Mineralocorticoid Blockade Reduces Vascular Injury in Stroke-Prone Hypertensive Rats

Ricardo Rocha, Praveen N. Chander, Kavita Khanna, Andrea Zuckerman, Charles T. Steer, Jr

Abstract—Chronic treatment of saline-drinking stroke-prone spontaneously hypertensive rats (SHRSP) with agents that interfere with the formation or actions of angiotensin II (Ang II) prevents the development of stroke and renal vascular damage. Ang II, in addition to its direct vascular effects, stimulates the synthesis and release of aldosterone. To assess the role of aldosterone in the development of pathologic changes in these rats, we implanted time-release pellets containing 200 mg of the mineralocorticoid receptor antagonist, spironolactone, into 14 SHRSP at 7 to 5 weeks of age. Eight SHRSP intermates received placebo pellets. Over the period of study (3 to 4 weeks), systolic blood pressure (SBP) was not different between the groups. Spironolactone did not enhance water and electrolyte excretion. All placebo-treated SHRSP developed marked proteinuria (150±6 mg/d) whereas in spironolactone-treated SHRSP, urinary protein excretion (UPE) averaged 39±9 mg/d (P<0.001). In a second study to assess effects on survival, 6 SHRSP received spironolactone (10 mg/kg/d) and 6 received vehicle. All but one of the control rats displayed signs of stroke and died by 16 weeks of age, while the spironolactone-treated SHRSP remained asymptomatic through 19 weeks of age (P<0.03). At 16 weeks of age, spironolactone-treated SHRSP were severely hypertensive (247±3 mm Hg), yet UPE remained at baseline levels. In contrast, preterminal UPE averaged 136±13 mg/d in control rats (P<0.001). In both studies, histopathologic examination revealed a marked protective effect of spironolactone against the development of malignant nephrosclerosis and cerebrovascular lesions. These observations indicate a vascular and end organ protective effect of spironolactone in the absence of lowered blood pressure in saline-drinking SHRSP and are consistent with a major role for mineralocorticoids as hormonal mediators of vascular injury (Hypertension. 1998;31[part 2]:451-458.)

Key Words: hypertension • kidney • malignant nephrosclerosis • spironolactone • stroke

In 1972, clinical studies by Brunner and coworkers identified plasma renin activity (PRA) and aldosterone as independent risk factors for heart attack and stroke.1 They found that among the patients who developed strokes and myocardial infarctions, all had normal or high PRA, and aldosterone secretion. Previous studies by our group and others have provided experimental evidence to support a role for the renin-angiotensin-aldosterone system (RAAS) in the development of vascular injury, as angiotensin converting enzyme (ACE) inhibitors7-9 and Ang II receptor antagonists7-9 prevented the development of stroke and malignant nephrosclerosis in SHRSP. Since these studies were conducted in salt-loaded SHRSP, which respond to these agents with minimal blood pressure lowering, they provided evidence for a pathophysiologic role for Ang II in the development of vascular lesions of malignant nephrosclerosis independent of severely elevated blood pressure. Consistent with a role for Ang II was the finding that SHRSP display a paradoxical increase in PRA with age, despite continued salt-loading.19,20 Although Ang II stimulates the synthesis and release of aldosterone,11,12 in addition to its direct vascular actions, a role for mineralocorticoids in the pathology of SHRSP has not previously been evaluated. This possibility is supported by the finding that lesions of malignant nephrosclerosis and stroke have been classically described in rats with mineralocorticoid hypertension induced by the chronic administration of deoxycorticosterone acetate (DOCA) and salt.13-15 The renal lesions that develop in these animals are characterized by fibromold necrosis of vessels and proliferative arteriopathy. Such lesions are identical to those that we16 and other investigators17,18 have observed in saline-drinking SHRSP and are also seen in Ang II-salt hypertensive rats.16 These observations have led us to hypothesize that mineralocorticoids play a role in the development of vascular injury in saline-drinking SHRSP. To test this hypothesis we chronically treated SHRSP with spironolactone to determine whether mineralocorticoid receptor blockade would alter the development of pathology in these animals.

Methods

Animals

Studies were conducted using male SHRSP/A3N (generations F-75 and F-77), n=34, from our local colony and were approved by the Institutional Animal Care and Use Committee. 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.
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 experimental protocols.

**Protocols**

**Protocol 1**  
Each SHRSP was housed in an individual metabolic cage (Nalgene) at 6.5 weeks of age. Rats were handled and weighed daily. At 7.5 weeks of age, time-release pellets containing 200 mg of spironolactone (Innovative Research of America) were implanted in 14 SHRSP and placebo pellets were implanted in 8 littermate controls. Pellets were implanted subcutaneously at the nape of the neck through a skin incision in animals receiving inhalation anesthesia with isoﬂurane. Pellets were shown to substantially reduce the actions and specific binding of aldosterone to tissues in vivo. After implantation, all animals were given Stroke-Prone Rodent Diet (539-288, Zeigler Bros Inc) and 1% NaCl drinking solution ad libitum. Twenty-four hour fluid and food intake, and urine output were measured before and following surgery and each week thereafter. Urine was collected for the determination of protein and electrolyte excretion, and heart rate were measured each week by tail-cuff plethysmography. Treatments were continued until 10.5 to 11.5 weeks of age. At that time, trunk blood was collected into chilled EDTA tubes after rapid decapitation of animals. The blood samples were centrifuged for 10 minutes at 4°C and 3000 rpm to obtain plasma, which was then stored below -20°C for later radioimmunoassay for PRA. The brain, heart, kidneys, and adrenal glands were removed, blotted dry, immediately weighed and fixed. Brains and kidneys were further processed for light microscopic evaluation.

**Protocol 2**  
In a second series, SHRSP received the same diet as described above, starting at 8.8 weeks of age. To assess the effects of mineralocorticoid receptor antagonism on survival, 6 SHRSP were injected each day with 10 mg/kg of spironolactone (Sigma Chemical Co) and 6 littermate control animals received an equal volume of the sesame oil vehicle (1 mL/kg/d). Weekly measurements of systolic arterial blood pressure were made by tail-cuff plethysmography. All animals were housed individually in metabolic cages at 10 weeks of age so that measurements of 24-hour food and saline intake and urine output could be obtained each week. Surviving SHRSP were decapitated at 19 weeks of age. Brains and kidneys from all animals were removed, preserved in fixative, and processed for light microscopic evaluation.

**Assays and Analyses**  
SBP and heart rate of awake animals were measured by tail-cuff plethysmography using a Natsume KN-210 manometer and tachometer (Peninsula Laboratorines Inc) Rats were warmed at 37°C for 10 minutes and allowed to rest quietly in a Lucite chamber before tail-cuff plethysmography. Urinary protein concentration was determined by the sulfosalicylic acid turbidity method, and urinary sodium and potassium concentrations were measured with an automated electrolyte analyzer (model 644, Ciba Corning). Urinary protein and electrolyte excretion was calculated as the product of the urinary concentration times the urine flow rate. PRA was determined by using the RAINEN radioimmunoassay kit (Dupont NEN Research Products). PRA activity was expressed as nanograms of Ang I formed per milliliter per hour.

**Histology**  
Brains were fixed in Bouin’s solution for a period of 48 hours and then enrobed in 10% phosphate-buffered formalin. Each brain was closed transversely at 5 to 7 levels and examined for gross abnormalities. Brain slices were then embedded in paraffin blocks and histologic sections (5 to 7 µm) from each were stained with hematoxylin and eosin (H & E) and examined for lesions by light microscopy without knowledge of the treatment as described previously. Cerebrovascular damage was evaluated using a grading system of 0 to 4, based on the extent and distribution of the lesions, without regard for the type or age of the lesion. A score of 4 indicated extensive lesions involving large areas at 3 or more levels, a score of 3 indicated large lesions at 2 levels or small lesions at 3 or more levels, a score of 2 indicated small lesions at 2 or more levels, a score of 1 indicated a single damaged area and a score of 0 was assigned when no abnormalities were observed.

Kidneys were preserved in 10% phosphate-buffered formalin. Coronal sections of kidney were cut at 3 to 4 mm, and lesions, if present, were sampled and embedded in paraffin. Histologic sections (2 to 3 µm) were stained with H & E and examined by light microscopy at 20X and 40X in a blinded fashion for lesions, as previously described. Glomerular damage was categorized as ischemic or thrombotic. Ischemic lesions were defined as retraction of glomerular tufts with or without appreciable mesangiolysis. Glomerular thrombotic lesions were defined as segmental to occasionally 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 mid-coronal section (mean±SE=224±4 glomeruli per animal, range=182 to 268 glomeruli). Vascular damage was assessed by counting the total number of arterial and arteriolar profiles in the same mid-coronal section showing thrombotic 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 mid-coronal section, and multiplied by a factor of 100.

**Statistical Analysis**  
Significant effects with respect to treatment and time were determined by two-way analysis of variance with data from only one grouping variable analyzed statistically. One-way analysis of variance followed by post-hoc analysis using the method of Bonferroni. The Kaplan-Meier method was used for comparison of cumulative percent survival curves. Ordinal data (brain lesion scores) were analyzed using the Mann-Whitney nonparametric test. Data were analyzed using version 2.01 of the GraphPad Prism statistical software package (GraphPad Software Inc). A value of P<0.05 was considered to be statistically significant. Data are reported as mean±SE.
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Figure 1. Line graphs showing (A) systolic arterial blood pressure (SBP), (B) urinary protein excretion (UPE), and (C) body weight (BW) in stroke-prone spontaneously hypertensive rats into which time-release pellets containing spironolactone (13-17 mg/kg/d, n=14) or placebo pellets (n=8) were implanted. Animals were maintained on 1% NaCl drinking solution and Stroke-Prone Rodent Diet starting at 7.5 weeks of age at which time the pellets were implanted subcutaneously. Values are mean±SE. Number in parentheses indicates the number of animals. *P< 0.05, ***P< 0.001 compared with placebo-treated controls.

Protocol 1

SHRSP from both groups showed a progressive increase in SBP with age (Fig 1A). By the end of the study, animals in both groups were severely hypertensive. No significant differences in SBP were observed between the groups. Likewise, heart rate was unchanged by chronic treatment with spironolactone and showed little change over the course of the study (data not shown). There was no difference in UPE between the groups through 9 weeks of age (Fig 1B). Thereafter, UPE increased markedly in SHRSP receiving placebo and averaged 150±6 mg/dl at 10.4 weeks of age. In contrast, UPE remained at low levels and averaged 22±5 mg/dl at 10.4 weeks of age in the spironolactone-treated group (P< 0.001). All animals were sacrificed in the ensuing week. Preterminal UPE averaged 39±9 mg/dl in the spironolactone-treated animals. Body weight (Fig 1C) increased progressively in both groups through 9.5 weeks of age at which time the weight of placebo-treated animals began to decline. Fig 2 shows the results for urine volume, sodium excretion, and potassium excretion. After placement of the animals on high salt/stroke prone diet, urine volume and sodium excretion increased markedly (P< 0.001) whereas potassium excretion remained unchanged. The urinary Na+/K+ ratio increased from 0.51±0.02 to 5.97±0.24 (P< 0.001). Urine volume, sodium excretion, and the urinary Na+/K+ ratio were comparable in both the spironolactone- and placebo-treated groups until 10.3 weeks of age, at which time urine output, sodium excretion, and the urinary Na+/K+ ratio were greater in the placebo-treated group. Urinary potassium excretion was not different between the groups during the study period. There were no significant differences in heart weight (1.4±0.05 versus 1.52±0.06 g), total kidney weight (2.56±0.14 versus 2.55±0.06 g), total adrenal weight (6.4±5.8 versus 57.6±5.2 mg) and brain weight (2.05±0.05 versus 2.0±0.04 g) between placebo- and spironolactone-treated SHRSP, respectively.

The Table summarizes the histopathologic findings in the brains and kidneys of SHRSP. The average cerebrovascular lesion score in spironolactone-treated animals was significantly less than in placebo-treated SHRSP. Microscopic examination revealed cerebrovascular lesions in the brains of all placebo-treated SHRSP. Lesions included moderate to severe edema.

Results

Figure 2. Bar graphs showing (A) urine output, (B) urinary sodium excretion (UNa+V) and (C) urinary potassium excretion (UK+V) in stroke-prone spontaneously hypertensive rats into which time-release pellets containing spironolactone (13-17 mg/kg/d, n=14) or placebo pellets (n=8) were implanted. Animals were maintained on 1% NaCl drinking solution and Stroke-Prone Rodent Diet starting at 7.5 weeks of age at which time the pellets were implanted subcutaneously. Values are mean±SE. Number in parentheses indicates the number of animals. **P< 0.01, compared with placebo-treated controls.

The Table summarizes the histopathologic findings in the brains and kidneys of SHRSP. The average cerebrovascular lesion score in spironolactone-treated animals was significantly less than in placebo-treated SHRSP. Microscopic examination revealed cerebrovascular lesions in the brains of all placebo-treated SHRSP. Lesions included moderate to severe edema.
and rarefaction, spongy, liquefactive necrosis, and hemorrhage. Brain samples from spironolactone-treated animals showed occasional lesions that were not as marked as in the placebo-treated group. Representative photomicrographs of renal cortex from SHRSP given either a placebo pellet or a time-release pellet containing spironolactone are shown in Figs 3A and 3B, respectively. Prominent glomerular and vascular lesions of thrombotic microangiopathy characteristic of malignant nephrosclerosis were noted in placebo-treated SHRSP. Vascular lesions were primarily confined to renal cortex and affected medium-sized to small interlobular arteries as well as arterioles. Vascular lesions consisted predominantly of the thrombotic type. Proliferative lesions were noted in lesser numbers. The remaining vessels without lesions showed minimal thickening. Rarely, an arcuate artery revealed focal fibrinoid necrosis. Glomeruli revealed predominantly ischemic retraction of capillary tufts with or without mesangiolysis. These were probably secondary to preglomerular vascular occlusion. A few additional glomeruli were markedly swollen and showed thrombotic lesions. In contrast to these changes in placebo-treated SHRSP, spironolactone-treated SHRSP exhibited a marked reduction in both renal vascular and glomerular lesions (Table). No appreciable leukocytic infiltrate was observed in the kidneys of either group.

PRA averaged 52±15 ng Ang I/mL/h in placebo-treated SHRSP, which is consistent with the paradoxical increases that are known to occur in salt-loaded SHRSP. PRA averaged 16±2 ng Ang I/mL/h in the spironolactone-treated SHRSP, which, although elevated, was significant less than in their placebo-treated littermates (P<0.01).

Protocol 2
In a separate experimental series, SHRSP were started on Stroke-Prone Rodent Diet and 1% NaCl drinking solution at 8 weeks of age and were chronically treated with either spironolactone (n=6) or vehicle (n=6). Fig 4A shows the cumulative percentage of spironolactone- and vehicle-treated animals surviving at different ages. Five out of the 6 SHRSP from the vehicle-treated group displayed signs of stroke beginning at 13.4 weeks of age and died before 16 weeks of age. In contrast, none of the spironolactone-treated SHRSP showed stroke signs and all survived until 19 weeks of age at which point the study was ended. Microscopic examination revealed cerebrovascular lesions in the brains of all vehicle-treated SHRSP, commensurate with the demonstration of stroke signs in these animals. Brains from spironolactone-treated animals showed occasional lesions of rarefaction and edema that were not as marked as in the controls. The average cerebrovascular lesion score in spironolactone-treated animals was significantly less than in the control group (0.8±0.6 versus 2.5±0.5, P<0.05). There was no significant difference in systolic blood pressure between the groups over the course of the study (Fig 4B). At 15 weeks of age, spironolactone-treated SHRSP developed severe hypertension, averaging levels of 259±5 mm Hg. Saline intake and urine volume tended to increase with time while food intake and body weight tended to decrease, however, there were no statistically significant differences between the groups (data not shown). Pretreatment UPE in these animals reached 136±13 mg/d while levels in spironolactone-treated animals remained at basal levels (17±4 mg/d, P<0.01). The occurrence of renal vascular and glomerular lesions was also markedly reduced in the kidneys of those SHRSP that were chronically treated with spironolactone. The percentage of lesioned glomeruli was 21±2% in the vehicle group and 6±4% in the group treated with spironolactone, P<0.01. The number of vascular profiles with lesions per 100 glomeruli counted was 28±4 in the vehicle group and 6±4 in the group treated with spironolactone, P<0.01.

Discussion
To determine whether endogenous mineralocorticoids play a role in the malignant nephrosclerosis and stroke that develops in saline-drinking SHRSP, we chronically administered a mineralocorticoid receptor antagonist to these animals. We found that a low dose of spironolactone, 10 mg/kg/d, markedly increased the survival of saline-drinking SHRSP as compared to the vehicle-treated group. In this experiment, mortality from stroke in the vehicle group also occurred at a somewhat later age relative to that seen in our previous studies. This delay is best explained by the fact that these animals were started on the high-salt/stroke-prone rodent diet at a later age.21 In a separate experimental series, we started the animals on high-salt/stroke-prone rodent diet at the usual time. Survival was not an endpoint in this study, nonetheless, 3 of the placebo-treated rats developed neurological signs of stroke and died early. Histopathologic analysis of the brains from both of these experiments demonstrated a significant reduction in cerebrovascular damage in those SHRSP given spironolactone. Previous studies by Roberts et al demonstrated that rats given DOCA and a 1% NaCl drinking solution developed neurological signs of stroke and cerebrovascular lesions. Recent studies by McLeod et al have demonstrated that chronic infusion of aldosterone can reverse the ability of captopril to prevent mortality and cerebrovascular injury in salt-loaded SHRSP.
Figure 3. Photomicrographs of representative hematoxylin and eosin-stained mid-coronal kidney sections from two 10.4 week-old stroke-prone spontaneously hypertensive rats maintained on Stroke-Prone Rodent Diet and 1% NaCl starting at 7.5 weeks of age (original magnification, x 130). (A) Renal cortex from an animal into which a placebo pellet was implanted at 7.5 weeks of age. The photomicrograph illustrates the presence of lesions of thrombotic microangiopathy affecting several glomeruli and blood vessels. A relatively normal glomerulus is seen towards the right lower quadrant. Ischemic retraction and obliteration of capillary tufts is seen in 1 glomerulus (small arrow). Segmental capillary tuft necrosis, widespread thrombosis, and cellular swelling, are evident in two additional swollen glomeruli (large arrows). Microvascular lesions consist of marked concentric medial hypertrophy (small arrowhead), and circumferential, transmural fibrinoid necrosis as observed in two small arteries (large arrowheads), one showing fragmented and extravasated erythrocytes, in addition. (B) Renal cortex from an animal into which a pellet containing 200 mg of spironolactone was implanted at 7.5 weeks of age. The photomicrograph illustrates the absence of significant glomerular or vascular pathology as seen above in the age-matched, placebo-treated littermate control.

The results of the present study are consistent with these findings and suggest that endogenous mineralocorticoids play a role in the development of cerebral lesions in saline-drinking SHRSP. Spironolactone also produced a marked protective effect against the development of renal vascular injury in saline-drinking SHRSP. In both experimental protocols, animals treated with the mineralocorticoid receptor antagonist developed less proteinuria and exhibited substantial reductions in the number of glomerular and vascular lesions. In previous studies, we found that chronic treatment with ACE inhibitors and the Ang II type 1 receptor antagonist, losartan, agents that would be expected to diminish aldosterone release, prevented lesions of malignant nephrosclerosis despite the
absence of a blood pressure lowering effect in saline-drinking SHRSP. Chronic ACE inhibitor treatment with enalapril also failed to lower arterial pressure in rats with DOCA-salt hypertension but, in contrast to SHRSP, did not protect against the development of proteinuria and malignant nephrosclerosis. 

Aldosterone has also been reported to play a role in the development of renal injury in the remnant kidney model of chronic renal failure. In that study, exogenous aldosterone administration completely reversed the ability of combined treatment with enalapril and losartan to attenuate the onset of renal damage, signified by proteinuria, at which time treatments were started. Values in parentheses indicate the number of animals.

The findings in the present study with spironolactone provide strong evidence for a major role of endogenous mineralocorticoids in the development of renal vascular pathology of saline-drinking SHRSP. A relationship between mineralocorticoids and malignant nephrosclerosis in rats was first demonstrated by Selye and coworkers in 1943. They reported that combined treatment with DOCA and a 1% NaCl-drinking solution produced severe hypertension and malignant nephrosclerosis while DOCA was comparatively inactive when NaCl intake was not excessive. The contribution of mineralocorticoids to vascular injury may have been particularly prominent under the conditions of our study, since SHRSP were maintained on a 1% NaCl-drinking solution.

In the present study PRA was markedly elevated in placebo-treated SHRSP, which is consistent with the paradoxical increase known to occur with salt-loading in these animals. PRA averaged 16.0 ± 2.0 ng Ang I/mL/h in spironolactone-treated SHRSP. Although this value was less than in placebo-treated SHRSP, it was substantially elevated compared to our previously reported values of 3.5 ± 1.0 ng Ang I/mL/h in WKY given standard diet and water, 0.6 ± 0.5 ng Ang I/mL/h in WKY given Stroke-Prone Rodent Diet and 1% NaCl, and 9.2 ± 2.5 ng Ang I/mL/h in SHRSP given standard diet and water. The higher level of PRA in placebo-treated SHRSP relative to that of spironolactone-treated SHRSP is probably due to the extensive renal damage that was observed in the former group (Table). Consistent with the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore the concept that the protective effect of spironolactone relates to mineralocorticoid receptor antagonism rather than effects on PRA is the finding that aldosterone can restore

The beneficial effects of spironolactone in saline-drinking SHRSP were also independent of major changes in water and electrolyte excretion. Chronic administration of mild diuretics is typically associated with only a transient increase in water and electrolyte excretion that is not sustained. In rats on a normal sodium intake, administration of spironolactone at a dose of 20 mg/kg/d for one week did not alter daily urinary potassium excretion. Increases in urinary sodium excretion (10%) and the urinary Na+/K+ ratio (15%) were observed only on the first day of treatment. We observed no differences in water and electrolyte excretion between the groups until the onset of renal damage, signified by proteinuria, at which time urine output and sodium excretion were higher in placebo-treated rats. Smeda and Tkachenko examined the effects of

![Figure 4. Line graphs showing (A) cumulative percent survival and (B) systolic blood pressure (SBP) of stroke-prone spontaneously hypertensive rats that were chronically treated with either spironolactone (10 mg/kg/d SC, n=6) or the saline oil vehicle (1 mL/kg/d SC, n=6) Animals were maintained on 1% NaCl drinking solution and Stroke-Prone Rodent Diet starting at 8.8 weeks of age at which time treatments were started. Values in parentheses indicate the number of animals.](http://hyper.ahajournals.org/DownloadedFrom)
Various diuretics on survival of salt-loaded SHRSP. They found that chronic treatment with chlorothiazide or amiloride offered no protection against stroke and concluded that increases or decreases in urinary potassium excretion do not affect the development of pathology in these animals. Also in this study, losartan treatment decreased survival of SHRSP; which was thought to be caused by activation of the RAAS. Previous studies have demonstrated a protective effect of high dietary potassium against the development of stroke in SHRSP, which was not associated with increases in plasma potassium levels. Thus, increases in serum potassium, per se, may not play a major role in the vascular protective effect of high dietary potassium in salt-loaded SHRSP. Studies by Volpe and coworkers demonstrated that the protective effect of high dietary potassium in salt-loaded SHRSP was most likely due to suppression of renin release and not duress and natruresis. Likewise, we found that chronic treatment with enalapril or captopril had no effect on water and electrolyte excretion by saline-drinking SHRSP but prevented end organ damage. Our results with spironolactone are commensurate with these findings and support the concept that the protective effect with this treatment is not due to major changes in water and electrolyte excretion.

The precise mechanism by which mineralocorticoids contribute to the development of vascular pathology in salt-drinking SHRSP remains unclear. Chronic treatment with spironolactone has been reported to prevent myocardial fibrosis in rats with hypertension induced by unilateral renal ischemia or chronic aldosterone infusion. It has been suggested that these pathophysiologic effects of aldosterone occur via nonpithelial mineralocorticoid receptors, have a time course of days to weeks rather than hours, reflect occupancy of only a small percentage of such receptors, and require salt loading. It has also been suggested that aldosterone may alter myocardial permeability so that fibrosis might be a secondary event accompanying the appearance of growth factors. The possibility that aldosterone exerts similar influences in other tissues and organs cannot be excluded and should be further investigated. Aldosterone and Ang II were found to increase protein kinase C activity in vascular smooth muscle cells and protein kinase C activation has been reported to increase vascular permeability. Ullan and coworkers demonstrated a direct relationship between the activity of aldosterone and Ang II in vascular smooth muscle cells. They found that aldosterone upregulates Ang II membrane receptors, thereby increasing the synthesis of mositol-1,4,5-trisphosphate and release of intracellular Ca++. This upregulation was inhibited to a considerable extent by spironolactone, suggesting that it was primarily mediated by the mineralocorticoid receptor. These findings are consistent with a synergistic interaction between Ang II and aldosterone in the production of vascular pathology, which was first proposed by Masson and coworkers. Thus, an interaction between Ang II and aldosterone may be important in the production of end-organ damage in SHRSP.

In summary, chronic treatment with the mineralocorticoid receptor antagonist, spironolactone, markedly diminished proteinuria, renal lesions of malignant nephrosclerosis and signs of stroke in saline-drinking SHRSP. Spironolactone treatment in these animals had little or no effect on systolic arterial blood pressure or water and electrolyte excretion. These results suggest that aldosterone, or a related factor with mineralocorticoid activity, plays a major role in the development of vascular injury in saline-drinking SHRSP.

Acknowledgments

The authors wish to thank James Funk and Jessica Brunner for technical assistance and Saramma George-Matthew for expert processing of tissue for histology. This work was supported by US Public Health Service grant HL-35522.

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Mineralocorticoid Blockade Reduces Vascular Injury in Stroke-Prone Hypertensive Rats

Hypertension. 1998;31:451-458
doi: 10.1161/01.HYP.31.1.451
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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