Aldosterone induces a vascular inflammatory phenotype in the rat heart

RICARDO ROCHA,1 AMY E. RUDOLPH,1 GREGORY E. FRIERDICHI, DENISE A. NACHOWIAK,1 BEVERLEY K. KEKEC,1 ERIC A. G. BLOMME,2 ELLEN G. MCMAHON,1 AND JOHN A. DELYANI1

1Division of Cardiovascular and Metabolic Diseases and 2Division of Global Toxicology, Pharmacia Corporation, Skokie, Illinois 60077 and St. Louis, Missouri 63141

Received 21 December 2001; accepted in final form 26 June 2002

Aldosterone induces a vascular inflammatory phenotype in the rat heart. Am J Physiol Heart Circ Physiol 283: H1802–H1810, 2002. First published June 27, 2002; 10.1152/ajpheart.01096.2001.—Vascular inflammation was examined as a potential mechanism of aldosterone-mediated myocardial injury in uninephrectomized rats receiving 1% NaCl (KCl to drink for 1, 2, or 4 wk and I) vehicle, 2) aldosterone infusion (0.75 μg/h), or 3) aldosterone infusion (0.75 μg/h) plus the selective aldosterone blocker eplerenone (100 mg·kg−1·day−1). Aldosterone induced severe hypertension at 4 wk (systolic blood pressure (SBP), 210 ± 7 mmHg vs. vehicle, 131 ± 3 mmHg, P < 0.001), which was partially attenuated by eplerenone (SBP, 180 ± 7 mmHg; P < 0.001 vs. aldosterone alone and vehicle). No significant increases in myocardial interstitial collagen fraction or hydroxyproline concentration were detected throughout the study. However, histopathological analysis of the heart revealed severe coronary inflammatory lesions, which were characterized by monocyte/macrophage infiltration and resulted in focal ischemic and necrotic changes. The histological evidence of coronary lesions was preceded by and associated with the elevation of cyclooxygenase-2 (up to ~4-fold), macrophage chemoattractant protein-1 (up to ~4-fold), and osteopontin (up to ~13-fold) mRNA expression. Eplerenone attenuated proinflammatory molecule expression in the rat heart and subsequent vascular and myocardial damage. Thus aldosterone and salt treatment in uninephrectomized rats led to severe hypertension and the development of a vascular inflammatory phenotype in the heart, which may represent one mechanism by which aldosterone contributes to myocardial disease.

cyclooxygenase-2; osteopontin; macrophage chemoattractant protein-1; eplerenone

MORE THAN 50 years ago, Selye (28) reported that exogenous administration of mineralocorticoids in the presence of a high-salt diet produced severe hypertension and vascular inflammatory lesions in the heart, kidney, brain, pancreas, and mesenteric arteries of rats. The damage observed in this model is generally presumed to be a consequence of elevations in intravascular volume and blood pressure derived from the mineralocorticoid effects on the renal epithelium leading to increased sodium and water retention (10). Clearly, elevated blood pressure per se has been shown to have deleterious effects on cardiovascular structures. However, the role of neurohormones, including aldosterone, as independent contributors to cardiovascular injury has also been suggested by multiple experimental and clinical studies. The recent identification of the mineralocorticoid receptor in the heart (18), blood vessels (30), and brain (35) raises the possibility that aldosterone may have additional direct actions on these target organs of hypertensive disease, independent of “classical” renal and blood pressure effects of mineralocorticoids. Recent data (21) showing improved survival with the addition of aldosterone antagonism to standard therapy in patients with severe heart failure, independent of hemodynamic effects, support this hypothesis.

Previous reports (23) have demonstrated that administration of spironolactone, a nonselective aldosterone blocker, prevented vascular damage in the kidney and brain of genetically hypertensive rats. This protective effect was achieved despite the persistence of severe hypertension, indicating that aldosterone may act on cardiovascular structures through additional nonhemodynamic mechanisms. There are two lines of evidence that suggest that the role of aldosterone in vascular injury is also independent of ANG II. First, aldosterone produced vascular injury in the presence of angiotensin-converting enzyme inhibition (25). Second, aldosterone antagonism results in marked vascular protection, even in the presence of ANG II infusions (24). Collectively, these results suggest that aldosterone mediates vascular injury through mechanisms independent of blood pressure or other components of the renin-angiotensin-aldosterone system.

Aldosterone has been shown to play a critical role in the development of myocardial fibrosis. Administration of aldosterone antagonists prevents the increases in collagen deposition characteristic of the myocardial disease process (26). Therefore, the identification of aldosterone’s role as a direct mediator of vascular injury provides a novel target for the prevention and treatment of cardiovascular disease.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: J. A. Delyani, Pharmacia Corp., 4901 Searle Pkwy., Skokie, IL 60077 (E-mail: John.A.Delyani@Pharmacia.com).

H1802 0363-6135/02 $5.00 Copyright © 2002 the American Physiological Society http://www.ajpheart.org
fibrosis that develops in aldosterone/salt-hypertensive rats after 6–8 wk of treatment (5, 33). More recently, it was proposed that the profibrotic effects of aldosterone in the heart are a consequence of myocardial ischemic and necrotic alterations rather than a direct effect of aldosterone on extracellular matrix deposition. This hypothesis was suggested after the observation that administration of eplerenone, a selective aldosterone blocker (6), as well as adrenalectomy, attenuated vascular lesions and myocardial necrosis in a model of ANG II-induced hypertension (26). However, the mechanisms by which aldosterone induces vascular damage in cardiovascular organs have not been elucidated.

In the present study, the early histopathological changes that occur in the heart during aldosterone/salt hypertension and the impact of eplerenone on these changes were investigated. In an effort to further understand the mechanisms of aldosterone-mediated myocardial damage, the molecular progression of myocardial injury and the accompanying inflammatory response were characterized for the first time. The results identify vascular inflammation as a pivotal contributor to aldosterone-induced myocardial pathology in this model.

**METHODS**

**Animals.** The present study was conducted in accordance with institutional guidelines for the humane treatment of animals using male Sprague-Dawley rats (225–250 g, n = 93), obtained from Harlan Sprague-Dawley (Indianapolis, IN). All animals were housed in a room lighted 12 h per day at an ambient temperature of 22 ± 1°C. Animals were allowed 1 wk to recover after arrival and had free access to rodent diet (Teklad 22/5, Harlan Teklad; Madison, WI) and tap water ad libitum until the initiation of the experiment.

**Experimental protocol.** The model of mineralocorticoid hypertension used in the present study followed the protocol previously described by Brilla and Weber (5). In this model, animals are uninephrectomized and given a 1% NaCl drinking solution to exacerbate, without qualitatively modifying, the hypertension and end-organ damage induced by mineralocorticoids (8, 28). Potassium chloride supplementation (0.3%) was added to the saline solution to prevent the potential hypokalemia associated with treatment. Rats were weighed and assigned to one of the following three groups: 1) vehicle (n = 32), 2) aldosterone (n = 31), or 3) aldosterone plus eplerenone (n = 30).

**Surgical procedure.** Laparotomy was performed in rats anesthetized with 1–2% isoflurane (Solvay Animal Health; Mendota Heights, MN). After left uninephrectomy, a radiotelemetry implant (Data Sciences International; St. Paul, MN) was inserted in the abdominal aorta ~2–3 mm above the bifurcation for continuous monitoring of blood pressure. The abdominal wall was then closed with 4-0 silk. Postoperative pain was controlled with a single subcutaneous dose of 0.1–0.5 mg/kg buprenorphine (Rickett and Colman; Richmond, VA).

**Treatment.** At the time of the surgery, an osmotic minipump (Alzet 2002, Alza; Palo Alto, CA) containing either vehicle (9% ethanol-87% propylene glycol-4% dH2O) or 1.0 mg/ml d-aldosterone (Sigma; St. Louis, MO) was inserted subcutaneously at the nape of the neck. Given the rate of infusion of the pumps, the approximate dose of aldosterone administered was 0.75 μg/h. Eplerenone was synthesized at Pharmacia and incorporated into the Teklad 22/5 rodent diet at a concentration of 1.0 mg/g of chow (~100 mg/kg/d). Previous analytic work demonstrated the stability of eplerenone in this diet, as well as the homogeneity obtained after preparation. This dose of eplerenone was selected from previous studies (24, 26), in which eplerenone significantly reduced blood pressure in hypertensive rats (unpublished data) and provided marked end-organ protective effects in the rat heart and kidney. One set of rats (n = 10/group) was treated for up to 28 days. To determine the early pathophysiological and molecular events that occur in response to aldosterone/salt administration, animals from each treatment regimen were euthanized after either 1, 2, or 4 wk of treatment (n = ~10/group).

**Euthanasia.** At the end of each experimental period, the animals were anesthetized with pentobarbital (65 mg/kg ip, Sigma). After cardiectomy, the hearts were rinsed in saline and blotted dry. A 2-mm coronal slab of the left ventricle (LV) (apex) was removed and frozen in dry ice/isopentane for molecular analysis; the remaining portion of the LV was fixed in 10% neutral-buffered formalin for hydroxyproline analysis and histology. The tissue section used for histology corresponded to the equatorial region of the left ventricle.

**Tissue processing and staining.** The equatorial regions of each LV were routinely processed and paraffin embedded. Sections (10 μm thick) were stained with the collagen-specific stain Picro-Sirius Red F3BA for determination of interstitial collagen volume fraction. Sections (5 μm) were stained with hematoxylin and eosin for histopathological analysis.

**Histopathological analysis.** Semiquantification of myocardial injury was performed, as described previously (26) with minor modification, by a trained pathologist that had no knowledge of the experimental groups during the evaluation of the tissues. Briefly, a scale from zero to four was used to score the level of myocardial injury in two sections from each heart. A score of zero represented no damage. A score of one represented the presence of vascular and perivascular inflammatory lesions without cardiomyocyte injury. A score of two was given when one clear area of myocardial necrosis was observed. Myocardial necrosis was defined as the presence of necrotic changes in cardiomyocytes, such as nuclear pyknosis or karyolysis, noncontracting marginal wavy fibers and hypereosinophilia of the cytoplasm, or clear evidence of destruction of the cardiomycyte membrane. When two or more separate areas of necrosis were found (implicating the presence of two different infarcted regions), hearts received a score of three. A score of four was assigned to hearts that demonstrated extensive areas of necrosis compromising ≥50% of the left ventricle.

**Immunohistochemistry.** Two sections (5 μm) from each heart were deparaaffinized in xylene, rehydrated in decreasing concentrations of ethanol, and endogenous peroxidase was blocked with 1.5% hydrogen peroxide for 20 min. All sections were then processed for antigen retrieval using citric acid solution pH 6.0. The primary antibody used for osteopontin was a MIIIIB101 monoclonal antibody (working dilution 1:100) developed by M. Solush and A. Franzen and obtained from the Developmental Studies Hybridoma Bank. The antibody was developed under the auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA. For identification of macrophages, an ED1 monoclonal antibody (MAB1435, working dilution 1:500) was obtained from Chemicon International
(Temecula, CA). T-cells were stained with a CD-3 polyclonal antibody (A0452, working dilution 1:300) from DAKO (Carpinteria, CA). Intracellular adhesion molecule-1 (ICAM-1) (M-19:sc-1511, working dilution 1:100) and vascular cell adhesion molecule-1 (VCAM-1) (C-19:sc-1504, working dilution 1:100) monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Positive staining was detected using horseradish peroxidase-conjugated secondary antibodies by incubating the sections in diamino-benzidine (DAKO). Isotype-matched IgGs at similar concentrations were used as primary antibodies for negative controls, and tissues known to express these targets were used as positive controls.

In situ hybridization for osteopontin. RNA probes were generated based on a sequence for rat osteopontin (GenBank accession no. NM 008608-1). Briefly, a cDNA fragment of rat osteopontin was generated by RT-PCR using the following primers: forward primer, 5'-TGG CAC ATT TGT CTT; reverse primer 3'-AGC CCA TCC AGTC. The cDNA fragment was inserted into the PCR II plasmid using the TA cloning kit (Invitrogen). Probes were labeled with an in vitro transcription kit using [33P]UTP and appropriate RNA polymerase. The cDNA fragment was inserted into the PCR II plasmid using the TA cloning kit (Invitrogen). Probes were labeled with an in vitro transcription kit using [33P]UTP and appropriate RNA polymerase. The cDNA fragment was inserted into the PCR II plasmid using the TA cloning kit (Invitrogen). Probes were labeled with an in vitro transcription kit using [33P]UTP and appropriate RNA polymerase.

Values are means ± SE.

**RESULTS**

**Blood pressure.** Blood pressure remained normal in saline-drinking controls throughout the experiment.
Aldosterone induced a progressive increase in blood pressure with time, with systolic blood pressure being significantly elevated by day 5 in both aldosterone- and aldosterone plus eplerenone-treated rats. Although significant reductions in blood pressure were apparent in animals receiving eplerenone from day 7 and throughout the study, the reduction was only partial (~50%) in that systolic blood pressure remained significantly elevated compared with saline-drinking controls.

**Myocardial fibrosis.** Interstitial collagen fraction was not statistically different at any time point among the experimental groups. The myocardial interstitial collagen of rats receiving vehicle, aldosterone/salt, and aldosterone/salt plus eplerenone at 4 wk was 6.2 ± 0.5%, 7.9 ± 1.0%, and 6.2 ± 0.5%, respectively. The slight elevation of interstitial collagen in the aldosterone/salt group at this time did not reach statistical significance ($P = 0.09$). Hydroxyproline levels also were not significantly different between the groups throughout the study (4.0 ± 0.7, 4.4 ± 0.6, and 4.1 ± 0.5 μg/mg, in rats receiving vehicle, aldosterone/salt, and aldosterone/salt plus eplerenone, respectively, for 4 wk). Thus significant myocardial interstitial fibrosis was not apparent for up to 4 wk of aldosterone/salt treatment.

**Myocardial histopathology.** Hearts from saline-drinking controls were histologically normal. No vascular or myocardial lesions were identified in hearts from rats receiving aldosterone/salt for 1 wk. In contrast, focal arterial and myocardial alterations were observed starting at 2 wk. Arterial changes were characterized by mild to moderate medial thickening with disorganization and pleomorphism of medial smooth muscle cells. Medial fibrinoid necrosis was occasionally present (Fig. 2, A and B). Perivascular leukocyte infiltration was commonly associated with morphological alterations in coronary arteries. Leukocytes also were found adhered in clusters to the endothelium in some coronary arteries. Cardiomyocyte degeneration and necrosis were sporadically observed in some animals, in association with extensive arterial inflammatory lesions, and accompanied by severe leukocyte infiltrates. These infiltrating cells were mostly macrophages, as indicated by their positive labeling with an ED1 antibody (Fig. 2C). Administration of eplerenone markedly attenuated myocardial injury at all time points (Fig. 2D). The semiquantitative scores for myocardial injury in animals treated for 4 wk are shown in Fig. 3.

**Gene expression of inflammatory mediators.** To investigate the molecular mechanisms of aldosterone/salt-mediated myocardial injury, the expression level of multiple proinflammatory molecules was quantitatively assessed. These data are summarized in Fig. 4. Consistent with the histological evidence of inflammatory changes induced by aldosterone in the heart, expression levels of cyclooxygenase-2 (COX-2) and monocyte chemotactic protein-1 (MCP-1) were similarly and significantly increased at all time points. Osteopontin expression also was markedly upregulated after 2 (~6-fold) and 4 wk (~13-fold) of aldosterone/salt treatment. Transforming growth factor-β1 mRNA levels were not upregulated at any of the time points examined. ICAM-1 mRNA expression was upregulated at 2 and 4 wk, although increases were modest. Gene expression for VCAM-1 was increased twofold at 4 wk; however, this increase did not reach statistical significance ($P = 0.06$). Consistent with the

---

**Fig. 2. Cardiac histopathology.** A: representative coronary and myocardial lesions induced by 4 wk of aldosterone/salt treatment (arrows; hematoxylin and eosin, original magnification ×100). B: ×4 magnification of Fig. 2A, inset, showing vascular inflammatory lesions. Fibrinoid necrosis of small coronary arteries (arrows) was accompanied by a severe perivascular inflammatory response (hematoxylin and eosin). C: coronary lesion in the heart of a rat that received aldosterone/salt treatment for 4 wk. Sections were immunostained with the macrophage-specific monoclonal antibody ED-1. The majority of cells infiltrating the lesions were identified as macrophages. D: myocardium of an animal receiving aldosterone/salt treatment for 4 wk, in the presence of the selective aldosterone blocker eplerenone, showing no lesions. This figure is also representative of histological sections from vehicle-infused control rats (data not shown).
histological evidence of a protective effect of eplerenone against inflammatory changes in the heart, eplerenone attenuated the molecular inflammatory response induced by aldosterone/salt treatment (Fig. 4).

**Immunohistochemistry.** The molecular analysis of the aldosterone/salt-induced proinflammatory response was further characterized using immunohistochemical analysis (Fig. 5). The majority of cells adhering to the endothelium and infiltrating the perivascular space stained positive for a monocyte/macrophage antibody (ED1) and negative for a T cell antibody (CD3). Associated with the marked leukocyte infiltration, significant expression of the chemoattractant cytokine, osteopontin, was evident in hearts from aldosterone/salt-treated rats, compared with the absence of osteopontin staining in hearts from saline-drinking controls. Osteopontin expression was primarily localized to medial cells of coronary arteries, but it was also present in macrophages in the perivascular space and areas of myocardial necrosis. No evidence of significant osteopontin expression was found in cardiomyocytes. In contrast, administration of eplerenone markedly blunted the aldosterone/salt-induced macrophage and osteopontin staining in the heart. ICAM-1 staining was identified in endothelial cells and in perivascular cells, whereas VCAM-1 immunostaining was largely limited to endothelial cells. The expression of these two molecules was consistently increased in vessels showing inflammatory changes such as monocyte clustering and adhesion to the coronary endothelium, or macrophage infiltration of the perivascular space (Fig. 5). ICAM-1 and VCAM-1 expression was indistinguishably low in saline-drinking controls and eplerenone-treated animals.

**In situ hybridization for osteopontin.** Previous studies (29) identified cardiomyocytes and endothelial cells as a source of production of osteopontin. However,
cardiac fibroblasts (1), as well as vascular smooth muscle cells and macrophages (11), have been suggested as additional potential sources for osteopontin in the heart. In an effort to localize osteopontin expression, in situ hybridization for osteopontin was performed. The majority of osteopontin mRNA was localized in the medial cells of coronary arteries (Fig. 6); however, osteopontin message was also identified in perivascular cells and cells infiltrating ischemic and necrotic areas. Osteopontin mRNA was not evident in cardiomyocytes or in unaffected interstitial areas. Consistent with the results of gene expression analysis and immunohistochemistry, coronary arteries from animals receiving eplerenone expressed negligible amounts of osteopontin (Fig. 6).

DISCUSSION

In the present study, the effects of hyperaldosteronism on myocardial injury in the setting of a high-salt diet were examined. There were three major findings of the present study. First, the data indicate that aldosterone/salt treatment induces leukocyte infiltration and injury of coronary arteries with associated ischemic and necrotic lesions of the adjacent myocardium. Second, we have identified the myocardial expression and progressive upregulation of the proinflammatory molecules osteopontin, MCP-1, and COX-2 in response to aldosterone/salt treatment. Third, the expression of proinflammatory molecules was diminished and vascular and cardiac pathology abrogated by treatment with the selective aldosterone blocker eplerenone, implicating a role for mineralocorticoid receptor stimulation in aldosterone/salt-induced myocardial injury. Although vascular inflammation seems to be the initial effect induced by aldosterone/salt treatment with the elevated myocardial expression of inflammatory molecules at 1 wk, the experimental design of the present study does not allow us to determine whether there is a cause-effect or even a temporal relationship between the inflammatory changes and the vascular damage.

COX-2, MCP-1, and osteopontin are not normally expressed in the heart (3, 12, 32). However, the present data indicate that these molecules can be expressed and upregulated before the onset of structural vascular injury. To our knowledge, this is the first study to identify myocardial expression of COX-2, MCP-1, and

Fig. 5. Immunohistochemistry. Representative photomicrographs of sections from uninephrectomized, saline-drinking rats receiving either vehicle, aldosterone infusion, or aldosterone infusion plus eplerenone for 4 wk (original magnification ×400). Sections were immunostained with specific antibodies against each molecule, as described in METHODS. OPN, osteopontin.
osteopontin during aldosterone/salt treatment. These results have important implications in the understanding of the mechanisms by which aldosterone participates in cardiovascular injury. Specifically, our results implicate vascular inflammation as a key contributor to the onset and progression of cardiovascular damage in hypertensive disease. In addition to the expression of proinflammatory mediators, leukocyte infiltration was evidenced in hearts from aldosterone/salt-treated animals. The primary inflammatory cells identified were monocyte/macrophages. The mechanisms by which aldosterone/salt treatment induces monocyte/macrophage recruitment and migration are unknown. However, our present findings indicate that in the setting of aldosterone excess accompanied by hypertension and elevated dietary salt intake, monocyte/macrophages are activated in the heart.

Little is known about the role of COX-2 in cardiovascular pathology. Previous reports implicate COX-2 in vascular smooth muscle cell proliferation (34), atherosclerosis (4), and heart failure (32). Interestingly, COX-2 expression was upregulated after 1 wk, a time when no vascular or myocardial damage was detected, and remained elevated until the end of the study. The increase in myocardial expression of COX-2 cannot be explained by the inflammatory response that naturally follows ischemic and necrotic injury. Administration of eplerenone reduced the aldosterone/salt-induced COX-2 upregulation in the heart at all time points. Although the effects of eplerenone on COX-2 expression implicate the participation of the aldosterone receptor, it is not clear whether this receptor can directly modulate COX-2 gene expression.

MCP-1 has the ability to recruit monocytes in areas of inflammation through induction of adhesion molecule expression, activation of calcium influx and stimulation of the secretion of interleukin (IL)-1 and -6 (13, 27). MCP-1 has been shown to play a role in myocardial injury observed during ANG II- and nitro-l-arginine methyl ester (l-NAME)-induced hypertension (14, 19). In addition, MCP-1 has been associated with the progression and severity of heart failure in rats (3) and humans (15). The present study demonstrated that aldosterone/salt treatment induced a progressive increase in MCP-1 expression in the heart. Similar to COX-2, this upregulation preceded the establishment of vascular injury. Thus MCP-1 stimulation represents another potential mechanism for the deleterious effects of aldosterone that deserves further investigation.

In addition to MCP-1, aldosterone/salt treatment induced the progressive upregulation of osteopontin expression in medial cells of coronary arteries. Osteopontin is an acidic, calcium-binding, phosphorylated protein found in the extracellular matrix of mineralized tissues or as a circulating cytokine (7). Its expression can be induced by multiple stimuli, including ANG II, endothelin, growth factors, and other cytokines (7). Interestingly, the osteopontin promoter contains a hormone response element that can be modulated by adrenal steroids (31), although it is unknown whether aldosterone can directly induce osteopontin expression. Osteopontin mediates type 1 immunity through mechanisms that involve stimulation of IL-12 in macrophages (2) and in T cells (20) and has been shown to stimulate migration and proliferation of vascular smooth muscle cells (9, 17). On the basis of our results and the known biological functions of osteopontin, we hypothesize that osteopontin plays a role in aldosterone/salt-induced vascular pathology through...
its effects on cell-mediated immunity and vascular remodeling.

The vascular adhesion molecules ICAM-1 and VCAM-1 have been shown to mediate rolling and attachment of activated monocytes to the endothelium (16). This step is critical for the process of transmigration of these cells into the perivascular interstitium in inflamed tissues. In the present study, the gene expression of ICAM-1 and VCAM-1 tended to increase in response to aldosterone/salt treatment, although the increases detected were rather modest. Because of the highly localized expression of these molecules to the coronary endothelium, it is possible that ICAM-1 and VCAM-1 message is diluted significantly in the left ventricular homogenate, masking more profound changes in activated endothelial cell expression. Further studies will be necessary to elucidate the contribution of ICAM-1 and VCAM-1 to aldosterone/salt-induced myocardial damage.

Previous studies (5, 22, 33) have demonstrated that mineralocorticoid/salt administration induces myocardial fibrosis in rats after a prolonged period of time, typically 6 to 8 wk. In the present study, no significant increases in interstitial collagen or hydroxyproline levels were observed throughout the study. Our results are consistent with those of Robert et al. (22) and Selye (28), indicating that the initial pathogenic event in mineralocorticoid/salt-induced myocardial damage is the inflammatory injury of blood vessels. This suggests that aldosterone/salt-induced myocardial fibrosis is, in large part, a consequence of the reparative process secondary to vascular and myocardial damage, rather than a primary effect of aldosterone. However, the possibility that aldosterone may have additional, direct profibrotic effects on cardiac fibroblasts (i.e., reactive fibrosis) at later time points cannot be excluded, as the present study primarily characterized the early events of aldosterone/salt-mediated damage.

Unlike other aldosterone receptor antagonists, eplerenone is highly selective for the mineralocorticoid receptor (6). Administration of eplerenone significantly reduced the hypertension induced by aldosterone/salt treatment. Although this effect was only partial, it may have contributed to the cardiac protection observed. Thus the present study does not exclude the possibility that the vascular inflammatory effects of aldosterone/salt treatment are due, in part, to the mechanical effects of blood pressure in the wall of coronary arteries. However, it should be noted that previous studies have shown that aldosterone blockade can provide end-organ protection in severely hypertensive rats in the absence of significant reductions in blood pressure. In rats made hypertensive by administration of the nitric oxide inhibitor L-NAME combined with a low-dose infusion of ANG II, administration of eplerenone as well as adrenalectomy prevented coronary vascular injury without significantly lowering blood pressure (26). Similarly, in genetically hypertensive rats, administration of spironolactone or eplerenone provided marked vascular protection in the brain and in the kidney despite the fact that blood pressure was not significantly reduced by either of the mineralocorticoid receptor antagonists (23, 24). This indicates that aldosterone blockade can afford vascular protection through blood pressure-independent mechanisms. This possibility is further supported by the presence of aldosterone receptors in the heart (18), blood vessels (30), and brain (35). Whether direct activation of these receptors by aldosterone contributes to the damage observed in nonepithelial tissues remains to be elucidated. The protection obtained with aldosterone antagonism could also be attributed, in part, to stimulation of sodium excretion and potassium retention. However, the present study was not intended to address the role of electrolyte changes on myocardial injury and further studies are required to answer this important question.

In summary, the combined administration of aldosterone and salt induced the development of a coronary inflammatory phenotype in the rat heart with no evidence of significant myocardial fibrosis for up to 4 wk. The lesions induced by aldosterone in the heart were preceded by the concurrent expression of COX-2, the proinflammatory cytokines MCP-1 and osteopontin. Thus myocardial injury occurring after prolonged exposure to high levels of aldosterone is likely to result from an initial inflammatory response followed by vascular and myocardial damage. Consistent with a role for aldosterone receptor stimulation, selective aldosterone blockade with eplerenone reduced blood pressure and effectively attenuated the vascular inflammatory changes and the subsequent tissue injury in the hearts of aldosterone/salt-treated rats. The present study offers novel insight into the molecular and pathological sequelae that characterize aldosterone/salt-mediated cardiovascular disease and provides a mechanism of action that supports the use of aldosterone antagonists as effective therapeutic agents in cardiovascular disease.

The authors thank Susan Knodle, Rachel Duan, and Andrew Lisowski for excellent work on the immunohistochemistry and in situ hybridization studies. We are also grateful for the administrative contributions of Lot Bercasio during the completion of this manuscript.

REFERENCES


AJP-Heart Circ Physiol • VOL 283 • NOVEMBER 2002 • www.ajpheart.org


