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Aldosterone alters the participation of endothelial factors in noradrenaline vasoconstriction differently in resistance arteries from normotensive and hypertensive rats

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ABSTRACT

This study analyzed the effect of aldosterone (0.05 mg/kg per day, 3 weeks) on vasoconstriction induced by noradrenaline in mesenteric resistance arteries from WKY rats and SHR. Contraction to noradrenaline was measured in mesenteric resistance arteries from untreated and aldosterone-treatedrats from both strains. Participation of nitric oxide (NO), superoxide anions, thromboxane A_2 (TxA_2) and prostacyclin in this response was determined. 6-keto-prostaglandin (PG)F1alpha and thromboxane B_2 (TxB_2) releases were determined by enzyme immunoassay. NO and superoxide anion release were also determined by fluorescence and chemiluminiscence, respectively. Aldosterone did not modify noradrenaline-induced contraction in either strain. In mesenteric resistance arteries from both aldosterone-treated groups, endothelium removal or preincubation with NO synthesis inhibitor L-NAME increased the noradrenaline-induced contraction, while incubation with the superoxide anion scavenger tempol decreased it. Preincubation with either the COX-1/2 or COX-2 inhibitor (indomethacin and NS-398, respectively) decreased the noradrenaline contraction in aldosterone-treated animals, while this response was not modified by COX-1 inhibitor SC-560. TxA_2 synthesis inhibitor (furegrelate), or TxA2 receptor antagonist (SQ 29 548) also decreased the noradrenaline contraction in aldosterone-treated animals. In untreated SHR, but not WKY rats, this response was increased by L-NAME, and reduced by tempol, indomethacin, NS-398 or SQ 29 548. Aldosterone treatment did not modify NO or TxB_2 release, but it did increase superoxide anion and 6-keto-PGF(1alpha) release in mesenteric resistance arteries from both strains. In conclusion, chronic aldosterone treatment reduces smooth muscle contraction to alpha-adrenergic stimuli, producing a new balance in the release of endothelium-derived prostanoids and NO.

1. 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, aldosterone has been reported to play a major role in the regulation of vascular tone as well as in vascular alterations associated to atherosclerosis, heart failure and some forms of hypertension (Pitt et al., 1999; Schiffrin, 2006).

The endothelium plays a pivotal role in the regulation of vascular tone by regulating the release of both relaxing and contracting factors under basal conditions and after stimulation with contractile agonists. The mechanisms by which aldosterone influences vascular function remain to be elucidated; nevertheless, it has been suggested that aldosterone can quantitatively and qualitatively alter the release of endothelial factors (Blanco-Rivero et al., 2005; Xavier et al., 2008). Several studies have demonstrated that aldosterone stimulates a vascular inflammatory response, and this could induce endothelial dysfunction and fibrosis (Blanco-Rivero et al., 2005; Neves et al., 2005; Xavier et al., 2008). In addition, an aldosterone-mediated increase in the release of various inflammatory agents has been described (Blanco-Rivero et al., 2005; Neves et al., 2005; Sanz-Rosa et al., 2005; Xavier et al., 2008). Recently we have reported that vasoconstrictor prostanoids are involved in the impaired endothelium-

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dependent vasodilation to acetylcholine induced by aldosterone in conductance and resistance vessels from normotensive and hypertensive rats (Blanco-Rivero et al., 2005; Xavier et al., 2008).

The renin–angiotensin–aldosterone system is involved in alterations of vascular function in hypertensive patients and the study of aldosterone effects on vascular contractility in resistance vessels could be especially relevant, particularly as resistance arteries have a major role in the regulation of vascular resistance and in the haemodynamic abnormalities associated with this pathology. The findings described above raise the possibility that aldosterone may induce variations in endothelial function that would promote changes in vascular contraction. Thus, the purpose of this study was to analyze the effect of aldosterone on noradrenaline-induced vasoconstriction in mesenteric resistance vessels from normotensive (WKY) and hypertensive (SHR) rats, and also study the possible role of endothelium-derived products in that putative aldosterone effect.

2. Methods

2.1. Animal housing

Male 6-month-old Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) weighing 250 to 300 g were obtained from colonies maintained at the animal quarters of the *Facultad de Medicina* of the *Universidad Autónoma de Madrid*. The investigations conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIHi Publication No. 85-23, revised 1996) and to the directives 609/86 CEE and R.D. 233/88 of the *Ministerio de Agricultura, Pesca y Alimentación* (registration No. EX-021U) of Spain.

Controlled time-release pellets (Innovative Research of America, U.S.A.), containing aldosterone (0.05 mg/kg per day) or a vehicle, were subcutaneously implanted. Animals were divided into four groups: WKY rats, WKY rats plus aldosterone, SHR, and SHR plus aldosterone (Blanco-Rivero et al., 2005; Xavier et al., 2008). Rats were fed *ad libitum* with standard rat chow (Safe A04, Panlab S.L., Spain). At the end of the treatment period (three weeks), systolic blood pressure (BP) was measured in awake rats by a tail-cuff method (Letica, Digital Pressure Meter, LE5000, Barcelona, Spain). Blood samples were collected by cardiac puncture before the animals were sacrificed. After centrifugation for 15 min at 1500 *g*, the serum was transferred to polypropylene tubes and then frozen at −80 °C.

2.2. Aldosterone, cortisol and noradrenaline levels

Serum levels of aldosterone, cortisol and noradrenaline were analyzed using aldosterone EIA kit, Cortisol EIA Kit (Cayman Chemical, Ann Arbor, Michigan, USA), and noradrenaline research EIA (Labor Diagnostica Nord. Gmbh & Co., Kg: Nordhorn, Germany) respectively. The assays were performed following the manufacturer's instructions. Results were expressed as pg/ml (for aldosterone and cortisol) or ng/ml (for noradrenaline).

2.3. Vessel preparation

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

For *ex vivo* 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).

2.4. Experimental protocols

After a 45 minute-equilibration period, each arterial segment was exposed twice to KCl (120 mmol/l) to assess its maximum contractility. Thirty minutes later, rings were contracted with a concentration of noradrenaline that induced approximately 50% of the KCl contraction, and then acetylcholine (1 μmol/l) was added to assess the integrity of the endothelium. After 60 min, cumulative concentration–response curves for noradrenaline (10 nmol/l–0.1 mmol/l) were generated.

The effect of the nonselective NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME, 0.1 mmol/l), as well as that of the superoxide dismutase mimetic 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (tempol, 0.1 μmol/l) on concentration–response curves for noradrenaline were investigated.

The possible role of cyclooxygenase-arachidonic acid metabolites in noradrenaline-induced contraction was investigated in segments pre-incubated with either indomethacin (a cyclooxygenase inhibitor, 10 μmol/l), 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole (SC-560, a COX-1 inhibitor, 1 μmol/l) or N-(2-cyclohexyloxy-4-nitrophenyl) methansulfonamide (NS-398, a COX-2 inhibitor, 10 μmol/l), furegrelate (a thromboxane synthase inhibitor, 10 μmol/l) or [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 (SQ 29 548, a thromboxane A_2 receptor (TP) antagonist, 1 μmol/l).

All drugs were added 30 min before performing the concentration–response curve to noradrenaline.

2.5. NO release

In order to study the effect of aldosterone on noradrenaline-induced nitric oxide (NO) release, the second, third and fourth branches of mesenteric artery from either control or aldosterone-treated WKY rats and SHR were incubated for 60 min in HEPES buffer containing in mmol/l: 119 NaCl, 20 HEPES, 4.6 KCl, 1 $MgSO_4 \cdot 7H_2O$, 0.15 $Na_2HPO_4 \cdot 12H_2O$, 0.4 KH_2PO4, 5 $NaHCO_3$, 1.2 $CaCl_2 \cdot 2H_2O$, and 5.2 glucose, at 37 °C (stabilization period). Afterward, arteries were incubated with the fluorescent probe 4,5-diaminofluorescein (2 μmol/l) for 30 min, and medium was collected to measure basal NO release. Once the organ bath was refilled, noradrenaline was added cumulatively (10 nmol/l–0.1 mmol/l) at 2 min intervals. The medium was collected only at the end of the concentration–response curve to noradrenaline. The fluorescence of the medium was measured at room temperature using a spectrofluorimeter (LS50 Perkin Elmer Instruments, FL WINLAB Software) with excitation wavelength set at 492 nm and emission wavelength at 515 nm. The stimulated NO release was calculated by subtracting the basal NO release from that evoked by noradrenaline. Also, blank measurement samples were collected from the medium without mesenteric segments in order to subtract background emission. Some assays were performed in the presence of L-NAME (0.1 mmol/l). The amount of NO released was expressed as arbitrary units per milligram of tissue.

2.6. Detection of superoxide anions

In order to study the effect of aldosterone on superoxide anion release, the second, third and fourth branches of mesenteric artery from