Participation of Prostacyclin in Endothelial Dysfunction Induced by Aldosterone in Normotensive and Hypertensive Rats

Javier Blanco-Rivero, Victoria Cachofeiro, Vicente Lahera, Rosa Aras-Lopez, Iván Márquez-Rodas, Mercedes Sálaices, Fabiano E. Xavier, Mercedes Ferrer, Gloria Balfagón

Abstract—The aim of the present study was to analyze the possible involvement of vasoconstrictors prostanoids on the reduced endothelium-dependent relaxations produced by chronic administration of aldosterone in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). For this purpose, acetylcholine (ACh) relaxations in aortic segments from both strains were analyzed in absence and presence of the cyclooxygenase-1 (COX-1) and COX-2 inhibitor indomethacin, the specific COX-2 inhibitor NS-398, the TP receptor antagonist (SQ 29 548), the thromboxane A2 (TXA2) synthase inhibitor furegrelate, and the prostacyclin (PGI2) synthesis inhibitor tranylcypromine (TCP). In addition, COX-2 protein expression was studied by Western blot analysis. Release of prostaglandin E2 (PGE2) and the metabolites of PGF2α, TXA2, and PGI2, 13,14-dihydro-15-keto PGF2α, TXB2, and 6-keto-PGF1α, respectively, were measured. Treatment with aldosterone did not modify blood pressure levels in any strain. However, aldosterone markedly reduced (P<0.05) ACh-induced relaxations in segments from both strains in a similar extent. Indomethacin, NS-398, SQ 29 548, and TCP enhanced (P<0.05) ACh relaxations in both strains treated with aldosterone. Aortic COX-2 protein expression was higher in both strains of rats treated with aldosterone. In normotensive animals, aldosterone increases the ACh-stimulated aortic production of 13,14-dihydro-15-keto PGF2α, PGE2, and 6-keto-PGF1α (P<0.05). In SHR, ACh only increased the 6-keto-PGF1α production (P<0.05). It could be concluded that chronic treatment with aldosterone was able to produce endothelial dysfunction through COX-2 activation in normotensive and hypertensive conditions. PGI2 seems to be the main factor accounting for endothelial dysfunction in hypertensive rats, whereas other prostanoids besides PGI2 appear to be involved in endothelial dysfunction under normotensive conditions. (Hypertension. 2005;46:107-112.)

Key Words: aldosterone ■ endothelium ■ prostacyclin ■ normotension ■ hypertension

Aldosterone is a mineralocorticoid that participates in electrolyte balance and plays an important physiological role in the long-term regulation of Na+ and K+ in the distal tubule and collecting duct. To date, several studies suggested that aldosterone plays a larger role than once appreciated in the regulation of vascular tone as well as in cardiovascular alterations such as endothelial dysfunction, vascular fibrosis, and inflammation, left ventricular hypertrophy, congestive heart failure, and cardiac arrhythmias. Furthermore, aldosterone has also been shown to be involved in the pathogenesis of hypertension. Diminished endothelium NO synthase expression or activity has been proposed as an important mechanism leading to reduced NO availability and endothelial dysfunction in aldosterone-treated animals. Furthermore, enhanced vascular production of reactive oxygen species, specifically superoxide anions, has been reported as another important mechanism contributing to endothelial dysfunction in aldosterone-treated animals. However, the role of vasoconstrictor prostanoids prostaglandin F2α (PGF2α) and thromboxane A2 (TXA2), as well as prostacyclin (PGI2) and PGE2, which can act as vasoconstrictors under certain conditions, is not well established in the endothelial dysfunction produced by aldosterone. Thus, the aim of the present study was to analyze the possible involvement of endothelium-derived vasoconstrictors prostanoids on the reduced en-
endothelium-dependent relaxations produced by chronic administration of aldosterone under normotensive and hypertensive conditions in rats.

Methods

Male 6-month-old Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) weighing 200 to 250 g were obtained from colonies maintained at the animal quarters of the Facultad de Medicina of the Universidad Autónoma de Madrid.

Rats were divided into 2 groups: untreated and aldosterone treated (0.05 mg/kg per day). Controlled time-release pellets (Innovative Research of America) containing aldosterone or vehicle were subcutaneously implanted. At the end of the treatment period (3 weeks), systolic blood pressure (BP) was measured by a tail-cuff method. After death by CO2 inhalation, aorta was carefully dissected out, cleaned of connective tissue, and placed in Krebs–Henseleit solution (KHS; in mmol/L: 115 NaCl, 2.5 CaCl2, 4.6 KCl, 1.2 KH2PO4, 1.2 MgSO4, 7/H2O, 25 NaHCO3, 11.1 glucose, and 0.03 Na, EDTA) at 4°C. Animals were kept in the animal facility of the Universidad Autónoma de Madrid (registration No. EX-021U) according to the directives 609/86 CEE and R.D. 233/88 of the Ministerio de Agricultura, Pesca y Alimentación.

Endothelium-Dependent Relaxations

Thoracic aorta was cut transversally in ring segments (4-mm long). Each ring was placed inside a 5-mL heated bath filled with KHS (37°C) bubbled with a 95% O2–5%CO2 mixture, pH 7.4, and suspended between 2 L-shaped stainless steel hooks. The top one was attached to a force transducer (Grass FT03C); in turn, this was connected to a model 7D Grass polygraph for measurement of isometric tension. Rings were allowed to equilibrate for 90 minutes, with changes of buffer every 15 minutes and with several adjustments of length until baseline tension stabilized at 1 g to obtain comparable acetylcholine (ACh) relaxation in both strains. In previous studies, we found that 2 g but not 1 g of resting tension evidenced reduction of ACh relaxation in SHR compared with WKY.25 When tension was stable, experiments were initiated by obtaining a reference contractile response to 75 mmol/L KCl.

Endothelium-dependent relaxations were studied by evaluating relaxations to ACh (10−6 to 10−3 mol/L) in noradrenaline precontracted rings (1.5±0.18 g). In pilot studies, we found that treatment of rats with aldosterone reduced endothelium-dependent relaxation to ACh.

To investigate the possible participation of prostanoids in the reduction of ACh relaxations produced by aldosterone treatment, aortic segments from both strains were incubated with the cyclooxygenase-1 (COX-1)/COX-2 inhibitor indomethacin (10−5 mol/L; Sigma-Aldrich). To clarify whether COX-1 or COX-2 was involved in the effect of aldosterone on ACh relaxation, aortic segments were incubated with the specific COX-2 inhibitor NS-398 (10−5 mol/L; Calbiochem-Novabiochem GmbH). In another set of experiments, segments were incubated with SQ 29 548 (TP receptor antagonist; 10−6 mol/L; ICN Iberica) to evaluate the possible participation of vasococontractor prostanoids. Finally, segments were incubated with the thromboxane (TXA2) synthase inhibitor furegrelate (10−7 mol/L; Sigma-Aldrich), the PGI2 synthase inhibitor tranilcyromine (TCP; 10−5 mol/L; Sigma-Aldrich), or the cytochrome P450 inhibitor 1-aminobenzotriazole (ABZ; 5×10−4 mol/L; Sigma-Aldrich) to analyze the possible involvement of these prostanoids in the effect of aldosterone on ACh relaxations.

Western Blot Analysis

Aorta samples from control and aldosterone-treated WKY and SHR were homogenized in a boiling buffer composed of 1 mmol/L sodium vanadate (Sigma-Aldrich), 1% SDS and pH 7.4, 0.01 mol/L Tris-HCl, and protein content was measured with a DC protein assay kit (Bio-Rad). Homogenates containing 20 μg protein were fractionated in a 10% SDS-PAGE and transferred to a polyvinylidene membrane (Bio-Rad). Membranes were blocked in 5% nonfat milk in Tris-HCl buffered saline 0.1% Tween 20 (TBS-T). Subsequent washes were done in TBS-T, and the membranes were incubated with antibody against COX-2 (1:1000; Cayman Chemical) or α-actin (1:3000; Sigma-Aldrich) proteins and individual horseradish peroxidase–conjugated secondary antibodies in blocking buffer. Immunoreactive proteins were detected by chemiluminescence with ECL-Plus (Amersham).

Prostanoid Production

To measure the release of PGE2 and the metabolites of PGF2α, TXA2, PGL2, 13,14-dihydro-15-keto PGF2α, thromboxane B2 (TXB2), and 6-keto-PGF1α, we used PGE2 enzyme immunoassay (EIA) Kit-Monoclonal, EIA kit, 13,14-dihydro-15-keto PGF2α, EIA kit (Cayman Chemical), TXB2, immunoassay, and 6-keto-PGF1α immunoassay (R & D Systems), respectively. Segments of rat aorta arteries were preincubated for 30 minutes in 5 mL of KHS at 37°C and continuously gassed with a 95% O2–5% CO2 mixture (stabilization period). Afterward, 2 washout periods of 7 minutes in a bath of 0.3 mL of KHS were made before incubation with ACh (10−10 to 10−5 mol/L). The different assays were made following manufacturer instructions. Results were expressed as pg prostanoid/mL mg tissue.

Statistical Analysis

Results are expressed as mean±SEM of the number of rats indicated. Differences were analyzed using Student t test for unpaired experiments or 2-way ANOVA to compare groups. A P value <0.05 was considered significant.

Results

Blood Pressure

As expected, SHR presented higher BP levels than WKY (175±2.3 versus 115±2.8 mm Hg; P<0.05). Chronic aldosterone administration did not substantially modify BP levels in any strain (SHR 181±6; WKY 121±7.9).

Endothelium-Dependent Relaxations

ACh-induced relaxations were comparable in untreated WKY and SHR (Emax WKY 76.0±5.7 versus SHR 73.0±2.8; percent noradrenaline contraction; negative logarithm of effective concentration 50 (pD2) WKY 7.7±0.2 versus SHR 7.6±0.1). Treatment with aldosterone markedly reduced (P<0.05) ACh-induced relaxations in both strains in a similar extent (Figure 1A and 1B).

Incubation of aortic rings with indomethacin or the COX-2 inhibitor NS-398 enhanced (P<0.05) ACh relaxations in both strains treated with aldosterone in a comparable manner (Figure 2A and 2B). Incubation with SQ 29 548 or ABZ also increased (P<0.05) ACh relaxations in both strains treated with aldosterone in a comparable manner (Figure 3A and 3B). However, incubation with the thromboxane synthase inhibitor furegrelate did not modify endothelium-dependent relaxations in any group treated with aldosterone (Figure 3A and 3B). Incubation with PGI2 synthesis inhibitor TCP increased (P<0.05) ACh relaxations in both strains treated with aldosterone (Figure 3A and 3B), this enhancement being higher in SHR.

COX-2 Protein Expression

COX-2 protein expression was higher in aortic segments from SHR compared with WKY. In both rat strains, this parameter was increased after chronic aldosterone treatment (Figure 4).
Prostanoid Production

In normotensive animals, aldosterone increased ACh-stimulated aortic production of 13,14-dihydro-15-keto PGF₂α, PGE₂, and 6-keto-PGF₁α (Figure 5A through 5C; P<0.05) and did not modify TXB₂ levels (untreated 68±14 pg/mL mg tissue; aldosterone 78±11 pg/mL mg tissue). However, in SHR, aldosterone did not modify 13,14-dihydro-15-keto PGF₂α (untreated 1.5±0.3 pg/mL mg tissue; aldosterone 1.2±0.2 pg/mL mg tissue); PGE₂ (untreated 8±0.4 pg/mL mg tissue; aldosterone 8.3±0.8 pg/mL mg tissue), and TXB₂ release (untreated 185±21 pg/mL mg tissue; aldosterone 179±33 pg/mL mg tissue) but increased the 6-keto-PGF₁α production induced by ACh (Figure 5D; P<0.05). Treatment with aldosterone increased 6-keto-PGF₁α levels in a similar extent in segments from both strains (WKY 1.83±0.23; SHR 1.89±0.3).

Discussion

The present results show that in our experimental conditions, aldosterone alters endothelial function because its chronic administration diminished the ACh-induced relaxation in normotensive and hypertensive animals. These data confirm previous observations indicating that aldosterone was able to produce endothelial dysfunction in experimental and clinical studies.20,26,27 This effect appears to be independent of previous BP levels because the reduction in ACh relaxation was similar in normotensive and hypertensive rats. Moreover, this deleterious effect induced by aldosterone on endothelial function is not mediated by hemodynamic changes induced by this mineralocorticoid because no differences in BP levels were observed between treated and untreated animals in both strains. Because an increase in BP associated with aldosterone administration has been reported,7,15 the lack of effect observed in this study could be a consequence of differences in experimental conditions (dose and period treatment).

Several mechanisms have been implicated in the endothelial dysfunction associated with different pathological situations (hypertension, diabetes, aging, and hypercholesterolemia)17,18,28–30 including a reduced NO availability and an increase in vasoconstrictor factors.28,29,31,32 Similarly, in aldosterone-treated animals, decreased NO levels have also been implicated in the alteration of endothelial function, mainly because of an increase in oxidative stress.22 In addition, the present results show that arachidonic acid-derived vasoconstrictor prostanoids are involved in endothelial dysfunction produced by aldosterone treatment in normotensive and hypertensive rats. This is demonstrated by the normalization of ACh relaxation in aortic segments of aldosterone-treated rats in the presence of the COX-1/COX-2 inhibitor indomethacin. Because the presence of the specific COX-2 inhibitor NS 398 increased the ACh response in a similar extent, then indomethacin, the vasoconstrictor factors are derived from COX-2. Supporting this role is the observation that COX-2 is overexpressed in the aorta from aldosterone-treated animals in normotensive and hypertensive rats, reaching higher levels in SHR. This is in agreement with
a previous study showing that the aldosterone antagonist eplerenone was able to reduce COX-2 expression in hearts from rats treated with angiotensin II and a high-salt diet. Therefore, the data suggest that there are interactions between aldosterone and COX that can be relevant in the regulation of vascular function.

It has been reported that through the activation of TP receptors, vasoconstrictor prostanoids such as TXA₂ can participate in the endothelial dysfunction associated with different cardiovascular risk factors. Incubation with SQ 29 548, a TP receptor antagonist, increased ACh relaxations in both strains of rats treated with aldosterone in a similar manner, suggesting the participation of vasoconstrictor prostanoids through TP receptors. However, the present results showed that in aorta from WKY and SHR treated with aldosterone, TXA₂ is not an important candidate responsible for the reduction of endothelium-dependent relaxations. This affirmation is based on the fact that the thromboxane synthase inhibitor furegrelate did not modify this response in any strain. These data are in agreement with previous results indicating that ACh did not release TXA₂ in aorta from normotensive and hypertensive rats. Likewise, ACh was unable to increase TXA₂ release in aorta from aldosterone-treated animal. Consequently, the participation of other prostanoids such as PGF₂α, PGE₂, and PGI₂, which can be synthesized through COX-2 and can act as vasoconstrictors under certain conditions, can be proposed. The present results showed that the presence of PGI₂ synthesis inhibitor TCP was able to increase the reduced ACh relaxation in both strains, although it was only normalized in SHR. Moreover, aldosterone increased aortic production of 6-keto-PGF₁α induced by ACh in both strains.

Therefore, it seems that PGI₂ plays a major role in the alterations in endothelial function produced by aldosterone in normotensive and hypertensive conditions, supporting that the vasoconstrictor action of PGI₂ observed could be relevant in hyperaldosteronism. TP receptor could be the main receptor implicated in this vasoconstrictor action of PGI₂ because SQ 29 548 increased ACh relaxation in aorta from both strains treated with aldosterone. However, PGI₂ or a metabolite of PGI₂ would activate EP₁ or EP₃ receptors that are coupled to contraction in vascular smooth muscle. In this sense, it has been described that EP₁ and EP₃ receptors bound IP ligands such as iloprost with Kᵢ values comparable to those for IP receptor. In addition, the participation of vasoconstrictor derivated from cytochrome P450 family can also be proposed because the presence of the cytochrome P450 inhibitor ABZ reversed the alterations in ACh relaxation in both strains. Because PGI₂ synthase is a member of the P450 family, it could be postulated that the effect of ABZ is mediated by PGI₂ synthesis inhibition.

It should be mentioned that under normotensive conditions, besides PGI₂, other vasoconstrictor prostanoids such as
PGF$_2\alpha$ and PGE$_2$ appear to participate in the reduced ACh-induced relaxations, as demonstrated by the results on prostanoid release. These differences could explain why endothelial dysfunction is comparable in both strains in addition to the higher expression of COX-2 in SHR.

In conclusion, the results of the study demonstrated that chronic treatment with aldosterone was able to produce endothelial dysfunction through COX-2 activation in normotensive and hypertensive conditions. PGI$_2$ seems to be the main factor accounting for endothelial dysfunction in hypertensive rats, whereas other prostanoids besides PGI$_2$ appear to be involved in endothelial dysfunction under normotensive conditions.

**Perspectives**

Aldosterone has been involved in vascular damage recently through direct effects on vascular wall at several levels (hypertrophy, inflammation, fibrosis, endothelial dysfunction, and atherosclerosis). The present results showed that vasoconstrictor prostanoids account for endothelial dysfunction induced by aldosterone in rats. Because endothelial dysfunction is involved in the development and complications of atherosclerosis, it could be hypothesized that treatment with anti-inflammatory drugs, and specifically COX-2 inhibitors, could ameliorate vascular damage and progression of atherosclerosis in patients with elevated aldosterone production.

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