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Aldosterone: A Mediator of Myocardial Necrosis and Renal Arteriopathy*

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ABSTRACT

To determine the role of aldosterone in mediating cardiovascular damage, we performed ablation/replacement experiments with aldosterone in a rat model of cardiac injury. Administration of angiotensin II and N^{ω}-nitro-L-arginine methyl ester (L-NAME; nitric oxide synthesis inhibitor) to male rats drinking 1% saline caused hypertension, severe biventricular myocardial necrosis, proteinuria, and fibrinoid necrosis of renal and cardiac vessels. Removal of aldosterone by adrenalectomy or through administration of the selective aldosterone antagonist eplerenone markedly reduced the cardiac and renal damage without significantly altering blood pressure. Aldosterone infusion in adrenalectomized, glucocorticoid-replaced L-NAME/angiotensin II-treated animals restored damage. Thus, we identified aldosterone as a critical mediator of L-NAME/angiotensine II induced vascular damage through mechanisms apparently independent of its effects on systolic blood pressure. (*Endocrinology* **141:** 3871–3878, 2000)

THE RENIN-ANGIOTENSIN-ALDOSTERONE system (RAAS) is a major regulator of the cardiovascular system. Abnormal activation of this system has been postulated to participate in the occurrence of end-organ damage in hypertensive patients (1, 2). Supporting this concept are clinical studies showing that treatment of hypertensive patients with either angiotensin-converting enzyme (ACE) inhibitors (3, 4) or angiotensin II type I (AT1) receptor antagonists (5, 6) provides significant protection against cardiovascular morbidity and mortality. Animal studies also demonstrate a significant contribution of ACE inhibitors and AT1 receptor antagonists in preventing renal (7–10), cerebral (7, 8, 11), and cardiovascular (8, 12) injury. Thus, it has been proposed that abnormal activation of the RAAS represents a cardiovascular risk factor (13).

Although many studies have investigated the role of angiotensin II (Ang II) in mediating cardiovascular damage, relatively little attention has been paid to the role of aldosterone, the end product of the RAAS. However, there are data to suggest that aldosterone may play an important role in the pathogenesis of cardiovascular disease that is independent of Ang II. Patients with primary aldosteronism, in which Ang II levels are usually very low, have a higher incidence of left ventricular hypertrophy (14), albuminuria (15), and stroke (16, 17) than do patients with essential hypertension. A recent study performed in patients classified with New York Heart Association class III and IV cardiac failure showed a 30% reduction in morbidity and mortality with the addition of the aldosterone antagonist spironolactone to conventional therapy including ACE inhibitors, loop diuretics, and digoxin (18). This decrease occurred with an average dose of spironolactone (26 mg/day) that did not have significant hemodynamic effects.

Experimental animal data support a role for aldosterone in mediating cardiovascular injury in the kidney and brain. In the stroke-prone spontaneously hypertensive rat (SHRSP), a genetic model of spontaneous hypertension, administration of either spironolactone (19) or an ACE inhibitor (7, 8, 20) greatly attenuated renal and cerebral vascular damage (20, 21). Likewise, in the remnant kidney hypertensive rat, administration of aldosterone reversed the renal protection given by blockade of the RAAS with combined ACE inhibition/AT1 receptor antagonist treatment (22). Thus, in the kidney and brain, aldosterone may have deleterious effects on the vasculature that may be independent of other components of the RAAS.

An important pathological effect of aldosterone in the heart has been reported in experimental models of mineralocorticoid hypertension. In these studies prolonged (6- to 8-week) exposure to aldosterone was associated with the development of myocardial fibrosis (23, 24). Although a direct effect of aldosterone on collagen deposition was initially proposed, *in vitro* studies have not consistently demonstrated an effect of aldosterone in modulating collagen gene expression (25, 26). Thus, the mechanisms by which aldo-

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sterone participates in the establishment of myocardial injury are unclear.

To explore the role of aldosterone in mediating early cardiovascular injury in the heart, we used an experimental model in rats that combines elevated blood pressure, moderately high salt intake, an activated RAAS, and suppressed nitric oxide production. This model involves chronic inhibition of nitric oxide synthase with N^{ω}-nitro-L-arginine methyl ester (L-NAME) for 14 days in 1% NaCl-drinking rats combined with a 3-day infusion of Ang II on days 11–14. In previous studies, administration of L-NAME alone for 4–6 weeks (27) or of L-NAME for 17 days coupled with a short-term Ang II infusion (28) caused the development of hypertension and myocardial fibrosis. Under both of these conditions, cardiac damage could be reduced by AT1 receptor antagonism. However, the role of aldosterone in mediating this damage was not studied.

In the present experiments we determined the early pathological effects of mineralocorticoids on the heart and kidney by performing ablation/replacement experiments with aldosterone in the 14-day L-NAME/Ang II/NaCl model of cardiac injury. Specifically, we tested whether reduction of mineralocorticoids by either adrenalectomy or pharmacological antagonism with eplerenone, a selective aldosterone receptor blocker (29, 30), would prevent cardiac and renal damage in this model and whether aldosterone replacement in adrenalectomized rats would restore damage. In addition, we determined what type of cardiac damage was induced by the L-NAME/Ang II/NaCl treatment and compared these changes to those that occurred in the kidney.

Materials and Methods

Animals

The present studies were conducted in accordance with institutional guidelines for the humane treatment of animals using male Wistar rats (n = 44), weighing 200–225 g, obtained from Charles River Laboratories, Inc. (Wilmington, MA). All animals were housed in a room lighted 12 h/day at an ambient temperature of 22 ± 1 C. Animals were allowed 1 week to recover after arrival and had free access to Purina Lab Chow 5001 (Ralston Purina Co., St. Louis, MO) and tap water until the initiation of the experiment.

Experimental protocol

Wistar rats were housed in individual metabolic cages and given 1% NaCl as drinking fluid *ad libitum*. Three days later, rats were placed on one of five dosing protocols. Group 1 (NaCl; n = 8) received 1% NaCl to drink. Group 2 (L-NAME/Ang II/NaCl; n = 8) received L-NAME for 14 days and 1% NaCl. On day 11 of L-NAME treatment, an osmotic minipump containing Ang II was implanted in each animal sc. Group 3 (L-NAME/Ang II/NaCl plus eplerenone; n = 8) received L-NAME/Ang II/NaCl and eplerenone (100 mg/kg/day p.o., days 0 to 14). Eplerenone was dissolved in 0.5% methylcellulose and administered twice a day by gavage. Two additional groups of NaCl-drinking rats were adrenalectomized (ADX) 3 days before initiation of L-NAME/Ang II treatment. Group 4 (L-NAME/Ang II/NaCl plus ADX; n = 11) received glucocorticoid replacement with dexamethasone starting immediately after the surgery. Group 5 (L-NAME/Ang II/NaCl plus ADX/ALDO; n = 9) received in addition to dexamethasone, aldosterone starting on day 0 simultaneously with L-NAME treatment. Dexamethasone was dissolved in sesame oil and administered as a single sc dose (12 μg/kg·day) every day. This dose of dexamethasone has been reported to maintain normal weight gain, glomerular filtration rate, and fasting plasma glucose and insulin levels in adrenalectomized rats (31). The experiment was concluded on day 14 of L-NAME treatment. Ang II and aldosterone were administered via Alzet osmotic minipumps (models 2001 and 2002, respectively, Alza Corp., Palo Alto, CA), which were implanted sc at the nape of the neck in animals anesthetized with isoflurane. The concentrations of Ang II and aldosterone used to fill the pumps were calculated based on the mean pump rate provided by the manufacturer, the body weight of the animals on the day before implantation of the pumps, and the dose planned. Ang II (human, 99% peptide purity) was purchased from American Peptide Co. (Sunnyvale, CA) and administered at a dose of 225 μg/kg·day as reported previously (28). The dose of aldosterone (40 μg/kg·day) is approximately 50% lower than the dose used previously in studies of aldosterone-induced cardiovascular injury (23, 24). This lower dose induced lesions in stroke-prone spontaneously hypertensive rats (20). Dexamethasone, aldosterone, and L-NAME were purchased from Sigma (St. Louis, MO). The concentration of L-NAME in the drinking water was adjusted daily to provide a dose of 40 mg/kg·day based on the daily fluid intake and the body weight of the rats.

Surgical procedure

Three days before initiation of L-NAME treatment, rats from groups 4 and 5 were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL; 60 mg/kg, ip). Bilateral adrenalectomy was performed using a dorsolumbar approach, making separate incisions on each side. Adrenalectomized animals received 1% NaCl *ad libitum* to drink after the surgical procedure. No postoperative deaths occurred.

Animals in all groups were handled and weighed daily and maintained in separate metabolic cages. Twenty-four-hour fluid intake, food intake, and urine output were measured daily. Systolic blood pressure was measured 3 days before initiation of L-NAME treatment and on days 1, 5, 9, and 13. On day 14 of L-NAME treatment, animals were decapitated, trunk blood was collected into chilled tubes containing EDTA, and the heart and kidneys were removed, blotted dry, and immediately weighed. The heart and the kidneys were stored in 10% phosphate-buffered formalin and later processed for light microscopic evaluation.

Assays and analyses

Systolic blood pressure was measured in awake animals by tail-cuff plethysmography using a Natsume KN-210 manometer and tachometer (Peninsula Laboratories, Inc., Belmont, CA). Rats were warmed at 37 C for 10 min and allowed to rest quietly in a Lucite chamber before measurement of blood pressure. Urinary protein concentration was determined in urine collected on the last day of the experiment using the sulfosalicylic acid turbidity method. Urinary protein excretion was calculated as the product of the urinary concentration times the urine output per 24 h. Plasma aldosterone concentration was determined using a standard RIA kit from Diagnostic Products (Los Angeles, CA). PRA was determined by RIA detection of generated angiotensin I (DiaSorin, Inc., Stillwater, MN).

Histology

Hearts were stained with the collagen-specific dye Sirius red for determination of fibrosis as reported previously (24). Interstitial collagen was determined using an automated image analyzer. The hearts were also stained with hematoxylin and eosin for light microscopic analysis. Two or three sections of the heart were analyzed for each animal. Sections were taken from different parts of the heart and contained both right and left ventricles. A scale from 0–4 was used to score the level of myocardial injury in each section, and an average score for each animal was obtained. A score of 0 represented no damage. A score of 1 represented the presence of myocytes demonstrating early necrotic changes such as nuclear pyknosis or karyolysis, noncontracting marginal wavy fibers, and eosinophilic staining of the cytoplasm associated with the presence of scattered neutrophilic infiltrates. A score of 2 was given when one clear area of necrosis (loss of myocardial cells with heavy neutrophilic infiltrates) was observed. An example of this type of lesion is shown in Fig. 3A. When two or more separate areas of necrosis were found (implicating the presence of two different myocardial infarctions in the same heart), but the areas were localized and compromised less

than 50% of the ventricular wall, the hearts received a score of 3. A score of 4 was assigned to hearts that demonstrated extensive areas of necrosis compromising more than 50% of either the left or the right ventricle.

Coronal sections of kidney were cut at 3–4 mm, and at least three or four of these were prepared as paraffin-embedded blocks. Histological sections (2–3 μm) were stained with periodic acid-Schiff reagent and examined by light microscopy at $\times$10 and $\times$40 by a pathologist who had no knowledge of the different experimental protocols. Glomerular damage, when present, was characterized as the presence of either segmental or global sclerosis with ischemic or thrombotic changes. Renal arterial and arteriolar damage was categorized as the presence of fibrinoid necrosis of the vascular wall. The renal arterial and arteriolar profiles presenting damage were counted, and the number of injured vessels per section was divided by the number of glomeruli in the same section to normalize for the amount of tissue examined. Renal vascular lesions were expressed as the number of injured vessels per 100 glomeruli.

Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Systolic blood pressure was analyzed using repeated measures ANOVA for time and treatment group with *post-hoc* analysis using Dunnett's test for comparisons against control. One-way ANOVA was used for normally distributed data with one grouping variable. *Post-hoc* analysis was performed using Newman-Keuls multiple comparison test. Data that were not normally distributed were analyzed with the Kruskal-Wallis test. Subsequently, selected pairwise comparisons were made using the exact Wilcoxon test. Data are reported as the mean $\pm$ SE for normally distributed data and as the median with upper and lower quartile values for data that were not normally distributed.

Results

Blood pressure

Baseline systolic blood pressure was similar in all treatment groups. By day 5 of L-NAME/NaCl treatment, animals in the adrenalectomized groups, but not those in the intact groups, showed a significant increase in systolic blood pressure compared with control animals ($P < 0.05$). Thereafter, all animals receiving L-NAME/Ang II/NaCl treatment showed similar increases in systolic blood pressure compared with the NaCl-drinking controls ($P < 0.001$). The extent of hypertension observed at the end of the experiment was not appreciably influenced by eplerenone treatment or adrenalectomy (Fig. 1).

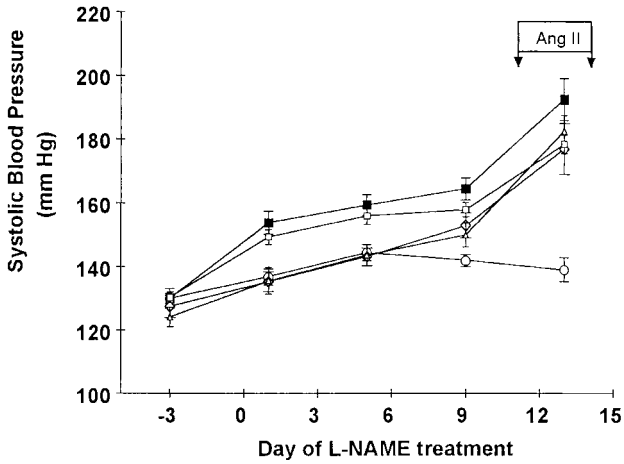

FIG. 1. Systolic blood pressure. Tail-cuff measurements of systolic blood pressure obtained before and after initiation of L-NAME treatment on days 1, 5, 9, and 13. Ang II was infused sc starting on day 11. Rats were killed on day 14. ○, NaCl; △, L-NAME/Ang II/NaCl; ◇, L-NAME/Ang II/NaCl plus eplerenone; □, L-NAME/Ang II/NaCl plus adrenalectomy; ■, L-NAME/Ang II/NaCl plus ADX/aldosterone. $P < 0.01$ in all groups *vs.* NaCl. Values are the mean $\pm$ SE.

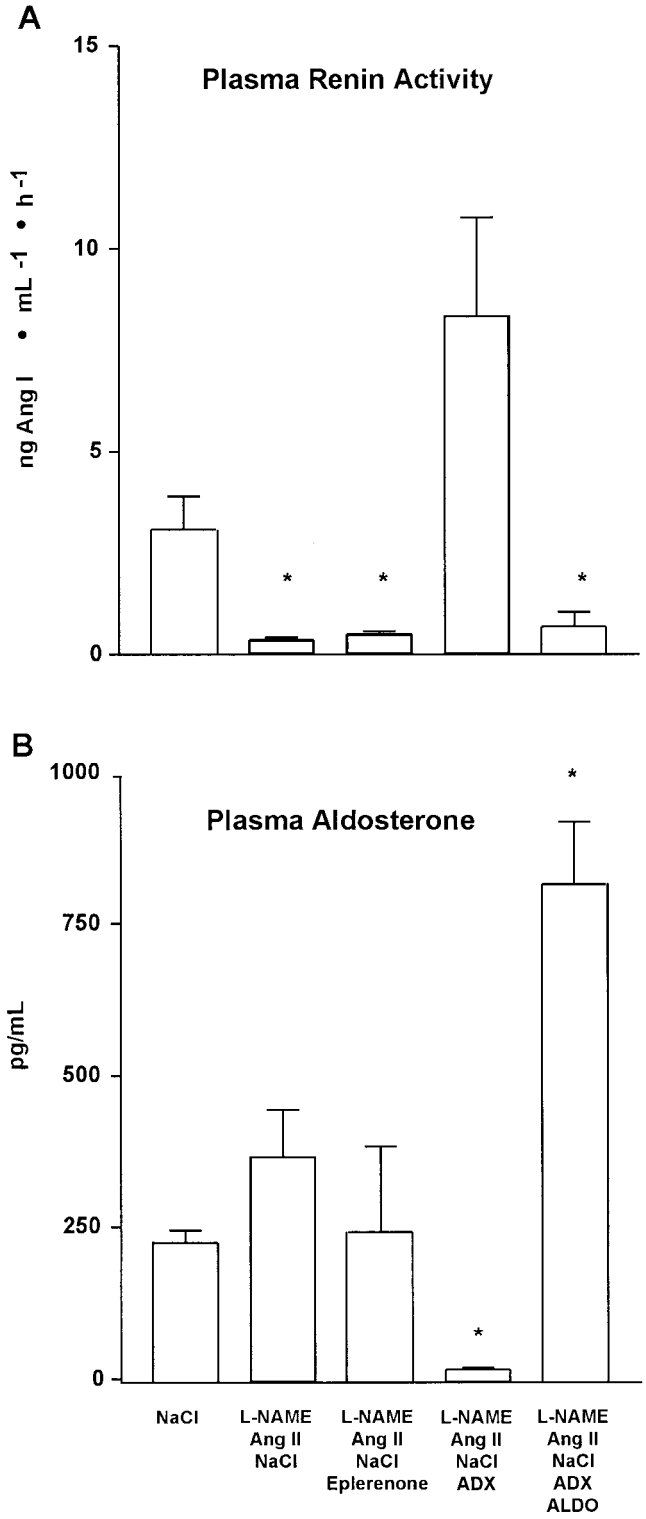

FIG. 2. PRA (A) and plasma aldosterone levels (B) determined after death. Values are the mean $\pm$ SE. *, $P < 0.01$ *vs.* NaCl.

PRA and aldosterone

Data for PRA and circulating aldosterone levels are shown in Fig. 2. L-NAME/Ang II/NaCl treatment significantly reduced PRA in intact animals compared with that in saline-drinking controls. The higher levels of PRA observed in L-NAME/Ang II/NaCl-treated rats that were adrenalectomized was prevented by the administration of aldosterone (Fig. 2A). Despite the marked inhibition of PRA observed in adrenal-intact animals treated with L-NAME/Ang II/NaCl or L-NAME/Ang II/NaCl plus eplerenone, plasma aldosterone was similar to that in saline-drinking controls (Fig. 2B). As anticipated, plasma aldosterone was reduced to undetectable levels in adrenalectomized rats, whereas adrenalectomized, aldosterone-infused rats had elevated aldosterone levels.

Cardiac damage

Summarized in Table 1 are data obtained at the end of the experiment. Body weight was not different among the three groups of adrenal-intact animals. However, both groups of adrenalectomized rats demonstrated significantly lower body weight compared with adrenal-intact groups. The ratio between total heart weight and total body weight was used as an index of cardiac hypertrophy. The cardiac hypertrophy index was higher in all groups of animals receiving L-NAME/Ang II/NaCl compared with that in NaCl-drinking controls (Table 1). Both eplerenone treatment and adrenalectomy significantly reduced cardiac hypertrophy compared with that observed in L-NAME/Ang II/NaCl-treated rats. Infusion of aldosterone reversed the effect of adrenalectomy on the cardiac hypertrophy index and restored it to a level that was not different from that in the L-NAME/Ang II/NaCl group.

Histological examination of the hearts revealed significant differences among the treatment groups ($P < 0.0001$; Figs. 3 and 4). L-NAME/Ang II/NaCl-treated rats developed vascular damage and myocardial necrosis. A representative photomicrograph of these lesions is shown in Fig. 3A. Myocardial necrosis was characterized by loss of cross-striation of myofibers, homogenization of cytoplasm, loss of cellular membranes, pyknosis and eventually karyolysis of nuclei, and influx of inflammatory cells, including polymorphonu-

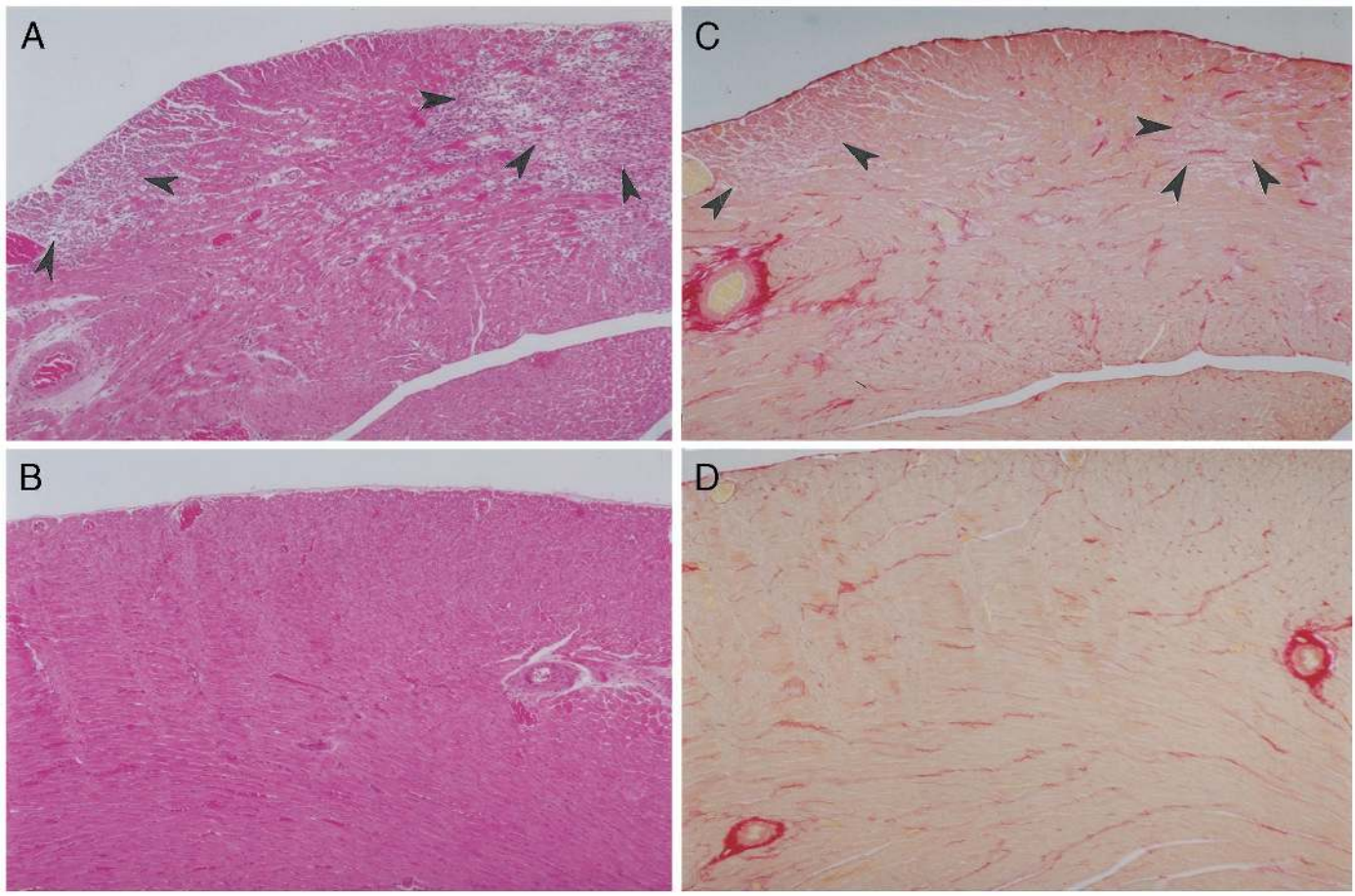

FIG. 3. Cardiac histopathology. A, Representative myocardial necrotic lesions (*arrowheads*) induced by L-NAME/Ang II/NaCl treatment (hematoxylin and eosin; magnification, ×40). These lesions were observed in both the left and right ventricles. B, Myocardium of an animal receiving L-NAME/Ang II/NaCl treatment in the presence of the mineralocorticoid receptor antagonist eplerenone, showing no necrotic lesions. This figure is also representative of histological sections from control, NaCl-drinking rats and from adrenalectomized animals receiving L-NAME/Ang II/NaCl. C and D, Staining of the hearts from A and B with the collagen-specific dye Sirius red did not reveal increased interstitial or reparative collagen deposition, even in areas where myocardial necrosis had occurred (*arrowheads* in C).

TABLE 1. Characteristics of the five treatment groups

Group	n	BW g	SBP mmHg	HW mg	HW/BW mg/g
NaCl	8	331 ± 4	139 ± 4	924 ± 2	2.79 ± .05
L-NAME/AngII/NaCl	8	311 ± 15	180 ± 5[b]	1150 ± 4[b]	3.87 ± .09[b]
L-Name/AngII/NaCl/ Eplerenone	8	330 ± 4	177 ± 8[b]	1144 ± 4[b]	3.46 ± .05[b,c]
L-NAME/AngII/NaCl/ ADX	11	278 ± 4[b,d]	176 ± 3[b]	880 ± 3[c]	3.20 ± .13[a,d]
L-NAME/AngII/NaCl/ ADX/ALDO	9	267 ± 6[b,d]	192 ± 7	951 ± 17[c]	3.57 ± .06[b,e]

Data obtained from rats in the different experimental groups at the end of the experiment.

[a] $P < 0.01$, [b] $P < 0.001$ *vs.* NaCl; [c] $P < 0.01$, [d] $P < 0.001$ *vs.* L-NAME/AngII/NaCl; [e] $P < 0.05$ *vs.* L-NAME/AngII/NaCl+ADX. Values are mean ± SE.

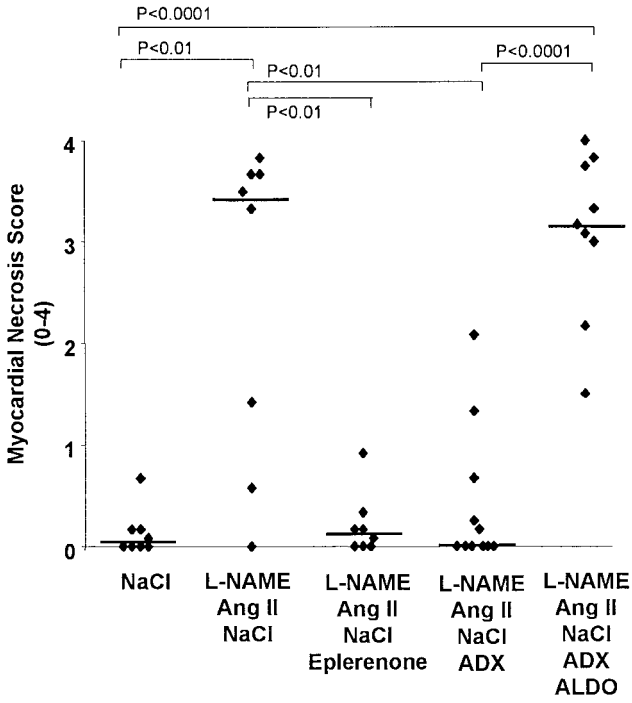

FIG. 4. Histopathological scores for myocardial necrosis. Two or three sections from each heart were examined under light microscopy and scored according to the extension of myocardial necrosis using a semiquantitative scale from 0–4, with 0 representing no damage and 4 representing severe damage. ♦, The average histopathological score for an animal. The *horizontal bar* represents the median value for the group.

clear cells and monocytes. Fibrinoid necrosis was present in small coronary arteries and arterioles (not shown). In contrast, cardiac injury in response to treatment with L-NAME/Ang II/NaCl was markedly reduced in those animals in which eplerenone was chronically administered or adrenalectomy was performed (Figs. 3B and 4). These two groups demonstrated levels of myocardial necrosis similar to those observed in the NaCl-drinking controls. The protective effect of adrenalectomy was completely reversed by the addition of an aldosterone infusion.

Staining with Sirius red (a collagen-specific dye) showed no increase in the interstitial collagen volume fraction in the heart in any of the groups receiving L-NAME/Ang II/NaCl treatment (data not shown). Furthermore, collagen deposition was not increased in areas of myocardial necrosis (Fig. 3, A and C, staining of adjacent sections with hematoxylin eosin and Sirius red, respectively).

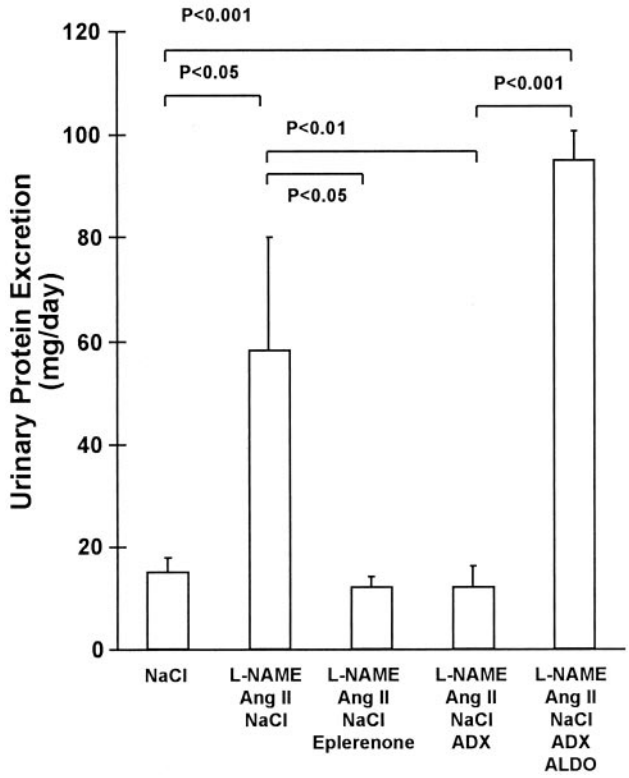

FIG. 5. Urinary protein excretion. Twenty-four-hour urinary protein measured in samples collected on the day of death (day 14). Values are the mean ± SE.

Renal damage

Urinary protein excretion (24 h) measured at the end of the 2-week treatment period was normal in the NaCl group (Fig. 5). Treatment with L-NAME/Ang II/NaCl markedly increased urinary protein excretion. Eplerenone treatment and adrenalectomy prevented the development of proteinuria in animals receiving L-NAME/Ang II/NaCl treatment. In contrast, administration of aldosterone to adrenalectomized rats completely restored the effects of L-NAME/Ang II/NaCl treatment on proteinuria.

Histopathological evaluation of the kidneys also demonstrated significant differences among the groups ($P < 0.001$; Figs. 6 and 7). Although renal arteriopathy was not found in kidneys from NaCl-drinking controls, animals receiving L-NAME/Ang II/NaCl treatment demonstrated severe renal vascular damage involving primarily arcuate and interlobular arteries and arterioles (Fig. 6). These vessels demonstrated fibrinoid necrosis of the vascular wall with medial thickening and proliferation of the perivascular connective tissue. A few isolated glomeruli had areas of focal thrombosis. Proteinaceous casts at the level of the distal tubules and reabsorption protein granules in proximal tubules frequently were observed in L-NAME/Ang II/NaCl-treated rats. Renal arteriopathy tended to be reduced in animals receiving eplerenone treatment. How-

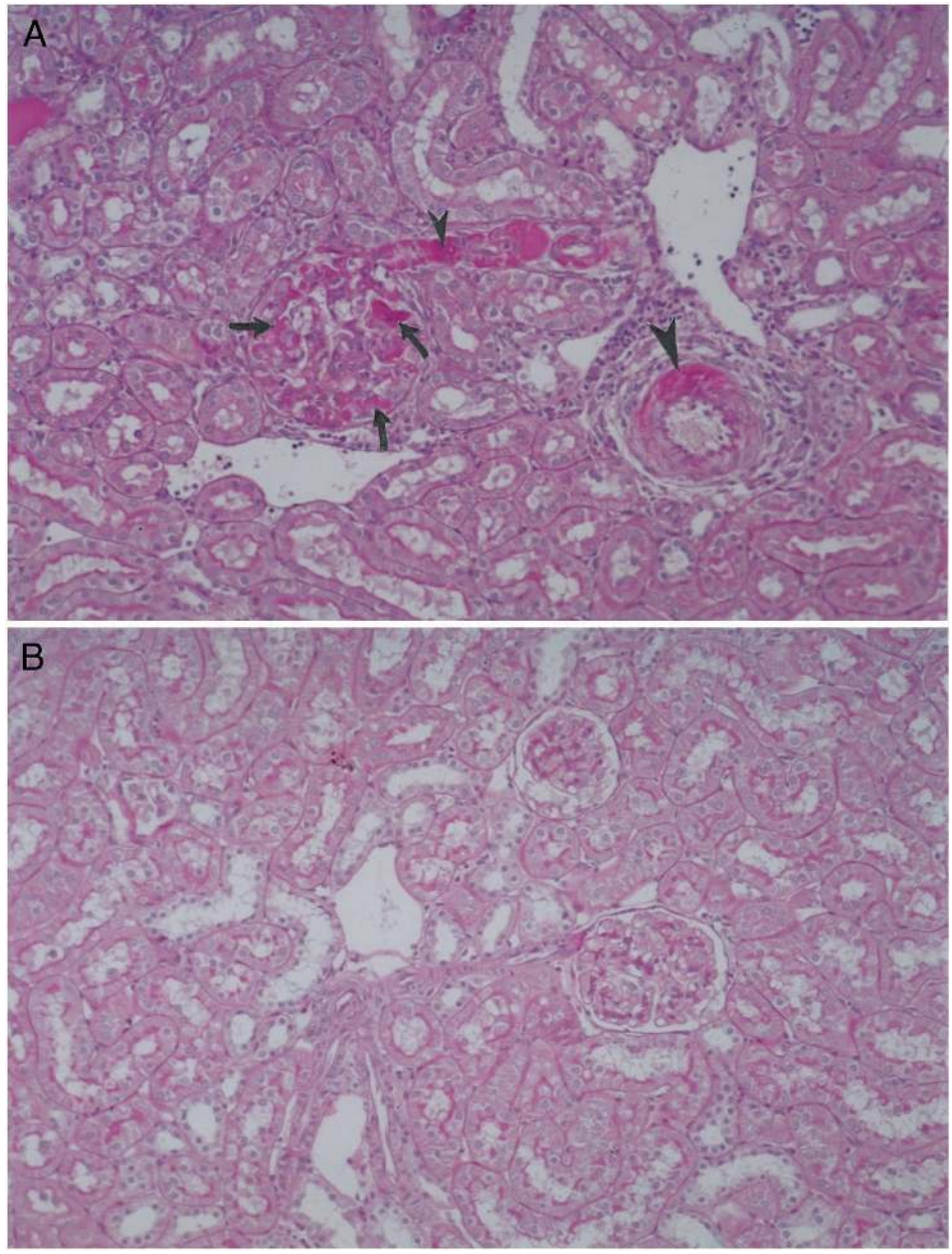

FIG. 6. A, Renal histopathology. Representative periodic acid-Schiff-stained midcoronal kidney section from an animal receiving L-NAME/Ang II/NaCl treatment (original magnification, ×40), showing microvascular lesions consisting of myointimal proliferation and circumferential, transmural fibrinoid necrosis in an afferent arteriole (*small arrowhead*) and a small interlobular artery (*large arrowhead*). In some cases the arteriolar lesions were extended to glomeruli. These glomeruli showed thrombotic lesions (*arrows*) with obliteration of the capillary lumen. B, Renal cortex of a rat receiving L-NAME/Ang II/NaCl plus eplerenone, in which arteriopathy was prevented. This figure is also representative of histological sections from control, NaCl-drinking rats and from adrenalectomized animals receiving L-NAME/Ang II/NaCl.

ever, this 60% reduction in damage compared with L-NAME/Ang II/NaCl-treated rats did not reach statistical significance upon analysis of the histopathological scores ($P = 0.1$). Adrenalectomy significantly reduced renal arteriopathy induced by L-NAME/Ang II/NaCl treatment to levels that were not significantly different from those in NaCl-drinking controls (Fig. 7). As was observed in the heart, when aldosterone was infused into adrenalectomized, L-NAME/Ang II/NaCl-treated rats, damage in the kidneys was significantly increased.

Discussion

The purpose of the present experiment was to evaluate the role of aldosterone in mediating early cardiovascular damage. We found that combined administration of Ang II and L-NAME, an inhibitor of nitric oxide synthesis, to rats on a high sodium diet caused the development of hypertension, cardiac hypertrophy, myocardial necrosis, proteinuria and renal arteriopathy. In contrast, there was no evidence of myocardial fibrosis, which is typically associated with chronic cardiovascular injury. Myocardial necrosis, proteinuria, and vascular lesions were prevented by adrenalectomy, which eliminated the presence of aldosterone. The protective effect of adrenalectomy was lost when adrenalectomized rats were infused with aldosterone. Similarly, aldosterone antagonism with eplerenone decreased cardiovascular damage, although this effect appeared to be more prominent in the heart than in the kidney. Thus, aldosterone appears to be required for the development of the acute cardiovascular lesions in L-NAME/Ang II/NaCl-treated rats.

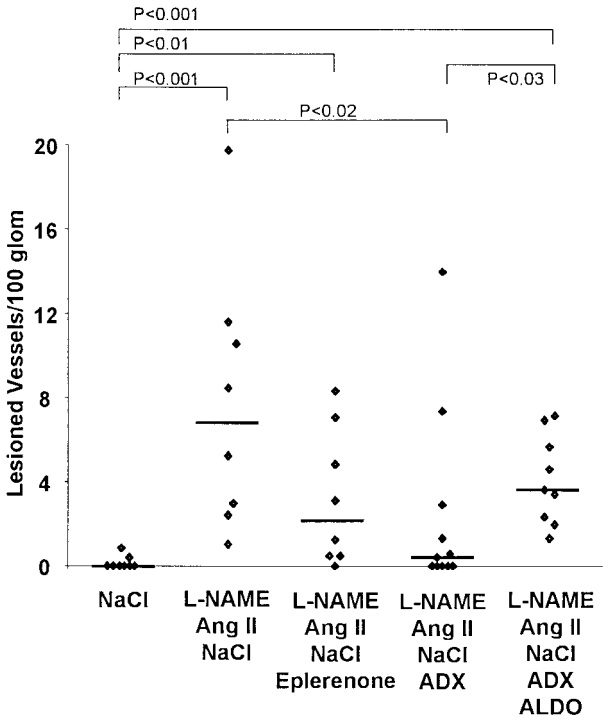

FIG. 7. Histopathological scores for renal vascular injury. Kidneys were examined under light microscopy. Renal arterial and arteriolar lesions were counted and expressed relative to the total number of glomeruli in the same section. ♦, The histopathological score for an animal. The *horizontal bar* represents the median value for the group.

Multiple studies in mineralocorticoid/salt hypertensive and renovascular hypertensive rats have suggested that aldosterone plays a critical role in the development of myocardial fibrosis (23, 24). These studies have led to the hypothesis that aldosterone has a direct effect on the synthesis of extracellular matrix proteins, which, under certain circumstances, may lead to the development of tissue fibrosis (23). However, studies attempting to show a direct effect of mineralocorticoids on extracellular matrix proteins have been inconclusive (25, 26). In the L-NAME/Ang II/NaCl model, aldosterone appears to play a critical role in the early development of vascular lesions in the small arteries and arterioles in the heart and kidney and in the development of myocardial necrosis. Myocardial interstitial fibrosis was not observed, nor was fibrosis observed in areas of myocardial damage. However, we anticipate that, as part of the reparative process, fibrosis would develop in these areas. Indeed, Hou and colleagues have shown that the L-NAME/Ang II treatment induces the expression of growth factors and extracellular matrix proteins in the heart (28). Based on the strong evidence that aldosterone provokes myocardial fibrosis when administered chronically *in vivo* (but not *in vitro*), and our present finding that aldosterone mediates early myocardial ischemic damage, we hypothesize that myocardial fibrosis is a consequence of aldosterone inducing vascular damage followed by myocardial ischemia/necrosis. Whether this effect is a direct effect of aldosterone interacting with mineralocorticoid receptors located in blood vessels (32) or in cardiomyocytes (33) or is an indirect effect mediated by other factors such as up-regulation of Ang II receptors (34, 35) or volume and electrolyte changes remains to be elucidated. Furthermore, it is not known whether eplerenone can antagonize the rapid, nongenomic effects of aldosterone.

The results of the present experiment are consistent with several studies examining the influence of mineralocorticoids on the vasculature of the kidney and brain. In the SHRSP, a genetic rat model that develops spontaneous malignant nephrosclerosis and stroke, thrombotic microangiopathy in the kidney and brain was reduced by adrenalectomy (36), spironolactone administration (19), or eplerenone administration (37). Adrenalectomy also reduced renal nephropathy in partially nephrectomized rats (38). Thus, there is accumulating evidence that aldosterone may be involved in the development of vascular damage in the brain, kidney, and heart. In the present study aldosterone antagonism by eplerenone was particularly beneficial in the heart compared with the kidney. This may reflect the greater vulnerability of the heart to ischemic injury and/or differences in the mechanisms of aldosterone-mediated injury in the two organs.

With L-NAME/NaCl treatment before the administration of Ang II, glucocorticoid-replaced adrenalectomized animals showed a more rapid increase in systolic blood pressure than did intact animals. The reason for this difference in blood pressure responsiveness is unclear, but could be related to the surgical procedure itself or to the lack of an adrenal factor. The present study design did not allow us to determine whether the presence of hypertension and/or elevated Ang II levels is a requirement for the development of aldosterone-mediated lesions. However, from our results it is clear that hypertension and high Ang II levels, in the absence of aldosterone, cause much less cardiovascular damage. This raises the question of whether some of the adverse cardiovascular effects traditionally attributed to Ang II may be mediated by aldosterone. In favor of the latter hypothesis is the fact that exogenous administration of aldosterone to hypertensive rats receiving ACE inhibition (20, 21) or combined ACE inhibition/AT1 antagonism (22) completely reverses the cardiovascular protection provided by suppression of the RAAS.

The beneficial effects of eplerenone or adrenalectomy were not related to reductions in systolic blood pressure. Other studies have shown a similar dissociation between blood pressure and end-organ damage induced under conditions of an activated RAAS. In the SHRSP, blockers of the RAAS prevented nephrosclerosis and stroke without reducing systolic blood pressure (8–11, 19, 20). In uninephrectomized, aldosterone/salt-treated rats, lowering systolic blood pressure by the administration of a mineralocorticoid receptor antagonist (RU28328) into the cerebral ventricles prevented the development of hypertension, but not that of myocardial fibrosis (24). Similarly, hypertension, but not myocardial fibrosis, was prevented with hydralazine in L-NAME-treated rats, whereas blocking the RAAS with an AT1 receptor antagonist reduced both systolic blood pressure and cardiac injury (39). Taken together, the above observations suggest that activation of the RAAS can mediate cardiovascular injury through mechanisms that are independent of a rise in systolic blood pressure.

In conclusion, the present experiments show that L-NAME/Ang II/NaCl treatment is highly effective in in-

ducing hypertension and end-organ damage at the level of the heart and the kidney. Manipulations that eliminate or antagonize aldosterone are effective in preventing such an effect, suggesting that the damaging cardiovascular effects of L-NAME/Ang II/NaCl treatment are mediated at least in part by aldosterone. Furthermore, as the removal of aldosterone did not appreciably alter systolic blood pressure, the damaging effect of aldosterone may be independent of its classic effect on sodium retention, volume expansion, and hypertension. Finally, our data suggest that the primary effect of aldosterone is not to promote fibrosis but to induce medial fibrinoid necrosis in small arteries and arterioles with subsequent tissue necrosis. Fibrosis may be a reparative process. Thus, this model of cardiac damage may provide a tool for beginning to understand the mechanisms by which aldosterone antagonism improves cardiac morbidity and mortality in patients with cardiac failure (18).

Acknowledgments

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