Role of Aldosterone in Renal Vascular Injury in Stroke-Prone Hypertensive Rats


Abstract—Stroke-prone spontaneously hypertensive rats (SHRSP) on 1% NaCl drinking solution and Stroke-Prone Rodent Diet develop severe hypertension and glomerular and vascular lesions characteristic of thrombotic microangiopathy seen in malignant nephrosclerosis. We recently reported that spironolactone, a mineralocorticoid receptor antagonist, markedly reduced proteinuria and malignant nephrosclerotic lesions in these animals. This observation, together with our previous findings that angiotensin-converting enzyme inhibitors prevent the development of vascular damage, suggests that mineralocorticoids, as part of the renin-angiotensin-aldosterone system, play a pathophysiological role in this model. In the present study, we examined whether chronic (2-week) infusion of aldosterone can reverse the renal vascular protective effects of captopril in SHRSP. SHRSP received vehicle (n=8); captopril alone (50 mg ⋅ kg⁻¹ ⋅ d⁻¹, orally) (n=10); aldosterone infusion alone (40 μg ⋅ kg⁻¹ ⋅ d⁻¹, SC) (n=7); or captopril and aldosterone at 20 (n=6) or 40 (n=7) μg ⋅ kg⁻¹ ⋅ d⁻¹. Systolic blood pressure was markedly elevated in all groups. Vehicle- and aldosterone-infused SHRSP developed severe proteinuria and comparable degrees of renal injury (21±3% and 29±3%, respectively) manifested as thrombotic and proliferative lesions in the arterioles and glomeruli. Captopril treatment reduced plasma aldosterone levels concomitant with marked reductions in proteinuria and the absence of histologic lesions of malignant nephrosclerosis. Aldosterone substitution at 20 or 40 μg ⋅ kg⁻¹ ⋅ d⁻¹ in captopril-treated SHRSP resulted in the development of severe renal lesions (16±3% and 21±2%, respectively) and proteinuria comparable with that observed in SHRSP given either aldosterone or vehicle alone. These findings support a major role for aldosterone in the development of malignant nephrosclerosis in saline-drinking SHRSP, independent of the effects of blood pressure.

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Key Words: hypertension • kidney • malignant nephrosclerosis • captopril • aldosterone

The participation of the renin-angiotensin-aldosterone system (RAAS) in the development of hypertensive vascular injury has been widely demonstrated. Numerous studies with angiotensin-converting enzyme (ACE) inhibitors and with angiotensin (Ang) II receptor antagonists have clearly identified Ang II as a major factor responsible for the development of end-organ damage in hypertensive vascular disease. However, mineralocorticoids may also play an important role, because animals with deoxycorticosterone acetate (DOCA)-salt hypertension typically develop severe vascular pathology, and resultant malignant nephrosclerosis, myocardial necrosis, and stroke, despite low levels of plasma renin activity. The renal lesions that develop in DOCA-salt hypertensive rats are characterized by fibrinoid necrosis of blood vessels and proliferative arteriopathy and are very similar to those seen in stroke-prone spontaneously hypertensive rats (SHRSP) receiving a high sodium chloride diet and in rats with Ang II-salt–induced hypertension. We have shown that chronic administration of agents that interfere with the formation or actions of Ang II will prevent the development of malignant nephrosclerosis and stroke in saline-drinking SHRSP. Because Ang II stimulates the synthesis and release of aldosterone, we recently investigated whether endogenous mineralocorticoids contribute to the development of vascular injury in these rats. We found that, similar to our findings with ACE inhibitors and Ang II receptor antagonists, blockade of the mineralocorticoid receptor (MR) with spironolactone markedly attenuated the development of vascular injury and provided end-organ protection in the absence of reductions in arterial blood pressure. These observations are consistent with a major role for endogenous mineralocorticoids as hormonal, or local, mediators of vascular injury in SHRSP.

Although the beneficial effects of ACE inhibitors typically have been attributed to reductions in the vascular actions of Ang II, these agents can also inhibit aldosterone release. In the present study, we evaluated the hypothesis that the renal vascular protective effects observed with ACE inhibitor therapy in SHRSP are the consequence of interference with endogenous aldosterone formation. If the above-mentioned hypothesis is true, chronic exogenous infusion of aldosterone should reverse the renal protective effect of ACE inhibition.

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If not, the vascular protective effect provided by ACE inhibition should be maintained despite exogenous administration of aldosterone. To evaluate these possibilities, we performed experiments in saline-drinking SHRSP chronically treated with captopril under experimental conditions that afford complete protection against renal microvascular injury as reported previously by us.7

Methods

Animals
Studies were conducted in accordance with institutional guidelines with use of male SHRSP/A3N (generations F 75 to 78), n = 38, from our local colony. All animals were housed in a room lighted 12 hours per day at an ambient temperature of 22±1°C in the Animal Care Facility at New York Medical College. Rats were weaned at 4 weeks of age and allowed free access to Purina Laboratory Chow 5001 (Ralston Purina Inc.) and tap water until the initiation of the experiment.

Protocol

SHRSP were housed in individual metabolic cages beginning at 7 weeks of age. All animals were given Stroke-Prone Rodent Diet (Catalog No. 39-288, Zeigler Bros Inc, Gardners, PA) and 1% NaCl drinking solution ad libitum starting at 7.5 weeks of age. At 8.2 weeks of age, all SHRSP received 1 of 4 dosing protocols: (1) an infusion of the vehicle (0.5% ethanol) used to dissolve aldosterone with no captopril (n = 8), (2) captopril alone (50 mg · kg⁻¹ · d⁻¹, orally; n = 10), (3) aldosterone infusion alone (40 µg · kg⁻¹ · d⁻¹, SC) with no captopril (n = 7), or (4) combined dosing with captopril and infusion of aldosterone at 20 (n = 6) or 40 µg · kg⁻¹ · d⁻¹ (n = 7). Aldosterone (d-aldosterone) and captopril were purchased from Sigma Chemical Co. Alzet osmotic minipumps (model 2002 Alza Co.) containing aldosterone or vehicle were implanted beneath the skin at the nape of the neck in SHRSP receiving inhalatory anesthesia with isofluorane (Ohmeda Caribe Inc.). The concentration of aldosterone used to fill the pumps was calculated based on the mean pump rate provided by the manufacturer, the body weight of the animals, and the dose intended. The doses of aldosterone were selected because they are similar to the minimal dose shown to significantly enhance plasma aldosterone levels in saline-drinking rats18 and are similar to or lower than those used by other investigators to induce the development of renal and cardiovascular injury in rats.19,20

Animals were handled and weighed daily. Twenty-four-hour fluid and food intake, and urine output were measured before and after surgery and each week thereafter. Urine samples were collected at different time points for the assessment of proteinuria and electrolyte excretion. Systolic blood pressure was measured each week. After 2 weeks of treatment, animals were decapitated, trunk blood was collected into chilled tubes containing EDTA, and kidneys were removed, blotted dry, and weighed immediately. Kidney sections were stored in fixative and later processed for light microscopic evaluation.

Assays and Analyses

Systolic blood pressure of awake animals was measured by tail-cuff plethysmography with a Natsume KN-210 manometer and tachometer (Peninsula Laboratories Inc.). Rats were warmed at 37°C for 10 minutes and allowed to rest quietly in a Lucite chamber before measurement of blood pressure. Urinary protein concentration was determined by the sulfosalicylic acid turbidity method and urinary protein excretion was calculated as the product of the urinary concentration times the urine flow rate. Plasma aldosterone concentration was determined by radioimmunoassay (Diagnostic Products Co.).

Histology

Kidneys were preserved in 10% phosphate-buffered formalin. Coronal sections of kidney were cut at 3 to 4 mm, and at least 3 to 4 such blocks were sampled and embedded in paraffin. Histologic sections (2 to 3 µm) were stained with hematoxylin and eosin and examined by light microscopy at 20 and 40 in a blinded fashion for lesions, as previously described.5–9 Glomerular damage was categorized as ischemic or thrombotic. Ischemic lesions were defined as retraction of glomerular capillary tufts with or without appreciable mesangiolysis. Glomerular thrombotic lesions were defined as segmental to global fibrinoid necrosis, focal thrombosis of glomerular capillaries, often accompanied by swelling and occasionally by proliferation of intracapillary (endothelial and mesangial) and extracapillary cells (crescents), and edematous expansion of mesangium without significant hypercellularity. The number of glomeruli exhibiting lesions in either category was enumerated from each kidney and was expressed as a percentage of the total number of glomeruli present per midcoronal section (mean±SE=218±5 glomeruli per animal; range=167 to 274 glomeruli). Vascular damage was assessed by counting the total number of arterial and arteriolar profiles in the same midcoronal section showing thombotic and/or proliferative arteriopathy. Vascular thrombotic lesions were defined as mural fibrinoid necrosis, extravasation of red blood cells, and luminal and mural thrombosis. Proliferative arteriopathy was characterized by proliferation of markedly swollen cells with large round to ovoid vesicular nuclei surrounded by mucinous extracellular matrix (“onion skinning”) often resulting in nodular thickening. Vascular damage was expressed as the number of arteries and arterioles with lesions per 100 glomeruli and was calculated by dividing the total number of vascular profiles with lesions by the total number of glomeruli in the same midcoronal section, and multiplied by 100.

Statistical Analysis

Significant effects with respect to treatment and time were determined by two-way ANOVA. Data with only one grouping variable were analyzed statistically by unpaired Student’s t tests or one-way ANOVA followed by post hoc analysis with use of the Newman-Keuls multiple comparison test. Data were analyzed with use of version 2.01 of the GraphPad Prism statistical software package obtained from GraphPad Software Inc. A value of P<0.05 was considered to be statistically significant. Data are reported as mean±SEM.

Results

All groups of SHRSP demonstrated a progressive increase in systolic blood pressure with age and were severely hypertensive 2 weeks after implantation of pumps (Figure 1). No significant differences in blood pressure were found up until 10.5 weeks of age. At that time, the blood pressure of the group infused with aldosterone alone was higher than that of the groups treated with captopril alone or captopril and aldosterone at 20 µg · kg⁻¹ · d⁻¹. No significant differences were found among the other groups.

Figure 2 shows data obtained at the end of the 2-week treatment period. Captopril reduced endogenous aldosterone levels (Figure 2A) and prevented the development of proteinuria (Figure 2B) compared with vehicle-control SHRSP. Histopathological analysis of kidneys from captopril-treated SHRSP showed neither glomerular (Figure 2C) nor renal vascular lesions (Figure 2D). Plasma aldosterone levels in aldosterone-infused animals were elevated relative to the vehicle controls, but were not significantly different from those observed in captopril-treated SHRSP receiving aldosterone infusion at 20 or 40 µg · kg⁻¹ · d⁻¹ (Figure 2A). SHRSP infused with vehicle or aldosterone alone developed severe proteinuria (Figure 2B) and similar degrees of glomerular and
renal vascular damage (Figure 2C, D). Despite concurrent ACE inhibitor therapy, captopril-treated SHRSP infused with aldosterone at either 20 or 40 mg·kg⁻¹·d⁻¹ (ALDO 20) or 40 mg·kg⁻¹·d⁻¹ (ALDO 40) were implanted subcutaneously at the nape of the neck. All animals were maintained on a 1% NaCl drinking solution and Stroke-Prone Rodent Diet and euthanatized 2 weeks later. **P<0.01 compared with captopril; #P<0.05 compared with captopril + ALDO 20 at 10.5 weeks of age. Values are mean±SEM. SBP indicates systolic arterial blood pressure; ALDO, aldosterone.

**Discussion**

We previously reported that agents that interfere with the formation or actions of Ang II will prevent the development of vascular injury in saline-drinking SHRSP.7–9 More recently we demonstrated that substantial vascular protection can also be achieved when these animals are chronically treated with the MR antagonist spironolactone.14 Because Ang II stimulates the synthesis and release of aldosterone, the above-mentioned observations suggest that aldosterone could play a pathophysiological role in saline-drinking SHRSP subsequent to activation of the RAAS. The present studies were performed to further evaluate this possibility. We found that aldosterone infusion could completely reverse the ability of ACE inhibitor therapy with captopril to protect against the development of proteinuria and renal vascular and glomerular lesions in these animals. This observation is consistent with a central role for aldosterone in the evolution of malignant nephrosclerotic injury in SHRSP and implicates activation of the RAAS as the initiating event.

Greene and coworkers17 have also evaluated the ability of aldosterone to reverse the renal protective effects of blockade of the RAAS in the 5/6 nephrectomy model of hypertension and glomerulosclerosis. In those studies, interruption of the RAAS was associated with substantial reductions in plasma aldosterone levels, systolic blood pressure, proteinuria, and renal lesions which were reversed when aldosterone was

![Figure 1.](https://hyper.ahajournals.org/)

**Figure 1.** Line graphs showing SBP in stroke-prone, spontaneously hypertensive rats receiving captopril or vehicle treatment since 8.2 weeks of age. At the same time, Alzet osmotic minipumps containing 0.5% ethanol or aldosterone solution prepared to administeraldosterone at 20 mg·kg⁻¹·d⁻¹ (ALDO 20) or 40 mg·kg⁻¹·d⁻¹ (ALDO 40) were implanted subcutaneously at the nape of the neck. All animals were maintained on a 1% NaCl drinking solution and Stroke-Prone Rodent Diet and euthanatized 2 weeks later. **P<0.01 compared with captopril; #P<0.05 compared with captopril + ALDO 20 at 10.5 weeks of age. Values are mean±SEM. SBP indicates systolic arterial blood pressure; ALDO, aldosterone.

![Figure 2.](https://hyper.ahajournals.org/)

**Figure 2.** Bar graphs showing plasma aldosterone (A), urinary protein excretion (B), glomerular lesions (C), and renal vascular lesions (D), in captopril-treated, saline-drinking stroke-prone spontaneously hypertensive rats at the end of 2 weeks of treatment with aldosterone or vehicle. Captopril or vehicle treatment was started at 8.2 weeks of age. At the same time, Alzet osmotic minipumps containing 0.5% ethanol or aldosterone solution prepared to administer aldosterone at 20 mg·kg⁻¹·d⁻¹ (ALDO 20) or 40 mg·kg⁻¹·d⁻¹ (ALDO 40) were implanted subcutaneously at the nape of the neck. Animals were euthanatized 2 weeks after treatment. **P<0.01, ***P<0.001 compared with captopril; ###P<0.01, ####P<0.001 compared with vehicle. Values are mean±SEM. capt indicates captopril; ALDO, aldosterone.
Figure 3. Representative photomicrographs (hematoxylin and eosin, ×130) of renal cortex from saline-drinking SHRSP after 2 weeks of treatment with (top) vehicle, (middle) captopril plus vehicle, and (bottom) captopril plus aldosterone at 40 μg · kg⁻¹ · d⁻¹. (top) Typical lesions of malignant nephrosclerosis consisting of ischemic or thrombotic glomeruli (large arrows), extensive mural fibrinoid deposits in microvessels (large arrowheads) focally associated with fragmented extravasated erythrocytes (small arrowheads) are present in vehicle-treated animals. (middle) After captopril treatment glomerular or vascular pathology virtually disappeared. (bottom) Captopril plus aldosterone leads to reversal of protection by captopril and malignant nephrosclerotic lesions comparable with vehicle-treated animals (top) can be seen again. Thrombotic glomeruli (large arrows) and ischemic glomeruli (small arrows) proliferative arteriopathy with fragmented extravasated erythrocytes (arrowheads).
infused concurrently. In a second set of studies, animals with remnant kidneys were treated with spironolactone. Whereas proteinuria diminished at 2 weeks, no differences were noted in urinary protein excretion and the number of sclerotic glomeruli at 4 weeks. Blood pressure showed a slight but significant lowering at this time. Unlike these observations, we have found that chronic treatment with spironolactone markedly protects SHRSP against the development of renal injury in the absence of blood pressure reduction. The explanation for the discrepancy in the degree of renal protection seen with mineralocorticoid receptor antagonism between these models is not clear but may relate to differences in the types of lesions that develop. Rats with remnant kidneys develop glomerular lesions of focal glomerulosclerosis, whereas SHRSP primarily develop vascular lesions of thrombotic microangiopathy. In TGR(mREN)27 rats, a model of severe hypertension that closely resembles the SHRSP, a recent preliminary study also found that spironolactone greatly prolongs survival without lowering blood pressure.

In our model of malignant hypertension, aldosterone completely restores renal lesion development in captopril-treated SHRSP in the absence of major changes in blood pressure. Systolic blood pressure in captopril-treated SHRSP receiving aldosterone at either 20 or 40 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) was not significantly different from the group treated with captopril alone, yet they developed extensive renal damage. This observation suggests that the vasculotoxic mechanism for aldosterone is independent of alterations in blood pressure. These results are consistent with our previous observations, and they support the hypothesis that severe elevation of blood pressure alone is not sufficient for development of vascular injury but also requires the concurrent participation of hormonal or local factors, with aldosterone as a major participant. Whether aldosterone can induce vascular injury in the absence of hypertension in SHRSP remains to be determined. However, intracerebroventricular infusion of a mineralocorticoid receptor antagonist in aldosterone-salt hypertensive rats blocked arterial blood pressure elevation, but not cardiac hypertrophy or fibrosis. This observation suggests that concurrent hypertension may not be an absolute requirement for aldosterone and high salt to induce vascular disorders. Whether a similar result can be observed in the microvasculature of the kidney remains to be established. Consonant with renal damage, many of the SHRSP receiving aldosterone, captopril plus aldosterone, or vehicle alone also exhibited clinical signs of stroke during the 2-week period of study, whereas none of the SHRSP receiving captopril treatment alone showed signs of stroke. These findings confirm the observation of MacLeod and collaborators that aldosterone can reverse the beneficial effects of captopril treatment against development of stroke in SHRSP. In addition to these findings in the brain, and our present observations on the kidney, several reports have implicated aldosterone in the development of pathophysiological changes in the aorta and the heart. Together, these observations suggest that the pathophysiological role of aldosterone can be elicited in several different target organs in hypertensive vascular disease.

The beneficial effects of ACE inhibitor therapy have been related to reductions in the vascular actions of Ang II. However, in the present study we observed complete restoration of renal injury in saline-drinking SHRSP receiving an exogenous infusion of aldosterone, despite continued ACE inhibitor therapy. This effect was observed with doses of either 20 or 40 \( \mu g \cdot kg^{-1} \cdot d^{-1} \), and was associated with the reestablishment of elevated plasma aldosterone levels. The protection obtained with captopril treatment, in turn, was associated with reduced plasma aldosterone levels. Previous experiments have also demonstrated a marked lowering of plasma aldosterone levels by captopril in SHRSP. These observations suggest that the beneficial effect of ACE inhibitors in saline-drinking SHRSP is related to the inhibitory effect of these agents on aldosterone release. However, in patients with cardiac failure, this inhibitory effect on aldosterone is not long-lasting because restoration of hyperaldosteronism occurs with time. This phenomenon has been referred to as aldosterone “escape” and has provided the basis for the current Randomized Aldactone Evaluation Study (RALES) to examine the effect of combining an aldosterone receptor antagonist with ACE inhibitor therapy. Whether aldosterone plays a pathophysiological role in other clinical settings remains to be established.

We previously found that spironolactone offers marked protection against renal and cerebral vascular injury in SHRSP independent of changes in SBR or water and electrolyte excretion. Enhanced expression of mRNA for the MR and/or aldosterone synthase has been demonstrated in blood vessels from SHRSP. These observations, taken together with our present findings, support a direct pathological effect of aldosterone at nonepithelial sites which is unrelated to alterations in blood pressure. However, further investigations are necessary to elucidate the actual mechanisms by which aldosterone induces vascular pathology in saline-drinking SHRSP.

In summary, captopril treatment reduces plasma aldosterone levels, preventing the development of proteinuria and renal vascular and glomerular injury in saline-drinking SHRSP. These effects can be fully reversed by chronic exogenous administration of aldosterone and are independent of major changes in arterial blood pressure. The results of the present study strongly support a toxic effect of aldosterone at the level of the renal microvasculature, which seems to be independent of other components of the RAAS.

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