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Aldosterone Plays a Pivotal Role in the Pathogenesis of Thrombotic Microangiopathy in SHRSP

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Abstract. Angiotensin-converting enzyme inhibitors and aldosterone receptor antagonists ameliorate malignant nephrosclerotic lesions of thrombotic microangiopathy in salt-loaded, stroke-prone, spontaneously hypertensive rats (SHRSP) without controlling hypertension. This suggests that angiotensin II (Ang II) and/or aldosterone (ALDO) plays a critical role in renal injury in this model. For evaluating their relative roles in the pathogenesis of thrombotic microangiopathy, SHRSP were adrenalectomized and infused with vehicle, Ang II, or ALDO or were sham-operated for adrenalectomy (SHAM). Saline-drinking rats were assigned to one of four groups: SHAM, adrenalectomy, adrenalectomy + Ang II (25 ng/min, subcutaneously), or adrenalectomy + ALDO (40 μg/kg per d, subcutaneously). All SHRSP received dexamethasone (12 μg/kg per d, subcutaneously). Adrenalectomy did not show changes in body weight, plasma creatinine, sodium and potassium, and daily urinary sodium and potassium excretion; did not prevent hypertension but prevented proteinuria (12 $\pm$ 1 *versus* 49 $\pm$ 3 mg/d; $P < 0.01$); and abrogated thrombotic microangiopathy and decreased plasma aldosterone (<16 *versus* 710 $\pm$ 91 pg/ml; $P < 0.001$) compared with SHAM. Systolic BP in adrenalectomy + Ang II and adrenalectomy + ALDO (238 $\pm$ 8 and 241 $\pm$ 9 mmHg, respectively) was similar to SHAM. Despite Ang II infusion, proteinuria (17 $\pm$ 9 mg/d) and thrombotic microangiopathy and plasma aldosterone (18 $\pm$ 18 pg/ml) remained low but daily urinary excretion of sodium and potassium were not different from adrenalectomy + ALDO. Adrenalectomy + ALDO showed plasma aldosterone levels of 735 $\pm$ 147 pg/ml; plasma potassium was lower; plasma creatinine and proteinuria (78 $\pm$ 7 mg/d) were greater and thrombotic microangiopathy lesions were comparable to SHAM. These results demonstrate a pivotal role for aldosterone in the development of thrombotic microangiopathy, independent of hypertension.

Malignant hypertension is characterized by markedly elevated BP, stimulation of the renin-angiotensin-aldosterone system (RAAS), and acute multiorgan failure associated with thrombotic microangiopathy (TMA). Stroke-prone spontaneously hypertensive rats (SHRSP), a genetic experimental model of malignant hypertension (1), on a high-salt intake rapidly develop severe hypertension with TMA and proteinuria and die of strokes. Consistent with a role for the RAAS in these animals was the finding that a paradoxical increase in plasma renin activity occurs with time, despite continued salt loading (2–4). Endothelial injury initiates the development of TMA. Historically, overstimulation of angiotensin II (Ang II) and/or shear stress of elevated BP has been implicated in the pathogenesis of TMA in malignant hypertension. Ang II, a potent vasoconstrictor, is believed to induce endothelial injury because of shear stress and/or a direct cytotoxic effect (5,6). In SHRSP, Ang II independent of severe hypertension is associated with TMA, as malignant nephrosclerosis in these animals can be entirely prevented by treatment with angiotensin-converting enzyme inhibitors (ACEI) (3,7,8) and Ang II receptor blockers (4,9,10) in the absence of appreciable BP lowering. Aldosterone (ALDO), however, is also released in response to Ang II, and normally these two hormones are concomitantly elevated during activation of the RAAS (11). Whereas Ang II has received much attention in the pathogenesis of TMA in malignant hypertension (12), a role for ALDO has not been well characterized. Mineralocorticoid receptors are present in endothelial cells (13,14) and vascular smooth muscle cells (15). We have previously reported that treatment with either a nonselective (15,16) or a selective (17,18) ALDO receptor antagonist largely attenuated cerebral and renal TMA and significantly prolonged the survival of SHRSP. Similar to our observations with ACEI, this effect was independent of BP lowering or any overt diuretic or natriuretic action, suggesting that this protection may not necessarily be elicited through the classic epithelial effects of ALDO on the distal nephron (16). The present study was designed to evaluate the relative role of ALDO and Ang II, the two major hormones of the RAAS, in the development of TMA, testing the hypothesis that they play independent roles in vascular injury. Because the adrenal gland is the major source of circulating mineralocorticoids, we used bilateral adrenalectomy to abolish endogenous ALDO production followed by glucocorticoid replacement plus exogenous

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infusions of either Ang II or ALDO for 2 wk, at which time kidneys were obtained for histologic evaluation.

Materials and Methods

Animals

Male SHRSP/A3N (generations F 76 to 78, 84; $n = 58$), obtained from our local colony, were used in these experiments. Studies were performed in accordance with NIH guidelines and were approved by the New York Medical College Institutional Animal Care and Use Committee. All animals were housed in a room lighted 12 h 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 wk of age and allowed free access to Purina Lab Chow 5001 (Ralston Purina, St. Louis, MO) and tap water until the experiments were initiated.

Protocol 1

At approximately 60 d of age, animals were placed on Stroke-Prone Rodent Diet (#39-288; Zeigler Bros., Gardners, PA) and 1% NaCl drinking solution *ad libitum*. Stroke-Prone Rodent Diet has lower potassium content than standard diet (0.7 *versus* 1.2%, respectively) and in conjunction with high salt intake accelerates the development of hypertensive end-organ pathology (7). Two to 7 d later, animals were anesthetized with pentobarbital (60 mg/kg, intraperitoneally) and bilateral adrenalectomy (ADX) was performed in 21 SHRSP through a flank incision. During this procedure, rats were assigned to one of three different groups—ADX, ADX + Ang II, or ADX + ALDO—and were infused, respectively, with 0.5% ethanol vehicle ($n = 7$), Ang II at 25 ng/min ($n = 6$), or aldosterone at 40 μg/kg per d ($n = 8$) via Alzet osmotic minipumps subcutaneously at the nape of the neck. The same surgical procedure was performed, but the adrenal glands were left intact in seven SHRSP that received vehicle (SHAM). Glucocorticoid replacement with dexamethasone (12 μg/kg per d in sesame oil) was instituted after surgery. This dose of dexamethasone maintains normal weight gain, GFR, and fasting plasma glucose and insulin levels in adrenalectomized rats (19). ALDO (D-aldosterone) and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO), and Ang II (human, peptide purity 99.9%) was purchased from American Peptide (Sunny Vale, CA). The doses of ALDO (20) and Ang II (18) were selected on the basis of our previous observations that they can reverse the protection provided by captopril in saline-drinking SHRSP. The concentrations used to fill the pumps were calculated on the basis of the mean pump rate provided by the manufacturer, the body weight of the animals, and the dose intended.

Two to 3 d before the animals were killed, they were placed in individual metabolic cages and 24-h urine samples were collected for the assessment of proteinuria. Systolic BP (SBP) was measured 24 h before the animals were killed. Animals were decapitated, and trunk blood was collected into chilled tubes that contained EDTA. The kidneys were then removed, cross-sectioned, fixed in formalin, and processed for light microscopy by standard techniques.

Protocol 2

A second series of SHRSP was housed in metabolic cages and given 1% NaCl drinking solution and Stroke-Prone Rodent Diet starting at 60 d of age. Bilateral adrenalectomy or sham operation was performed at 67 d of age, at which time 2-wk Alzet osmotic minipumps were implanted subcutaneously at the nape of the neck using the identical procedures as described in protocol 1 (SHAM = 8, ADX = 7, ADX + Ang II = 8, ADX + ALDO = 7). Twenty-four-hour food intake, urinary volume and urinary sodium and potassium excretion, and body weight were measured each day. The study was terminated 2 wk later at 81 d of age, at which time the animals were anesthetized with pentobarbital (60 mg/kg, intraperitoneally) and blood was drawn carefully from the abdominal aorta into heparin-coated syringes for later measurement of plasma sodium, potassium, and creatinine levels.

Assays and Analyses

Tail-cuff plethysmography was performed to determine SBP of awake animals using a Natsume KN-210 manometer and tachometer (Peninsula Laboratories, Belmont, CA). Rats were warmed at 37°C for 10 min and allowed to rest quietly in a Lucite chamber before measurement of BP. 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 ALDO concentration was determined by standard RIA (Diagnostic Products Co., Los Angeles, CA). Plasma and urinary sodium and potassium concentrations were measured using an IL 943 flame photometer (Instrumentation Laboratory, Lexington, MA). Plasma creatinine concentration was measured using reagent kit 541 from Sigma and a Sclavo Unifast II analyzer (Sienna, Italy).

Histology

Coronal sections of kidney, cut at 3 to 4 mm, were preserved in 10% phosphate-buffered formalin, and at least three such tissues were sampled from different regions and embedded in paraffin. Histologic sections (2 to 3 μm) were stained with hematoxylin and eosin and examined by light microscopy at 200 and 400× in a blinded manner for lesions, as described previously (3,8,9,16,20,21). Vascular damage was assessed by counting the total number of arterial and arteriolar profiles per midcoronal section showing thrombotic and/or proliferative arteriopathy. Vessels with thrombotic lesions showed mural fibrinoid necrosis with fragmented and extravasated erythrocytes and luminal obliteration with thrombosis. Proliferative arteriopathy was characterized by nodular mural thickening as a result of proliferation of markedly swollen myointimal cells often superimposed on thrombotic lesions. Vascular damage was expressed as the number of lesioned arteries and arterioles per 100 glomeruli and calculated by dividing the total number of lesioned vascular profiles by the total number of glomeruli in the same midcoronal section and multiplied by 100. Total glomerular damage was assessed by evaluating TMA lesions characterized by thrombonecrosis of glomeruli (thrombotic) and by separately counting retracted capillary tufts with or without mesangiolysis. The latter, termed ischemic, were a consequence of vascular obliteration or resolved thrombotic lesions. Glomerular thrombotic lesions showed in addition to segmental or global thrombosis or necrosis of capillary tufts, cellular swelling, and frequently also fragmented erythrocytes. These lesions were often an extension of similar pathology affecting the adjacent arterioles. The number of glomeruli exhibiting lesions in either category was enumerated from each kidney and expressed as a percentage of the total number of glomeruli present per midcoronal section (mean ± SEM = 218 ± 5 glomeruli per animal; range = 167 to 274 glomeruli). Tubules were semiquantitatively assessed for casts and ischemic profiles as a percentage of the total by evaluating all sections. Ischemic tubules were characterized by relatively small diameter, intact but wrinkled basement membranes, and were lined by simplified epithelium.

Statistical Analyses

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 *t* tests or one-way ANOVA followed by *post hoc* analysis using the Newman-Keuls multiple comparison test. Data were analyzed using version 2.01 of the GraphPad Prism statistical software package obtained from GraphPad Software (San Diego, CA). $P < 0.05$ was considered statistically significant. Data are reported as mean ± SEM.

Results

The effects of ADX on BP are shown in Figure 1. ADX did not prevent hypertension but decreased SBP as compared with SHAM (208 ± 6 *versus* 244 ± 5 mmHg; $P < 0.01$). Infusion of either Ang II or ALDO restored SBP (238 ± 8 and 241 ± 9 mmHg, respectively) to levels that were not different from SHAM but were significantly different from ADX ($P < 0.001$).

Figure 2 shows the results of 24-h urinary protein excretion measured before the animals were killed. ADX prevented the increase in urinary protein excretion observed in SHAM (12 ± 1 *versus* 49 ± 3 mg/d; $P < 0.01$). This effect was totally reversed by the infusion of ALDO, with urinary protein excretion even greater than that seen in SHAM (78 ± 7 mg/d; $P < 0.001$ ADX *versus* ADX + ALDO). In contrast, the infusion of Ang II did not overcome the protective effect of ADX against the development of proteinuria in saline-drinking SHRSP (17 ± 9 mg/d in ADX + Ang II *versus* 12 ± 1 mg/d in ADX). Thus, urinary protein excretion levels in these animals remained markedly reduced (17 ± 9 mg/d) relative to SHAM ($P < 0.001$) or ALDO ($P < 0.001$).

Figure 3 shows ALDO levels measured in the plasma obtained upon termination of the study. SHAM demonstrated plasma ALDO levels of 710 ± 91 pg/ml. Plasma ALDO was at or below the limit of detection in vehicle-infused, ADX rats (<16 pg/ml; $P < 0.001$ *versus* SHAM). As would be expected,

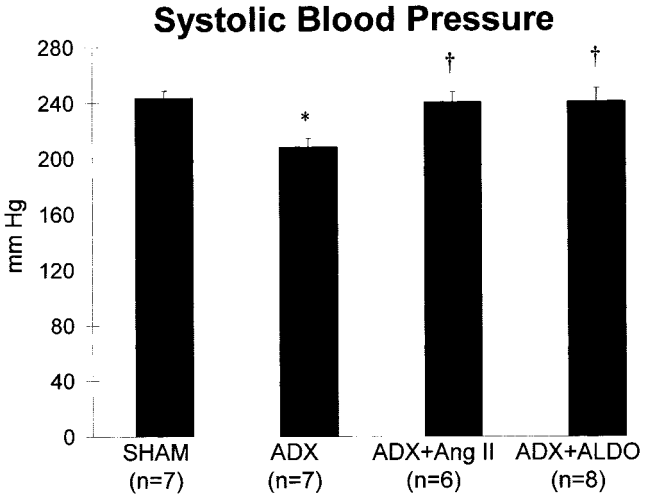

Figure 1. Preterminal systolic BP in saline-drinking stroke-prone spontaneously hypertensive rats (SHRSP): SHAM, ADX, ADX + Ang II (25 ng/min), and ADX + ALDO (40 μg/kg per d). Animals were adrenalectomized or sham operated at 62 to 67 d of age and infused with Ang II or ALDO for 2 wk. *$P < 0.01$ compared with SHAM; †$P < 0.001$ compared with ADX. Values are mean ± SEM.

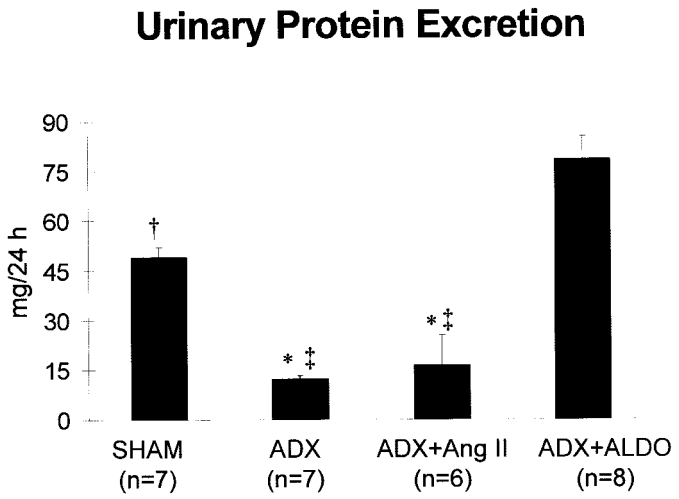

Figure 2. Urinary protein excretion in saline-drinking SHRSP upon termination of the study. Treatments and results for statistical comparisons are as described in the legend to Figure 1. *$P < 0.01$ compared with SHAM; †$P < 0.01$, ‡$P < 0.001$ compared with ADX + ALDO. Values are mean ± SEM.

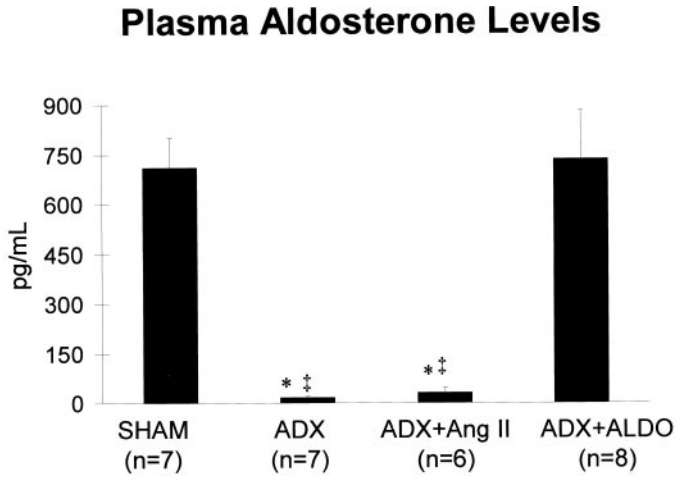

Figure 3. Plasma ALDO levels in saline-drinking SHRSP upon termination of the study. Treatments and results for statistical comparisons are as described in the legend to Figure 1. *$P < 0.01$ compared with SHAM, ‡$P < 0.01$ compared with ADX + ALDO. Values are mean ± SEM.

Ang II–infused, adrenalectomized animals demonstrated markedly suppressed plasma ALDO levels ($P < 0.001$ *versus* SHAM). Plasma ALDO levels were above the limit of detection in only one of the six animals receiving Ang II infusion. ALDO infusion restored plasma levels to 735 ± 147 pg/ml, which did not differ from SHAM but were significantly elevated compared with ADX ($P < 0.001$) or ADX + Ang II ($P < 0.001$).

Histopathology results are summarized in Table 1. Widespread and severe lesions of TMA affecting microvessels (20.2 ± 2.2 *versus* 1.4 ± 1.4%; $P < 0.01$) and glomeruli—thrombotic (2.9 ± 0.3 *versus* 0.2 ± 0.2%; $P < 0.01$) and ischemic

Table 1. Renal pathology

Parameter	SHAM ($n = 7$)	ADX ($n = 7$)	ADX + Ang II ($n = 6$)	ADX + ALDO ($n = 8$)
TMA				
Glomerular (%)	14.3 ± 2.6	0.6 ± 0.6[b]	1.8 ± 1.6[b]	8.2 ± 1.7[a,d,e]
Thrombotic (%)	2.9 ± 0.3	0.2 ± 0.2[b]	0.1 ± 0.1[b]	1.8 ± 0.9[c,e]
Ischemic (%)	11.4 ± 2.4	0.4 ± 0.4[b]	1.7 ± 1.6[b]	6.4 ± 1.1[a,c,e]
Vascular (%)	20.2 ± 2.2	1.4 ± 1.4[b]	2.7 ± 2.7[b]	15.1 ± 2.9[d,f]
Ischemic tubules (%)	35 ± 3	3 ± 3[b]	2 ± 2[b]	27 ± 7[d,f]
Tubular casts (%)	3.4 ± 0.8	0.1 ± 0.1[b]	0.3 ± 0.3[b]	3.4 ± 0.6[d,f]

Values are mean ± SEM.
[a] $P < 0.05$, [b] $P < 0.01$ compared with SHAM.
[c] $P < 0.05$, [d] $P < 0.01$ compared with ADX.
[e] $P < 0.05$, [f] $P < 0.01$ compared with ADX + Ang II.

(11.4 ± 2.4 *versus* 0.4 ± 0.4%; $P < 0.01$)—were observed in SHAM *versus* ADX, respectively, and are illustrated in Figure 4A. Tubules surrounding the lesioned glomeruli and vessels frequently revealed ischemic changes or contained tubular casts. Tubular casts and proteinuria were generally proportional to the degree of glomerular damage. The lesions were almost completely prevented by ADX as illustrated in Figure 4B. It is interesting that despite the restoration of severe hypertension, Ang II infusion failed to induce significant renal injury in ADX + Ang II (Figure 4C). In fact, these animals demonstrated almost complete absence of glomerular and vascular lesions of TMA ($P < 0.01$ *versus* SHAM; Table 1) except in one of six animals with a few scattered lesions. This was also the only animal that revealed a detectable level of ALDO, although no overt adrenal regeneration was evident. Protection provided by adrenalectomy was completely lost, however, with ALDO replacement, as evidenced by the development of renal lesions in ADX + ALDO comparable to those seen in SHAM (Figure 4D). TMA in SHAM and ADX + ALDO was most prominent in the juxtamedullary or deeper nephrons as described in our previous studies.

Table 2 shows the results for plasma values and body weight obtained in a separate series of animals (protocol 2). There was no difference in plasma sodium among the groups. Plasma potassium and creatinine were unaffected by ADX alone or ADX + Ang II. However, plasma potassium was markedly diminished in ADX + ALDO relative to all other groups, and plasma creatinine was increased relative to ADX and ADX + Ang II. Body weight was similar among the groups at the beginning and the end of the study. The change in body weight over the course of the study was greater in ADX + ALDO (−18 ± 4) than SHAM (−3 ± 7; $P < 0.05$), but there were no significant differences among the other groups. Figure 5 shows urinary sodium and potassium excretion on each day of the study. There were differences only on a few occasions between the groups; however, no consistent pattern emerged. ADX + Ang II *versus* ADX + ALDO differed in sodium excretion only on two occasions despite significant differences in the renal lesions. Similarly, daily potassium excretion was different between ADX + Ang II *versus* ADX + ALDO only on the last 2 d, when food intake by ADX + ALDO had declined (data not shown).

Discussion

Malignant nephrosclerosis, characterized by TMA, is considered secondary to shear stress from markedly elevated BP and RAAS activation. Ang II has been conventionally implicated as the primary mediator of this hypertensive damage. From recent studies, a role for ALDO in renal injury has also emerged (15–18,20,22,23). In the present study, we evaluated their relative role in the pathogenesis of TMA in animals in which endogenous ALDO production was completely abolished by bilateral surgical ablation of the adrenal glands, followed by infusion of Ang II or ALDO.

Consonant with previous observations (1–4,9,10,16–18,20,21), SHAM developed severe hypertension, proteinuria and renal TMA, and tubular ischemic damage and casts. Bilateral adrenalectomy virtually prevented the development of proteinuria and TMA, despite the persistence of significant hypertension, although SBP was slightly but significantly lower in ADX than in SHAM. Plasma ALDO levels were barely detectable in ADX, which corroborates the successful surgical ablation and lack of regeneration of adrenal tissue or systemic elaboration from an overexpressed extra-adrenal tissue source.

To evaluate whether exogenously infused Ang II in the absence of circulating ALDO could reverse the renal protection afforded by adrenalectomy, we administered Ang II (25 ng/min). This dose, when chronically infused into adrenal-intact, captopril-treated, saline-drinking SHRSP, reversed the prevention of TMA by ACEI without increment in SBP (18). Eplerenone, a selective ALDO receptor antagonist, in turn abrogated Ang II–induced renal TMA, suggesting that it was mediated by ALDO. This study required further confirmation, as in these adrenal intact animals both Ang II and ALDO would be in the circulation. In the present study, Ang II infusion in the absence of detectable plasma ALDO levels failed to restore proteinuria and development of renal TMA, despite full restoration of SBP levels to those observed in SHAM. Elevation in SBP of ADX + Ang II compared with ADX is not surprising in view of the well-known, potent,

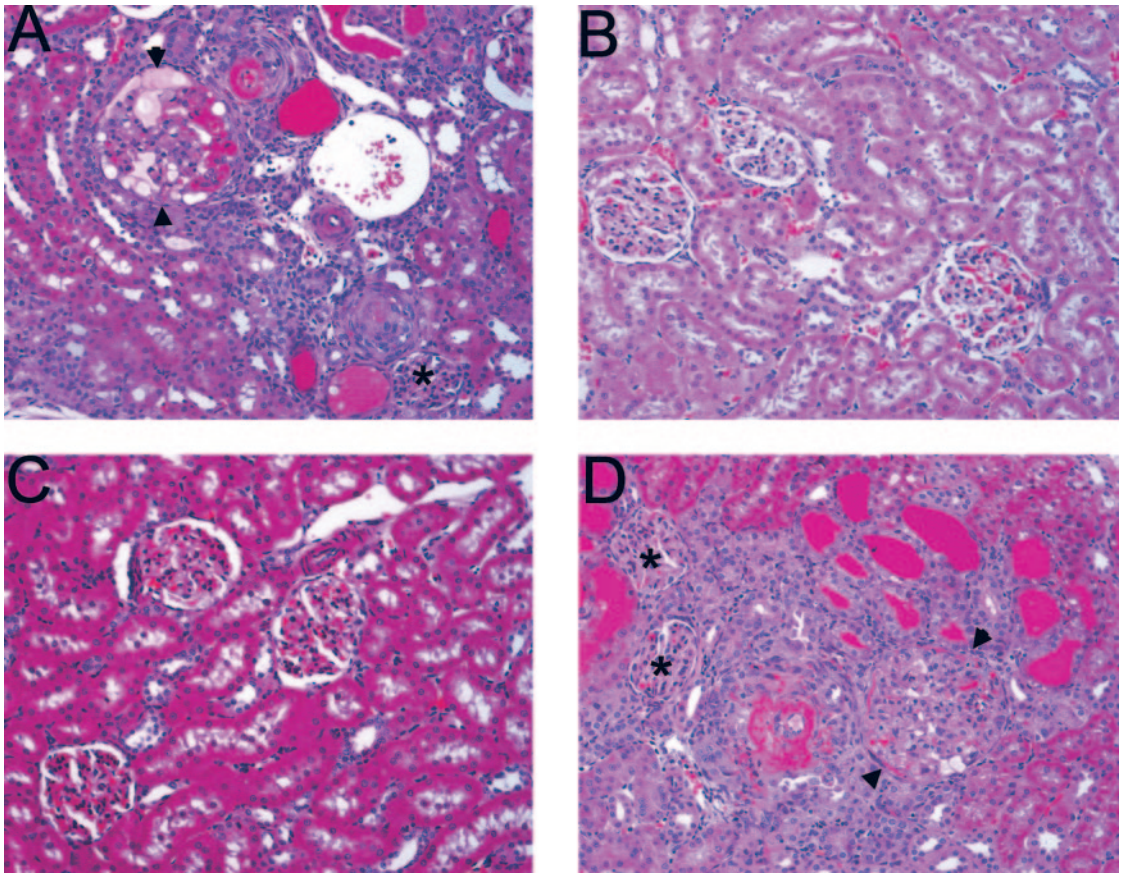

Figure 4. Representative photomicrographs of renal cortex from bilaterally adrenalectomized or sham-operated, saline-drinking SHRSP. (A) Sham-operated, vehicle-infused SHRSP (SHAM) demonstrate typical lesions of thrombotic microangiopathy (TMA) associated with malignant nephrosclerosis. There is a large swollen glomerulus (arrowheads) with segmental thrombosis and focally stranded fragmented erythrocytes. An adjacent arteriole is obliterated with intraluminal and mural fibrinoid deposits. Another glomerulus (*) with ischemic retraction is adjacent to an obliterated arteriole showing marked concentric proliferative arteriolopathy. Note also moderate medial thickening of an arteriole without discrete lesions of TMA between the other two arterioles. Surrounding tubules show ischemic change or hyaline casts accompanied by mild interstitial inflammation. (B) Adrenalectomized SHRSP (ADX) infused with vehicle alone are devoid of any glomerular, vascular, or tubular pathology. (C) Adrenalectomized, saline-drinking SHRSP infused with Ang II (25 ng/min; ADX + Ang II) reveal no lesions of TMA either. (D) Lesions of malignant nephrosclerosis reappear in adrenalectomized SHRSP that are infused with aldosterone (40 μg/kg per d; ADX + ALDO) instead of Ang II. Note a swollen glomerulus (arrowheads) with fibrinoid necrosis and fragmented erythrocytes and two small ischemic glomeruli (*). An arteriole in the center reveals thrombonecrotic lesions accompanied by proliferative arteriopathy. Surrounding parenchyma reveals tubules with ischemic retraction or hyaline casts and mild interstitial inflammation similar to changes seen in SHAM. Magnification, $\times$100, hematoxylin and eosin.

direct, vasoconstricting effect of Ang II (24). In conclusion, Ang II, in the absence of circulating ALDO, fails to induce renal TMA, despite markedly elevated SBP.

The present findings support our previous observations of dissociation between hypertension and TMA in saline-drinking SHRSP (3,7–9,15–18). Such dissociation is also observed in patients with acute scleroderma crisis, in whom TMA identical to malignant nephrosclerosis can be present in nonhypertensive individuals (25). Malignant nephrosclerosis, independent of BP lowering with triple therapy, has been reported in rats double transgenic for human renin and angiotensinogen genes with elevated Ang II (26). Chuslip and Kincaid-Smith (6) also reported that RAAS stimulation, independent of severity of BP, was responsible for TMA.

We previously reported that ALDO induces TMA in saline-drinking SHRSP protected by ACEI (20). The role of ALDO relative to Ang II, however, could not be addressed. Our present findings specifically implicate ALDO in the pathogenesis of TMA in malignant hypertension. In contrast to Ang II, when ALDO was replaced in ADX, not only SBP but also proteinuria and TMA were restored. Indeed, proteinuria was even greater than in SHAM and was accompanied by hypokalemia, which likely further aggravated the ALDO-induced renal injury as evidenced by elevated plasma creatinine. Mineralocorticoid-induced TMA, virtually identical to that seen in salt-loaded SHRSP but independent of Ang II effect, is seen in the well-established model of deoxycorticosterone acetate–salt hypertension (27). Renin levels in this model remain markedly suppressed even during the malignant phase of hypertension (28). Treatment with the ACEI enalapril (29,30) or the Ang II subtype 1 receptor blocker losartan (30) fails

Table 2. Plasma values and body weight

Parameter	SHAM ($n = 8$)	ADX ($n = 7$)	ADX + Ang II ($n = 8$)	ADX + ALDO ($n = 7$)
Sodium (mEq/L)	141 ± 1	141 ± 1	141 ± 1	143 ± 2
Potassium (mEq/L)	3.2 ± 0.3	3.5 ± 0.3	3.8 ± 0.3	2.1 ± 0.2[a,c,e]
Creatinine (mg%)	0.70 ± 0.07	0.57 ± 0.08	0.54 ± 0.12	0.88 ± 0.21[b,d]
Initial body wt (g)	250 ± 6	253 ± 6	247 ± 5	244 ± 7
Final body wt (g)	247 ± 5	243 ± 6	238 ± 7	227 ± 9

Values are mean ± SEM.
[a] $P < 0.01$ compared with SHAM.
[b] $P < 0.05$, [c] $P < 0.01$ compared with ADX.
[d] $P < 0.05$, [e] $P < 0.01$ compared with ADX + Ang II.

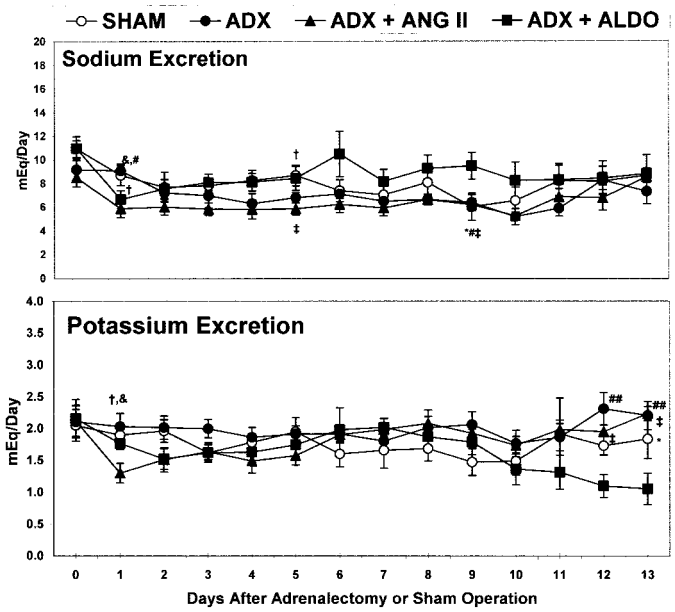

Figure 5. Daily urinary sodium and potassium excretion in saline-drinking SHRSP: SHAM, ADX, ADX + Ang II (25 ng/min), and ADX + ALDO (40 μg/kg per d). Animals were adrenalectomized or sham operated at 67 d of age (day 0) and infused with Ang II or ALDO for 2 wk. For SHAM, †$P < 0.05$ compared with ADX + Ang II; *$P < 0.05$ compared with ADX + ALDO. For ADX, &$P < 0.05$ compared with ADX + Ang II; #$P < 0.05$, ##$P < 0.01$ compared with ADX + ALDO. For ADX + Ang II: ‡$P < 0.05$ compared with ADX + ALDO. Values are mean ± SEM.

to prevent proteinuria and TMA in salt-loaded, mineralocorticoid-treated animals. Spironolactone, however, protects against radiation-induced TMA (31) in an animal model in which the RAAS is known to be stimulated. ALDO has also been shown to induce TMA in rats with nitro-L-arginine methyl ester/Ang II/salt arteriolopathy (32). These findings support the view that ALDO-induced microvascular injury occurs without overt participation of and downstream from Ang II.

The effect of adrenalectomy on the development of renal injury has not been widely studied. Quan *et al.* (33), demonstrated that adrenalectomy afforded marked renal protection independent of the corticosterone maintenance level in ablative nephropathy. ALDO replacement was not performed in that study, and the mechanism behind this protective effect remained unclear. It can be argued that hypovolemia and/or hyperkalemia induced by adrenalectomy may play a role. In saline-drinking SHRSP, ADX was protective despite the lack of difference in body weight and in plasma sodium and potassium compared with SHAM. These findings may relate to the reduced potassium content of the Stroke-Prone Rodent Diet in conjunction with high-salt intake. Nonetheless, the lack of hyperkalemic response in ADX or ADX + Ang II compared with SHAM does not suggest that the renal protective effect in these two groups is mediated via hyperkalemia. Moreover, no major differences were noted in the daily urinary potassium excretion. A decrease in potassium excretion in ADX + ALDO compared with the other groups on the last 2 d of study could be correlated to reduced food intake and considerable morbidity in these animals. Likewise, daily urinary sodium excretion did not differ considerably or in a consistent pattern among the groups. Although plasma sodium and daily urinary excretion of sodium were similar in ADX + Ang II and ADX + ALDO, there were marked differences in renal lesions between these groups. These findings suggest that the induction of TMA or renal protective effects observed in these animals were not volume dependent.

Although our findings suggest an important role for ALDO in end-organ damage, it would be important to recognize that high circulating ALDO levels alone may not necessarily result in TMA. For example, patients with Bartter's syndrome and Yanomama Indians (34) remain normotensive without significant cardiovascular events, despite markedly elevated circulating levels of renin and ALDO as a result of marked tubular salt wasting and exceedingly low dietary salt intake, respectively. In these conditions, excessive secretion of ALDO, therefore, is reactive, to maintain normal electrolyte and fluid homeostasis. In primary hyperaldosteronism, however, Conn *et al.* (35), in their original report, observed a high incidence of vascular pathologic changes and an 85% incidence of proteinuria among 145 patients with an ALDO-producing adrenal adenoma. Malignant hypertension and strokes have also been occasionally reported in patients with Conn's syndrome (36–38). The induction of TMA occurs most readily in experimental models of malignant hypertension in which inappropriate

salt loading is associated with high ALDO (30) or other mineralocorticoids such as deoxycorticosterone acetate (27). TMA, however, is not always dependent on salt excess and has been reported to occur in the Ren-2 transgenic rat model of hypertension in the absence of salt loading (26,39) or rats made hypertensive by unilateral renal artery stenosis that are volume depleted (40). Our study confirms that ALDO but not Ang II, in the presence of inappropriate salt loading and severe hypertension, induces TMA.

The mechanism for ALDO-induced vascular injury is not well understood. ALDO may interfere with vascular nitric oxide production and/or bioactivity (41–43). A proinflammatory role for ALDO has also been suggested in the coronary microvasculopathy in the salt-AngII model of hypertension (44). The vascular lesions in this model are similar to the renovascular lesions in SHRSP and show infiltration by ED-1–positive monocytes; enhanced expression of vascular cell adhesion molecule-1 in the endothelium; and of COX-2, osteopontin, and monocyte chemoattractant protein-1 mRNA in the media. Vasculopathy and the associated proinflammatory expressions were attenuated by eplerenone, suggesting a pathogenetic role for aldosterone. Likewise, coronary vasculopathy and associated enhanced expression of the inflammatory transcription factors NF-kβ and activator protein-1 were attenuated by mineralocorticoid blockade in rats double-transgenic for the human renin and angiotensinogen genes (45). Furthermore, a thrombogenic potential of ALDO is suggested by its stimulation of plasminogen activator inhibitor-1 (31). ALDO-mediated microvascular injury may also be induced via reduced neuronal uptake of norepinephrine (46) and superoxide and/or hydroxyl radicals (47). There thus is cumulative evidence that ALDO-induced vascular injury may result from its prothrombotic and/or proinflammatory vascular effects.

Ang II and ALDO may mediate end-organ damage in a distinct yet interdependent manner. In conclusion, our studies indicate that ALDO plays a unique and pivotal role, independent of severe hypertension, in the pathogenesis of TMA in malignant nephrosclerosis.

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