Blood Pressure Variability Activates Cardiac Mineralocorticoid Receptor and Induces Cardiac Remodeling in Hypertensive Rats

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Background: Hypertensive patients with large blood pressure variability (BPV) have aggravated target organ damage. Because the aldosterone/mineralocorticoid receptor (MR) system is a possible mechanism of hypertensive organ damage, we investigated in spontaneously hypertensive rats (SHRs) whether a specific MR blocker, eplerenone, would prevent BPV-induced aggravation of hypertensive cardiac remodeling.

Methods and Results: A rat model of a combination of hypertension and large BPV was created by performing bilateral sinoaortic denervation (SAD) in SHRs. SAD increased BPV without changing mean BP. SAD induced perivascular macrophage infiltration and aggravated myocardial fibrosis and cardiac hypertrophy, resulting in LV systolic dysfunction. Immunohistostaining revealed SAD-induced translocation of MRs into the nuclei (ie, MR activation) of the intramyocardial arterial medial cells and cardiac myocytes. SAD increased phosphorylation of p21-activated kinase1 (PAK1), a regulator of MR nuclear translocation. Chronic administration of a subdepressor dose of eplerenone prevented MR translocation, macrophage infiltration, myocardial fibrosis, cardiac hypertrophy, and LV dysfunction, while not affecting BPV. Circulating levels of aldosterone and cortisol were not changed by SAD.

Conclusions: Eplerenone inhibited the aggravation of cardiac inflammation and hypertensive cardiac remodeling, and thereby prevented progression of LV dysfunction in SHRs with large BPV. This suggests that the PAK1-MR pathway plays a role in cardiac inflammation and remodeling induced by large BPV superimposed on hypertension, independent of circulating aldosterone.   (*Circ J* 2013; **77:** 1474–1481)

Key Words: Blood pressure variability; Cardiac hypertrophy; Fibrosis; Inflammation; Mineralocorticoids

T he aldosterone/mineralocorticoid receptor (MR) system has been highlighted as a mechanism of hypertensive organ damage.[1] It has long been considered that aldosterone-induced hypertensive organ damage is dependent on blood pressure (BP) elevation per se, which is attributed to increased sodium and water retention mediated by MR activation in the renal tubular epithelium.[2] There is increasing evidence of the direct action of aldosterone on target organs in hypertensive disease: MRs have been identified in the heart and blood vessels.[3,4] In saline-treated stroke-prone spontaneously hypertensive rats (SHRs), a nonselective MR blocker,

spironolactone, reduced proteinuria and the incidence of stroke, independently of its BP-lowering effect.[5] Rocha et al demonstrated that a combination of high-salt diet and extrinsic aldosterone administration induced severe hypertension and vascular inflammation in the heart and that a selective MR blocker, eplerenone, blocked the cardiac inflammation and remodeling.[6] Recently, it has been shown that MR activation causes target organ damage even at normal or low levels of circulating aldosterone.[7]

Nowadays, more attention is being paid to the increase in short-lasting or more sustained BP variability (BPV) as a mech-

anism of hypertensive target organ damage, in addition to elevated mean BP.[8–10] Although large BPV aggravates cardiac remodeling in hypertensive patients,[8,11,12] the mechanism whereby large BPV aggravates hypertensive cardiac remodeling remains poorly understood. Recently, we created a rat model of a combination of hypertension and large BPV by performing bilateral sinoaortic denervation (SAD) in SHRs.[13] Using this model, we have shown for the first time that chronic cardiac inflammation plays an important role in the BPV-induced aggravation of hypertensive cardiac remodeling and systolic dysfunction, and that the cardiac angiotensin II system is involved in the mechanism of chronic inflammatory changes. However, we did not investigate the role of the aldosterone/MR system in BPV-induced cardiac remodeling.

Accordingly, we hypothesized that the MR activation would induce cardiac inflammation and thereby would aggravate cardiac remodeling in hypertension associated with large BPV. To address this issue, we investigated the effect of eplerenone on the BPV-induced aggravation of hypertensive cardiac remodeling in SHRs.

Methods

All experiments were conducted in accordance with the Regulation for Animal Experimentation at Kurume University. Male SHRs were purchased from SLC Inc (Shizuoka, Japan) and housed under standard conditions of humidity, room temperature and a 12:12-h dark-light cycle. They were provided with free access to tap water and normal-salt chow (0.26% NaCl).

Study Groups
SHRs were randomly assigned to the following 4 groups (n=14 per group): Sham+vehicle (undergoing sham operation and receiving vehicle); Sham+eplerenone group (receiving sham operation and eplerenone); SAD+vehicle group (receiving SAD and vehicle); and SAD+eplerenone group (receiving SAD and eplerenone). At 12 weeks of age, the rats underwent SAD or the sham operation and 7 days later, $25\,mg \cdot kg^{-1} \cdot day^{-1}$ eplerenone (Pfizer Inc, New York, NY, USA) or vehicle was administered orally every day. The dose of eplerenone was the maximum that would not reduce BP in SHRs during the observation period.

Bilateral SAD
At 12 weeks old, SHRs underwent bilateral SAD as previously described.[13,14] Briefly, rats were anesthetized intraperitoneally with a mixture of ketamine (50 mg/kg), medetomidine (0.5 mg/kg), and atropine sulfate (0.5 mg/kg). The aortic depressor and superior laryngeal nerves were cut bilaterally and the superior cervical ganglia and cervical sympathetic trunks were resected. Then, the carotid bifurcation and the external and internal carotid arteries were stripped off the surrounding connective tissues, followed by the application of 10% phenol in absolute ethanol.

Telemetric Hemodynamic Recording
At 6 weeks after SAD operation (18 weeks old), rats underwent implantation of a BP radiotelemeter (model TA 11 PA-C 40, Data Sciences International, Minneapolis, MN, USA) under intraperitoneal anesthesia with a mixture of ketamine (50 mg/kg) and medetomidine (0.5 mg/kg).[13] Thereafter, rats were housed individually in hemodynamic monitoring cages. At 7 days after telemeter implantation (19 weeks old), BP and heart rate were monitored for 24 h under unrestricted, conscious conditions. The 24-h average and coefficient of variance of mean BP and heart rate were calculated as descriptive statistics of the distribution variability.[13]

Echocardiographic Study
After hemodynamic monitoring, echocardiographic measurement of left ventricular (LV) mass and LV fractional shortening was performed using a commercially available echocardiographic machine equipped with a 17.5-MHz transducer (Vevo770 High-Resolution Imaging System, VisualSonics Inc, Toronto, Canada) under anesthesia with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg).[15]

Morphometric Analysis and Immunohistostaining
On the day after echocardiographic monitoring, rats were killed with an overdose of ketamine and xylazine injected intraperitoneally. Blood was drawn from the right atrium for measurement of serum aldosterone and cortisol at a commercially available laboratory (SRL Co, Fukuoka, Japan). After rats were perfuse-fixed with 4% neutrally buffered paraformaldehyde at 100 mmHg, the LV was isolated from both atria and the right ventricular free wall, then weighed, and immediately embedded in paraffin. The paraffinized sections were subjected to morphometry and immunohistostaining.[15] The shortest transverse myocyte diameter and the percent area of myocardial fibrosis were measured in 3 independent hematoxylin-eosin-stained sections and Mallory-Azan-stained sections of each rat, respectively.[15,16] Immunohistostaining against ED-1 (Chemicon International, Temecula, CA, USA) or phosphorylated form of p21-activated kinase-1 (PAK1) at Ser144 (Abcam, Cambridge, UK) was performed as previously described.[17–20] ED-1-labeled macrophages were counted at ×200 magnification in 3 independent entire cross-sections of each rat.[21,22]

Immunofluorescence Study
Immediately after hemodynamic monitoring, rats were killed with an overdose of ketamine and xylazine by intraperitoneal injection and then perfused with ice-cold saline for 5 min. The unfixed LV was isolated, snap-frozen in liquid nitrogen, and stored at –80°C until use. Cryosections were used for the immunofluorescence study against MR (Abcam) with counterstaining using DAPI (DOJINDO, Kumamoto, Japan), as described previously.[23]

Real-Time Reverse-Transcription-Polymerase Chain Reaction Analysis
Unfixed LV samples were homogenized in lysis buffer using FastPrep homogenizer (MP-Biomedicals, Irvine, CA, USA). Aliquots of tissue homogenate were used for total RNA extraction and RT, as described previously.[24,25] Equal amounts of the resulting cDNA underwent real-time PCR for rat type-B natriuretic peptide (BNP), transforming growth factor-β1 (TGF-β), sgk-1, and β-actin commercially available primers (Applied Biosystems, Tokyo, Japan).[15,26] Expression level of the target gene was normalized by the β-actin level in each sample.

Immunoblotting
After homogenization in lysis buffer, the total protein was extracted, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subjected to immunoblotting.[24,27] Blots were probed with an antibody against phosphorylated PAK1 (p-PAK1) (Abcam), and re-probed with an antibody against PAK1/3/5 (Cell Signaling Technology, Boston, MA, USA). The signals were detected with an enhanced chemiluminescence reagent kit (Pharmacia Biotech).[26,28] The intensity of the immunoreactive bands was quantified by an image ana-
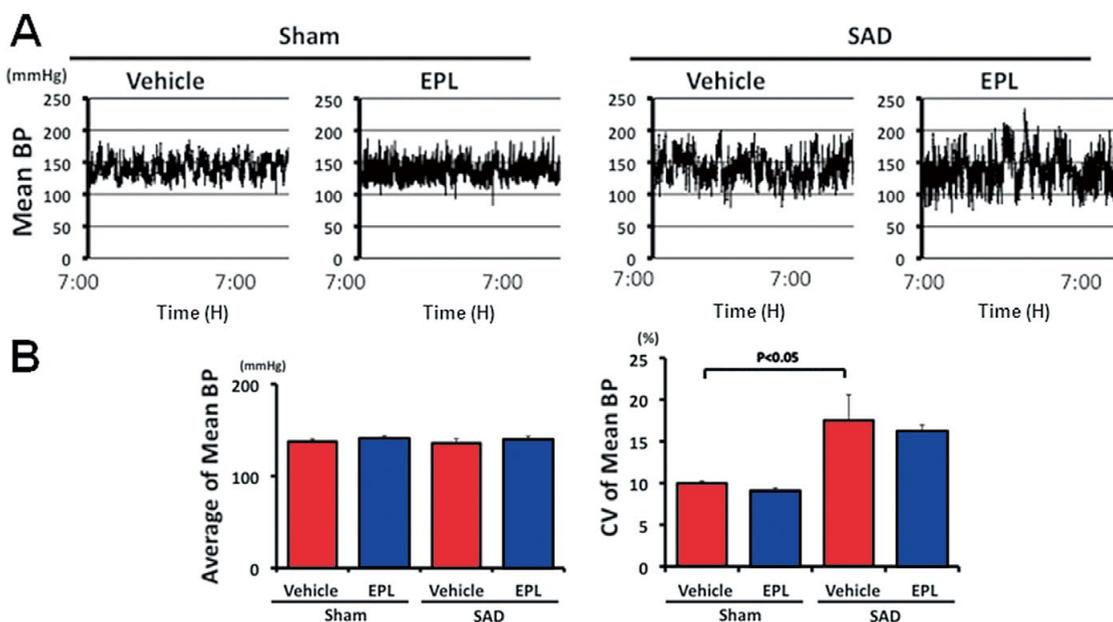
Figure 1. Effects of bilateral sinoaortic denervation (SAD) and eplerenone (EPL) on mean blood pressure (BP) and variability (BPV) in SHRs at 7 weeks after SAD. (**A**) Representative 24-h telemetric recordings of mean BP. (**B**) Pooled data of the effects of SAD and eplerenone on the average (**Left**) and coefficient of variance (CV) (**Right**) of mean BP in SHRs. Bar=1×SD (n=14). SHR, spontaneously hypertensive rat.

Table. General Characteristics of the Experimental Groups at 7 Weeks After Operation

	Sham		SAD	
	Vehicle	**Eplerenone**	**Vehicle**	**Eplerenone**
LV hypertrophy				
Body weight (g)	347±16	346±22	316±31	308±32
LV weight/tibial length (mg/mm)	2.93±0.18	2.95±0.24	3.16±0.22**	3.03±0.25**,†
Heart rate (beats/min)				
Average	297±29	321±21	314±27	318±39
Coefficient of variance	13.2±1.3	12.8±1.6	11.0±2.5	11.2±1.4
Circulating levels				
Aldosterone (pg/ml)	163±77	275±227	197±236	510±457
Cortisol (ng/ml)	486±208	523±168	356±181	485±106

Data are mean±SD. **$P<0.01$ vs. Sham; †$P<0.05$ vs. vehicle.
Because of skewed distributions, the natural logarithmic transformations were performed for circulating aldosterone and cortisol levels.
LV, left ventricular; SAD, sinoaortic denervation.

lyze system (ATTO Co, Tokyo, Japan). The expression level of p-PAK1 was normalized by the PAK1 level.
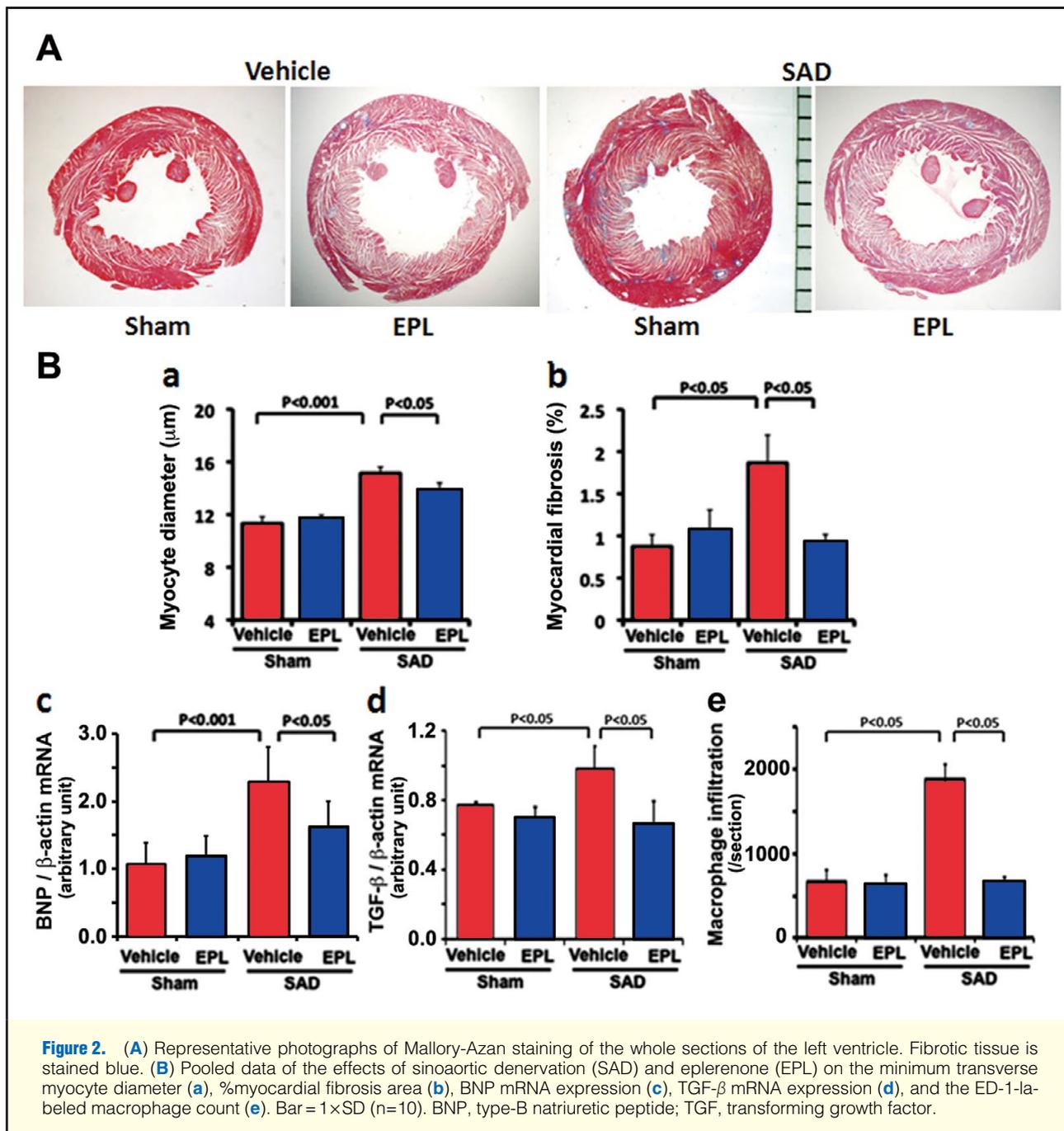
Statistical Analysis

Data are expressed as mean±SD. Quantitative histological analysis was performed by 2 observers in a blinded manner. The inter- or intraobserver variability was <5% in each experiment. One-way ANOVA followed by Scheffé's F test was performed for the comparisons. $P<0.05$ was considered statistically significant.

Results

Effects on Mean BP and BPV

Figure 1A shows representative 24-h telemetric recordings of mean BP at 7 weeks after SAD or Sham operation. Mean BP levels were similar in the 4 groups (Figure 1B). SAD increased the coefficient of the variance of mean BP. Thus, the SAD+ vehicle group was considered as the model of hypertension with large BPV. A subdepressor dose of eplerenone did not affect the average or variability of mean BP. The average and variability of heart rate did not differ among the 4 groups (Table).

Figure 2. (**A**) Representative photographs of Mallory-Azan staining of the whole sections of the left ventricle. Fibrotic tissue is stained blue. (**B**) Pooled data of the effects of sinoaortic denervation (SAD) and eplerenone (EPL) on the minimum transverse myocyte diameter (**a**), %myocardial fibrosis area (**b**), BNP mRNA expression (**c**), TGF-β mRNA expression (**d**), and the ED-1-labeled macrophage count (**e**). Bar = 1×SD (n=10). BNP, type-B natriuretic peptide; TGF, transforming growth factor.

Effects on Cardiac Hypertrophy

There was no difference in the ratio of LV wet weight to tibial length (LVW/TL) and cardiac myocyte diameter between the Sham+vehicle and Sham+eplerenone groups (**Table**, **Figure 2**). SAD increased the LVW/TL and myocyte diameter in SHRs, associated with BNP mRNA upregulation (**Figure 2B-a,2B-c**). Eplerenone reduced the SAD-induced increases in LVW/TL and myocyte diameter, as well as the BNP expression level.

Effects on Fibro-Inflammatory Changes

Mild perivascular fibrosis was observed in the Sham+vehicle group (**Figure 2A,2B-b**). SAD enhanced perivascular fibrosis and interstitial reparative fibrosis, associated with TGF-β mRNA upregulation (**Figure 2B-b,2B-d**). Eplerenone prevented the SAD-induced myocardial fibrosis and TGF-β induction. SAD induced perivascular macrophage infiltration in the heart, which was prevented by eplerenone (**Figure 2B-e**).

Effects on Echocardiographic Data

SAD increased the echocardiographic LV mass index (**Figure 3**) and eplerenone prevented the SAD-induced aggravation of LV hypertrophy. LV fractional shortening did not differ between the Sham+vehicle and Sham+eplerenone groups. SAD reduced LV fractional shortening, and eplerenone prevented the SAD-induced LV systolic dysfunction.
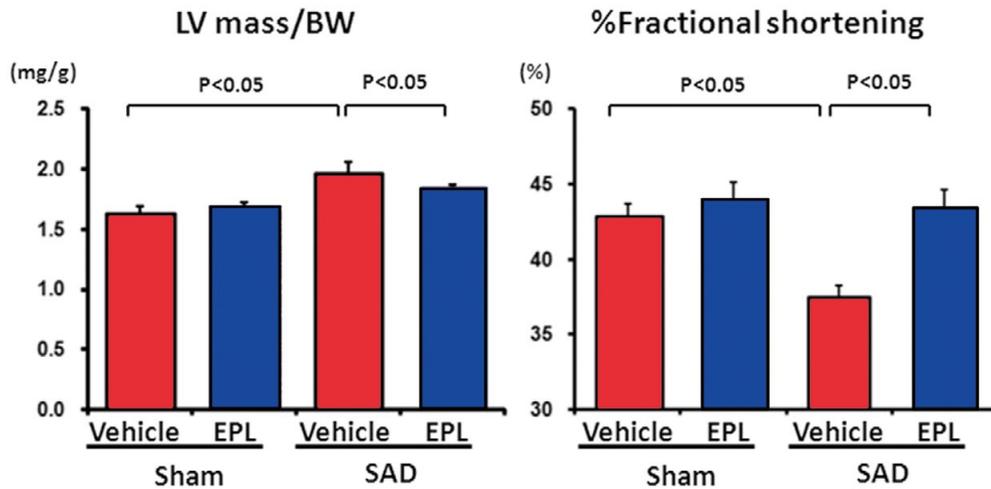
Figure 3. Echocardiographic data. Pooled data of the effects of sinoaortic denervation (SAD) and eplerenone (EPL) on the ratio of left ventricular mass to body weight (LV mass/BW) and %fractional shortening of the LV. Bar = 1×SD (n=10).
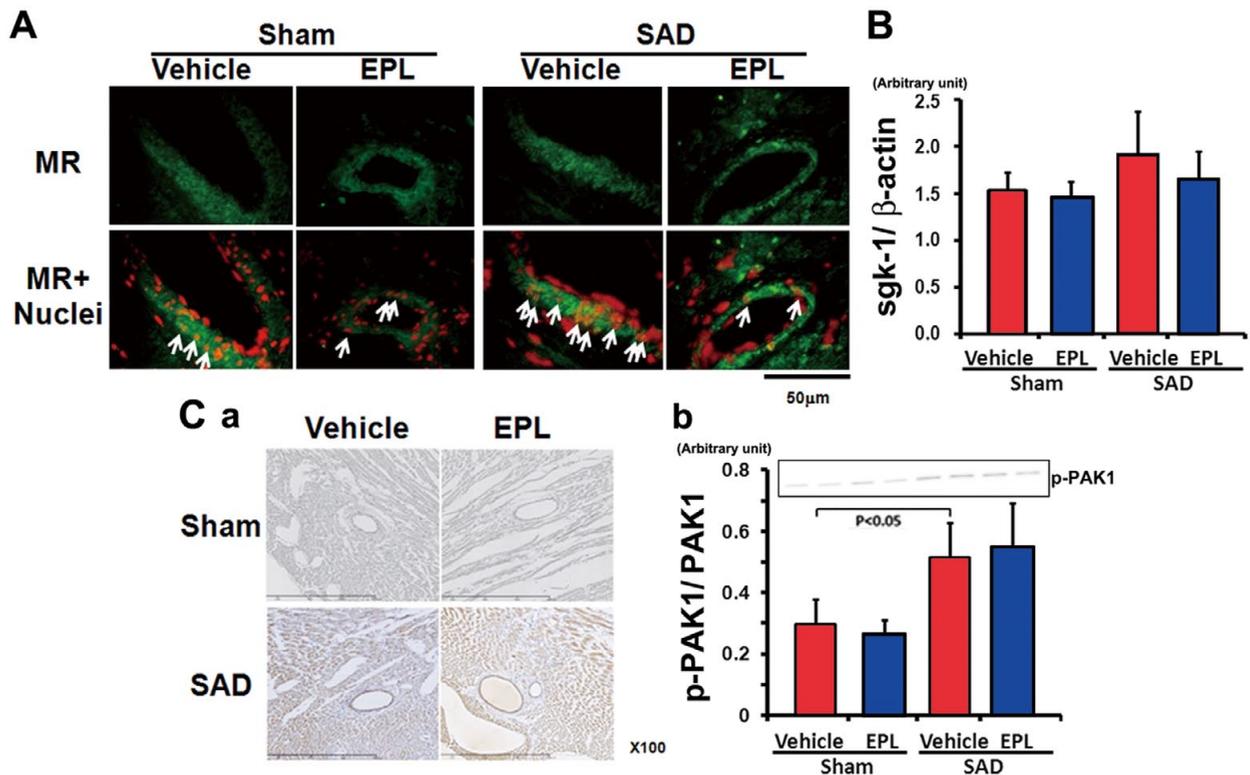
Figure 4. (**A**) Representative immunofluorescence microphotographs demonstrating the effects of sinoaortic denervation (SAD) and eplerenone (EPL) on activation of the aldosterone/mineralocorticoid receptor (MR). (**Top**) MR staining; (**Bottom**) merged image with MR and nuclear staining. MR: green; DAPI-stained nuclei, orange; MR translocated into the nuclei (activated MR), yellow (white arrows). (**B**) Pooled data of the effects of SAD and EPL on sgk-1 mRNA expression. (**C-a**) Representative immuno-fluorescence microphotographs for phosphorylated PAK1, the active form of PAK1 (brown color). (**C-b**) Pooled data of the effects of SAD and EPL on phosphorylated PAK1 (p-PAK1). Inset: representative immunoblotting for p-PAK1. Bar = 1×SD (n=6).

Effects on MR Activation

Neither SAD nor eplerenone changed the MR mRNA expression levels in the heart (data not shown). The immunofluorescence study revealed that MRs mainly existed in the cytosol of the medial cells of intramyocardial arteries and in cardiac myocytes (**Figure 4A**). A small number of cells showed MR immunoreactivity in the nucleus. SAD translocated MRs into the nucleus, indicating MR activation. Eplerenone prevented the SAD-induced MR nuclear translocation.

To further examine the effects of SAD on MR activation, the mRNA expression levels of sgk-1, a known downstream molecule of MR activation, were investigated. SAD increased sgk-1 expression levels and eplerenone attenuated the SAD-induced sgk-1 upregulation, although the changes did not reach statistical significance (**Figure 4B**).

Effects on PAK1 Activation

Next, we investigated the levels of phosphorylation of PAK1. Immunohistostaining showed no apparent PAK1 phosphorylation in the Sham+vehicle and Sham+eplerenone groups (**Figure 4C-a**). SAD increased the level of PAK1 phosphorylation in the intramyocardial arteries and myocardium. Eplerenone did not change the SAD-induced PAK1 phosphorylation. Immunoblotting confirmed that SAD increased PAK1 phosphorylation, which was unchanged by eplerenone (**Figure 4C-b**).

Circulating Levels of Aldosterone and Cortisol

There were no differences between the Sham+vehicle and SAD+vehicle groups in the circulating levels of aldosterone and cortisol (**Table**). Eplerenone had no significant effects on these, although the aldosterone levels tended to be higher in eplerenone-treated animals.

Discussion

The present study has shown for the first time that a combination of hypertension and large BPV induces MR activation of the intramyocardial arterial cells and cardiac myocytes, without changes in circulating aldosterone levels. It also demonstrated that a subdepressor dose of eplerenone not only blocked the BPV-induced MR activation but also ameliorated chronic cardiac fibro-inflammatory changes, aggravation of hypertensive cardiac remodeling, and LV dysfunction, while not affecting the mean BP and its BPV, in the SHR+SAD group.

Recently, we showed that SHR+SAD is a model of a combination of chronic hypertension and large BPV.[13] In this model, large BPV aggravates hypertensive cardiac remodeling by activating chronic cardiac fibro-inflammatory changes, independently of activation of systemic inflammation, systemic renin-angiotensin system, and sympathetic nerve system. Thus, we used this model to investigate the role of the aldosterone/MR system in BPV-induced aggravation of hypertensive cardiac remodeling. To distinguish the direct MR blocking effect from its BP-lowering effect, the maximum dose of eplerenone that would not reduce BP was used in this study.

The most important findings of this study are that the increase in BPV was associated with MR activation in the heart (**Figure 4A,4B**) and that a subdepressor dose of eplerenone prevented the BPV-induced aggravation of hypertensive cardiac remodeling (cardiac hypertrophy and myocardial fibrosis) and LV dysfunction without affecting BPV (**Figures 1–3**). The prevention of cardiac remodeling by eplerenone was associated with inhibition of the SAD-induced upregulation of both BNP, the molecular marker of cardiomyocyte hypertrophy, and TGF-β, a fibro-inflammatory cytokine (**Figure 2B-c,2B-d**).

These effects of eplerenone were attributable to a MR blocking action because eplerenone inhibited the BPV-induced MR nuclear translocation (ie, an indicator of MR activation; **Figure 4A**).[3,4] The observation that eplerenone reduced SAD-induced upregulation of sgk-1, a target gene of MR activation, also supports this hypothesis, although the changes were not statistically significant (**Figure 4B**). It has been shown that MR activation provokes the association of the MR with the dynein/dynactin motor protein complex, which induces the transportation of the MR toward the nucleus and the binding of the MR to a hormone response element to regulate the expression of its target genes.[29] Because spironolactone has been shown to reduce the MR nuclear translocation by inhibiting the formation of the motor protein complex,[30] the same mechanism is considered to operate for eplerenone. SAD had no effects on the mRNA expression levels of MR, irrespective of eplerenone treatment, suggesting that upregulation of MR was not involved in the mechanism of SAD-induced MR activation.

Eplerenone prevented both the cardiac hypertrophy and fibrosis induced by large BPV (**Figure 2**). Recently, we showed that chronic inflammation manifested by macrophage infiltration and TGF-β induction plays a role in the BPV-induced aggravation of cardiac hypertrophy and fibrosis in this model.[13] In the present study, SAD induced macrophage infiltration and TGF-β upregulation in SHRs, and the fibro-inflammatory changes were prevented by eplerenone (**Figure 2B-c, 2B-d**). These findings suggest that inhibition of chronic fibro-inflammatory changes is the mechanism whereby eplerenone prevents the BPV-induced aggravation of cardiac hypertrophy and fibrosis in the hypertensive heart. It is considered that the large BPV-induced deterioration of LV systolic function was ameliorated because eplerenone prevented myocyte damage and loss, with resultant reparative cardiac fibrosis (**Figures 2, 3 Right**).

There is increasing evidence of cross-talk between the MR and angiotensin II type 1 receptor (AT1R) pathways during cardiovascular remodeling, although the molecular mechanisms remain unclear: in vascular smooth muscle cells (VSMCs), MR activation amplifies angiotensin II-induced signals, such as ERK1/2, and upregulates AT1R expression,[31,32] whereas activation of AT1R is required for activation of aldosterone-induced signaling pathways in VSMCs.[33] Cross-talk between MR and AT1R in the heart has been also suggested because cardiomyocyte-specific MR overexpression enhances cardiac hypertrophy and fibrosis without changing BP levels in mice treated with chronic angiotensin II infusion.[34] We have demonstrated that activation of cardiac AT1R plays a role in SAD-induced chronic inflammation and aggravation of hypertensive remodeling in this model.[13] It is noteworthy that SAD upregulated cardiac AT1R expression and increased cardiac ERK1/2 activity in SHR.[13,14] Thus, it is possible that cross-talk between MR and AT1R has a role in the SAD-induced aggravation of hypertensive remodeling.

The present study results suggest a role for aldosterone-independent MR activation in cardiac inflammation in SHRs with large BPV, because circulating levels of aldosterone were unchanged (**Table**) and the cardiac aldosterone content was below the detectable range in this model (data not shown). Cortisol is another candidate ligand for the MRs, because cortisol can bind them in cardiac myocytes that do not have 11β-hydroxysteroid dehydrogenase, a cortisol-degrading enzyme.[3] However, SAD did not change the circulating cortisol levels (**Table**). Thus, it was less likely that cortisol participated in the MR activation in this model.

Study Limitations

From this study, the mechanism of MR activation by large BPV still remains unknown. There is increasing evidence of ligand-independent activation of the MRs.[35,36] It is noteworthy that PAK1 was activated by large BPV in SHRs (**Figure 4C**). PAK1 is known as a serine/threonine kinase that regulates cytoskeletal remodeling and cell motility.[37] Recently, it has been shown that PAK1 regulates the activity of steroid receptors and promotes nuclear translocation of the MRs.[38,39] Also, PAK1 is an effector protein of a small G-protein Rac1, which is a ligand-independent activator of the MRs.[40] As shown in **Figure 4C**, eplerenone did not affect BPV-induced PAK1 phosphorylation. Taken together, the findings suggest that PAK1 phosphorylation may be upstream of BPV-induced MR activation in SHRs. Further investigation is needed to address this issue.

Conclusions

Increased BPV induced cardiac MR activation and eplerenone inhibited the BPV-induced aggravation of cardiac remodeling and LV dysfunction without changing mean BP or BPV in SHRs. Inhibition of chronic cardiac inflammation may be the mechanism whereby eplerenone prevents BPV-induced aggravation of hypertensive cardiac remodeling. A large BPV is a characteristic feature of hypertensive patients with advanced atherosclerosis, especially with carotid atherosclerosis, or in the elderly. Therefore, eplerenone may be useful for preventing the worsening of hypertensive cardiac remodeling in hypertensive patients with large BPV.

References

1. Zia AA, Kamalov G, Newman KP, McGee JE, Bhattacharya SK, Ahokas RA, et al. From aldosteronism to oxidative stress: The role of excessive intracellular calcium accumulation. *Hypertens Res* 2010; **33:** 1091–1101.
2. Gavras H, Brunner HR, Laragh JH, Vaughan ED Jr, Koss M, Cote LJ, et al. Malignant hypertension resulting from deoxycorticosterone acetate and salt excess: Role of renin and sodium in vascular changes. *Circ Res* 1975; **36:** 300–309.
3. Fuller PJ, Young MJ. Mechanisms of mineralocorticoid action. *Hypertension* 2005; **46:** 1227–1235.
4. Schiffrin EL. Effects of aldosterone on tha vasculature. *Hypertension* 2006; **47:** 312–318.
5. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT Jr. Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. *Hypertension* 1998; **31:** 451–458.
6. Rocha R, Rudolph AE, Frierdich GE, Nachowiak DA, Kekec BK, Blomme EA, et al. Aldosterone induces a vascular inflammatory phenotype in the rat heart. *Am J Physiol Heart Circ Physiol* 2002; **283:** H1802–H1810.
7. Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxi-

cal activation of the mineralocorticoid receptor: Role of oxidative stress. *Hypertension* 2007; **50:** 877–883.
8. Parati G, Pomidossi G, Albini F, Malaspina D, Mancia G. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. *J Hypertens* 1987; **5:** 93–98.
9. Nair AP, Timoh T, Fuster V. Contemporary medical management of systolic heart failure. *Circ J* 2012; **76:** 268–277.
10. Hirai Y, Geleijnse JM, Adachi H, Imaizumi T, Kromhout D. Systolic blood pressure predicts cardiovascular mortality in a farming but not in a fishing community: A 40-year follow up of the Japanese cohorts of the seven countries study. *Circ J* 2011; **75:** 1890–1896.
11. Sega R, Corrao G, Bombelli M, Beltrame L, Facchetti R, Grassi G, et al. Blood pressure variability and organ damage in a general population: Results from the PAMELA study (Pressioni Arteriose Monitorate E Loro Associazioni). *Hypertension* 2002; **39:** 710–714.
12. Tatasciore A, Zimarino M, Renda G, Zurro M, Soccio M, Prontera C, et al. Awake blood pressure variability, inflammatory markers and target organ damage in newly diagnosed hypertension. *Hypertens Res* 2008; **31:** 2137–2146.
13. Kudo H, Kai H, Kajimoto H, Koga M, Takayama N, Mori T, et al. Exaggerated blood pressure variability superimposed on hypertension aggravates cardiac remodeling in rats via angiotensin II system-mediated chronic inflammation. *Hypertension* 2009; **54:** 832–838.
14. Takayama N, Kai H, Kudo H, Yasuoka S, Mori T, Anegawa T, et al. Simvastatin prevents large blood pressure variability-induced aggravation of cardiac hypertrophy in hypertensive rats by inhibiting RhoA/Ras-ERK pathways. *Hypertens Res* 2011; **34:** 341–347.
15. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, et al. Transforming growth factor-β function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation* 2002; **106:** 130–135.
16. Kai H, Muraishi A, Sugiu Y, Nishi H, Seki Y, Kuwahara F, et al. Expression of proto-oncogenes and gene mutation of sarcomeric proteins in patients with hypertrophic cardiomyopathy. *Circ Res* 1998; **83:** 594–601.
17. Kuwahara F, Kai H, Tokuda K, Niiyama H, Tahara N, Kusaba K, et al. Roles of intercellular adhesion molecule-1 in hypertensive cardiac remodeling. *Hypertension* 2003; **41:** 819–823.
18. Kuwahara F, Kai H, Tokuda K, Shibata R, Kusaba K, Tahara N, et al. Hypoxia-inducible factor-1α/vascular endothelial growth factor pathway for adventitial vasa vasorum formation in hypertensive rat aorta. *Hypertension* 2002; **39:** 46–50.
19. Kai H, Kuwahara F, Tokuda K, Shibata R, Kusaba K, Niiyama H, et al. Coexistence of hypercholesterolemia and hypertension impairs adventitial vascularization. *Hypertension* 2002; **39:** 455–459.
20. Kuwahara F, Kai H, Tokuda K, Takeya M, Takeshita A, Egashira K, et al. Hypertensive myocardial fibrosis and diastolic dysfunction: Another model of inflammation. *Hypertension* 2004; **43:** 739–745.
21. Tokuda K, Kai H, Kuwahara F, Yasukawa H, Tahara N, Kudo H, et al. Pressure-independent effects of angiotensin II on hypertensive myocardial fibrosis. *Hypertension* 2004; **43:** 499–503.
22. Mori T, Kai H, Kajimoto H, Koga M, Kudo H, Takayama N, et al. Enhanced cardiac inflammation and fibrosis in ovariectomized hypertensive rats: A possible mechanism of diastolic dysfunction in postmenopausal women. *Hypertens Res* 2011; **34:** 496–502.
23. Takemiya K, Kai H, Yasukawa H, Tahara N, Imaizumi T. Mesenchymal stem cell-based prostacyclin synthase gene therapy for pulmonary hypertension rats. *Basic Res Cardiol* 2010; **105:** 409–417.
24. Kai H, Griendling KK, Lassegue B, Ollerenshaw JD, Runge MS, Alexander RW. Agonist-induced phosphorylation of the vascular type 1 angiotensin II receptors. *Hypertension* 1994; **24:** 523–527.
25. Kusaba K, Kai H, Koga M, Takayama N, Ikeda A, Yasukawa H, et al. Inhibition of intrinsic interferon-γ function prevents neointima formation after balloon injury. *Hypertension* 2007; **49:** 909–915.
26. Koga M, Kai H, Yasukawa H, Yamamoto T, Kawai Y, Kato S, et al. Inhibition of progression and stabilization of plaques by therapeutic interferon-γ function blocking in ApoE-KO mice. *Circ Res* 2007; **101:** 348–356.
27. Tahara N, Kai H, Niiyama H, Mori T, Sugi Y, Takayama N, et al. Repeated gene transfers of naked prostacyclin synthase plasmid into skeletal muscles attenuate monocrotaline-induced pulmonary hypertension and prolong survival in rats. *Human Gene Ther* 2004; **15:** 1270–1278.
28. Kai H, Griendling KK, Lassègue B, Fukui T, Minieri CA, Alexander RW. Prolonged exposure to agonist results in a reduction in the levels of the Gq/G11 α-subunit in cultured vascular smooth muscle cells. *Mol Pharmacol* 1996; **49:** 96–104.
29. Echeverría PC, Mazaira G, Erlejman A, Gomez-Sanchez C, Piwien Pilipuk G, Galigniana MD. Nuclear import of the glucocorticoid receptor-hsp90 complex through the nuclear pore complex is mediated

by its interaction with Nup62 and importin beta. *Mol Cell Biol* 2009; **29:** 4788–4797.

30. Galigniana M. Steroid receptor coupling becomes nuclear. *Chem Biol* 2012; **19:** 662–663.

31. Rautureau Y, Paradis P, Schiffrin EL. Cross-talk between aldosterone and angiotensin signaling in vascular smooth muscle cells. *Steroids* 2011; **76:** 834–839.

32. Lemarié CA, Paradis P, Schiffrin EL. New insights on signaling cascades induced by cross-talk between angiotensin II and aldosterone. *J Mol Med* 2008; **86:** 673–678.

33. Lemarié CA, Simeone SM, Nikonova A, Ebrahimian T, Deschênes ME, Coffman TM, et al. Aldosterone-induced activation of signaling pathways requires activity of angiotensin type 1a receptors. *Circ Res* 2011; **105:** 852–859.

34. Di Zhang A, Nguyen Dinh Cat A, Soukaseum C, Escoubet B, Cherfa A, Messaoudi S, et al. Cross-talk between mineralocorticoid and angiotensin II signaling for cardiac remodeling. *Hypertension* 2008; **52:** 1060–1067.

35. Masaad C, Houard N, Lombes M, Barouki R. Modulation of human mineralocirticoid receptor function by protein kinase A. *Mol Endocrinol* 1999; **13:** 57–65.

36. Funder JW. Is aldosterone bad for the heart? *Trends Endocrinol Metab* 2004; **15:** 139–142.

37. Kumar R, Gururaj AE, Barnes CJ. p21-activated kinases in cancer. *Nat Rev Cancer* 2006; **6:** 459–471.

38. Wang RA, Mazumdar A, Vadlamudi RK, Kumar R. P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *EMBO J* 2002; **21:** 5437–5447.

39. Zhao L, Ma QL, Calon F, Harris-White ME, Yang F, Lim GP, et al. Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. *Nat Neurosci* 2006; **9:** 234–242.

40. Shibata S, Mu S, Kawarazaki H, Muraoka K, Ishizawa K, Yoshida S, et al. Rac1 GTPase in rodent kidneys is essential for salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. *J Clin Invest* 2011; **121:** 3233–3243.