Role of Mineralocorticoid Receptor on Experimental Cerebral Aneurysms in Rats

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Abstract—Activation of the renin-angiotensin (Ang)-aldosterone system is involved in the pathology of vascular diseases. Although the blockade of the mineralocorticoid receptor protects against vascular diseases, its role in cerebral aneurysms remains to be elucidated. We treated female rats subjected to renal hypertension, increased hemodynamic stress, and estrogen deficiency for 3 months with the mineralocorticoid receptor blocker eplerenone (30 or 100 mg/kg per day) or vehicle (vehicle control). Eplerenone reduced the incidence of cerebral aneurysms and saline intake without lowering of the blood pressure. In the aneurysmal wall, the production of Ang II and nitrotyrosine was increased. The mRNA levels of Ang-converting enzyme 1 and NADPH oxidase subunits NOX4, Rac1, monocyte chemoattractant protein 1, and matrix metalloproteinase 9 were increased. Eplerenone brought about a reduction in these molecules, suggesting that mineralocorticoid receptor blockade suppresses cerebral aneurysm formation by inhibiting oxidative stress, inflammatory factors, local renin-Ang system activation, and saline intake. Other female rats implanted with pellets of the mineralocorticoid receptor agonist deoxycorticosterone acetate manifested a high incidence of cerebral aneurysm formation and the upregulation of molecules related to oxidative stress, inflammatory factors, and the local renin-Ang system; their saline intake was increased. We demonstrate that mineralocorticoid receptor activation at least partly contributes to the pathogenesis of cerebral aneurysms. **(*Hypertension*. 2009;54:552-557.)**

Key Words: cerebral ■ aneurysm ■ inflammation ■ mineralocorticoid receptor ■ oxidative stress

Rupture of cerebral artery aneurysms results in catastrophic subarachnoid hemorrhage and a high risk for morbidity and mortality.[1] On the basis of epidemiological data showing a high incidence of cerebral aneurysms in postmenopausal women, we subjected female rats to increased hemodynamic stress, hypertension, and estrogen deficiency (by oophorectomy); in these animals, the incidence of cerebral aneurysms was high.[2] Treatment with 17β-estradiol or an angiotensin (Ang) II type 1 receptor blocker reduced this incidence.[2] Elsewhere we suggested that endothelial injury is an initial event in the pathogenesis of cerebral aneurysms and that an increase in Ang II and NADPH oxidase subunits is involved.[2] Also, inflammation and degradation of the extracellular matrix in the vascular wall play a role in the development of cerebral aneurysms.[3–5]

The renin-Ang-aldosterone system is involved in the pathophysiology of cardiovascular and kidney diseases, activation of the mineralocorticoid receptor (MR) is especially highlighted in recent studies.[6–10] The identification of a new site of MR expression in nonepithelial tissues, such as the heart,[11] vasculature,[12] and brain,[13] suggested the presence in these tissues of potential new MR target genes with unexpected biological functions. In aortic endothelial cells, aldosterone increased the expression of Ang-converting enzyme (ACE) genes that may be involved in the development of vascular injury.[14] Eplerenone, a selective MR antagonist, performs beneficial actions, such as antihypertensive, anti-inflammatory effects,[15] the prevention of cardiac fibrosis,[16] and the suppression of NADPH oxidase activity[8,15] and matrix metalloproteinase (MMP).[17] However, the relationship between MR and cerebral aneurysms remains unclear.

We show that, in female rats, MR blockade by eplerenone inhibited the progression of cerebral aneurysms via its antioxidant and anti-inflammatory effects, suppressed local renin-Ang system (RAS) activation, and decreased their salt intake in a blood pressure–independent manner. We also demonstrate that MR activation by the MR agonist deoxycorticosterone acetate (DOCA) contributed to the formation of cerebral aneurysms.

Materials and Methods

For detailed descriptions of Materials and Methods, please see the online Data Supplement at http://hyper.ahajournals.org.

Results

MR Blockade Suppresses the Progression of Experimentally Induced Cerebral Aneurysms

Based on our morphological findings, we classified the left anterior cerebral artery-olfactory artery (ACA-OA) bifurca-

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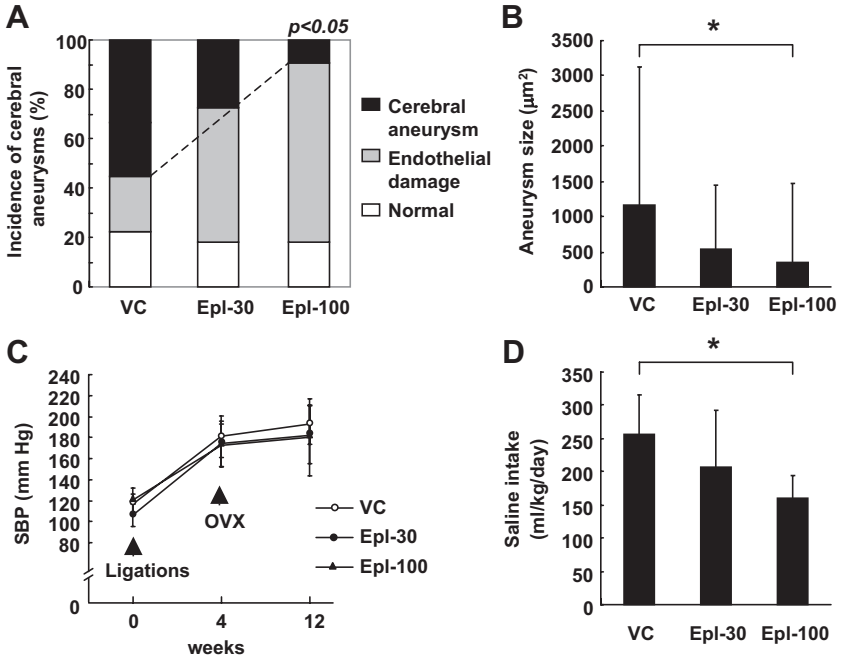

Figure 1. Effects of eplerenone on cerebral aneurysm formation in rats. A, Aneurysmal changes at the left ACA-OA bifurcation of rats treated with VC (n=17) or eplerenone (30 mg/kg per day, Epl-30 group, n=11; 100 mg/kg per day, Epl-100 group, n=11) were morphologically evaluated by scanning electron microscopy using vascular corrosion casts. Data were analyzed with the Fisher's exact test. Epl indicates eplerenone. B, To determine the aneurysm size, on each image we outlined regions of interest corresponding with areas with aneurysmal involvement. Analysis was with National Institutes of Health image software. Data (mean±SD) were analyzed by 1-way ANOVA followed by Scheffe's test. *$P<0.05$ vs VC. C, Time course of SBP changes in VC (n=17), Epl-30 (n=11), and Epl-100 rats (n=11). OVX indicates oophorectomy. D, Saline intake of VC and eplerenone-treated rats (each group n=8). Data (mean±SD) were analyzed by 1-way ANOVA followed by Scheffe's test. *$P<0.05$ vs VC.

tion as normal, as exhibiting endothelial damage, and as manifesting cerebral aneurysm (Figure S1 in the online Data Supplement). As shown in Figure 1A, 59% of the vehicle controls (VC) developed cerebral aneurysms at the left ACA-OA bifurcation. The rate of aneurysm development in eplerenone-treated rats (30 mg/kg per day, Epl-30 group; 100 mg/kg per day, Epl-100 group) was reduced in a dose-dependent manner (27% in Epl-30, P value not significant; 9.1% in Epl-100 versus VC, $P<0.05$), and the average aneurysm size was significantly reduced in the Epl-100 group ($P<0.05$; Figure 1B), suggesting that MR blockade contributed to the suppression of cerebral aneurysms.

There was no significant difference in the systolic blood pressure (SBP) between eplerenone-treated and VC rats (Figure 1C). The saline intake of Epl-100 was significantly lower than of VC rats (Figure 1D), and the plasma aldosterone level was significantly higher in eplerenone-treated than VC rats (Figure S2).

MR Blockade Reduces Oxidative Stress and Inflammatory Reaction in the Cerebral Vascular Wall

Next, we investigated the molecular mechanisms of cerebral aneurysm formation and the effect of MR blockade. Immunohistochemically, the levels of nitrotyrosine, NOX4, and Rac1 representing an oxidative stress index were higher in the endothelium of cerebral aneurysms than in sham-operated rats (Figure 2A); eplerenone reduced their expression levels. In VC rats, macrophages were primarily located in the aneurysmal wall (Figure 2B). The area per 150×150 μm that contained macrophages was smaller in eplerenone-treated than VC rats ($P<0.05$; Figure 2C). Monocyte chemoattractant protein 1 (MCP-1) and MMP-9, as proinflammatory molecules, were abundantly expressed in the aneurysmal wall (Figure 2B). Their expression was low in eplerenone-treated rats and almost undetectable in sham-operated rats. The mRNA levels of NOX4, Rac1, MCP-1, and MMP-9 were significantly higher in VC- than in sham-operated rats (Figure 3A through 3D); they were reduced by eplerenone ($P<0.05$).

Immunohistochemical staining of cerebral arteries from VC rats demonstrated MR expression in the endothelium and smooth muscle cells of aneurysms and parent arteries (Figure S3A). We detected the MR gene in cerebral arteries from VC rats by quantitative real-time PCR (Figure S3B). Eplerenone reduced the protein and mRNA level of MR (Figure S3A and S3B).

MR Blockade Reduces Activation of the Local RAS in the Cerebral Vascular Wall

We examined the effect of MR blockade on the local RAS in cerebral aneurysms. Immunohistochemically, Ang II was highly expressed in the endothelium (Figure 2B); eplerenone reduced its expression.

ACE1 and ACE2 regulate the Ang II levels.[18] The mRNA level of ACE1 was significantly higher in VC rats than sham-operated rats (Figure 3E); it was significantly decreased by eplerenone ($P<0.05$). The mRNA level of ACE2 was not different among the 3 groups (Figure 3F), suggesting that MR blockade partly reduced the activation of the local RAS in the aneurysmal wall via the downregulation of ACE1. We hypothesized that oxidative stress, inflammation, activation of the local RAS, and saline intake via MR activation are associated with cerebral aneurysms.

DOCA Salt Induces Cerebral Aneurysms Despite a Moderate Blood Pressure Increase

To test whether the activation of MR contributes to cerebral aneurysm formation, we used DOCA, an MR agonist, instead of inducing renal hypertension (rHT) by ligation. The incidence of cerebral aneurysms in DOCA rats was similar to that in rHT rats (69% versus 59%; Figure 4A). The aneurysm size was not different between the 2 groups (Figure 4B). SBP in

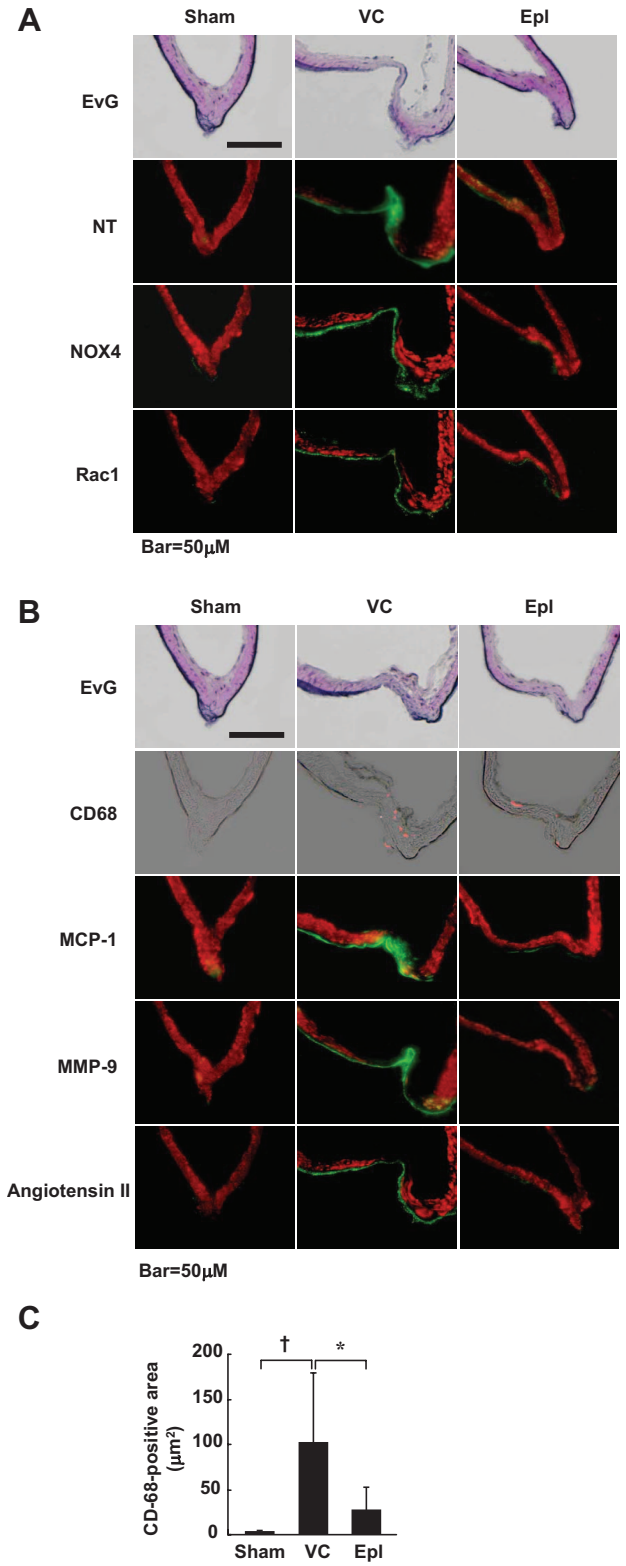

Figure 2. Elastica van Gieson (EvG) and immunohistochemical staining for nitrotyrosine (NT), NOX4, and Rac1 (green). Red shows staining of smooth muscle α-actin. A, EvG and immunohistochemical staining for CD68 (a macrophage marker), MCP-1, MMP-9, and Ang II (green). Red shows staining for smooth muscle α-actin. B. CD68-positive cells per 150-μm^2 field around the aneurysm were counted (sham, n=15; VC, n=15; Epl, n=13). Data (mean±SD) were analyzed by 1-way ANOVA followed by Scheffe's test: *$P<0.05$; †$P<0.01$ vs VC.

DOCA rats was moderately elevated compared with sham-operated rats, and it was lower than in rHT rats ($P<0.05$; Figure 4C). The saline intake of DOCA rats was significantly increased (Figure 4D) compared with that of rHT rats. The plasma aldosterone level was significantly lower in DOCA rats than in sham-operated rats (Figure S4); plasma aldosterone was not different between DOCA and rHT rats. Based on our observation that, in DOCA rats, the incidence of cerebral aneurysms was almost the same as in rHT rats, we posit that MR activation is involved in the pathogenesis of cerebral aneurysms.

MR Activation Induces the Upregulation of Molecules Related to Oxidative Stress, Inflammation, and the Local RAS in the Aneurysmal Wall

We examined whether oxidative stress, inflammation, and the local RAS contribute to the pathogenesis of cerebral aneurysms in DOCA rats. The mRNA levels of NOX4, p22phox, MCP-1, and MMP-9 were significantly higher in DOCA rats than in sham-operated rats (Figure 5A through 5D); the ACE1 mRNA level was also higher in DOCA rats (Figure 5E). On the other hand, the level of ACE2 mRNA was lower in DOCA rats than in sham-operated rats (Figure 5F).

Discussion

We report 2 new insights. First, blockade of MR activation by eplerenone suppressed the formation of cerebral aneurysms in rats. Second, the inhibition mechanisms may involve attenuation of oxidative stress and vascular inflammation, as well as inhibition of the activation of the local RAS. The reduced salt intake of rats subjected to MR blockade may be associated with the lower incidence of cerebral aneurysms.

Human studies have shown a link between elevated plasma aldosterone and stroke risk, especially the risk for hemorrhagic stroke.[19,20] A comparison of patients with essential hypertension and primary hyperaldosteronism suggested that the increased incidence of cerebral hemorrhage in the latter group is blood pressure–independent.[21] In stroke-prone spontaneously hypertensive rats fed a high-salt diet, MR antagonists prevented spontaneous hemorrhagic stroke without lowering the blood pressure.[22,23] Moreover, autosomal-dominant polycystic kidney disease was strongly associated with the development of cerebral aneurysms; these patients exhibited significantly higher plasma aldosterone concentrations in the supine and upright positions and after ACE inhibition.[24] These studies imply that aldosterone or MR activation is linked to hemorrhagic stroke or cerebral aneurysm development.

Estrogen deficiency induced reactive oxygen species generation and endothelial damage leading to cerebral aneurysm formation; in combination with hypertension, their effects were enhanced.[2] Here we demonstrate that the protein and gene expression of NADPH oxidase subunits was increased in parallel with the increased expression of nitrotyrosine in the aneurysmal wall. We also found that inflammatory molecules and macrophage infiltration were increased in the aneurysmal wall. MCP-1 is a major factor promoting the accumulation of macrophages in atherosclerosis[25] and abdominal aortic aneurysms,[26] and it is involved in the patho-

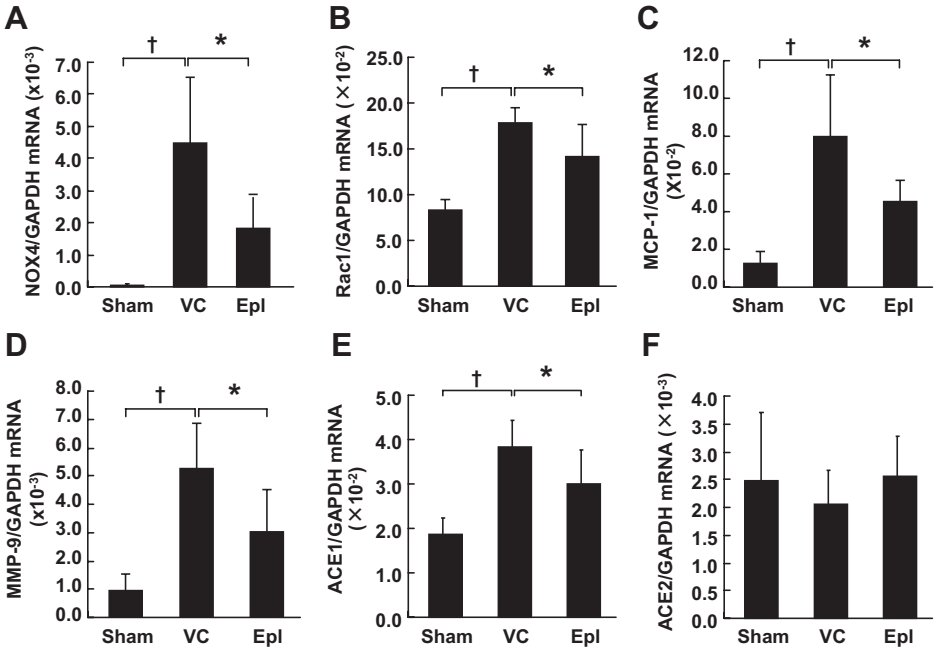

Figure 3. The mRNA levels of NOX4 (A), Rac1 (B), MCP-1 (C), MMP-9 (D), ACE1 (E), and ACE2 (F) were analyzed by quantitative real-time PCR (each group, n=7). Values were corrected by GAPDH and expressed as the mean±SD. *$P<0.05$; †$P<0.01$ vs VC.

genesis of cerebral aneurysms.[5] Macrophage-derived MMP-9 can degrade components of the extracellular matrix in vascular walls,[3,4] thereby promoting the progression of cerebral aneurysms. Our results, that MR blockade reduced the protein and gene expression of these molecules, suggested that MR blockade suppressed cerebral aneurysm development by inhibiting oxidative stress and inflammatory factors.

We demonstrated that MR activation with DOCA resulted in a high incidence of cerebral aneurysms, and the mRNA levels of oxidative stress and inflammatory genes were increased despite a moderate increase in blood pressure. Oxidative stress and inflammation are associated with RAS activation.[27] We found that Ang II production was increased in the aneurysm wall and that the mRNA level of ACE1 was increased in rHT and DOCA rats. MR blockade reduced the production of Ang II and the mRNA level of ACE1. Our results suggest that MR activation partly induced (and that MR blockade partly reduced) local RAS activation.

rHT is related to the activation of systemic RAS. In the DOCA-salt model, hypertension is generated by plasma volume expansion secondary to an increased sodium load and is associated with a dramatic reduction of plasma renin and Ang II concentrations.[28] In preliminary studies, plasma renin activity was one tenth lower in DOCA rats than in rHT rats (data now shown), suggesting that the SBP response to DOCA is listless compared with the response in the presence of rHT. Although it had no marked effect on blood pressure, MR blockade reduced the incidence of aneurysmal changes and suppressed increases in the aneurysmal size. Therefore, the mechanisms underlying the suppression of cerebral aneurysm development would be independent of the antihypertensive effect of MR blockade.

MR blockade did not normalize the expression of genes, suggesting that the residual elevation of genes may be attributable to the increased blood pressure. We do not know what portion of the increase in gene expression is attributable

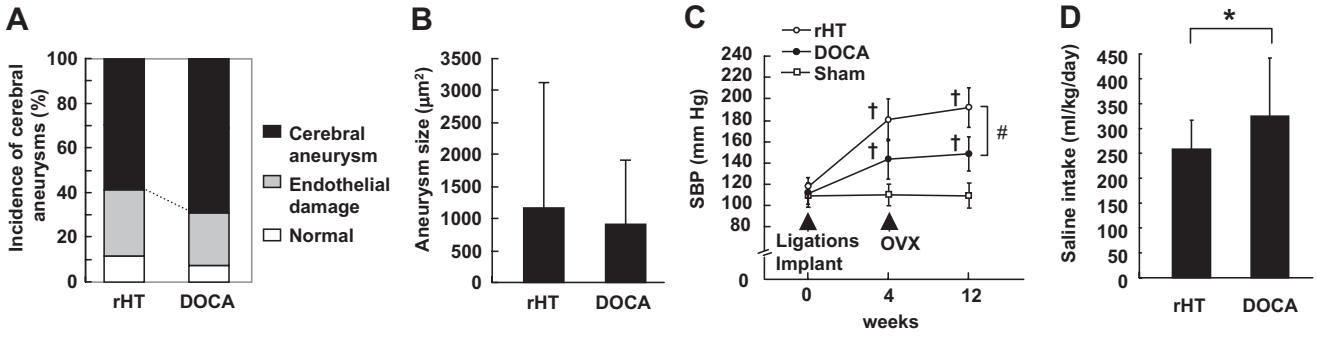

Figure 4. A, Aneurysmal changes of rHT (n=17) and DOCA rats (n=13). They were morphologically evaluated by scanning electron microscopy using vascular corrosion casts. Data were analyzed with the Fisher's exact test. B, Size of the aneurysms. Data (mean±SD) were analyzed by the Mann–Whitney *U* test. C, Time-course of SBP changes in sham-operated (n=15), rHT (n=17), and DOCA rats (n=13). Data (mean±SD) were analyzed by 1-way ANOVA followed by Scheffe's test. †$P<0.01$ vs sham, #$P<0.01$ vs rHT. D, Saline intake of rHT and DOCA rats (each group, n=8). Data (mean±SD) were analyzed by the Mann–Whitney *U* test. *$P<0.05$ vs rHT.

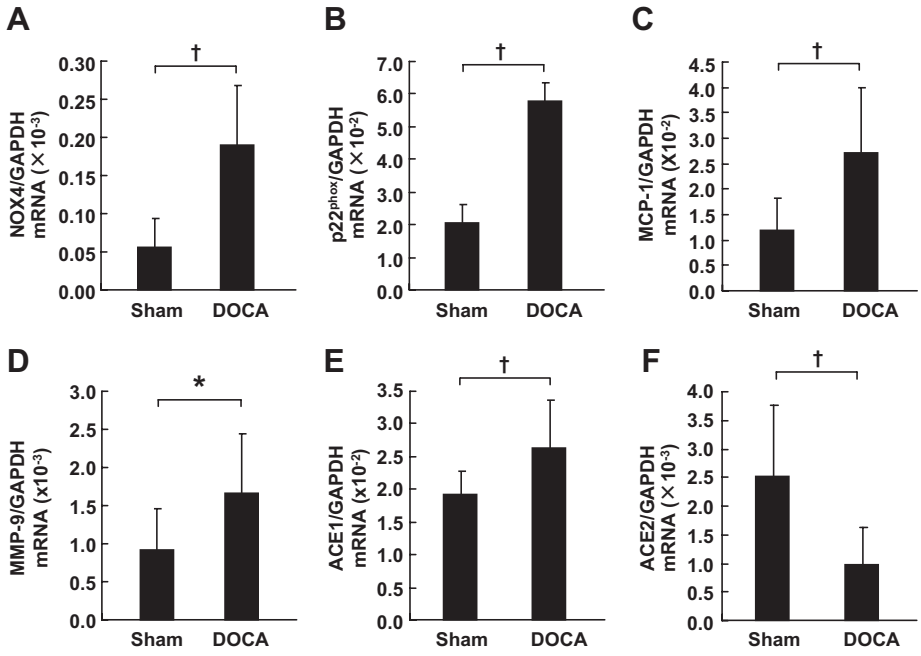

Figure 5. The mRNA levels of NOX4 (A), $p22^{phox}$ (B), MCP-1 (C), MMP-9 (D), ACE1 (E), and ACE2 (F) assayed by quantitative real-time PCR (sham, n=7; DOCA, n=6). The mRNA levels of genes except ACE2 were significantly higher in DOCA than in sham-operated rats. Values were corrected by GAPDH and expressed as the mean±SD. *$P<0.05$; †$P<0.01$ vs sham.

to blood pressure and what portion to direct MR activation. Cross-talk between MR activation and estrogen deficiency may be an important factor in promoting oxidative stress and the inflammatory state[29] and may lead to the formation of cerebral aneurysms.

A synergistic relationship between salt-induced and MR-mediated tissue injury has been suggested[30,31]; salt-loading enhanced reactive oxygen species generation and MR activation in target tissues.[31] In an ecological analysis of the correlation between urinary sodium excretion and stroke mortality in Western Europe, the relationship between 24-hour urinary sodium excretion and stroke mortality was much stronger than between blood pressure and stroke mortality.[32] Zhang et al[33] showed that the formation of cerebral aneurysms in stroke-prone spontaneously hypertensive rat was significantly increased by sodium loading. In our rats subjected to MR blockade, the saline intake was reduced, whereas in rats with DOCA-induced MR activation, it was increased significantly in parallel with an increase in the incidence of cerebral aneurysms. In previous studies, treatment with MR antagonists increased or did not affect urinary sodium excretion.[22,34,35] Thunhorst et al[36] showed that DOCA treatment progressively increased sodium ingestion, and the sodium intake exceeded the urinary sodium excretion; they suggested that the mechanisms underlying these effects involve actions on the brain that affect the central binding of Ang II and mineralocorticoid. Furthermore, Titze et al[37] showed that DOCA-salt led to a total-body sodium excess. Our and other findings suggest that MR activation increases sodium retention and that high sodium retention may have additive harmful effects on the formation of cerebral aneurysms.

Rigsby et al[38] and Dorrance et al[39] indicated that MR blockade by spironolactone reduced the cerebral infarction size in male stroke-prone spontaneously hypertensive rats, and MR activation with DOCA increased the size of cerebral infarcts; they suggested that MR activation results in inward hypertrophic remodeling of the cerebral vasculature and an increase in infarct size. However, cerebral aneurysms involve outward remodeling, and the vascular aneurysmal wall tends to be thin rather than thick. Moreover, cerebral aneurysms commonly arise at the arterial bifurcation where abnormal hemodynamic stress affects the vascular wall[40]; this may contribute to the differences in the reported results.

Perspectives

We first demonstrated that, in female rats, MR blockade reduced the incidence of cerebral aneurysm formation concomitant with a decrease in oxidative stress, inflammation, and the local RAS and that MR activation plays an important role in the formation of cerebral aneurysms. There are some limitations in our study. Because we did not use aged rats, our results do not reflect the possible effects of ageing. MR blockade was not sufficiently strong to completely prevent aneurysmal growth. Because the development of cerebral aneurysms involves multiple factors, other pathogenic mechanisms must be investigated. It remains unclear whether MR activation is associated with the rupture of cerebral aneurysms, because there are no reliable animal models of subarachnoid hemorrhage as a consequence of aneurysmal rupture. In future studies, we need to establish a reliable experimental subarachnoid hemorrhage model and investigate the efficacy of MR blockade in preventing subarachnoid hemorrhage.

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Disclosures

None.

References

1. Stegmayr B, Eriksson M, Asplund K. Declining mortality from subarachnoid hemorrhage: changes in incidence and case fatality from 1985 through 2000. *Stroke*. 2004;35:2059–2063.
2. Tamura T, Jamous MA, Kitazato KT, Yagi K, Tada Y, Uno M, Nagahiro S. Endothelial damage due to impaired nitric oxide bioavailability triggers cerebral aneurysm formation in female rats. *J Hypertens*. 2009;27:1284–1292.
3. Jamous MA, Nagahiro S, Kitazato KT, Tamura T, Aziz HA, Shono M, Satoh K. Endothelial injury and inflammatory response induced by hemodynamic changes preceding intracranial aneurysm formation: experimental study in rats. *J Neurosurg*. 2007;107:405–411.
4. Aoki T, Kataoka H, Morimoto M, Nozaki K, Hashimoto N. Macrophage-derived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. *Stroke*. 2007;38:162–169.
5. Aoki T, Kataoka H, Ishibashi R, Nozaki K, Egashira K, Hashimoto N. Impact of monocyte chemoattractant protein-1 deficiency on cerebral aneurysm formation. *Stroke*. 2009;40:942–951.
6. Funder JW. Mineralocorticoid receptors: distribution and activation. *Heart Fail Rev*. 2005;10:15–22.
7. Guo C, Martinez-Vasquez D, Mendez GP, Toniolo MF, Yao TM, Oestreicher EM, Kikuchi T, Lapointe N, Pojoga L, Williams GH, Ricchiuti V, Adler GK. Mineralocorticoid receptor antagonist reduces renal injury in rodent models of types 1 and 2 diabetes mellitus. *Endocrinology*. 2006;147:5363–5373.
8. Nishiyama A, Abe Y. Molecular mechanisms and therapeutic strategies of chronic renal injury: renoprotective effects of aldosterone blockade. *J Pharmacol Sci*. 2006;100:9–16.
9. Brown NJ. Aldosterone and end-organ damage. *Curr Opin Nephrol Hypertens*. 2005;14:235–241.
10. Yu Y, Wei SG, Zhang ZH, Gomez-Sanchez E, Weiss RM, Felder RB. Does aldosterone upregulate the brain renin-angiotensin system in rats with heart failure? *Hypertension*. 2008;51:727–733.
11. Barnett CA, Pritchett EL. Detection of corticosteroid type I binding sites in heart. *Mol Cell Endocrinol*. 1988;56:191–198.
12. Lombes M, Oblin ME, Gasc JM, Baulieu EE, Farman N, Bonvalet JP. Immunohistochemical and biochemical evidence for a cardiovascular mineralocorticoid receptor. *Circ Res*. 1992;71:503–510.
13. Han F, Ozawa H, Matsuda K, Nishi M, Kawata M. Colocalization of mineralocorticoid receptor and glucocorticoid receptor in the hippocampus and hypothalamus. *Neurosci Res*. 2005;51:371–381.
14. Sugiyama T, Yoshimoto T, Tsuchiya K, Gochou N, Hirono Y, Tateno T, Fukai N, Shichiri M, Hirata Y. Aldosterone induces angiotensin converting enzyme gene expression via a JAK2-dependent pathway in rat endothelial cells. *Endocrinology*. 2005;146:3900–3906.
15. Nagata K, Obata K, Xu J, Ichihara S, Noda A, Kimata H, Kato T, Izawa H, Murohara T, Yokota M. Mineralocorticoid receptor antagonism attenuates cardiac hypertrophy and failure in low-aldosterone hypertensive rats. *Hypertension*. 2006;47:656–664.
16. Young M, Funder JW. Eplerenone, but not steroid withdrawal, reverses cardiac fibrosis in deoxycorticosterone/salt-treated rats. *Endocrinology*. 2004;145:3153–3157.
17. Rastogi S, Mishra S, Zaca V, Alesh I, Gupta RC, Goldstein S, Sabbah HN. Effect of long-term monotherapy with the aldosterone receptor blocker eplerenone on cytoskeletal proteins and matrix metalloproteinases in dogs with heart failure. *Cardiovasc Drugs Ther*. 2007;21:415–422.
18. Danilczyk U, Eriksson U, Crackower MA, Penninger JM. A story of two ACEs. *J Mol Med*. 2003;81:227–234.
19. Litchfield WR, Anderson BF, Weiss RJ, Lifton RP, Dluhy RG. Intracranial aneurysm and hemorrhagic stroke in glucocorticoid-remediable aldosteronism. *Hypertension*. 1998;31:445–450.
20. McMahon GT, Dluhy RG. Glucocorticoid-remediable aldosteronism. *Cardiol Rev*. 2004;12:44–48.
21. Takeda R, Matsubara T, Miyamori I, Hatakeyama H, Morise T; for the Research Committee of Disorders of Adrenal Hormones in Japan. Vascular complications in patients with aldosterone producing adenoma in Japan: comparative study with essential hypertension. *J Endocrinol Invest*. 1995;18:370–373.
22. 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.
23. Rocha R, Stier CT Jr. Pathophysiological effects of aldosterone in cardiovascular tissues. *Trends Endocrinol Metab*. 2001;12:308–314.
24. Chapman AB, Johnson A, Gabow PA, Schrier RW. The renin-angiotensin-aldosterone system and autosomal dominant polycystic kidney disease. *N Engl J Med*. 1990;323:1091–1096.
25. Egashira K. Molecular mechanisms mediating inflammation in vascular disease: special reference to monocyte chemoattractant protein-1. *Hypertension*. 2003;41:834–841.
26. Middleton RK, Bown MJ, Lloyd GM, Jones JL, London NJ, Sayers RD. Characterisation of interleukin-8 and monocyte chemoattractant protein-1 expression within the abdominal aortic aneurysm and their association with mural inflammation. *Eur J Vasc Endovasc Surg*. 2009;37:46–55.
27. Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. *Trends Pharmacol Sci*. 2008;29:367–374.
28. Schenk J, McNeill JH. The pathogenesis of DOCA-salt hypertension. *J Pharmacol Toxicol Methods*. 1992;27:161–170.
29. Kawanishi H, Hasegawa Y, Nakano D, Ohkita M, Takaoka M, Ohno Y, Matsumura Y. Involvement of the endothelin ET(B) receptor in gender differences in deoxycorticosterone acetate-salt-induced hypertension. *Clin Exp Pharmacol Physiol*. 2007;34:280–285.
30. Funder JW. Aldosterone, mineralocorticoid receptors and vascular inflammation. *Mol Cell Endocrinol*. 2004;217:263–269.
31. Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxical activation of the mineralocorticoid receptor: Role of oxidative stress. *Hypertension*. 2007;50:877–883.
32. Perry IJ, Beevers DG. Salt intake and stroke: a possible direct effect. *J Hum Hypertens*. 1992;6:23–25.
33. Zhang W, Liu AJ, Yi-Ming W, Liu JG, Shen FM, Su DF. Pressor and non-pressor effects of sodium loading on stroke in stroke-prone spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol*. 2008;35:83–88.
34. Brandish PE, Chen H, Szczerba P, Hershey JC. Development of a simplified assay for determination of the antimineralocorticoid activity of compounds dosed in rats. *J Pharmacol Toxicol Methods*. 2008;57:155–160.
35. Rocha R, Martin-Berger CL, Yang P, Scherrer R, Delyani J, McMahon E. Selective aldosterone blockade prevents angiotensin ii/salt-induced vascular inflammation in the rat heart. *Endocrinology*. 2002;143:4828–4836.
36. Thunhorst RL, Beltz TG, Johnson AK. Glucocorticoids increase salt appetite by promoting water and sodium excretion. *Am J Physiol Regul Integr Comp Physiol*. 2007;293:R1444–R1451.
37. Titze J, Bauer K, Schafflhuber M, Dietsch P, Lang R, Schwind KH, Luft FC, Eckardt KU, Hilgers KF. Internal sodium balance in DOCA-salt rats: a body composition study. *Am J Physiol Renal Physiol*. 2005;289:F793–F802.
38. Rigsby CS, Pollock DM, Dorrance AM. Spironolactone improves structure and increases tone in the cerebral vasculature of male spontaneously hypertensive stroke-prone rats. *Microvasc Res*. 2007;73:198–205.
39. Dorrance AM, Rupp NC, Nogueira EF. Mineralocorticoid receptor activation causes cerebral vessel remodeling and exacerbates the damage caused by cerebral ischemia. *Hypertension*. 2006;47:590–595.
40. Ellegala DB, Day AL. Ruptured cerebral aneurysms. *N Engl J Med*. 2005;352:121–124.