British Journal of Pharmacology (2008) 154, 1225–1235

0007–1188/08 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Aldosterone induces endothelial dysfunction in resistance arteries from normotensive and hypertensive rats by increasing thromboxane A_2 and prostacyclin

FE Xavier[3], R Aras-López[1], I Arroyo-Villa[1], L del Campo[1], M Salaices[2], LV Rossoni[4], M Ferrer[1] and G Balfagón[1]

[1]*Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain;* [2]*Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain;* [3]*Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, Brazil and* [4]*Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*

Background and purpose: The present study was designed to assess whether cyclooxygenase-2 (COX-2) activation is involved in the effects of chronic aldosterone treatment on endothelial function of mesenteric resistance arteries (MRA) from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR).
Experimental approach: Relaxation to acetylcholine was measured in MRA from both untreated and aldosterone-treated strains. Vasomotor responses to prostacyclin and U46619 were also analysed. Release of 6-oxo-prostaglandin (PG)$F_{1\alpha}$ and thromboxane B_2 (TxB_2) was determined by enzyme immunoassay. COX-2 protein expression was measured by western blot.
Key results: Aldosterone reduced acetylcholine relaxation in MRA from both strains. In MRA from both aldosterone-treated strains the COX-1/2 or COX-2 inhibitor (indomethacin and NS-398, respectively), TxA_2 synthesis inhibitor (furegrelate), prostacyclin synthesis inhibitor (tranylcypromine) or TxA_2/ PGH_2 receptor antagonist (SQ 29 548), but not COX-1 inhibitor SC-560, increased acetylcholine relaxation. In untreated rats this response was increased only in SHR. Prostacyclin elicited a biphasic vasomotor response: lower concentrations elicited relaxation, whereas higher concentrations elicited contraction that was reduced by SQ 29 548. Aldosterone increased the acetylcholine-stimulated production of 6-oxo-$PGF_{1\alpha}$ and TxB_2 in MRA from both strains. COX-2 expression was higher in both strains of rats treated with aldosterone.
Conclusions and implications: Chronic treatment with aldosterone impaired endothelial function in MRA under normotensive and hypertensive conditions by increasing COX-2-derived prostacyclin and thromboxane A_2. As endothelial dysfunction participates in the pathogenesis of many cardiovascular disorders we hypothesize that anti-inflammatory drugs, specifically COX-2 inhibitors, could ameliorate vascular damage in patients with elevated aldosterone production.
British Journal of Pharmacology (2008) **154,** 1225–1235; doi:10.1038/bjp.2008.200; published online 26 May 2008

Keywords: aldosterone; hypertension; resistance arteries; endothelial dysfunction; COX-2; prostanoids

Abbreviations: 6-oxo-$PGF_{1\alpha}$, 6-oxo prostaglandin 1_α; DEA–NO, diethylamine-nitric oxide; E_{max}, maximum response; KHS, Krebs Henseleit solution; SHR, spontaneously hypertensive rats; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2; WKY, Wistar-Kyoto

Introduction

Aldosterone is the main circulating mineralocorticoid in humans, and it is normally produced in response to volume depletion and angiotensin stimulation. It participates in the electrolyte balance and plays an important physiological role in the long-term regulation of Na^+ and K^+ in the distal tubules and collecting ducts of the kidney (Giebisch, 1998; Palmer, 1999; Giebisch and Wang, 2000; Palmer and Frindt, 2000). In addition, it has also been shown that aldosterone plays a major role in the regulation of the vascular tone and in the vascular alterations associated with atherosclerosis, heart failure and some forms of hypertension (Pitt *et al.*, 1999; Schiffrin, 2006). Furthermore, in primary or secondary

Correspondence: Dr G Balfagón, Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, C/Arzobispo Morcillo, 4, Madrid 28029, Spain.
E-mail: gloria.balfagon@uam.es
Received 3 March 2008; accepted 28 March 2008; published online 26 May 2008

aldosteronism, aldosterone production continues despite volume expansion and elevating arterial blood pressure (Stowasser, 2001).

Endothelial dysfunction is characterized by a shift in the actions of vascular endothelium towards reduced vasodilation, increased vasoconstriction, a proinflammatory state and prothrombic events (Feletou and Vanhoutte, 2006). Several studies have demonstrated that aldosterone stimulates a vascular inflammatory response that could induce endothelial dysfunction and fibrosis (Blanco-Rivero *et al.*, 2005; Neves *et al.*, 2005). An aldosterone-mediated increase in the activity of various inflammatory agents, including COX-2-derived products, leukocyte chemoattractants and adhesion molecules, has been demonstrated in some experimental models of cardiovascular diseases (Blanco-Rivero *et al.*, 2005; Neves *et al.*, 2005; Sanz-Rosa *et al.*, 2005). Diminished endothelium NOS expression or activity has also been proposed as an important mechanism leading to the endothelial dysfunction induced by aldosterone (Ikeda *et al.*, 1995). Previously, we have reported that vasoconstrictor prostanoids, mainly prostacyclin, acting as a vasoconstrictor factor, participate as mediators in the endothelial dysfunction induced by aldosterone in aorta from normotensive and hypertensive rats (Blanco-Rivero *et al.*, 2005). However, at present, there are no published studies on the contribution of COX-derived products to the vascular effects of aldosterone on resistance arteries. As the renin–angiotensin–aldosterone system is involved in alterations of vascular function in hypertensive patients, the study of aldosterone effects on vascular function in resistance vessels could be especially relevant, particularly as resistance arteries have a much greater role in the regulation of vascular resistance and in the haemodynamic abnormalities associated with hypertension.

Thus, the purpose of this study was to analyse the probable endothelial dysfunction produced by aldosterone in mesenteric resistance vessels under normotensive and hypertensive conditions, as well as the possible role of COX-1- and COX-2-derived prostanoids, prostacyclin and TxA_2 (thromboxane A_2), in that effect of aldosterone.

Methods

Animals

All animal procedures in this investigation conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85–23, revised 1996) and the directives 609/86 CEE and RD 233/88 of the Ministerio de Agricultura, Pesca y Alimentación (registration no. EX-021U) of Spain.

Six-month-old male Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) weighing 250–300 g were obtained from colonies maintained at the animal quarters of the Facultad de Medicina of the Universidad Autónoma de Madrid.

Controlled time-release pellets (Innovative Research of America, Sarasota, FL, USA) containing aldosterone or vehicle were subcutaneously implanted. Animals were divided into four groups: WKY, WKY plus aldosterone (0.05 mg kg^{-1} per day), SHR, SHR plus aldosterone. At the end of the treatment period (3 weeks), systolic blood pressure was measured in awake rats by a tail-cuff method (Letica, Digital Pressure Meter, LE5000, Barcelona, Spain).

Vessel preparation

After death by CO_2 inhalation, the mesenteric vascular bed was removed and placed in cold (4 °C) Henseleit solution (KHS (in mM): 115 NaCl, 2.5 $CaCl_2$, 4.6 KCl, 1.2 KH_2PO_4, 1.2, $MgSO_4.7H_2O$, 25 $NaHCO_3$, 11.1 glucose and 0.03 EDTA).

For reactivity experiments, the third-order branch of the mesenteric arcade (untreated: SHR 262 ± 5.1, $n=8$ vs WKY 301 ± 4.8 μm diameter, $n=10$, $P<0.05$; aldosterone-treated: SHR 254 ± 7.0, $n=7$ vs WKY 318 ± 5.4 μm diameter, $n=9$, $P<0.05$) was dissected from the mesenteric bed, cleaned of connective tissue and cut in segments of approximately 2 mm in length. Two tungsten wires (40-μm diameter) were introduced through the lumen of the segments and mounted in a small vessel dual-chamber myograph (Danish Myo Technology A/S, Aarhus, Denmark) to measure isometric tension according to the method described by Mulvany and Halpern (1977). After a 30-min equilibration period in oxygenated KHS at 37 °C and pH = 7.4, segments were stretched to their optimal lumen diameter for active tension development. This was determined based on the internal circumference–wall tension ratio of the segments by setting their internal circumference, L_0, to 90% of what the vessels would have if they were exposed to a passive tension equivalent to that produced by a transmural pressure of 100 mm Hg (Mulvany and Halpern, 1977). Afterwards, segments were washed with KHS and left to equilibrate for 30 min; segment contractility was then tested by an initial exposure to a high-K^+ (120 mM) solution.

Experimental protocols

Endothelium-dependent and -independent relaxations were studied by evaluating relaxations to acetylcholine (0.1 nM–1 μM) or diethylamine-nitric oxide (DEA–NO) (0.1 nM–100 μM), respectively, in arterial rings submaximally precontracted with noradrenaline (10 μM). This concentration produced responses that were more stable than those produced by lower concentrations. For example, at 1 μM noradrenaline, the tonic phase of the contractions was not sufficiently sustained to generate a cumulative concentration–response curve to a vasodilator agent, such as acetylcholine or DEA–NO.

In a pilot study, we found that a 3-week aldosterone treatment reduced endothelium-dependent relaxation to acetylcholine in small mesenteric arteries from normotensive and hypertensive rats. Therefore, the possible role of arachidonic acid metabolites of COX was investigated in segments from aldosterone-treated and -untreated SHR and WKY rats. Arterial segments were preincubated with either indomethacin (a COX inhibitor, 10 μM), SC-560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole) (a COX-1 inhibitor, 1 μM), *N*-(2-cyclohexyloxy-4-nitrophenyl) methansulphonamide (NS-398, a COX-2 inhibitor, 10 μM), furegrelate (a thromboxane synthase inhibitor, 10 μM), SQ 29 548 (1*S*-[1*a*,2*a*(*Z*),3*a*,4*a*]]-7-[3-[[2-(phenylamino)

carbonyl]hydrazino]methyl]-7-oxabicyclo [2.2.1] hept-2-yl]-5-heptanoic acid) (a thromboxane receptor (TP) antagonist, 1 μM) or tranylcypromine (TCP, a prostacyclin synthesis inhibitor, 10 μM) before generating concentration–response curves to acetylcholine. All drugs were added 30 min before the concentration–response curve to acetylcholine. Because we observed that relaxation to acetylcholine is blunted in a time-dependent manner in single arterial segment not exposed to inhibitors, experiments were performed in unpaired segments.

In another set of experiments, the vasoactive response to prostacyclin (0.01 nM–10 μM) was analysed in noradrenaline-precontracted segments. To analyse the participation of TP receptors in response to prostacyclin, segments were preincubated with SQ 29 548 (1 μM). To analyse possible alterations produced by the aldosterone treatment in vascular response mediated by activation of TP or α adrenoceptors, concentration–response curves to the TP receptor agonist (15)-hydroxy-11α,9α-(epoxymethano) prosta-5,13-dienoic acid (U46619, 1 nM–3 μM) or noradrenaline (10 nM–0.1 mM), respectively, were obtained in segments from both experimental groups.

Prostanoid production

To measure the release of TxA_2 and prostacyclin, we used a thromboxane B_2 (TxB_2) or 6-oxo-$PGF_{1\alpha}$ enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) respectively. The second, third and fourth branches of mesenteric artery were preincubated for 45 min in 200 μl of KHS at 37 °C and continuously gassed with a 95% O_2–5% CO_2 mixture (stabilization period). Afterwards, three wash-out periods of 7 min in a bath of 200 μl of KHS were run before incubation with acetylcholine (0.1 nM–1 μM). The different assays were performed following the manufacturer's instructions. Results were expressed as pg mL^{-1} per mg wet tissue.

Western blot analysis

Mesenteric resistance arteries from untreated and aldosterone-treated WKY and SHR were homogenized in a boiling buffer composed of 1 mM sodium vanadate (Sigma-Aldrich, St Louis, MO, USA), 1% sodium lauryl sulphate and pH 7.4, 0.01 M Tris-HCl, and protein content was measured with a DC protein assay kit (Bio-Rad, Hercules, CA, USA). Homogenates containing 60 μg protein were fractionated in a 10% sodium lauryl sulphate-polyacrylamide gel electrophoresis and transferred to a polyvinylfluoride membrane (Bio-Rad). Membranes were blocked in 5% non-fat milk in Tris-HCl-buffered saline-0.1% Tween 20. Subsequent washes were done in Tris-HCl-buffered saline-Tween 20, and the membranes were then incubated with antibody against COX-2 (1:200; Cayman Chemical) and after washing, they were incubated with anti-mouse IgG antibody conjugated to horseradish peroxidase (1:2000; Transduction Laboratories, San Jose, CA, USA). The immunocomplexes were detected using an enhanced horseradish peroxidase/luminol chemiluminiscence system (ECL Plus; Amersham International plc, Little Chalfont, UK) and subjected to autoradiography (Hyperfilm ECL; Amersham International plc). Signals on the immunoblot were quantified using a computer program (NIH Image V1.56). The same membranes were used to determine α-actin expression using a mouse monoclonal antibody (1:3000; Boehringer Mannheim, Mannheim, Germany).

Statistical analysis

Relaxation responses to acetylcholine and DEA–NO were expressed as the percentage of relaxation of the maximum contractile response induced by noradrenaline. Vascular responses to prostacyclin were expressed as a percentage of the tone previously obtained with noradrenaline. U46619 contractile response was expressed as a percentage of the maximum response produced by KCl. For each concentration–response curve, the maximum effect (E_{max}) and the log of the concentration of agonist that produced half of the E_{max} ($\log EC_{50}$) were calculated using non-linear regression analysis (GraphPad Prism Software, San Diego, CA, USA). Results of COX-2 expression are expressed as the ratio between optical density for COX-2 and for α-actin.

All values are expressed as means ± s.e.mean of the number of animals used in each experiment. Results were analysed using Student's *t*-test or by two-way ANOVA for comparison between groups. When ANOVA showed a significant treatment effect, Bonferroni's *post hoc* test was used to compare individual means. Differences were considered statistically significant at $P<0.05$.

Drugs

Drugs used were noradrenaline hydrochloride, acetylcholine chloride, furegrelate, tranylcypromine, indomethacin, SC-560, DEA–NO (Sigma, St Louis, MO, USA), NS-398 (Calbiochem-Novabiochem GmbH, San Diego, CA, USA) and SQ 29 548 (Cayman Chemical). Stock solutions of drugs were made in distilled water, except for noradrenaline, which was dissolved in an NaCl (0.9%)–ascorbic acid (0.01% w/v) solution, and indomethacin, which was dissolved in 1.5 mM $NaHCO_3$. These solutions were kept at −20 °C and appropriate dilutions were made on the day of the experiment.

Results

Blood pressure

As expected, SHR presented higher blood pressure levels than WKY (SHR 171 ± 5.0 vs WKY 118 ± 3.0 mm Hg; $P<0.05$, $N=8$–10). Chronic aldosterone administration did not significantly modify this parameter in any strain (SHR-aldo 180 ± 4.0 vs WKY-aldo 120 ± 8.0 mm Hg, $N=7$–9).

Vascular response to KCl, noradrenaline and endothelium-dependent and -independent relaxations

KCl (120 mM) evoked similar contractions in vessels from both untreated and aldosterone-treated WKY and SHR (WKY 3.25 ± 0.02 mN mm^{-1}, SHR 3.19 ± 0.04 mN mm^{-1}; WKY-aldo 3.19 ± 0.05; SHR-aldo 3.21 ± 0.08; $P>0.05$). Similarly, vasoconstriction to noradrenaline was not different between

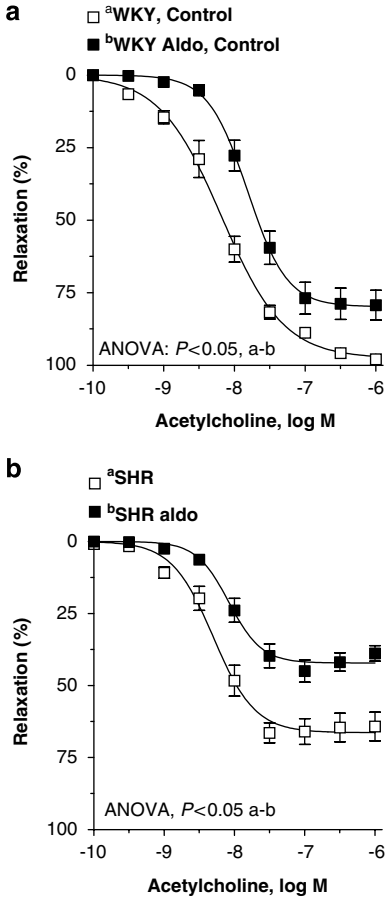

Figure 1 Effect of chronic treatment with aldosterone (Aldo) on the concentration-dependent relaxation to acetylcholine in mesenteric resistance segments from WKY (**a**) and SHR (**b**). Each point represents the mean of seven to eight experiments ± s.e.mean. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

WKY and SHR; this response was not changed by aldosterone treatment (E_{max}: WKY 110 ± 0.12, WKY-aldo 110 ± 0.27; SHR 113 ± 1.40, SHR-aldo 114 ± 0.79, $P>0.05$; $\log EC_{50}$: −5.72 ± 0.001; WKY-aldo −5.84 ± 0.003; SHR −5.72 ± 0.02; SHR-aldo −5.60 ± 0.009, $P>0.05$).

Acetylcholine induced endothelium-dependent vasodilator responses that were greater in segments from WKY than in those from SHR (Figure 1, Table 1). Aldosterone treatment markedly impaired vasodilation to acetylcholine in resistance mesenteric arteries from either strains (Figure 1, Table 1).

In arterial segments from untreated rats, incubation with either indomethacin or NS-398 increased vasorelaxation to acetylcholine only in segments from SHR (Figures 2a and c, Table 1). This effect was similar in presence of indomethacin or NS-398. In both aldosterone-treated WKY and SHR, pretreatment of mesenteric resistance arteries with indomethacin or NS-398 resulted in a potentiation of the concentration–response curve to acetylcholine (Figures 2b and d, Table 1). In these arteries, the effect of indomethacin and NS-398 was comparable, but was greater in SHR than in WKY. In segments from all experimental groups, preincubation with SC-560 did not modify acetylcholine-induced

Table 1 Changes in the maximum response (E_{max}, expressed as a percentage of relaxation) and $\log EC_{50}$ to acetylcholine of mesenteric resistance arteries from untreated and aldosterone-treated WKY and SHR

	Untreated		*Aldosterone-treated*	
	E_{max} *(%)*	*log* EC_{50}	E_{max} *(%)*	*log* EC_{50}
Control				
WKY	100 ± 1.15	−8.15 ± 0.02	79.0 ± 0.60*	−7.81 ± 0.01*
SHR	66.3 ± 1.60^{+}	−8.30 ± 0.04	42.2 ± 1.04*	−8.07 ± 0.03
Indomethacin				
WKY	96.5 ± 1.0	−8.11 ± 0.02	93.8 ± 1.19$^{\#}$	−8.43 ± 0.03$^{\#}$
SHR	93.0 ± 1.5$^{\#}$	−8.32 ± 0.03	95.4 ± 2.28$^{\#}$	−8.23 ± 0.05
NS-398				
WKY	99.6 ± 2.96	−8.12 ± 0.06	92.3 ± 1.62$^{\#}$	−8.51 ± 0.06$^{\#}$
SHR	90.0 ± 0.56$^{\#}$	−8.44 ± 0.01	90.6 ± 2.27$^{\#}$	−8.28 ± 0.05
Furegrelate				
WKY	100 ± 1.91	−8.17 ± 0.04	95.0 ± 0.63$^{\#}$	−8.26 ± 0.02$^{\#}$
SHR	82.4 ± 1.21$^{\#}$	−8.11 ± 0.03	72.8 ± 1.36$^{\#}$	−8.23 ± 0.03
SQ 29 548				
WKY	100 ± 2.15	−8.17 ± 0.05	93.1 ± 1.19$^{\#}$	−7.83 ± 0.02
SHR	81.1 ± 1.40$^{\#}$	−8.12 ± 0.03	60.0 ± 1.31$^{\#}$	−8.00 ± 0.03
Tranylcypromine				
WKY	99.7 ± 2.34	−8.03 ± 0.04	92.0 ± 0.93$^{\#}$	−8.31 ± 0.02$^{\#}$
SHR	65.0 ± 0.59	−8.15 ± 0.01	77.8 ± 1.38$^{\#}$	−8.27 ± 0.03$^{\#}$

Abbreviations: E_{max}, maximum response; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.
ANOVA: *$P<0.05$ vs untreated, $^{+}P<0.05$ vs WKY, $^{\#}P<0.05$ vs WKY/SHR control.

vasodilation (Figure 2). Results obtained in presence of the TxA_2 synthesis inhibitor, furegrelate, were comparable to those in presence of indomethacin or NS-398 (Figure 3, Table 1). In the presence of SQ 29 548, vasodilation to acetylcholine was also increased in segments from untreated SHR (Table 1) and in segments from both aldosterone-treated strains (Table 1). Incubation with the prostacyclin synthesis inhibitor tranylcypromine did not modify endothelium-dependent relaxations in segments from untreated WKY or SHR (Figures 3a and c, Table 1). In segments from both strains treated with aldosterone, tranylcypromine increased the acetylcholine relaxation (Figures 3b and d, Table 1); however, this effect was greater in SHR than in WKY. All inhibitors used did not affect basal or noradrenaline-induced tone of arteries from either untreated or aldosterone-treated WKY and SHR (results not shown).

Relaxation to the NO donor, DEA–NO, was similar in segments from WKY and SHR (E_{max}: SHR 85.6 ± 3.3, WKY 81.5 ± 3.5%, $P>0.05$; $\log EC_{50}$: SHR −6.29 ± 0.13 vs WKY −6.13 ± 0.16, $P>0.05$) (Figure 4) and remained unmodified after aldosterone treatment (E_{max}: SHR aldo 84.3 ± 1.73%, WKY-aldo 73.0 ± 7.4, $P>0.05$; $\log EC_{50}$: SHR-aldo −6.34 ± 0.07 vs WKY-aldo −6.13 ± 0.25, $P>0.05$) (Figure 4).

Vasomotion to prostacyclin

Exogenous prostacyclin induced a concentration-dependent relaxation in mesenteric resistance arteries from WKY rats (Figure 5b, Table 2). In segments from SHR and in both strains when treated with aldosterone, prostacyclin

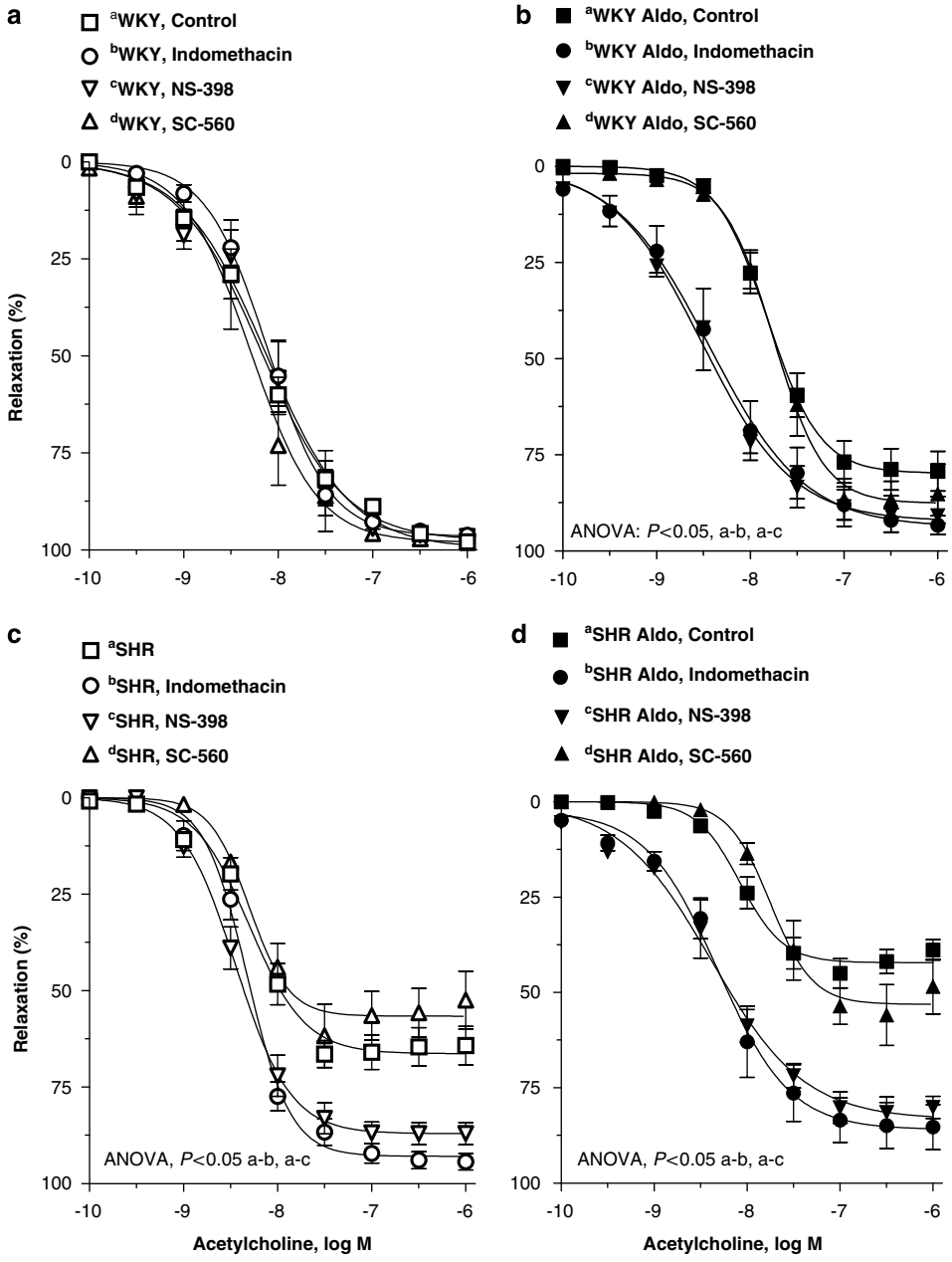

Figure 2 Effect of indomethacin, NS-398 or SC-560 on the concentration-dependent relaxation to acetylcholine in mesenteric resistance segments from control WKY (**a**) and SHR (**c**), aldosterone-treated WKY (**b**) and SHR (**d**). Results are expressed as mean ± s.e.mean. $N = 6$–8 animals in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

concentrations below 0.1 μM induced concentration-dependent relaxation whereas concentrations equal or above 0.1 μM have a biphasic effect, characterized by an initial contracting response followed by a relaxing phase (Figures 5a, c, d and e, Table 2).

The observed contractile effect of prostacyclin was greater in segments from SHR treated with aldosterone than in the aldosterone-treated WKY rats or untreated SHR (Table 2). In mesenteric segments from these experimental groups, incubation with SQ 29 548 markedly reduced vasoconstriction induced by prostacyclin (Figures 5c, d and e, Table 2). The relaxing effect of prostacyclin was greater in segments from WKY than in segments from SHR (Table 2). In both strains, relaxation to prostacyclin was reduced by aldosterone treatment (Table 2). Incubation with SQ 29 548 increased the relaxant response to prostacyclin in segments from untreated SHR and in both strains when treated with aldosterone, but not in segments from untreated WKY rats (Figures 5c, d and e, Table 2).

Vascular response to the TP receptor agonist, U46619

In mesenteric resistance arteries from SHR, the contractile response to U46619 was greater than in those from WKY rats.

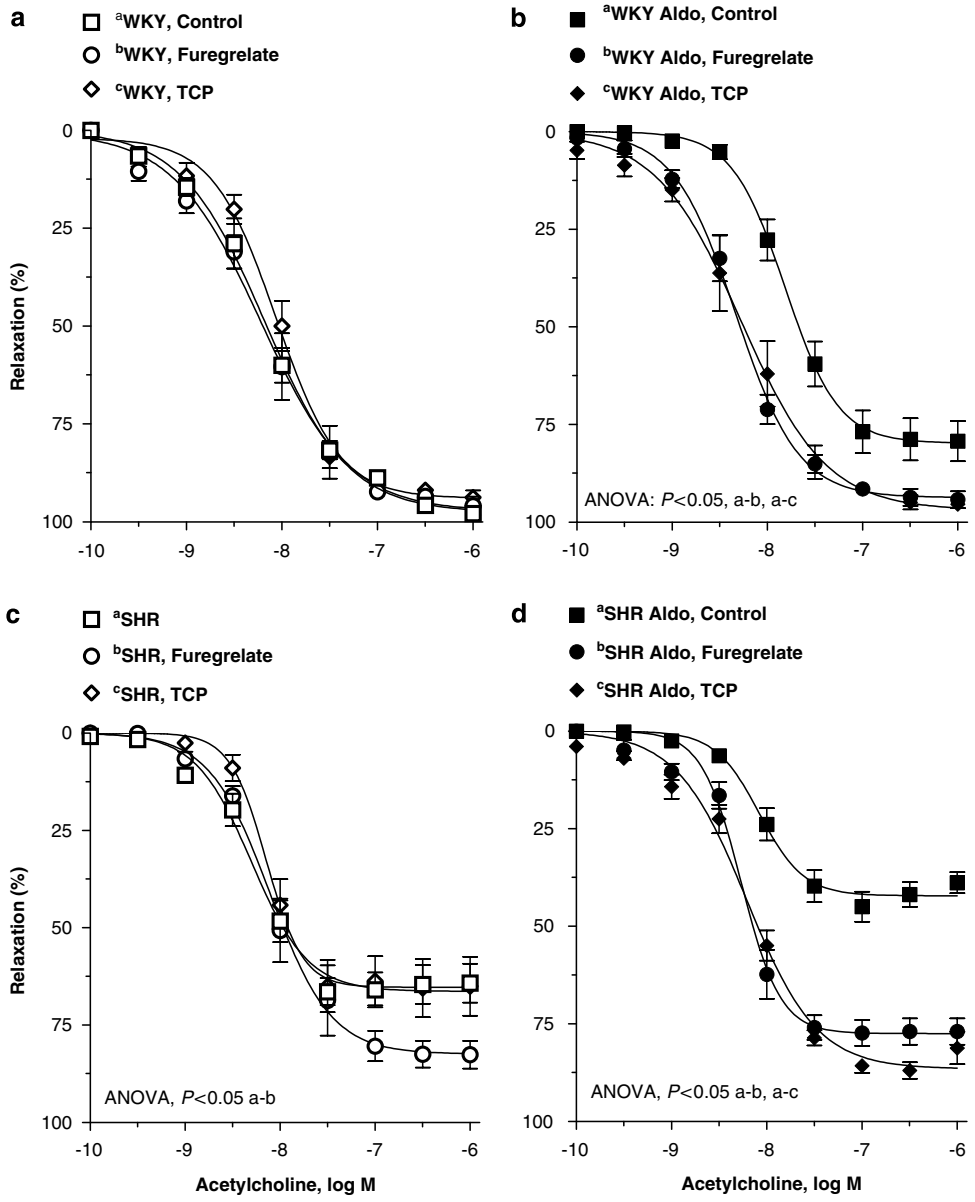

Figure 3 Effect of furegrelate or tranylcypromine (TCP) on the concentration-dependent relaxation to acetylcholine in mesenteric resistance segments from control WKY (**a**) and SHR (**c**), aldosterone-treated WKY (**b**) and SHR (**d**). Results are expressed as mean ± s.e.mean. $N = 6$–8 animals in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

In both strains, this response was increased by chronic aldosterone treatment (E_{max}: untreated, SHR 72.6 ± 2.2, WKY 66.2 ± 1.0; aldosterone-treated, SHR 102 ± 2.0, WKY 72.0 ± 1.8%, $P < 0.05$; log EC_{50}: untreated, SHR −7.15 ± 0.05 vs WKY −6.55 ± 0.01; aldosterone-treated, SHR −7.05 ± 0.06, WKY −6.97 ± 0.03, $P < 0.05$) (Figure 6).

Prostanoid production

In mesenteric resistance arteries from all experimental groups, acetylcholine increased the production of both 6-oxo-$PGF_{1\alpha}$ and TxB_2 (Figure 7). The acetylcholine-stimulated 6-oxo-$PGF_{1\alpha}$ levels were similar in arteries from untreated WKY and SHR and were increased by aldosterone treatment (untreated, SHR 4752 ± 883 vs WKY 4953 ± 1608; aldosterone-treated, SHR 19 528 ± 2759 vs WKY 15 477 ± 2501 pg mL^{-1} per mg tissue, $P < 0.05$) (Figure 7). In mesenteric arteries from SHR, the levels of acetylcholine-stimulated TxB_2 were higher than in those from WKY rats. In both strains, aldosterone treatment increased TxB_2 production (untreated, SHR 73.2 ± 7.90 vs WKY 47.7 ± 3.16; aldosterone-treated, SHR 152 ± 14.0 vs WKY 120 ± 12.0 pg mL^{-1} per mg tissue, $P < 0.05$) (Figure 7).

COX-2 protein expression

Basal COX-2 protein expression was higher in mesenteric resistance arteries from SHR compared with WKY. In both rat

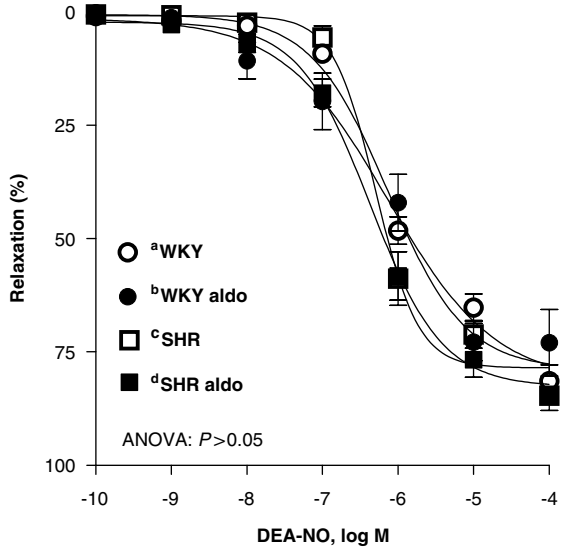

Figure 4 Effect of chronic treatment with aldosterone (aldo) on the concentration-dependent relaxation to DEA–NO in mesenteric resistance segments from WKY and SHR. Each point represents the mean of four experiments ± s.e.mean. DEA–NO, diethylamine-nitric oxide; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

strains, this variable was increased after chronic aldosterone treatment (Figure 8).

Discussion and conclusions

In several pathological conditions, including hypertension, atherosclerosis and heart failure, aldosterone promotes vascular damage through the formation of reactive oxygen species and proinflammatory agents (Duprez *et al*., 2000; Stier *et al*., 2002; Funder, 2004). In this study, we observed that a 3-week aldosterone treatment altered endothelial function in mesenteric resistance vessels from normotensive and hypertensive rats because its chronic administration diminished the acetylcholine-induced relaxation in these vessels without any effect on relaxant response to DEA–NO. This effect confirms previous studies indicating that this mineralocorticoid produces endothelial dysfunction in these vessels (Virdis *et al*., 2002). However, in contrast to earlier reports by Virdis *et al*. (2002), our findings demonstrated that the effects of aldosterone on the endothelial-dependent responses of mesenteric resistance arteries appear to be independent of haemodynamic changes, because no differences in blood pressure were observed between aldosterone-treated and -untreated animals. As we have previously reported (Blanco-Rivero *et al*., 2005), the reasons for these differences are not clear, although they could be explained by differences in the strains used.

Several mechanisms have been implicated in the endothelial dysfunction associated with different pathological states, including reduced vasodilator bioavailability due to increased oxidative stress and an increase in vasoconstrictor factors (Feletou and Vanhoutte, 2006). In resistance mesenteric arteries from aldosterone-infused rats, it has been reported that the observed endothelial dysfunction seems to be mediated by increases in NADPH oxidase-dependent oxidative stress (Virdis *et al*., 2002). However, in a previous study, we demonstrated that chronic aldosterone treatment induced endothelial dysfunction in aorta from normotensive and hypertensive rats by increasing COX-2-dependent vasoconstriction (Blanco-Rivero *et al*., 2005). Similarly, the results presented here show that COX-2-derived arachidonic acid metabolites in mesenteric resistance arteries are also involved in the endothelial dysfunction produced by aldosterone treatment in normotensive and hypertensive rats. In vessels from aldosterone-treated rats, indomethacin or NS-398, but not SC-560, improved the vasodilator response to acetylcholine, indicating that COX-2, but not COX-1, contributes to the pathogenesis of aldosterone-induced endothelial dysfunction in mesenteric resistance vessels. Supporting these results is the fact that COX-2 is overexpressed in mesenteric resistance arteries from normotensive and hypertensive aldosterone-treated animals, reaching higher levels in SHR. These agree with our previous study in rat aorta under similar experimental conditions (Blanco-Rivero *et al*., 2005).

It has been reported that activation of TP receptors allows vasoconstrictor prostanoids such as TxA_2 and prostacyclin to participate in endothelial dysfunction associated with different cardiovascular risk factors (Carvalho *et al*., 1997; Alvarez *et al*., 2005; Blanco-Rivero *et al*., 2005; Gluais *et al*., 2005). In the present study, incubation with furegrelate or SQ 29 548 increased acetylcholine-induced relaxation in both WKY and SHR treated with aldosterone, this effect being higher in segments from SHR. Different from those reported in conductance vessels (Blanco-Rivero *et al*., 2005), results presented here suggest the participation of TxA_2 in the endothelial dysfunction induced by aldosterone in resistance arteries from WKY and SHR. In untreated rats, SQ 29 548 or furegrelate only increased acetylcholine response in segments from SHR, indicating the participation of TxA_2 in the endothelial dysfunction induced by the hypertensive state in resistance vessels (Carvalho *et al*., 1997; Suzuki *et al*., 2000). Moreover, the TxA_2 release and the contraction induced by the TxA_2 analogue, U46619, were higher in mesenteric resistance arteries from SHR than in arteries from WKY rats. Both parameters were increased by aldosterone treatment, contrasting to the results observed in aorta from the same strains, where aldosterone treatment did not change acetylcholine-stimulated TxA_2 levels (Blanco-Rivero *et al*., 2005). To our knowledge, this is the first report showing that chronic aldosterone treatment increases the acetylcholine-stimulated TxA_2 release and its vasoconstrictor response in resistance vessels from WKY and SHR. Our findings are in line with some studies suggesting that aldosterone sensitizes the vasculature to vasoconstrictors (Schiffrin, 2006); however, under our experimental conditions, this effect seems to be specific for TP receptor activation, as the vasoconstriction induced by noradrenaline or KCl remained unmodified, after aldosterone treatment.

Numerous studies have demonstrated that prostacyclin promotes vasodilation in various vascular beds by stimulating prostacyclin (IP) receptors and thereby increasing the intracellular cyclic-AMP concentration (Wise and Jones, 1996). However, it is often forgotten that prostacyclin can

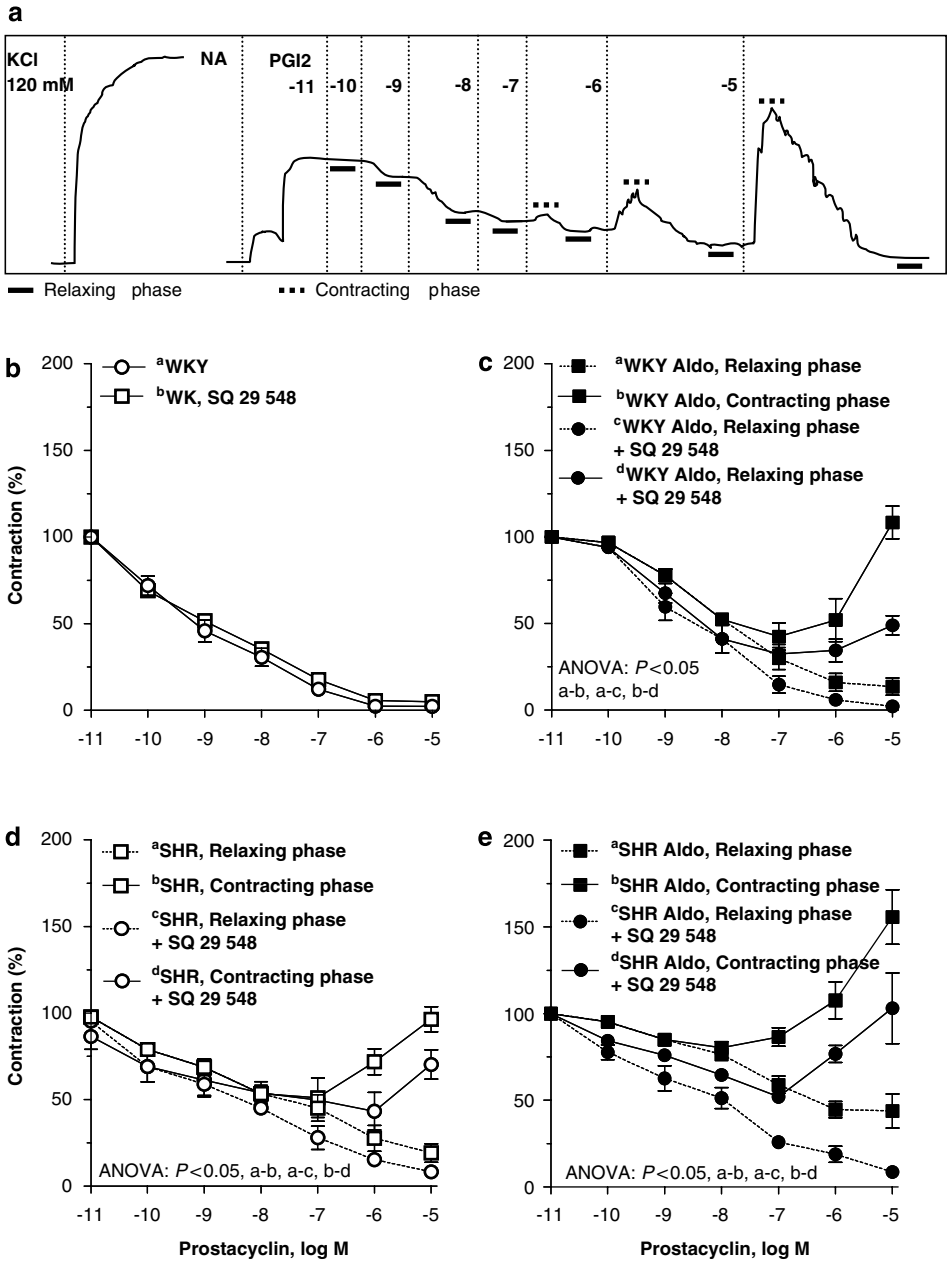

Figure 5 Typical tracings of tension recordings of resistance mesenteric arteries exposed to prostacyclin (**a**); effect of SQ 29 548 on the concentration-dependent response to prostacyclin in segments from control WKY (**b**) and SHR (**d**) and aldosterone-treated WKY (**c**) and SHR (**e**). Results (mean ± s.e.mean) are expressed as per cent of previous contraction to noradrenaline. $N = 5–6$ animals in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

also promote vasoconstriction via TP receptors (Levy, 1978, 1980; Davis *et al*., 1980; Williams *et al*., 1994; Zhao *et al*., 1996; Gluais *et al*., 2005). To assess the involvement of prostacyclin in the effect of aldosterone in resistance vessels from WKY and SHR, we used the prostacyclin synthase inhibitor, tranylcypromine. In vessels from aldosterone-treated WKY and SHR, the blunted endothelium-dependent relaxation was improved by exposure to tranylcypromine, an effect that was higher in arteries from SHR. These results therefore indicate that prostacyclin, acting as a contracting factor, mediates the effect of aldosterone in resistance vessels from normotensive and hypertensive rats. In keeping with these functional results, acetylcholine-stimulated release of 6-oxo-$PGF_{1\alpha}$ was higher in mesenteric resistance vessels from aldosterone-treated WKY and SHR, similar to that previously reported for rat aorta (Blanco-Rivero *et al*., 2005). Taken together, our findings provide the first demonstration of participation of increased TxA_2 release together with prostacyclin on changes of endothelial function produced by aldosterone in resistance vessels from normotensive and hypertensive conditions, supporting the possible relevance of these prostanoids in hyperaldosteronism.

Experiments with exogenous prostacyclin revealed that, different from reports on WKY and SHR aorta (Gluais *et al*.,

2005), prostacyclin was able to promote relaxation in mesenteric resistance arteries from both strains. This response was smaller in segments from SHR than in those from WKY rats and in both strains it was reduced by aldosterone. Previous studies have demonstrated that vasodilation to prostacyclin may be 'masked' by its contractile effect through activation of TP receptors (Zhao *et al.*, 1996). Results presented here demonstrated that in the presence of SQ 29 548, the vasodilation to prostacyclin was similar in segments from all experimental groups, supporting the hypothesis that the impaired vasodilation to prostacyclin in segments from SHR and in both strains when treated with aldosterone is related to its contractile effect through activation of TP receptors instead of dysfunction linked to the IP receptor itself. The present study also demonstrates that, in contrast to earlier reports on WKY aorta (Gluais *et al.*, 2005), exogenous prostacyclin failed to produce contraction in mesenteric resistance arteries from WKY rats. However, in arteries from hypertensive rats, a vasoconstrictor effect to this prostanoid was also observed. Aldosterone treatment allowed prostacyclin to induce contraction in segments from WKY rats and increased this response in segments from SHR. In both cases, the contractile responses were transient, as

Table 2 Maximum contracting and relaxing responses (expressed as a percentage of the tone previously obtained with noradrenaline) to prostacyclin in mesenteric resistance arteries from untreated and aldosterone-treated WKY and SHR

	Untreated		*Aldosterone-treated*	
	Contracting response	*Relaxing response*	*Contracting response*	*Relaxing response*
WKY				
Control	—	2.22 ± 1.05	108 ± 9.50	13.53 ± 4.90*
SQ 29 548	—	4.84 ± 1.86	49.9 ± 5.50$^{\#}$	2.06 ± 1.46$^{\#}$
SHR				
Control	93.32 ± 7.30	19.8 ± 5.27†	156 ± 15.0†	43.75 ± 9.80*
SQ 29 548	70.25 ± 8.44$^{\#}$	8.33 ± 1.24$^{\#}$	103 ± 20.0$^{\#}$	8.69 ± 2.66$^{\#}$

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.
ANOVA: *$P<0.05$ vs untreated, $^{\dagger}P<0.05$ vs WKY control/SQ 29 548, $^{\#}P<0.05$ vs WKY/SHR control.

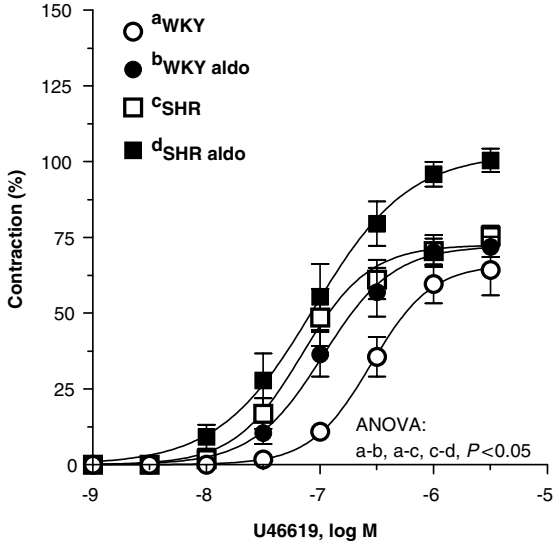

Figure 6 Effect of chronic treatment with aldosterone (aldo) on the concentration-dependent contraction to U46619 in mesenteric resistance segments from WKYand SHR. Results (mean ± s.e.mean) are expressed as per cent of previous contraction to KCl. $N = 5$–8 animals in each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

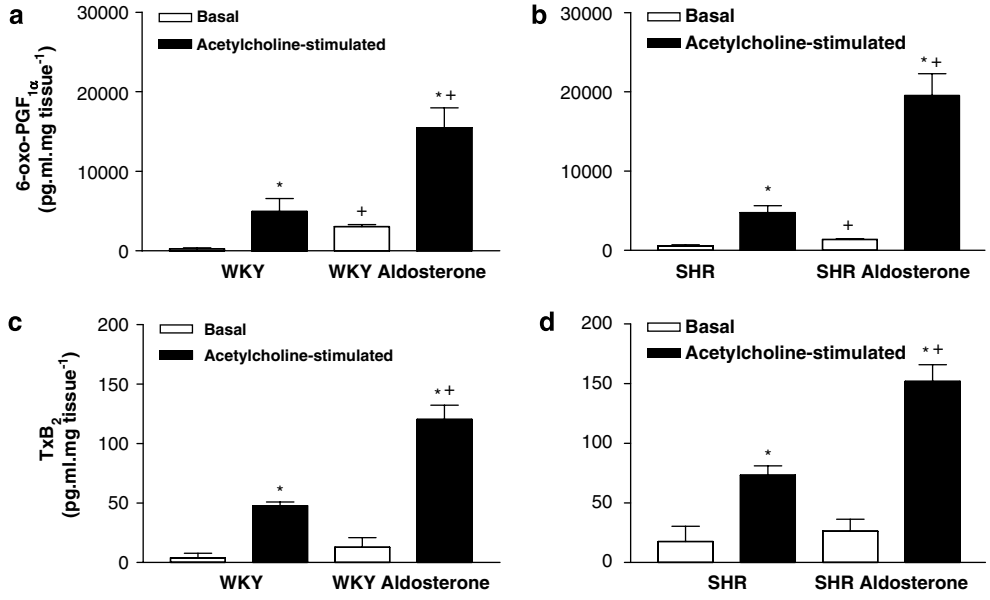

Figure 7 Effect of chronic treatment with aldosterone on the basal and acetylcholine-stimulated production of 6-oxo-$PGF_{1\alpha}$ from WKY (**a**) and SHR (**b**) and on the production of TxB_2 from WKY (**c**) and SHR (**d**). $N = 4$–5 animals in each group. SHR, spontaneously hypertensive rats; TxB_2, thromboxane B_2; WKY, Wistar-Kyoto; 6-oxo-$PGF_{1\alpha}$, 6-oxo prostaglandin 1_{α}.

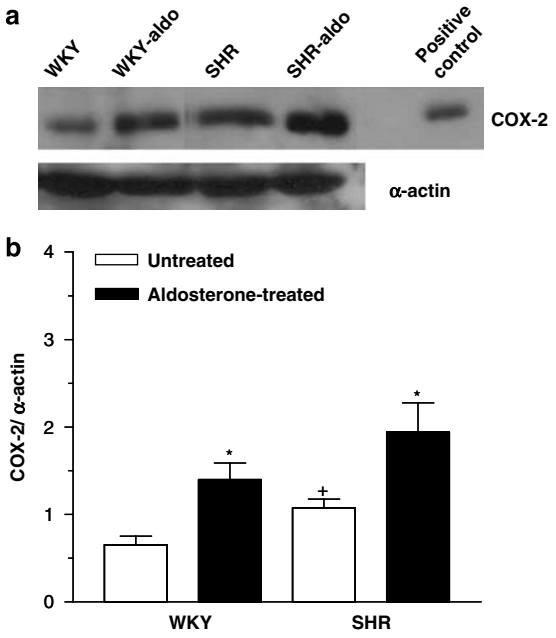

Figure 8 Representative western blot for COX-2 expression in resistance mesenteric arteries from control and aldosterone-treated WKY and SHR (**a**). Figure is representative of four separate experiments. (**b**) Densitometric analysis of the western blot for COX-2 protein expression. Results (means ± s.e.mean) are expressed as the ratio between the signal for the COX-2 protein and the signal for α-actin. $^{+}P<0.05$ WKY vs SHR; $^{*}P<0.05$ untreated vs aldosterone-treated. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

also reported in rat aorta (Gluais *et al*., 2005), and were reduced by SQ 29 548, indicating that this response is mediated in part by TP receptor activation (Williams *et al*., 1994; Zhao *et al*., 1996; Gluais *et al*., 2005). To our knowledge, this is the first study to demonstrate the actions of prostacyclin in resistance mesenteric arteries from normotensive and hypertensive rats as well as its contribution to the vascular effects of aldosterone in these vessels.

Our results, in concert with a prior report (Blanco-Rivero *et al*., 2005) show that an increase in COX-2-derived products, especially TxA_2 and prostacyclin, is one mechanism linked to impairment of endothelial function induced by aldosterone in resistance vessels. Because endothelial dysfunction is involved in the pathogenesis of hypertension, heart failure and atherosclerosis, it could be hypothesized that treatment with anti-inflammatory drugs, specifically COX-2 inhibitors, could ameliorate vascular damage and the progression of that damage in patients with elevated aldosterone production.

In conclusion, the results of the present study demonstrated that chronic treatment with aldosterone was able to produce endothelial dysfunction in mesenteric resistance vessels, through COX-2 activation in normotensive and hypertensive conditions. In both strains, increased TxA_2 and prostacyclin release and vasoconstrictor response seem to be the main factors involved. Nevertheless, further studies are necessary to clearly elucidate the mechanism by which aldosterone increases COX-2 expression in vessels from normotensive and hypertensive rats.

Acknowledgements

This research was supported by the Fondo de Investigaciones Sanitarias (PI051767), the Comisión de Ciencia y Tecnología (SAF-2006-07888) and Banco de Santander-UAM.

Conflict of interest

The authors state no conflict of interest.

References

Alvarez Y, Briones AM, Balfagon G, Alonso MJ, Salaices M (2005). Hypertension increases the participation of vasoconstrictor prostanoids from cyclooxygenase-2 in phenylephrine responses. *J Hypertens* **23**: 767–777.

Blanco-Rivero J, Cachofeiro V, Lahera V, Aras-Lopez R, Marquez-Rodas I, Salaices M *et al*. (2005). Participation of prostacyclin in endothelial dysfunction induced by aldosterone in normotensive and hypertensive rats. *Hypertension* **46**: 107–112.

Carvalho MH, Fortes ZB, Nigro D, Oliveira MA, Scivoletto R (1997). The role of thromboxane A_2 in the altered microvascular reactivity in two-kidney, one-clip hypertension. *Endothelium* **5**: 167–178.

Davis K, Ginnsburg R, Bristow M, Harrison DC (1980). Biphasic action of prostacyclin in the human coronary artery. *Clin Res* **28**: 165A.

Duprez D, De Buyzere M, Rietzschel ER, Clement DL (2000). Aldosterone and vascular damage. *Curr Hypertens Rep* **2**: 327–334.

Feletou M, Vanhoutte PM (2006). Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol* **291**: 985–1002.

Funder JW (2004). Aldosterone, mineralocorticoid receptors and vascular inflammation. *Mol Cell Endocrinol* **217**: 263–269.

Giebisch G (1998). Renal potassium transport: mechanisms and regulation. *Am J Physiol* **274**: 817–833.

Giebisch G, Wang W (2000). Renal tubule potassium channels: function, regulation and structure. *Acta Physiol Scand* **170**: 153–173.

Gluais P, Lonchampt M, Morrow JD, Vanhoutte PM, Feletou M (2005). Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. *Br J Pharmacol* **146**: 834–845.

Ikeda U, Kanbe T, Nakayama I, Kawahara Y, Yokoyama M, Shimada K (1995). Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells induced by interleukin-1 beta. *Eur J Pharmacol* **290**: 69–73.

Levy JV (1978). Contractile responses to prostacyclin of isolated human saphenous and rat venous tissue. *Prostaglandins* **16**: 93–97.

Levy JV (1980). Prostacyclin-induced contraction of isolated aortic strips from normal and spontaneously hypertensive rats (SHR). *Prostaglandins* **19**: 517–525.

Mulvany MJ, Halpern W (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res* **41**: 19–26.

Neves MF, Amiri F, Virdis A, Diep QN, Schiffrin EL (2005). Role of aldosterone in angiotensin II-induced cardiac and aortic inflammation, fibrosis, and hypertrophy. *Can J Physiol Pharmacol* **83**: 999–1006.

Palmer LG (1999). Potassium secretion and the regulation of distal nephron K channels. *Am J Physiol* **277**: 821–825.

Palmer LG, Frindt G (2000). Aldosterone and potassium secretion by the cortical collecting duct. *Kidney Int* **57**: 1324–1328.

Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A *et al*. (1999). The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized aldactone evaluation study investigators. *N Engl J Med* **341**: 709–717.

Sanz-Rosa D, Cediel E, de las Heras N, Miana M, Balfagon G, Lahera V *et al*. (2005). Participation of aldosterone in the vascular

inflammatory response of spontaneously hypertensive rats: role of the NFkappaB/IkappaB system. *J Hypertens* **23**: 1167–1172.
Schiffrin EL (2006). Effects of aldosterone on the vasculature. *Hypertension* **47**: 312–318.
Stier CT, Chander PN, Rocha R (2002). Aldosterone as a mediator in cardiovascular injury. *Cardiol Rev* **10**: 97–107.
Stowasser M (2001). New perspectives on the role of aldosterone excess in cardiovascular disease. *Clin Exp Pharmacol Physiol* **28**: 783–791.
Suzuki H, Ikezaki H, Hong D, Rubinstein I (2000). PGH(2)-TxA(2)-receptor blockade restores vasoreactivity in a new rodent model of genetic hypertension. *J Appl Physiol* **88**: 1983–1988.
Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL (2002). Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension* **40**: 504–510.
Williams SP, Dorn GW, Rapoport RM (1994). Prostaglandin I_2 mediates contraction and relaxation of vascular smooth muscle. *Am J Physiol* **267**: 796–803.
Wise H, Jones RL (1996). Focus on prostacyclin and its novel mimetics. *Trends Pharmacol Sci* **17**: 17–21.
Zhao YJ, Wang J, Tod ML, Rubin LJ, Yuan XJ (1996). Pulmonary vasoconstrictor effects of prostacyclin in rats: potential role of thromboxane receptors. *J Appl Physiol* **81**: 2595–2603.