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The possible roles of mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase type 2 in cardiac fibrosis in the spontaneously hypertensive rat☆

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Abstract

In hypertension, aldosterone has been demonstrated to play a crucial role in cardiac fibrosis, which generally increases cardiac morbidity and death. However, few studies have reported the expression of the mineralocorticoid receptor (MR) and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the heart under hypertensive conditions. Therefore, in this study, spontaneously hypertensive rats (SHR) were examined to elucidate the possible actions of mineralocorticoids via binding to MR. Wister Kyoto Rat (WKY), SHR, stroke-prone SHR (SHRSP), and malignant SHRSP (M-SHRSP) were used. Total RNA was extracted from the left ventricle of these rats, and examined for the expression levels of MR, 11β-HSD2 and Collagen types 1 and 3 using reverse transcription real-time quantitative polymerase chain reaction employing the Light Cycler Instrument. Blood pressure was significantly different among each group. The mean mRNA levels for MR, 11β-HSD2 and Collagen types 1 and 3 in M-SHRSP were found to be significantly increased compared to those of WKY, whereas no significant differences in mRNA levels were detected among SHR and SHRSP. Findings from the present study appear to demonstrate that MR and 11β-HSD2 mRNA significantly rise in the left ventricle of M-SHRSP and increase of these mRNA is one of the cause of cardiac fibrosis.

Keywords: SHR; Hypertension; Cardiac fibrosis; Mineralocorticoid receptor; 11β-Hydroxysteroid dehydrogenase type 2

1. Introduction

In hypertension, left ventricular hypertrophy resulting from structural remodeling of the myocardium such as myocytic hypertrophy, intersititial fibrosis and structural alterations of the coronary microcirculation [1] have been shown to greatly contribute to cardiac morbidity and subsequent death of patients [2–4].

Myocardial fibrosis has always been associated with increased levels of circulating aldosterone or local stimulation of the renin–angiotensin–aldosterone system [5]. However, little is known on the correlation among other factors such as MR, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) which has been shown to protect MR from occupancy by glucocorticoids [6,7], arterial blood pressure and cardiac fibrosis.

In the present study, we examined spontaneously hypertensive rats (SHR) which were classified based on the severity of hypertension, for the relative mRNA expression of MR, 11β-HSD2, and Collagen type 1 (Collagen 1) and Collagen type 3 (Collagen 3), both of which have been demonstrated to be closely associated with myocardial fibrosis [8] using semi-quantitative PCR.

2. Materials and methods

2.1. Animals

Wister Kyoto (WKY), SHR, stroke-prone SHR (SHRSP) and malignant SHRSP (M-SHRSP) rats were employed in this study. These three strains of hypertensive rats were established from WKY [9]. The animals were bred and fed with the blood pressure of each strain carefully maintained

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at its respective fixed level in the Kinki University School of Medicine. Each animal was sacrificed at the age of 15 weeks, when the blood pressure achieved peak levels [10].

2.2. RNA isolation and cDNA synthesis

Total RNA was extracted by homogenizing frozen tissues from these specimens using TRIzol reagent (Gibco-BRL, Grand Island, NY) followed by a phenol-chloroform phase extraction and isopropanol precipitation. The SUPERSCRIPT Preamplification system reverse transcription kit (Gibco-BRL, Grand Island, NY) was used to synthesize and amplify first strand complementary DNA (cDNA). The cDNA was synthesized from 2 μg total RNA using 25 ng/μl Oligo (dT) Primer (Life Technologies Inc., Gaintherburg, ND) on a PTC-200 Peltier Thermal Cycler DNA Engine (MJ Reserch Inc., Watertown, MA).

2.3. Real-time quantitative polymerase chain reaction (qPCR)

Rat gene-specific PCR primers for MR, 11β-HSD2, Collagen 1 and 3, and the house-keeping gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) are listed in Table 1. Real-time PCR was carried out with the LightCycler Instrument (Roche Molecular Biochemicals, Mannheim, Germany) using the DNA binding dye SYBR Green I for detection of PCR products. The expression levels of target genes were evaluated by the ratio of the target mRNA to that of GAPDH mRNA and evaluated as a ratio compared to that of each positive control. As a positive control, frozen tissues of kidney were used for MR and 11β-HSD2, and those of skin were used for Collagen 1 and 3.

2.4. Statistical analysis

When Kruskal–Wallis showed a significant difference between groups, pairwise comparisons of each group versus the control group were performed using Mann–Whitney *U* test. Significance was set at $P < 0.05$.

Table 1
Primer sequences in rats

Gene	Accession no.	Position	Primer sequence
GAPDH	M17701	638–658	5′-aca tca tcc ctg cat cca ct-3′
		876–896	5′-ggg agt tgc tgt tga agt ca-3′
MR	NM013131	1573–1593	5′-aga aag gtg ctc acg acg tt-3′
		1772–1792	5′-cgc ctg aac atg agt gct tg-3′
11β-HSD2	U22424	1084–1103	5′-ccc gtt gta gat gcc atc a-3′
		1304–1324	5′-agc tga tac tgt ggg gga ag-3′
Collagen 1	Z78279	4184–4202	5′-tca cct aca gca cgc ttg-3′
		4411–4429	5′-ggt ctg ttt cca ggg ttg-3′
Collagen 3	X70369	1801–1819	5′-ata tca aac acg caa ggc-3′
		1956–1976	5′-gat taa agc aag agg aac ac-3′

3. Results

3.1. Hypertension model

Blood pressure was significantly different among each group. (WKY: 139.6±2.7 mmHg; SHR: 177.2±6.1 mmHg; SHRSP: 218.6 ± 32.7 mmHg; and M-SHRSP: 255.6 ± 8.8 mmHg).

3.2. Expression of MR mRNA

The average level of MR mRNA in M-SHRSP increased significantly (12.3-hold) compared to that of WKY, whereas SHR and SHRSP did not, as shown in Fig. 1 ($P = 0.0011$, Kruskal-Wallis; $P = 0.0090$, Mann–Whitney *U* test).

3.3. Expression of 11β-HSD2 mRNA

Fig. 2 demonstrated that 11β-HSD2 mRNA yielded a similar pattern with respect to MR mRNA. The average levels of 11β-HSD2 in M-SHRSP were 3.03-fold compared to that of WKY. ($P = 0.0365$, Kruskal–Wallis; $P = 0.0433$, Mann–Whitney *U* test)

3.4. Expression of Collagen 1 and 3 mRNA

The patterns of abundance of Collagen 1 mRNA and those of Collagen 3 mRNA were similar (Figs. 3 and 4, respectively) and correlated with those of MR mRNA. The average levels of Collagen 1 and 3 mRNAs in M-SHRSP were significantly elevated, 42.0-fold and 7.67-fold, respectively, compared to rats in the WKY group. ($P = 0.0242$, Kruskal–Wallis; $P = 0.0209$, Mann–Whitney *U* test and $P = 0.0149$, Kruskal–Wallis; $P = 0.0209$, Mann–Whitney *U* test, respectively).

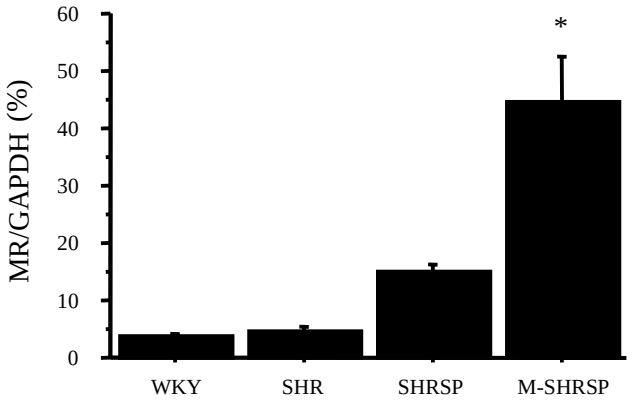

Fig. 1. Levels of mRNA expression for MR in WKY, SHR, SHRSP and M-SHRSP rats (mean ± S.E.; $n = 6$, respectively). Expression levels are summarized as a ratio of GAPDH. $P = 0.0011$, Kruskal–Wallis; $^{*}P = 0.0090$ vs. WKY, Mann–Whitney *U* test.

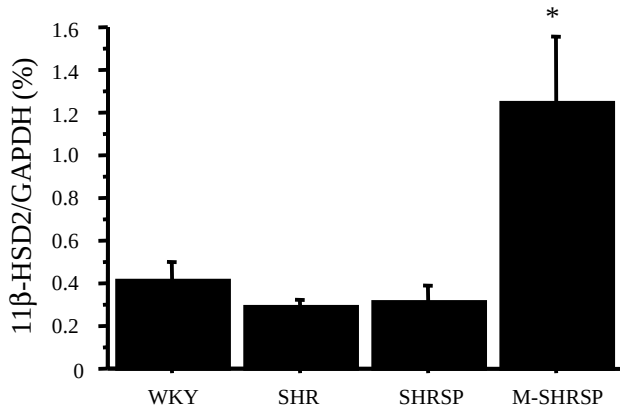

Fig. 2. Levels of mRNA expression for 11β-HSD2 in WKY, SHR, SHRSP and M-SHRSP rats (mean ± S.E.; $n = 6$, respectively). Expression levels are summarized as a ratio of GAPDH. $P = 0.0365$, Kruskal–Wallis; $^{*}P = 0.0433$ vs. WKY, Mann–Whitney U test.

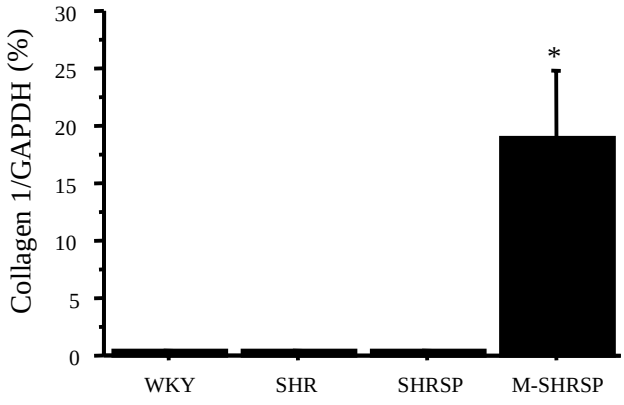

Fig. 3. Levels of mRNA expression for Collagen 1 in WKY, SHR, SHRSP and M-SHRSP rats (mean ± S.E.; $n = 6$, respectively). Expression levels are summarized as a ratio of GAPDH. ($P = 0.0242$, Kruskal–Wallis; $^{*}P = 0.0209$ vs. WKY, Mann–Whitney U test).

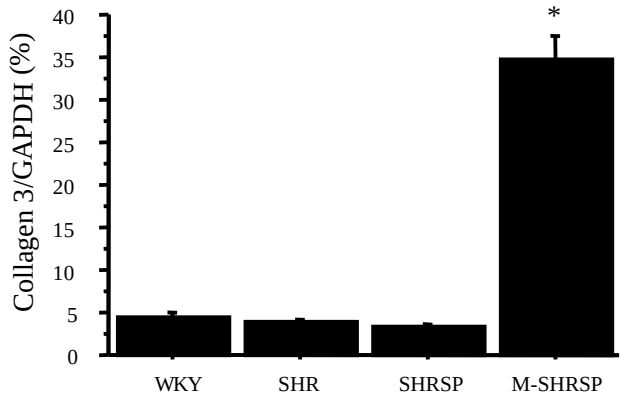

Fig. 4. Levels of mRNA expression for Collagen 3 in WKY, SHR, SHRSP and M-SHRSP (mean ± S.E.; $n = 6$, respectively). Expression levels are summarized as a ratio of GAPDH. $P = 0.0149$, Kruskal–Wallis; $^{*}P = 0.0209$ vs. WKY, Mann–Whitney U test.

4. Discussion

The present study demonstrated that MR mRNA increased steeply in the left ventricle of M-SHRSP (Fig. 1), which has been reported to have significantly high plasma aldosterone concentration [11]. Aldosterone produces a dose-dependent increase of MR and its mRNA levels in cultured primary hippocampal neurons [12]. Therefore, the presence of positive feedback mechanism of aldosterone to MR expression may take place in the heart.

11β-HSD2 mRNA also increased in the same manner as MR mRNA (Fig. 2). Cortisol occupies MR as antagonists of aldosterone in nonepithelial tissue like heart [13–15], and this enzyme plays a crucial role in the human heart to promote cortisol metabolism and to confer aldosterone specificity on MR [7]. Increment of only MR therefore does not necessarily represent augmentation of aldosterone action, but increment of both MR and 11β-HSD2 strengthen effects of aldosterone to the heart.

In conclusion, findings of the present study appear to demonstrate that MR and 11β-HSD2 mRNA significantly rise in the left ventricle of M-SHRSP and increase of these mRNA one of the cause of cardiac fibrosis determined by the amounts of collagen and related to severity of hypertension.

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