical studies for α-smooth muscle actin and isoforms of smooth muscle myosin, and histological studies of kidneys

At 20 weeks of age, kidneys were removed under pentobarbital anesthesia, fixed overnight in 95% ethanol with 0.5% acetate at 4 ℃, and then embedded in paraffin. Then all tissue specimens were serially sectioned to a thickness of 4 μm and stained with periodic acid -Schiff (PAS). In general, the immunohistochemical procedure was done according to the manufacturer's instructions (DAKO LSAB Kit, DAKO Co Denmark). Briefly, the sections, deparaffinized with xylene, were sequentially exposed to 0.3% hydroperoxide for 5 minutes to inhibit endogenous peroxidase activity and normal goat serum for 5 minutes to prevent nonspecific staining. The sections were incubated overnight at 4 ℃ with mouse monoclonal IgG antibody to α- smooth muscle actin (American Research Products Inc., USA) with no dilution, and with rabbit polyclonal antibodies to SM1 and SM2 (gifts from Dr. Robert S. Adelstein, National Institutes of Health, USA) [15] . Antisera were diluted at 1 to 200 for SM1 and SM2 with 0.05 M Tris HCl buffer, pH 7.6, containing 0.15 M NaCl and 0.05% Tween-20. The sections were thoroughly washed with the same buffer, and then incubated at room temperature for 2 hours with either biotin- labelled goat anti-mouse IgG (Zymed Laboratories, Inc., San Francisco, CA, USA) against antibody to smooth muscle actin or with biotin-labelled goat anti-rabbit IgG (Zymed Laboratories, Inc., San Francisco, CA, USA) against antibodies to SM1 and SM2, as a second antibody. These biotinylated antibodies were bound by streptoavidin-conjugated peroxidase, which was visualized by a diaminobenzidine reaction with a hydroperoxide substrate. Counterstaining was done with 0.1% hematoxylin for 5 minutes. As negative controls, the sections were treated identically to the samples except that preimmunized rabbit normal serum or mouse normal IgG was used as a primary antibody. Afferent arterioles were differentiated from efferent arterioles by comparing their diameters in continuous sections [16] .

Quantification of SM1 and SM2 expression

To quantify the staining of α-smooth muscle actin, SM1, and SM2 in rat renal arterioles, about 100 randomly chosen glomeruli were independently examined three times for each kidney specimen with a magnification of 100, and the mean values were used for

the analysis [17-19].

I counted paraglomerular arteriole (PGA), which was located adjacent to a glomerulus and stained with α-smooth muscle actin. The PGA was assumed to include both afferent and efferent arterioles. Number of the PGA on a section was counted and the percent PGA was calculated as follows : Percent PGA = [(number of PGA)/(number of examined glomeruli)] × 100. SM1- and SM2-positive arterioles in PGAs were also counted, and SM1 and SM2 staining scores were calculated as follows: SM1 staining score = (number of SM1-positive arterioles)/(number of PGA); SM2 staining score = (number of SM2-positive arterioles) / (number of PGA).

All of the quantification procedures were done by three independent investigators blinded to the specimen, and were averaged for each animal.

Statistical analysis

Data were evaluated by an analysis of variance. Statistical significance was taken as $p<0.05$. All values are expressed as means ± SE.

Results

Hemodynamic data

BP was significantly lower in both enalapril-treated SHR and WKY than in vehicle SHR, and was not significantly different between WKY and enalapril-treated SHR (Table 1). Neither heart rate nor body weight differed significantly among the three groups.

Histological findings in glomeruli

Light-microscopic examination with PAS stain revealed little differences in the structure of afferent arterioles among the three

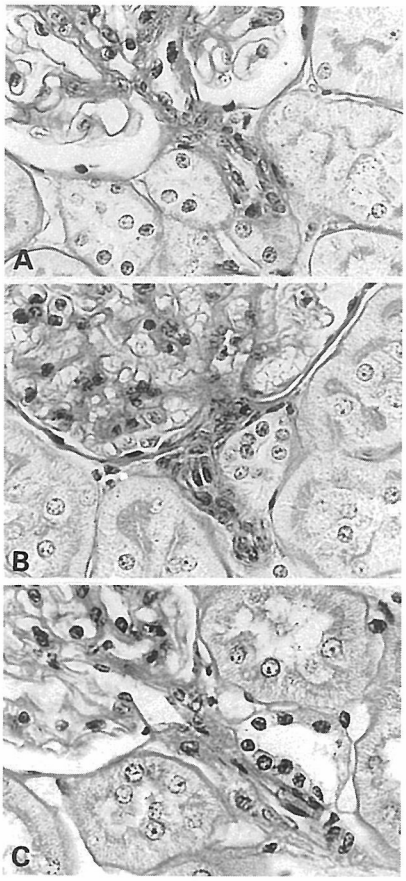

Fig. 1. *Photomicrographs of PAS-stained specimens of afferent arterioles obtained from vehicle SHR, enalapril-treated SHR, and from WKY* (× 400). (A: WKY;B: vehicle SHR; C: enalapril-treated SHR).

Table 1. Hemodynamic data

	WKY	Vehicle SHR	enalapril-treated SHR
Number of experiments	6	6	7
Heart rate, bpm	330±29	325±24	330±15
Blood pressure, mmHg	122± 6	166±10[a]	123± 3
Body weight, g	352±12	335±21	347±16

All data were obtained from 20-week-old rats. Enalapril (10 mg/kg/day) was given for 6 weeks. [a]$p<0.01$ versus WKY and enalapril-treated SHR. There was no difference in blood pressure between WKY and enalapril-treated SHR.

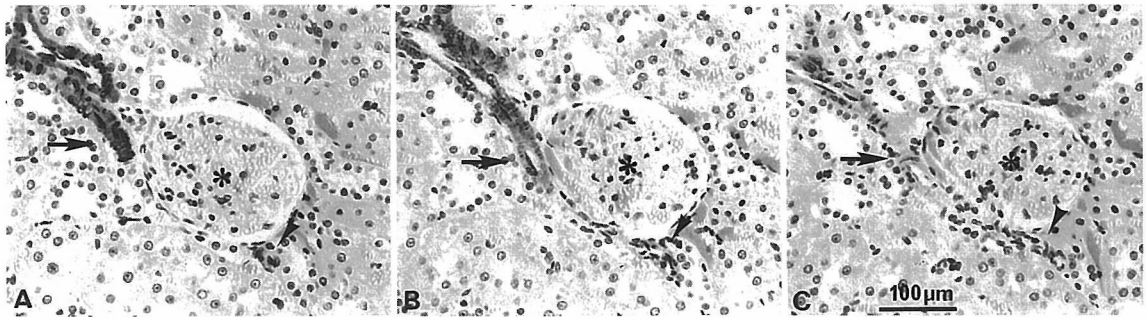

Fig. 2. *Immunohistochemical localization of α-smooth muscle actin, SM1, and SM2 in renal afferent and efferent arterioles in WKY (× 200, counterstained with hematoxylin).* (A: α-smooth muscle actin, B: SM1, and C: SM2). Arrows indicate afferent arterioles and arrowheads indicate efferent arterioles in the specimens. Asterisks show glomeruli.

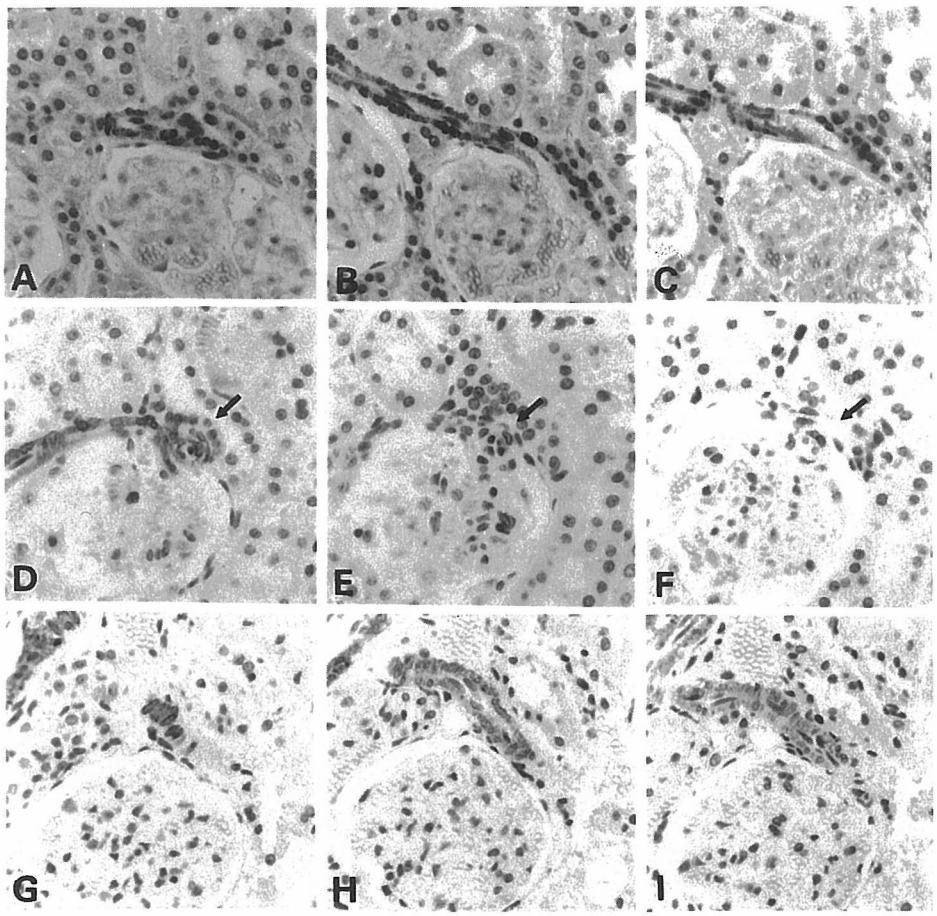

Fig. 3. *Comparison of the immunohistochemical staining of α-smooth muscle actin (A, D, and G), SM1 (B, E, and H) and SM2 (C, F, and I) in renal afferent arterioles (× 200, counterstained with hematoxylin).* (WKY: A, B, and C; vehicle SHR: D, E, and F; enalapril-treated SHR: G, H, and I). Arrows indicate afferent arterioles in the specimens from vehicle SHR (D, E, and F).

groups (Fig. 1). In addition, glomerular sclerosis was not apparent and the interstitium was relatively intact in all groups.

Expression of α-smooth muscle actin, SM1, and SM2

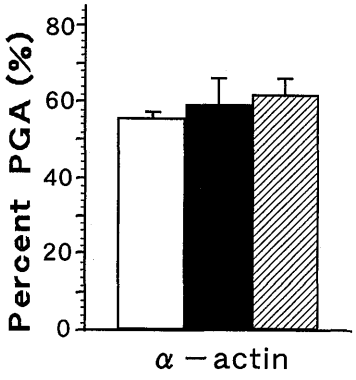

Fig. 4. *Percent PGA in WKY, vehicle, and enalapril-treated SHR.* The arterioles counted are α-smooth muscle actin positive arterioles adjacent to glomeruli. Values are means±SE. (□): WKY; (■): vehicle SHR; (▨): enalapril-treated SHR.

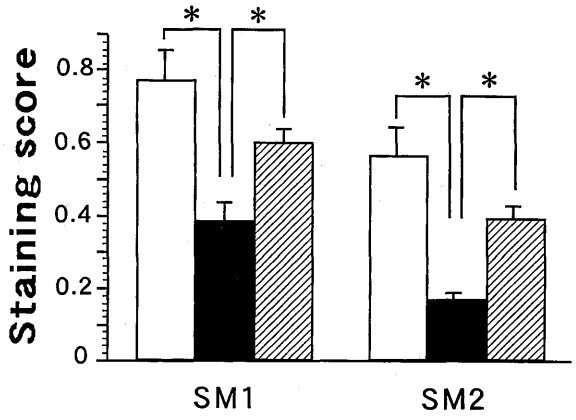

Fig. 5. *SM1 and SM2 staining scores.* SM1 and SM2 staining scores indicate the degree of expression of SM1 and SM2 in afferent arterioles. Values are means ± SE. (□): WKY; (■): vehicle SHR; (▨): enalapril-treated SHR. *; $p<0.05$

SM1 and α-smooth muscle actin were co-expressed in both efferent and afferent arterioles in all groups. The expression of SM2 in afferent arterioles was more intense in the WKY and enalapril-treated SHR than the vehicle SHR (Fig. 3). SM2 was not expressed in efferent arterioles in all three groups, as shown in Figure 2.

Staining scores

The percent PGA did not differ significantly among the three groups (Fig. 4).

Both SM1 and SM2 staining scores were significantly higher in WKY and enalapril-treated SHR than in the vehicle SHR ($p<0.05$). On the other hand. there was no significant difference in SM1 and SM2 staining score between WKY and enalapril-treated SHR (Fig. 5). In addition, there was about 70% decrease in the staining score for SM2 in vehicle SHR compared with WKY. On the other hand, the staining score for SM1 in vehicle SHR was about 50% decrease compared with WKY (Fig. 5).

Discussion

In the present study, based upon the light-microscopic observation, there were no structural changes in the renal afferent arterioles among three groups at 20 weeks, although blood pressure was already significantly increased in vehicle SHR compared with both WKY and enalapril-treated SHR. On the other hand, immunohistochemical analysis demonstrated that, in both WKY and SHR, α-smooth muscle actin was stained in efferent renal arterioles as well as afferent renal arterioles. Both SM1 and SM2 were expressed in afferent arterioles, but only SM1 was recognized in efferent arterioles in both groups. Furthermore, I demonstrated that the expression of SM1 and SM2 in afferent arterioles was significantly reduced in vehicle SHR in comparison with WKY and enalapril-treated SHR (Fig. 3).

There are some reports, suggesting dysfunction such as abnormal high resistance in renal afferent arterioles in SHR [3, 4, 20] . It is also reported that the decrease in the vasoconstrictive responsiveness of afferent arterioles caused by the changes in renal arterial perfusion pressure is greater in 14- to 16-week-old stroke-prone SHR than in WKY [5] . In addition. Holstein-Rathlou et al showed that the changes in tubular pressure due to the dynamic behavior of the tubuloglomerular feedback system are highly irregular in SHR, but not in WKY [6, 21] . These results suggest that the afferent arteriole, which is a major regulator of glomerular

capillary blood flow and an effector of both myogenic response and the tubuloglomerular feedback system, can be adversely affected by high blood pressure [6, 21] .

Although the significance of SM1 and SM2 in rat renal arterioles has not been clarified yet, Kim et al suggested that SM2 might contribute to the regulation of vascular tone and contractile properties of the ductus arteriosus of rabbits [22] . Kimura et al also reported that SM1 and α-smooth muscle actin were co-expressed both in preglomerular vessels (including the afferent arterioles) and in efferent arterioles, whereas SM2 was expressed only in the preglomerular vessels with Sprague-Dawley rats, rabbits and human. Furthermore, they showed that phenotypic differences in smooth muscle MHC isoforms between pre- and postglomerular arterioles may underlie the differences in contractile properties that are important in the regulation of glomerular filtration [13] . My immunohistochemical study agreed with Kimura et al in WKY. In addition, in this study, I also demonstrated the same tendency in the expression of SM1 and SM2 in SHR. However, the difference in the staining score for SM2 between WKY and vehicle SHR was about over 1.5 times larger than that for SM1, suggesting that well-differentiated smooth muscle cells in afferent arterioles could be less in vehicle SHR than WKY.

ACEIs can prevent glomerulosclerosis caused by abnormal glomerular hemodynamics in SHR [14] . In addition, Harrap et al showed that a short-term treatment with an ACEI in 6 to 10-week-old SHR reduced blood pressure over the long term, and that concomitant administration of angiotensin II prevented this effect, suggesting that angiotensin II or arterial pressure caused structural changes in resistant vessels [23] . These results indicate that ACEIs may prevent the development of arteriolar sclerosis in afferent arterioles. Therefore, I examined the expression of SM1 and SM2 in afferent arterioles from enalapril-treated SHR and compared it to the data from vehicle SHR and from WKY.

The present immunohistochemical study showed no differences in the expression of α-smooth muscle actin. In contrast, there was a significant difference in the expression of SM1 and SM2 in afferent arterioles among three groups . In addition, SM2 was little expressed in afferent arterioles from vehicle SHR, but expressed in afferent arterioles from WKY and enalapril-treated SHR (Fig. 3, 5). The expression of SM1 also had similar tendency to SM2 (Fig. 3, 5). These results indicate that treatment with an enalapril might upregulate the expression of SM1 and SM2 in afferent arterioles in SHR toward the normal (WKY) level compared with vehicle SHR. Since both SM1 and SM2 are major contractile proteins involved in smooth muscle contraction. ACEIs might either inhibit the downregulation or stimulate the expression of SM1 and SM2 in afferent arterioles to maintain the vascular constrictive responsiveness to perfusion pressure in SHR.

Several studies demonstrated that ACEIs might act on afferent arterioles by either decreasing vascular resistance, inhibiting angiotensin II [23] , increasing the level of bradykinin [24] , or directly inhibiting the calcium flux in smooth muscle cells [25] . My study demonstrated a possibility that these mechanisms to regulate the contraction of afferent arteriole may be associated with the expression of SM1 and SM2.

In conclusion, pressure load and the antihypertensive treatment with enalapril resulted in the changes of the expression of smooth muscle MHC isoforms in renal afferent arterioles, suggesting that smooth muscle MHC isoforms in renal afferent arterioles may play an important role in the regulation of glomerular function in hypertension. Further study is necessary to clarify the significance of SM1 and SM2 in renal afferent arterioles in hypertension.

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