ORIGINAL ARTICLE

Activation of mineralocorticoid receptors in the rostral ventrolateral medulla is involved in hypertensive mechanisms in stroke-prone spontaneously hypertensive rats

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Mineralocorticoid receptor (MR) is recognized as a target for therapeutic intervention in hypertension and heart failure. MRs in the central nervous system are thought to have an important role in blood pressure regulation. Thus, we examined whether activation of the MR pathway in the rostral ventrolateral medulla (RVLM) of the brainstem contributes to the neural mechanism of hypertension in stroke-prone spontaneously hypertensive rats (SHRSPs). We microinjected eplerenone, aldosterone or Na^+-rich artificial cerebrospinal fluid (aCSF) into the RVLM of anesthetized Wistar–Kyoto (WKY) rats and SHRSPs. Arterial pressure (AP), heart rate (HR) and renal sympathetic nerve activity (RSNA) were recorded. The expressions of the MR protein and the serum- and glucocorticoid-regulated kinase protein (Sgk1), which is a marker of MR activity, in the RVLM were measured by western blot analysis. Bilateral microinjection of eplerenone into the RVLM decreased AP and RSNA in WKY rats and SHRSPs, and the decreases in those variables were significantly greater in SHRSPs than WKY rats. Microinjection of aldosterone or Na^+-rich aCSF into the RVLM increased AP and RSNA dose-dependently. The increases in those variables were significantly greater in SHRSPs than in WKY rats. The pressor responses of aldosterone or Na^+-rich aCSF were attenuated by the prior injection of eplerenone in SHRSPs. Sgk1 expression levels in the RVLM were significantly greater in SHRSPs than in WKY rats. These findings suggest that activation of MRs in the RVLM enhances sympathetic activity, thereby contributing to the neural mechanism of hypertension in the SHRSP.
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Keywords: blood pressure; mineralocorticoid receptor; rostral ventrolateral medulla; sympathetic nervous system

INTRODUCTION

Accumulating evidence indicates that the sympathetic nervous system has an important role in the pathogenesis of hypertension.[1–3] Mineralocorticoid receptor (MR) is recognized as a target for therapeutic intervention in hypertension and heart failure.[4,5] The beneficial effects of the MR antagonist spironolactone or the more specific antagonist eplerenone on end-organ damage have been studied in animal models.[6–10] It has been reported that MRs regulate epithelial Na channels (ENaCs) that are important for the regulation of sodium transport and the maintenance of extracellular fluid volume and arterial pressure (AP) in the kidney.[11] Recent studies have reported the distribution of MRs or ENaCs in the choroid plexus, ependyma and neurons, such as those in the supraoptic nucleus, paraventricular nucleus and nucleus tractus solitarius.[12,13] In addition, the expression of MR mRNA has been reported in the brainstem,[14] including the rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla.[15,16] These findings suggest that MRs in the central nervous system, especially in neurons, regulate Na^+ influx via ENaCs, leading to hypertension and sympathoexcitation. However, it remains to be determined whether activation of MRs in the brainstem contributes to blood pressure regulation via the sympathetic nervous system.

The RVLM of the brainstem is one of the most important vasomotor centers that determines sympathetic nervous system activity, and it is essential for the maintenance of basal vasomotor tone.[3,17,18] There is a evidence that increased activity of the RVLM leads to sympathetic hyperactivity in various model of hypertension.[18–20] For example, both the pressor response to the microinjection of angiotensin II into the RVLM and the stimulation of AT_1

receptors were enhanced in spontaneously hypertensive rats (SHRs).[21] The activation of endogenous AT_1 receptors has also been demonstrated in SHRs and stroke-prone spontaneously hypertensive rats (SHRSPs).[22,23] It has been reported that microinjection of aldosterone into the RVLM increases AP, and that the MR blocker spironolactone decreases AP.[24] The purpose of the present study was thus to determine whether the MR pathway in the RVLM contributes to the neural regulation of blood pressure through the sympathetic nervous system, and whether the activation of endogenous MRs in the RVLM leads to sympathetic hyperactivity and hypertension in SHRSPs. Furthermore, we examined neuronal sensitivity to Na^+ in the RVLM, because activation of MRs would induce ENaC upregulation.[11] SHRSPs are widely used as a model of hypertension, because they develop exaggerated blood pressure elevation with high sympathetic nervous system activity, thereby causing stoke and death.[23] In addition, oral administration of eplerenone prevented salt-induced cardiac fibrosis in SHRSPs.[6] For this purpose, we investigated the effects of aldosterone, Na^+-rich artificial cerebrospinal fluid (aCSF) and the MR blocker eplerenone administered into the RVLM on arterial blood pressure, heart rate (HR) and renal sympathetic nerve activity (RSNA). The expression of MRs and serum- and glucocorticoid-regulated kinase (Sgk1) expression levels, which indicate MR activity, were also evaluated.

METHODS

This study was reviewed and approved by the Animal Experiments Ethics Committee, Kyushu University Graduate School of Medical Sciences, and was conducted according to the Guidelines for Animal Experiments of Kyushu University.

Animals and general procedures

Male Wistar–Kyoto rats (WKY/Izm) and SHRSP/Izm (12–16 weeks old; SLC Japan, Hamamatsu, Japan) were used. Food and tap water were available ad libitum throughout the study. The rats were kept in a temperature- and humidity-controlled room with a 12-h light period between 0800 hours to 2000 hours.

Microinjection into the RVLM and recording of blood pressure, HR and RSNA

SHRSPs and WKY rats were initially anesthetized with sodium pentobarbital ($50 \, mg \, kg^{-1}$ i.p., followed by a maintenance dosage of $20 \, mg \, kg^{-1}$ per h i.v.). A catheter was inserted into the femoral artery to record AP and HR. A tracheal cannula was connected to a ventilator, and the rats were artificially ventilated. Body temperature was monitored with a rectal thermometer and maintained in the range of 36.5–37.5 °C with a heating pad. The left renal nerve was exposed using a left retroperitoneal flank incision. A pair of stainless steel bipolar electrodes was placed beneath the renal nerve to record multifiber RSNA.[25] All signals were recorded on a computer using a PowerLab system (AD Instruments, Nagoya, Japan). The signal from the electrodes was amplified, passed through a band pass filter and then rectified and integrated (resetting every 0.1 s). The rats were placed in a stereotaxic frame, and the dorsal surface of the medulla was surgically exposed to allow for positioning of the microinjection pipettes in the RVLM (with the pipette angled rostrally 18 °C, 1.8 mm lateral, 3.5 mm below the calamus scriptorius), as previously described.[26] The microinjections (all microinjections were in a volume of 50–100 nl unless otherwise indicated) into the RVLM were made according to the following protocols: (1) bilateral microinjections of the MR blocker eplerenone (100 pmol each); (2) unilateral microinjection of aldosterone (10 pmol-1 nmol); (3) unilateral microinjection of aldosterone (100 pmol) 30 min after bilateral microinjections of eplerenone (100 pmol each); (4) unilateral microinjection of Na^+-rich aCSF (0.15–0.2 M); and (5) unilateral microinjection of Na^+-rich aCSF (0.2 M in 50 nl) 30 min after bilateral microinjections of eplerenone (100 pmol each).

aCSF (which contained (in $mmol \, l^{-1}$) 121 NaCl, 3.4 KCl, 1.2 $MgCl_2$, 0.6 NaH_2PO_4, 29 $NaHCO_3$ and 3.4 glucose) was used as a vehicle, and Na^+-rich

aCSF was prepared by adjusting the $[Na^+]$ of aCSF with additional NaCl.[27] Aldosterone was obtained from Sigma-Aldrich (St Louis, MO, USA). The MR blocker eplerenone was a gift from the Pfizer Pharmaceutical Company, (New York, NY, USA). Drug doses were based on previous reports[24,27] or our preliminary experiments. Before microinjection of the drugs, the RVLM was identified by monitoring the mean arterial pressure (MAP) after injecting a small dose (1 nmol) of L-glutamate. For bilateral injections, injections were first made on one side, and then the pipette was moved to the contralateral side; the two injections were made ∼3 min apart.

Western blot analysis for the MR and Sgk1 in the RVLM

To obtain RVLM tissues, the rats were deeply anesthetized with sodium pentobarbital ($100 \, mg \, kg^{-1}$ i.p.) and transcardially perfused with phosphate-buffered saline. The brain was quickly removed. The RVLM tissue was homogenized and then sonicated in a lysing buffer containing $40 \, mmol \, l^{-1}$ of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1% Triton X-100, 10% glycerol, $1 \, mmol \, l^{-1}$ phenylmethanesulfonyl fluoride and 1 protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis, IN, USA). The tissue lysate was centrifuged at 6000 r.p.m. for 5 min at 4 °C in a microcentrifuge. The lysate was collected, and the protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA). Aliquots of protein (50 μg) from each sample were separated on a 7.5% sodium dodecyl sulfate-polyacrylamide gel. Subsequently, the separated proteins were transferred onto polyvinylidene difluoride membranes (Immobilon-P membrane; Millipore, Billerica, MA, USA). The membranes were incubated with goat IgG polyclonal antibody against MR (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), with rabbit IgG polyclonal antibody against SGK1 (Abcam, Tokyo, Japan) and with rabbit IgG polyclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (1:1000; Santa Cruz Biotechnology) for 24 to 48 h. The membranes were then washed and incubated with horseradish peroxidase-conjugated horse anti-goat IgG or anti-rabbit antibody (1:10000; Santa Cruz Biotechnology) for 40 min. Immunoreactivity was detected by autoradiography using enhanced chemiluminescence and a western blotting detection kit (Amersham, Piscataway, NJ, USA).

Statistical analysis

All values are expressed as the mean ± s.e.m. The changes in MAP, HR and RSNA values during the eplerenone microinjection studies and the MR and Sgk1 receptor expression were compared between SHRSPs and WKY rats using an unpaired t-test. Intergroup differences in MAP and RSNA after aldosterone and Na^+-rich aCSF microinjection were compared using two-way analysis of variance. P values of < 0.05 were considered statistically significant.

RESULTS

Effect of blockade of MR in the RVLM on arterial pressure, HR and RSNA

The basal MAP and HR were significantly higher in SHRSPs than in WKY rats (183.1 ± 4.1 vs. 103.2 ± 2.7 mm Hg, 355.7 ± 3.1 vs. 318.1 ± 3.3 b.p.m., $P < 0.01$, $n = 5$ for each). Bilateral microinjection of the MR blocker eplerenone into the RVLM induced a significant decrease in MAP, HR and RSNA in both SHRSPs and WKY rats. The magnitudes of the decreases in AP and RSNA were significantly greater in SHRSPs than in WKY rats (ΔMAP, -31.5 ± 2.7 vs. -12.0 ± 1.2 mm Hg, $P < 0.01$; RSNA Δbaseline, -14.6 ± 2.1 vs. $-4.3 \pm 0.5\%$, $P < 0.01$, $n = 5$ for each; Figures 1a and b). In contrast, the decrease in HR was not significantly different between SHRSPs and WKY rats (ΔHR, -22.9 ± 7.8 vs. -17.4 ± 9.2 b.p.m.; NS, $n = 5$). These changes occurred several minutes after injection, peaked at 40–60 min, and gradually recovered over time, but they lasted more than 2 h.

Effect of microinjection of aldosterone into the RVLM on arterial pressure, HR and RSNA

The basal MAP and HR were significantly higher in SHRSPs than in WKY rats (185.7 ± 5.6 vs. 91.9 ± 2.7 mm Hg, 356.5 ± 4.2 vs.
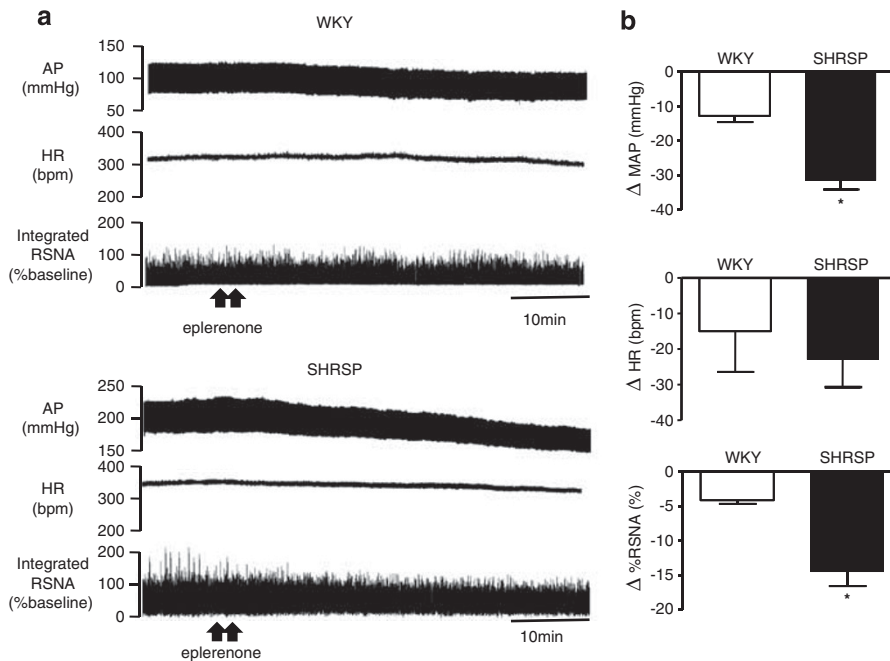
Figure 1 The responses of arterial pressure (AP), heart rate (HR) and renal sympathetic nerve activity (RSNA) to bilateral microinjection of the mineralocorticoid receptor (MR) blocker eplerenone into the RVLM of Wistar–Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSPs). (**a**) Raw data of the changes in AP, HR and RSNA evoked by bilateral microinjection of eplerenone in WKY rats and SHRSPs. (**b**) Group data of the changes in mean arterial pressure (MAP), HR and RSNA in response to bilateral microinjection of eplerenone (100 pmol). Values are expressed as the mean ± s.e.m. *$P<0.05$ ($n=5$ for each).
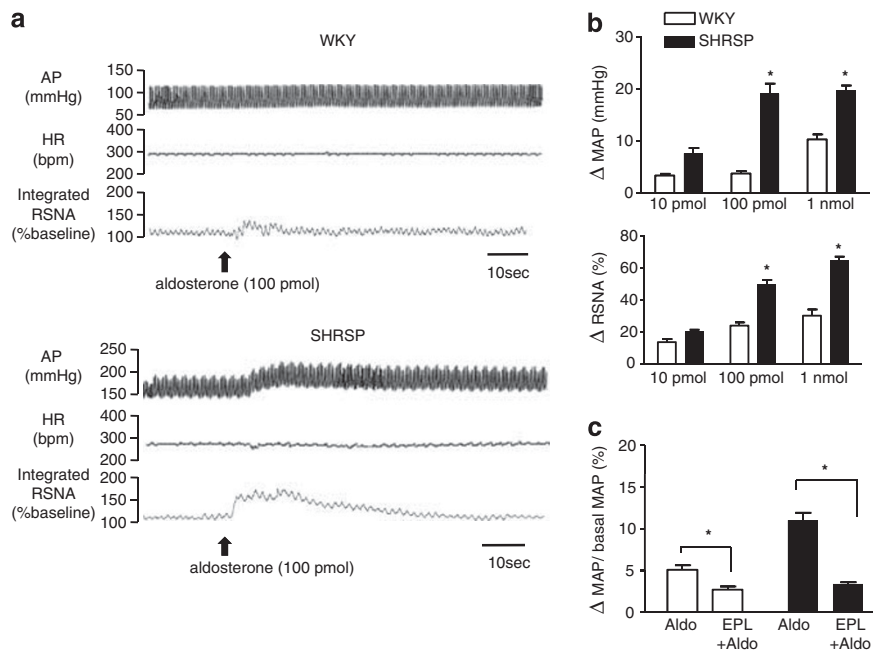
Figure 2 The responses of arterial pressure (AP), heart rate (HR) and renal sympathetic nerve activity (RSNA) to microinjection of aldosterone into the rostral ventrolateral medulla (RVLM). (**a**) Raw data of the changes in AP, HR and RSNA after unilateral injection of aldosterone (10 pmol-1 nmol) into the RVLM in Wistar–Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSPs). (**b**) Group data of the changes in mean arterial pressure (MAP) and HR evoked by unilateral microinjection of aldosterone (10 pmol–1 nmol) into the RVLM in WKY rats and SHRSPs. (**c**) Group data of the changes in MAP in response to microinjection of aldosterone (100 pmol) without pretreatment (Aldo) and microinjection of aldosterone with pretreatment of eplerenone (100 pmol) (Aldo + EPL) in WKY rats and SHRSPs. Values are expressed as the mean ± s.e.m. *$P<0.05$ ($n=4$ for each).

302 ± 5.1 b.p.m., $P < 0.01$, $n = 4$ for each). Unilateral microinjection of aldosterone into the RVLM significantly increased MAP and RSNA in both SHRSPs and WKY rats. The pressor response induced by aldosterone occurred in a dose-dependent manner (Figures 2a and b). No significant changes in HR were observed in either strain of rats (data not shown). The magnitude of the increases in these variables was significantly greater in SHRSPs than in WKY rats ($P < 0.05$, $n = 4$ for each; Figure 2b). Pretreatment with eplerenone nearly prevented the aldosterone-induced pressor responses in SHRSPs and WKY rats. However, the blocking effect of MRs was significantly greater in SHRSPs ($P < 0.05$, $n = 5$ for each; Figure 2c).
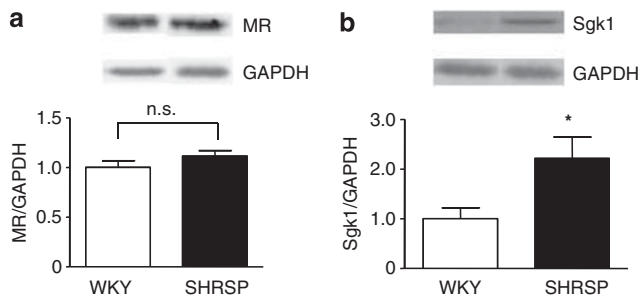
Figure 3 Western blot analysis demonstrating (a) mineralocorticoid receptor (MR) and (b) serum- and glucocorticoid-regulated kinase (Sgk1) expression in the rostral ventrolateral medulla (RVLM) in 12-week-old Wistar–Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSPs). The densitometric average was normalized to the values obtained from the analysis of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Expressions are shown relative to those seen in WKY rats, which were assigned a value of 1. Values are expressed as the mean ± s.e.m. *$P < 0.05$ ($n = 4$ for each).

MR and Sgk1 expression levels in the RVLM

The protein expression levels of MRs in the RVLM did not differ between SHRSPs and WKY rats. However, the levels of Sgk1, which is induced by MRs and is a marker of the activity of MRs, were significantly greater in SHRSPs than in WKY rats ($P < 0.05$, $n = 4$ for each; Figure 3).

Effect of microinjection of Na$^+$-rich aCSF into the RVLM on arterial pressure, HR and RSNA

Similarly, the basal MAP and HR were significantly higher in SHRSPs than in WKY rats (180.8. ± 4.1 vs. 87.1 ± 9.9 mm Hg, 357.9 ± 5.6 vs. 312.5 ± 6.4 b.p.m., $P < 0.01$, $n = 5$ for each). Microinjection of Na$^+$-rich aCSF into the RVLM increased both MAP and RSNA in a dose-dependent manner (Figures 4a and b), whereas microinjection of 0.15 M aCSF into the RVLM caused no significant changes in MAP, HR or RSNA. The magnitudes of the increases in these variables were significantly greater in SHRSPs than in WKY rats ($P < 0.05$, $n = 5$ for each; Figure 4b). In addition, pretreatment with bilateral microinjection of eplerenone into the RVLM significantly attenuated the Na$^+$-rich aCSF-induced pressor responses in SHRSPs, but did not significantly change them in WKY rats ($P < 0.05$, $n = 5$ for each; Figure 4c).

DISCUSSION

The findings of this study were as follows: (1) blockade of MRs in the RVLM decreased MAP and RSNA in both SHRSPs and WKY rats, but the decreases were apparently greater in SHRSPs than in WKY rats; (2) microinjection of aldosterone or Na$^+$-rich aCSF into the RVLM increased MAP via sympathetic nerve activity in both SHRSPs and WKY rats, but the increases were greater in SHRSPs than in WKY rats;
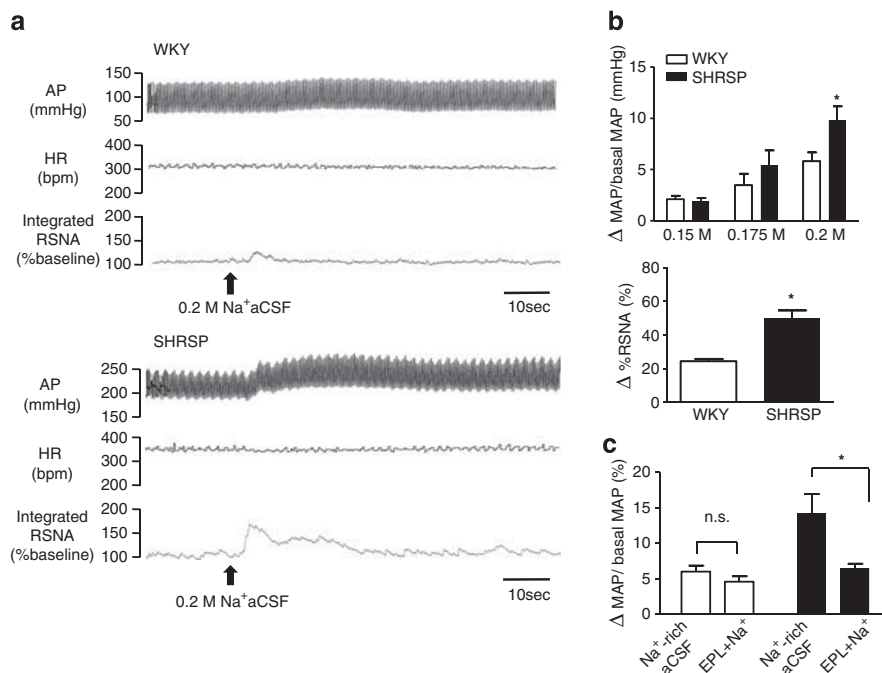
Figure 4 The response to unilateral microinjection of Na$^+$-rich artificial cerebrospinal fluid (aCSF) (0.15–0.2 M) into the rostral ventrolateral medulla (RVLM). (a) Raw data of the changes in mean arterial pressure (AP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in Wistar–Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSPs). (b) Group data of the changes in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) evoked by unilateral microinjection of Na$^+$-aCSF (0.2 M) into the RVLM in WKY rats and SHRSPs. (c) Group data of the changes in mean MAP in response to microinjection of Na$^+$-rich-aCSF (0.2 M) without pretreatment (Na$^+$-rich aCSF) and microinjection of Na$^+$ rich-aCSF (0.2 M) with pretreatment of eplerenone (100 pmol) (EPL + Na) in WKY rats and SHRSPs. Values are expressed as the mean ± s.e.m. *$P < 0.05$ ($n = 5$ for each).

(3) in SHRSPs, the prior injection of an MR blocker completely prevented the aldosterone-induced pressor response, but it only partially prevented the Na^+-rich aCSF-induced pressor response; and (4) the protein expression levels of MRs in the RVLM did not differ between SHRSPs and WKY rats. However, the Sgk1 expression levels were significantly greater in SHRSPs than in WKY rats. Taken together, our findings indicate that activation of MRs in the RVLM contributes to the neural mechanisms of hypertension in SHRSPs.

The most important finding of this study was that microinjection of the MR-specific blocker eplerenone into the RVLM induced decreases in MAP and RSNA in WKY rats and SHRSPs, and these decreases were greater in SHRSPs. MRs in the brain have been shown to be involved in sympathetic nerve activation in salt-sensitive hypertensive rats,[28] in a rat model of myocardial infarction[29] and in other salt-sensitive models.[30] The blockade of MRs in the brain has been suggested to decrease sympathetic nerve activation and result in a decrease in the blood pressure rise from high salt intake in salt-sensitive rats.[28] This decrease has also been associated with an improvement in the cardiac function of mice with pressure overload.[30] In the present study, we confirmed that endogenous blockade of MRs in the RVLM decreased blood pressure via sympathoinhibition, with greater decreases in SHRSPs. However, the decreases in HR were not significantly different, because it is difficult to assess HR in an acute experiment with anesthetics. To investigate the long-term effects of endogenous MR blockade on blood pressure or RSNA, we microinjected eplerenone into the RVLM bilaterally. It has been reported that Sgk1 is induced by aldosterone and upregulates ENaC levels and activity in the kidney,[11,31] and that this aldosterone-induced upregulation is dependent on MRs.[32] Therefore, these studies suggest that Sgk1 can be classified as an aldosterone-effector kinase and a marker of MR signaling. In the RVLM, the protein expression of MRs did not differ between the two strains, but Sgk1 was significantly higher in SHRSPs than in WKY rats. The enhanced protein expression of Sgk1 in SHRSPs in the present study might indicate an enhancement of MR stimulation in the RVLM. We observed that endogenous blockade of MRs by eplerenone induced a greater depressor response through sympathoinhibition in SHRSPs than in WKY rats, and exogenous stimulation of MR by aldosterone elicited a greater pressor response through sympathoexcitation in SHRSPs than in WKY rats. Altogether, these findings suggest that activation of MRs in the RVLM is enhanced and contributes to elevation of the AP in SHRSPs.

The effects of aldosterone have been ascribed to a genomic mechanism of binding to its receptors, followed by translocation of the steroid receptor complex to the nucleus, where it acts as a transcriptional regulator. However, recent studies suggest that effects may be because of non-genomic actions of aldosterone, which occur more rapidly after binding MRs.[33,34] For example, the rapid action of aldosterone in the kidney[33] or vasculature[34] has been demonstrated within a few minutes. Therefore, it is possible that acute inhibition of MR activation might be caused by non-genomic mechanism in the RVLM. However, we cannot exclude the possibility that genomic action is also involved in our observation, because the depressor response evoked by eplerenone lasted more than 2 h. In addition, it should be noted that the depressor and sympathoinhibitory responses occurred several minutes after the bilateral microinjection of eplerenone into the RVLM, and that the pressor and sympathoexcitatory responses occurred within seconds after the microinjection of aldosterone. In general, exogenously administered neurotransmitter/neuromodulators into the RVLM have been shown to elicit rapid action of blood pressure and sympathetic nerve activity. In contrast, the blockade of endogenous receptors in the RVLM gradually evokes the responses of blood pressure and sympathetic nerve activity.[35] In this context, the time course of the responses is not surprising, although we still do not have a clear explanation for this phenomenon. Further study is needed to clarify whether endogenous activation of MR is involved in hypertensive mechanisms both in the genomic and non-genomic action of aldosterone.

Our observations are consistent with previous reports that microinjection of aldosterone into the RVLM increases MAP.[24] It has been reported that intracerebroventricular (ICV) infusion of aldosterone increased MAP[36] and that these sympathoexcitatory and central pressor effects of aldosterone can be blocked by ICV infusion of an MR blocker. In the present study, microinjection of the MR blocker eplerenone into the RVLM also prevented an aldosterone-induced pressor response. These results suggest that aldosterone is likely to be an endogenous ligand of MRs in the RVLM. It has also been reported that central administration of aldosterone appears to depend on the MR–ENaC–ouabain pathway and, ultimately, AT_1 receptor stimulation.[37] The sympathoexcitatory and pressor responses to central infusion of aldosterone and Na^+-rich aCSF can be prevented by central infusion of the sodium channel blocker benzamil or an ouabain blocker,[27,38] and the pressor responses elicited by central infusion of aldosterone or ouabain can be blocked by an AT_1 blocker.[27,39] In the RVLM, microinjection of an ouabain-like compound[40] or angiotensin II[21] elicits a pressor response and sympathoexcitation. Collectively, these data suggest that aldosterone in the RVLM might activate central mechanism(s) involving the MR–ENaC–ouabain pathway, thereby causing sympathetic hyperactivity and hypertension.

Several studies have reported that aldosterone shows poor penetration of the blood-brain barrier compared with other steroid hormones.[12] However, the enzymes for steroid biosynthesis are present in the central nervous system[41] and RVLM,[15] and aldosterone can be detected in the tissues of various brain regions in vitro[41] and in vivo.[42] In particular, it should be noted that Gomez-Sanchez et al. reported that aldosterone was detectable in the whole brain of adrenalectomized rats, despite the fact that plasma aldosterone was undetectable.[41] These finding suggest that aldosterone is produced locally in the RVLM. In the present study, such locally produced aldosterone might have been enhanced and might have activated MRs in SHRSPs. Alternatively, there might be an aldosterone-independent MR activation pathway in the RVLM. It has been reported that MRs were activated by Rac1, which was independent of aldosterone in the kidney.[43] Further studies are needed to clarify the precise mechanisms involved in the activation of MR in the RVLM.

In this study, microinjection of Na^+-rich aCSF into the RVLM caused concentration-related increases in MAP and RSNA, whereas microinjection of 0.15 M aCSF at the same volume generated no significant increases. It has been reported that acute ICV infusion[44] or microinjection into the paraventricular nucleus of Na^+-rich aCSF[27] causes sympathetic hyperactivity and hypertension in normotensive rats, and pressor responses are enhanced in salt-sensitive rats compared with salt-resistant rats.[44] Abrams et al.[45] hypothesized that when MRs bind ligand, there is a subsequent upregulation of ENaCs, which would increase the membrane permeability to sodium in the brain. This response, in the face of transient increases in sodium levels, would lead to membrane depolarization and an increase in neural activity, driving sympathetic outflow and increasing MAP. It has also been reported that in the paraventricular nucleus, it is possible for an increase in intracellular Na^+ caused by a larger extra-/intracellular gradient to increase intracellular Ca^{2+} through the Na^+/Ca^{2+} channel exchanger, and thereby increase Ang II release.[27] Considering these

observations, it is possible that Na$^+$-rich aCSF in the RVLM increases sympathetic nerve activity and MAP.

The pressor effect was greater in SHRSPs than in WKY rats, and this difference between the strains might have been related to the different neural responsiveness to Na$^+$-rich aCSF via ENaCs in the RVLM. This pressor effect was partially blocked by the MR blocker eplerenone in SHRSPs, indicating that the effect of Na$^+$ might be mediated by MR activation. ICV infusion of the MR blockers, spironolactone[46] or benzamil,[47] prevented Na$^+$-induced sympathoexcitatory and pressor responses in WKY rats. It has also been reported that ICV infusion of eplerenone attenuated ENaC expression in mice with pressure overload.[30] Taken together, these findings suggest that MRs mediate Na$^+$ via ENaCs or transporters on the cell surface of neurons in the RVLM.

Study limitations

MRs are largely occupied by the glucocorticoid corticosterone,[48] which is present in a higher concentration than aldosterone in the brain.[41] The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which is distributed the brainstem, including the nucleus tractus solitarius,[49] rapidly converts corticosterone to an inactive metabolite. Thus, the coexpression of 11β-HSD2 with MRs may identify brain regions that are particularly sensitive to aldosterone. Although the precise expression of 11β-HSD2 in the RVLM has not yet been determined, we found that aldosterone in the RVLM increased blood pressure, and this pressor response was prevented by the MR blocker eplerenone. Therefore, our findings suggest that aldosterone acts on the MRs in the RVLM. We still cannot exclude the possibility that corticosterone, instead of aldosterone, may act on the MRs in the RVLM. Together with the origin of aldosterone in the RVLM as well as the central nervous system, the study regarding ligand-specifying mechanisms has just begun. In addition, we did not determine whether ENaC activity is involved in the neural responsiveness to Na$^+$-rich aCSF in the RVLM, because we did not measure ENaC activation in the RVLM. However, it is possible that MRs and ENaCs in the RVLM may be involved in this mechanism.

In conclusion, these findings indicate that MRs in the RVLM contribute to the neural mechanisms of hypertension via sympathetic nerve activity, and that increased activity of MRs may be involved in the elevation of blood pressure in SHRSPs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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