

Spironolactone Improves Angiotensin-Induced Vascular Changes and Oxidative Stress

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Abstract—Angiotensin II plays an important role in vascular remodeling. We investigated the role of aldosterone, which is stimulated by angiotensin II, as a mediator of angiotensin II–induced vascular structural and functional alterations. Sprague-Dawley rats (n=8 to 12/group) received angiotensin II (120 ng/kg per minute, subcutaneously) for 14 days ± spironolactone or hydralazine (25 mg/kg per day). An additional group received aldosterone (750 ng/h, subcutaneously) ± spironolactone. Systolic blood pressure was increased by angiotensin II ($P<0.001$) and reduced by spironolactone and hydralazine ($P<0.001$). Aldosterone-induced increase of blood pressure was reduced by spironolactone ($P<0.05$). In mesenteric small arteries studied on a pressurized myograph, media/lumen ratio was increased ($P<0.001$) and acetylcholine-mediated relaxation was impaired in angiotensin II–infused rats ($P<0.001$); both were partially improved by spironolactone ($P<0.05$) but not by hydralazine. Aldosterone-induced increase of media/lumen ratio ($P<0.001$) and impaired response to acetylcholine ($P<0.001$) were normalized by spironolactone. Response to sodium nitroprusside was similar in all groups. Aortic NADPH oxidase activity was increased ($P<0.01$) by angiotensin II and reduced by spironolactone and hydralazine. Aldosterone also increased ($P<0.05$) activation of NADPH oxidase, an effect abolished by spironolactone. Plasma thiobarbituric acid–reactive substances (a marker of oxidative stress), higher in angiotensin II and aldosterone rats ($P<0.001$), were normalized by spironolactone. In conclusion, spironolactone, which inhibited aldosterone actions, partially corrected structural and functional angiotensin II–induced abnormalities. These effects were associated with reduced vascular NADPH oxidase activity and decreased plasma markers of oxidative stress. Our findings suggest that aldosterone may mediate some of angiotensin II–induced vascular effects in hypertension, in part via increased oxidative stress. (*Hypertension*. 2002;40:●●●-●●●.)

Key Words: aldosterone ■ angiotensin II ■ endothelium ■ hypertension, arterial ■ oxidative stress
■ remodeling ■ rats



Increased peripheral resistance, a hallmark of essential hypertension, results predominantly from structural and functional alterations (vascular remodeling and endothelial dysfunction, respectively) in small-resistance arteries.^{1,2} Increased media to lumen ratio (M/L), which characterizes vascular remodeling, can result from a reduced outer diameter that narrows the lumen without net growth (eutrophic remodeling) or from a thicker media encroaching on the lumen (hypertrophic remodeling).³ Endothelial dysfunction is mainly assessed as an impaired acetylcholine-induced, endothelium-dependent relaxation.⁴ These alterations are associated with and may be secondary to increased production of reactive oxygen species (ROS).^{5–7} A large body of evidence indicates that angiotensin (Ang) II, the principal effector of the renin-angiotensin-aldosterone system (RAAS), plays a critical role in the development of structural and functional vascular changes,^{8,9} mainly through an increased generation of vascular ROS via NADH/NADPH oxidase activation.^{10,11} Because Ang II, in addition to its direct vascular actions,

stimulates the synthesis and release of aldosterone,¹² it may be possible that some of the Ang II–induced vascular effects are mediated via aldosterone. This is supported by in vivo and in vitro studies showing upregulation of Ang II receptors by aldosterone.^{13–16} This upregulation may amplify the Ang II hypertrophic response, an effect inhibited by spironolactone, a mineralocorticoid receptor antagonist.¹⁵ Moreover, it has been documented that both human endothelial cells and smooth muscle cells are a source of aldosterone, therefore suggesting that locally generated mineralocorticoid could play a role.¹⁷ Subsequent in vivo studies provided evidence that aldosterone contributes to the promotion of cardiovascular end-organ damage in hypertensive animal models characterized by abnormal activation of the RAAS. Indeed, MacLeod et al¹⁸ demonstrated that exogenous administration of aldosterone reduced the protective effect of captopril against stroke in stroke-prone spontaneously hypertensive rats (SHRSP). Furthermore, Rocha et al¹⁹ observed that spironolactone markedly reduced the incidence of renal vascular

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lesions in saline-drinking SHRSP. Also, it has been documented that spironolactone ameliorated the cardiac hypertrophy, inflammation, and extracellular matrix production in Ang II-induced cardiac injury.²⁰ In the present study, we hypothesized that aldosterone could play a direct role in the development of Ang II-mediated vascular abnormalities of resistance arteries by mediating part of the effects of Ang II. To test this, we evaluated the effect of spironolactone on structural and functional alterations of resistance mesenteric arteries in Ang II-infused rats. In addition, the effects on vascular NADPH oxidase activity as well as on plasma thiobarbituric acid-reactive substances (TBARS), a marker of oxidative stress, were also evaluated.

Methods

Animal Experiments

The study protocol was approved by the Animal Care Committee of the Clinical Research Institute of Montreal and was conducted in accordance with the recommendations of the Canadian Council of Animal Care. Rats were housed under conditions of constant humidity and temperature and subjected to 12-hour light/dark cycles. Male Sprague-Dawley rats (Charles River, St Constant, Quebec, Canada), aged 7 to 8 weeks and weighing 250 to 300 g, were studied. Rats, under anesthesia with ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) given intramuscularly, were implanted subcutaneously with Alzet osmotic minipumps (Alza Corp) that infused 120 ng/kg per minute $I\text{Ie}^3$ -Ang II (Peninsula) or 750 ng/h d-aldosterone (Sigma Chemical Co), both dissolved in 0.9% saline. Sham-infused rats served as controls. In 2 additional groups of Ang II-infused and aldosterone-infused rats, 25 mg/kg per day spironolactone (ICN, Biomedicals Inc) was given in the food. Finally, a group of Ang II-infused rats was treated with hydralazine (Sigma Chemical Co, 25 mg/kg per day, in food) to evaluate effects of systolic blood pressure (SBP) lowering. Rats were treated for 2 weeks. SBP was measured weekly by the tail-cuff method and recorded on a Model 7 polygraph fitted with a 7-P8 preamplifier and PCPB photoelectric pulse sensor (Grass Instruments Co). The average of 3 pressure readings was obtained. Rats were killed by decapitation.

Preparation and Study of Small Arteries

Third order superior mesenteric arteries (≈ 2 mm in length) were dissected out and placed in cold physiological salt solution (PSS) containing (mmol/L): NaCl 120, NaHCO_3 25, KCl 4.7, KH_2PO_4 1.18, MgSO_4 1.18, CaCl_2 2.5, EDTA 0.026, and glucose 5.5. They were mounted on 2-glass microcannulae in a pressurized myograph chamber.²¹ Intraluminal pressure was set to 45 mm Hg with a servocontrolled pump. Vessels were then equilibrated for 1 hour with PSS that was bubbled with 95% air and 5% CO_2 to give a pH of 7.4 and were heated to 37°C. Media and lumen dimensions were measured with the intraluminal pressure maintained at 45 mm Hg, as previously described.²¹ Endothelium-dependent and -independent relaxations were assessed by measuring the dilatory responses to cumulative doses of acetylcholine (10^{-9} to 10^{-4} mol/L) and sodium nitroprusside (10^{-8} to 10^{-4} mol/L), respectively, in vessels precontracted with norepinephrine (10^{-5} mol/L).

The media cross-sectional area (CSA) was evaluated as previously described.²¹ The growth index was calculated as $(\text{CSA}_h - \text{CSA}_n) / \text{CSA}_n$, where CSA_n and CSA_h were media CSA of normotensive and hypertensive vessels, respectively.²² The remodeling index was calculated as $100 \times [(D_i)_n - (D_i)_{\text{remodel}}] / [(D_i)_n - (D_i)_h]$ where (D_i) indicates internal diameter; n, normal vessels; h, hypertensive vessels. $(D_i)_{\text{remodel}} = [(D_e)_h^2 - (4 \times \text{CSA}_h / \pi)]^{0.5}$, where $(D_e)_h$ is the external diameter of hypertensive vessels and CSA_n was the CSA of normotensive vessels.²²

Measurement of Vascular Superoxide and NADPH Oxidase Activity

Aortic segments were prepared as previously described.²³ Activity of NADPH oxidase was measured in a luminescence assay with 5 $\mu\text{mol/L}$ lucigenin as the electron acceptor and 100 $\mu\text{mol/L}$ NADPH as the substrate. The reaction was started by the addition of NADPH to tissue sample. Basal superoxide anion ($\cdot\text{O}_2^-$) was measured in the absence of exogenously added NADPH. Luminescence was measured every 1.8 seconds for 3 minutes in a luminometer (AutoLumat LB 953, Berthold). A buffer blank was subtracted from each reading. Activity was expressed as counts/min per milligram dry tissue weight. To verify that the lucigenin signal reflected $\cdot\text{O}_2^-$ generation, diphenylene iodonium (DPI), a flavoprotein inhibitor, and tempol (10^{-5} mol/L), a superoxide dismutase mimetic, were added to some samples. DPI and tempol completely abolished the NADPH-induced increase in chemiluminescence (data not shown).

Plasma TBARS Measurements

Plasma TBARS were measured by a colorimetric method based on a previously described method.²⁴ TBARS values were expressed in nmol/mL malondialdehyde (MDA) equivalents. Briefly, plasma was mixed with 2% butylated hydroxytoluene and quintanilla reagent (26 mmol/L thiobarbituric acid and 918 mmol/L trichloroacetic acid). The mixture reaction was boiled for 15 minutes. Thereafter, the reaction mixture was cooled and centrifuged at 3000g for 10 minutes. The soluble phase was measured with a spectrophotometer at a wavelength of 535 nm. In parallel, MDA standards (Sigma Chemical Co) were diluted in the range of 0 to 4 $\mu\text{mol/L}$.

Measurement of Plasma Renin Activity and Aldosterone

Plasma renin activity (PRA) was measured by radioimmunoassay (RIA) as previously described.²⁵ Similarly, plasma aldosterone was measured using an aldosterone RIA kit (ICN Diagnostics).

Data Analysis

Results are presented as mean \pm SEM and analyzed by 1-way ANOVA followed by Newman-Keuls test. A value of $P < 0.05$ was considered statistically significant.

Results

SBP, Body and Heart Weight, PRA, and Aldosterone

The SBP increase induced by Ang II infusion ($P < 0.001$ versus control) was significantly reduced by spironolactone and hydralazine ($P < 0.001$ versus Ang II). The aldosterone-mediated increase of SBP was significantly reduced ($P < 0.05$ versus aldosterone), but not normalized, by spironolactone ($P < 0.001$ versus control) (Table). Body weight was similar in all groups (Table). Relative heart weight (normalized for body weight) was similar among groups (Table).

As expected, PRA was significantly depressed in Ang II-infused and in aldosterone-infused rats ($P < 0.001$ versus control). This decrease was unaffected by hydralazine or spironolactone (Table). Plasma aldosterone values were significantly increased in Ang II-infused and in aldosterone-infused rats ($P < 0.01$ versus control) and unaffected by hydralazine or spironolactone (Table).

Morphology and Endothelial Function of Mesenteric Resistance Arteries

Ang II infusion decreased lumen diameter and increased media thickness of mesenteric resistance arteries (Table), resulting in an increase in media/lumen ratio compared with controls ($7.1 \pm 0.3\%$ and $4.8 \pm 0.2\%$, respectively; $P < 0.001$)

Physiological Parameters and Morphological Characteristics of Resistance Arteries from Ang II-Infused or Aldosterone-Infused Rats With or Without Spironolactone or Hydralazine

Parameter	Control (n=12)	Ang II (n=12)	Ang II+Spiro (n=12)	Ang II+Hyd (n=9)	Aldo (n=8)	Aldo+Spiro (n=11)
Body weight, g	315±11	313±11	314±14	313±7	336±8	330±5
Heart weight/100 g BW	0.342±0.02	0.368±0.02	0.368±0.01	0.368±0.01	0.340±0.01	0.324±0.01
SBP, mm Hg	115±2	174±4*	153±8*†	125±2††	143±3*†	133±3‡§
PRA, ng Ang I/mL per hour	2.45±0.37	0.24±0.04*	ND	ND	ND	0.86±0.4*
Aldosterone, pg/mL	203±67	592±81‡	730±83‡	607±98‡	613±108‡	690±88‡
Lumen diameter, μm	257±6	231±12‡	248±8	228±7‡	228±10‡	261±11§
Media thickness, μm	12.1±0.2	16.3±0.7*	15.5±0.4*	17.0±0.5*	14.8±0.9‡	13.8±0.9‡
Media CSA, $10^3 \times \mu\text{m}^2$	10.3±0.3	12.8±1.1	12.9±0.7	13.2±0.7	11.4±1.1	12.0±1.1

Spiro indicates spironolactone; Hyd, hydralazine; Aldo, aldosterone; BW, body weight; ND, not detectable (values <0.01 ng Ang I/ml per hour); CSA, cross-sectional area.

* $P < 0.001$ vs control; † $P < 0.001$ vs Ang II; ‡ $P < 0.05$ vs control; § $P < 0.05$ vs Aldo.

(Figure 1). This effect was partially reversed by spironolactone ($6.3 \pm 0.2\%$, $P < 0.05$ versus Ang II, $P < 0.001$ versus control) but not by hydralazine ($7.5 \pm 0.2\%$) (Figure 1). After Ang II infusion, the growth and the remodeling indices were 24.3% and 75.3%, respectively, indicating some degree of hypertrophic remodeling, even though the slight increase in CSA had not achieved statistical significance (Table). Spironolactone treatment of Ang II-infused rats resulted in a remodeling index of 28.9%, whereas the growth index remained at 25.2%, suggesting that spironolactone prevented an eutrophic component of remodeling. Aldosterone infusion, which decreased lumen diameter and increased media thickness, induced an increase in M/L ($6.5 \pm 0.2\%$, $P < 0.001$ versus control). This alteration was prevented by spironolactone administration ($5.3 \pm 0.4\%$) (Figure 1). Differences in media CSA of resistance arteries did not achieve statistical significance between groups (Table).

Vasodilatory responses to the maximal dose of acetylcholine (10^{-4} mol/L) were diminished in Ang II-infused rats ($67.7 \pm 4.9\%$) compared with controls ($99.4 \pm 0.4\%$, $P < 0.001$)

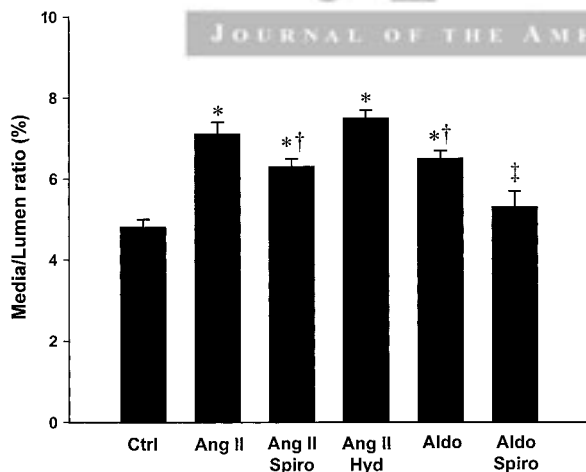


Figure 1. Media/lumen ratio of mesenteric arteries from Ang II-infused or aldosterone (Aldo)-infused rats treated with or without spironolactone (Spiro) or hydralazine (Hyd). Results are mean \pm SEM. * $P < 0.001$ versus control (Ctrl), † $P < 0.05$ versus Ang II, ‡ $P < 0.01$ versus Aldo.

(Figure 2A). This was partially prevented by spironolactone ($84.8 \pm 3.4\%$; $P < 0.05$ versus Ang II, $P < 0.001$ versus control), but not by hydralazine ($67.5 \pm 2.9\%$) (Figure 2A). Aldosterone-infused rats also showed a reduced vascular response to acetylcholine ($79.8 \pm 2.2\%$; $P < 0.001$ versus control), which was normalized by spironolactone ($99.5 \pm 0.7\%$) (Figure 2A). Endothelium-independent relaxation by sodium nitroprusside was similar in all groups (Figure 2B).

Vascular $\cdot\text{O}_2^-$ and NADPH Oxidase Activity

Basal $\cdot\text{O}_2^-$ was significantly increased ($P < 0.01$) in Ang II-infused rats compared with controls, an effect prevented by spironolactone and hydralazine (Figure 3A). Although basal $\cdot\text{O}_2^-$ tended to be increased in aldosterone-infused rats, significance was not achieved (Figure 3A).

Activity of NADPH oxidase in aorta, expressed as 10^3 cpm/mg dry tissue weight, was significantly increased in Ang II-infused rats compared with controls (3096 ± 142 versus 1800 ± 28 , $P < 0.01$), with the increase inhibited by both spironolactone and hydralazine (1947 ± 44 and 2096 ± 81 , respectively) (Figure 3B). Aldosterone infusion induced a significant ($P < 0.05$) activation of NADPH oxidase (2300 ± 59), compared with control rats, with the effect abrogated by spironolactone administration (1459 ± 24) (Figure 3B).

Plasma TBARS

Plasma TBARS were significantly higher in Ang II-infused rats compared with controls (4.3 ± 0.3 versus 2.6 ± 0.1 nmol/mL, $P < 0.001$), with the increase inhibited by spironolactone (2.5 ± 0.2 nmol/mL) (Figure 3C) but not by hydralazine, TBARS (3.7 ± 0.1 nmol/mL). Aldosterone infusion induced a significant ($P < 0.001$) elevation of plasma TBARS (4.3 ± 0.2 nmol/mL) compared with controls, with the effect abolished by spironolactone treatment (3.0 ± 0.2 nmol/mL) (Figure 3C).

Discussion

The present results demonstrate for the first time that the aldosterone receptor antagonist spironolactone partially reduced the increased media/lumen ratio and the endothelial dysfunction of resistance arteries in Ang II-infused rats.

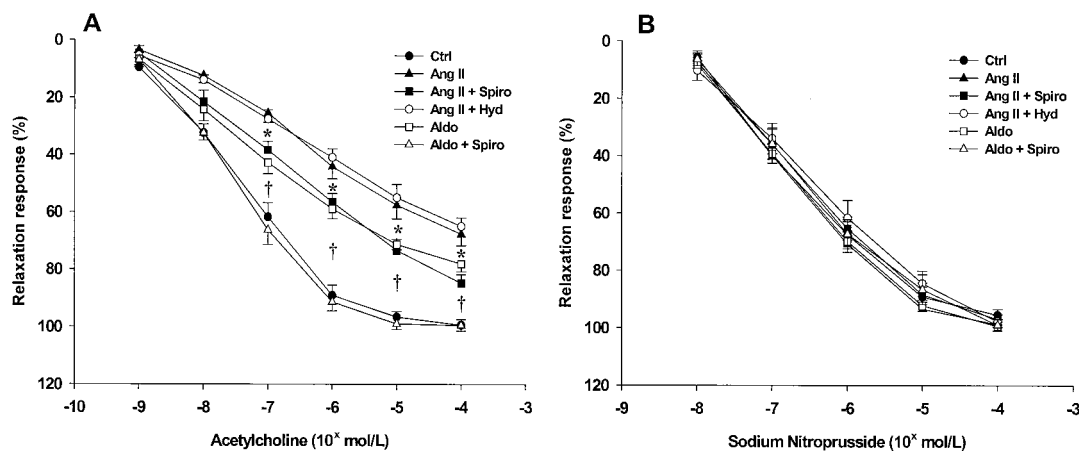


Figure 2. A, Endothelium-dependent relaxation to acetylcholine (%) in mesenteric arteries from Ang II-infused or aldosterone-infused rats treated with or without spironolactone or hydralazine. Results are mean \pm SEM. * P < 0.05, † P < 0.001. B, Endothelium-independent relaxation in response to sodium nitroprusside (%). Results are mean \pm SEM.

Thus, aldosterone participates, at least in part, in the development of structural and functional vascular alterations induced by Ang II.

It is widely accepted that Ang II, mainly through the Ang II type 1 (AT₁) receptor, plays a central role in the pathophysiology of vascular remodeling through smooth muscle growth^{8,26} and collagen deposition,²⁷ leading to hypertrophic remodeling.^{8,26} We found a growth index of 24.3%, suggesting some degree of hypertrophic remodeling, although CSA changes did not achieve statistical significance. Thus, there appears to have occurred some growth and some eutrophic remodeling (increased M/L with unchanged CSA) after Ang II infusion in this set of experiments. This is not surprising, because AT₁-receptor stimulation is accompanied secondarily by an increased apoptotic rate in the arterial wall,²⁸ which may counterbalance cell proliferation, thereby explaining the maintenance of media volume under some experimental conditions.²⁶ Accordingly, spironolactone prevented remodeling predominantly eutrophically (remodeling index of 28.9% versus 75.3% in Ang II-infused rats).

BP lowering does not appear to affect vascular structure in our study because hydralazine attenuated BP rise induced by Ang II, but not arterial remodeling. Previous studies reported that hydralazine inhibited the pressor effect of higher doses of Ang II (200 and 435 ng/kg per minute) without affecting vascular changes.^{29,30} In large arteries, structural changes induced by 100 ng/kg per minute Ang II for 2 weeks³¹ were, however, abolished by minoxidil, suggesting an effect of pressure. A likely explanation of these discrepant results could be the different arterial bed (conduit versus resistance vessels) as well as the different antihypertensive agent. We cannot exclude that minoxidil, a K_{ATP} channel activator, could have specific effects on the arterial wall beyond lowering BP. The authors, furthermore, did not observe Ang II-induced structural alterations in small mesenteric vessels, an effect that is likely the consequence of the different experimental technique.

Although a trend to an increase in heart weight in Ang II rats was observed in our study, it did not achieve statistical significance, in contrast to previous evidence by Griffin et

al.²⁹ This discrepancy could be due to the higher doses of Ang II used by these authors and the higher BP values reached. Supporting the hypothesis that the hemodynamic load per se plays a role in inducing the cardiac hypertrophy, in contrast to effects on small arteries, is the finding that hydralazine abolished cardiac hypertrophy.²⁹

Growing evidence indicates that aldosterone contributes to cardiovascular damage. Besides the traditional concept that aldosterone is synthesized only in the adrenal cortex, both human endothelial and smooth muscle cells express corticoid receptors and produce aldosterone.¹⁷ Aldosterone synthesized in the vasculature may participate in the development of vascular hypertrophy together with Ang II.³² In animal models of hypertension, spironolactone reduced cerebral and renal vascular lesions and ameliorated cardiac hypertrophy, inflammation, and extracellular matrix production, supporting the hypothesis that aldosterone is involved in cardiovascular injury.^{19,20} Primary aldosteronism patients exhibit vascular remodeling of small subcutaneous resistance arteries.³³ Furthermore, Park et al³⁴ showed increased M/L of mesenteric arteries in aldosterone-infused rats. Our study is, however, the first to indicate participation of aldosterone in the development of structural remodeling of resistance arteries induced by Ang II. This is confirmed in aldosterone-infused rats, which showed vascular remodeling that could be prevented with spironolactone. Of importance, spironolactone only partially reduced the Ang II-induced vascular remodeling, indicating a residual direct and specific effect of Ang II on the vascular wall that has been well demonstrated.⁸ Our results showing that aldosterone partially contributes to structural alterations induced by Ang II are in agreement with those of Rizzoni et al³³ indicating that patients with primary aldosteronism had fewer vascular structural changes compared with patients with renovascular hypertension, who have a markedly activated RAAS. Our results are further supported by Fiebeler et al,²⁰ who showed that spironolactone ameliorated, but did not normalize, cardiac hypertrophy and inflammation in rats transgenic for the human renin and angiotensinogen genes.

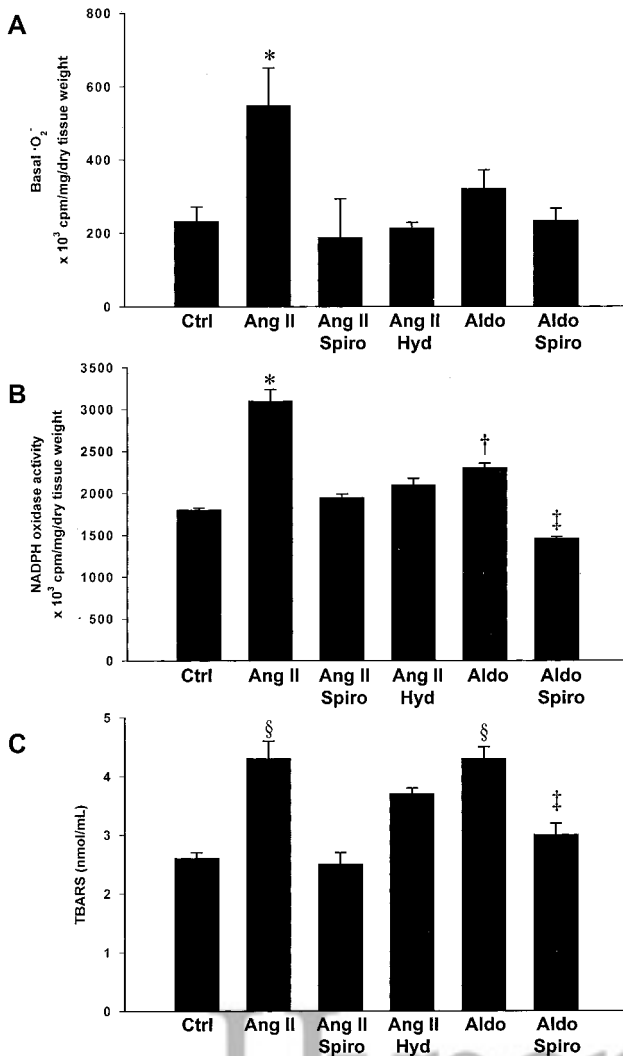


Figure 3. A, Basal $\cdot\text{O}_2^-$ levels in aortic segments from Ang II-infused or aldosterone-infused rats treated with or without spironolactone or hydralazine. Results are mean \pm SEM. * $P < 0.01$ versus other groups. B, Activity of aortic NADPH oxidase. Results are mean \pm SEM. * $P < 0.01$ versus other groups; $\ddagger P < 0.05$ versus control; $\ddagger P < 0.05$ versus Aldo. C, Plasma TBARS values. Results are mean \pm SEM. $\S P < 0.001$ versus control and Ang II Spiro groups; $\ddagger P < 0.05$ versus Aldo.

Blood vessels from Ang II-infused rats displayed impaired endothelium-dependent relaxation, as previously documented.¹⁰ The novel finding of the present study is that spironolactone administration in Ang II-infused rats improved the vascular response to acetylcholine. This effect was endothelium-specific, because endothelium-independent vasodilation to sodium nitroprusside was unaffected. That aldosterone participates in the vascular functional abnormalities induced by Ang II is confirmed by our results in blood vessels from aldosterone-infused rats, in which vasodilating responses to acetylcholine were also prevented by spironolactone. Decreased BP could also contribute to improvement of endothelial dysfunction. However, hydralazine, which lowered BP, did not influence endothelial function, suggesting that BP lowering by itself does not participate importantly in our findings. Our data agree with a recent human study in which

spironolactone improved endothelium-dependent vasodilation in the forearm microcirculation of patients with chronic heart failure, possibly by improving nitric oxide bioavailability.³⁵ The aldosterone-induced impairment of endothelial function is in accordance with data obtained in salt-sensitive Dahl rats, in which a reduced endothelium-dependent relaxation to acetylcholine was present.³⁶ A similar finding has also been observed in patients with primary aldosteronism, showing reduced vascular response to acetylcholine in the forearm microcirculation³⁷ and in subcutaneous resistance arteries.³³ However, there are no previous data demonstrating that mineralocorticoid blockade attenuates the Ang II-induced endothelial dysfunction in small vessels. The present study is the first to suggest that, similarly to structural changes of resistance mesenteric vessels, the effect on vascular function, previously attributed to a direct action of Ang II, may be mediated in part by aldosterone.

Although molecular mechanisms whereby aldosterone participates in Ang II-mediated vascular changes were not investigated in the present study, our results on aortic superoxide and NADPH oxidase activity provide preliminary mechanistic insight. Ang II increased basal $\cdot\text{O}_2^-$ and NADPH-induced generation of $\cdot\text{O}_2^-$. These effects were abolished by spironolactone. Although basal $\cdot\text{O}_2^-$ was not significantly increased in aldosterone-infused rats, NADPH oxidase activity was enhanced. Taken together, these findings suggest that aldosterone plays a role in activation of NADPH oxidase by Ang II. Of importance, although spironolactone inhibited NADPH oxidase activity, it only partially corrected Ang II-induced vascular changes, suggesting that redox-independent pathways also underlie Ang II-induced effects. In our study the Ang II-induced NADPH oxidase activation was blocked by hydralazine. This finding, previously observed by Fukui et al³⁸ and Munzel et al,³⁹ raises further possibilities: first, that blood pressure per se might regulate the NADPH oxidase activity; and second, that hydralazine has antioxidant properties. Although these hypotheses cannot be distinguished in the present study, it seems unlikely that blood pressure itself affected NADPH oxidase activity, because a prior study failed to observe activation of this oxidase after norepinephrine-induced hypertension.¹⁰ The picture is further complicated if we consider that hydralazine, although it reduced NADPH oxidase activity, failed to reverse Ang II-induced structural and functional abnormalities. A possible explanation of this discrepancy could be that the beneficial effect of hydralazine on this source of ROS is counterbalanced by activation of the sympathetic nervous system, per se implicated in vascular remodeling.⁴⁰ Although elucidation of these aspects requires further investigation, we can speculate that the aldosterone component of Ang II-induced vascular damage is associated with activation of NADPH oxidase and generation of superoxide. Our results with plasma TBARS are in line with this hypothesis. Plasma TBARS are considered a systemic marker of lipid peroxidation and consequently of oxidative stress. Similarly to NADPH oxidase activity, plasma TBARS, which were higher in Ang II-infused rats compared with controls, were significantly reduced by spironolactone and modestly by hydralazine. These

findings reinforce the possibility that increased oxidative stress exerted by Ang II is mediated partly by aldosterone.

In conclusion, spironolactone partially prevented structural abnormalities and endothelial dysfunction of mesenteric resistance arteries in Ang II-infused rats. Spironolactone also inhibited Ang II-induced activation of aortic NADPH oxidase and reduced plasma TBARS levels. These findings suggest that the vascular damage exerted by Ang II is mediated, at least in part, via stimulation of aldosterone and receptor activation. Activation of NADPH oxidase and generation of ROS may play a role in these effects.

Perspectives

This study indicates that the mineralocorticoid receptor antagonist spironolactone partially reduced the structural and functional vascular changes and inhibited the generation of oxidative stress mediated by Ang II, suggesting that aldosterone may mediate part of Ang II-induced oxidative stress and associated vascular damage. These findings have important clinical implications. The mechanism of action of spironolactone proposed in our study could explain some of the beneficial effects of spironolactone reported in the RALES study.⁴¹ Further studies using more selective aldosterone receptor blockers will elucidate molecular processes whereby aldosterone mediates Ang II actions.

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