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Vascular dysfunction and fibrosis in stroke-prone spontaneously hypertensive rats: The aldosterone-mineralocorticoid receptor-Nox1 axis

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ABSTRACT

Aims: We questioned whether aldosterone and oxidative stress play a role in vascular damage in severe hypertension and investigated the role of Nox1 in this process.
Materials and methods: We studied mesenteric arteries, aortas and vascular smooth muscle cells (VSMC) from WKY and SHRSP rats. Vascular effects of eplerenone or canrenoic acid (CA) (mineralocorticoid receptor (MR) blockers), ML171 (Nox1 inhibitor) and EHT1864 (Rac1/2 inhibitor) were assessed. Nox1-knockout mice were also studied. Vessels and VSMCs were probed for Noxs, reactive oxygen species (ROS) and pro-fibrotic/inflammatory signaling.
Key findings: Blood pressure and plasma levels of aldosterone and galectin-3 were increased in SHRSP versus WKY. Acetylcholine-induced vasorelaxation was decreased (61% vs 115%) and phenylephrine-induced contraction increased in SHRSP versus WKY (E_{max} 132.8% vs 96.9%, $p < 0.05$). Eplerenone, ML171 and EHT1864 attenuated hypercontractility in SHRSP. Vascular expression of collagen, fibronectin, TGFβ, MCP-1, RANTES, MMP2, MMP9 and p66Shc was increased in SHRSP versus WKY. These changes were associated with increased ROS generation, 3-nitrotyrosine expression and Nox1 upregulation. Activation of vascular p66Shc and increased expression of Nox1 and collagen I were prevented by CA in SHRSP. Nox1 expression was increased in aldosterone-stimulated WKY VSMCs, an effect that was amplified in SHRSP VSMCs (5.2vs9.9 fold-increase). ML171 prevented aldosterone-induced VSMC Nox1-ROS production. Aldosterone increased vascular expression of fibronectin and PAI-1 in wild-type mice but not in Nox1-knockout mice.
Significance: Our findings suggest that aldosterone, which is increased in SHRSP, induces vascular damage through MR-Nox1-p66Shc-mediated processes that modulate pro-fibrotic and pro-inflammatory signaling pathways.

1. Introduction

Hypertension-associated vascular damage is characterised by functional, structural and mechanical alterations comprising hypercontractility, endothelial dysfunction, inflammation, calcification and fibrosis [1,2]. Molecular mechanisms underlying vascular changes in hypertension are incompletely understood, but a role for the mineralocorticoid hormone aldosterone, has been suggested. Aldosterone acts through genomic and non-genomic pathways to regulate blood pressure and electrolytic homeostasis. Accumulating evidence suggests that aldosterone also has direct vascular effects and that it is a potent pro-fibrotic agent in cardiovascular remodeling in hypertension [3]. Evidence from animal models and clinical trials in patients with heart failure demonstrate that blockade of the mineralocorticoid receptor (MR) through which aldosterone signals, is cardio- and vaso-protective [4,5]. In the context of vascular function, a role for aldosterone-MR signaling remains controversial because aldosterone has been shown to induce both vasorelaxation and vasoconstriction [6]. Recently, a role for reactive oxygen species (ROS) has been suggested to mediate the detrimental effects of aldosterone in the vasculature [3].

Oxidative stress has been strongly implicated in many of the molecular processes associated with endothelial dysfunction, structural remodeling and vascular inflammation in hypertension [7–9]. In the vascular wall, NADPH oxidases (Noxs) are the predominant source of ROS and are upregulated in hypertension [7–9]. Nox isoforms 1,2,4

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Table 1
Primers targeted to rat genes for qRT-PCR analysis.

Rat gene	Forward primer	Reverse primer
Nox1	TCCCTTTGCTTCCTTCTTGA	CCAGCCAGTGAGGAAGAGTC
NoxA1	TTACTGTGCCCCTGAAGGTC	CTCGGGCTTTGTAGCTGAAC
NoxO1	TCCAGACGTTTGCCTTCTCT	CGTGTCAACAATGGAGCATC
Nox2	ACCCTTTCACCCTGACCTCT	TCCCAGCTCCCACTAACATC
Nox4	CCAGAATGAGGATCCCAGAA	AGCAGCAGCAGCATGTAGAA
P22phox	TTGTTGCAGGAGTGCTCATC	CAGGGACAGCAGTAAGTGGA
P47phox	AGCTCCCAGGTGGTATGATG	ATCTTTGGCCGTCAGGTATG
MMP2	AGCTCCCGGAAAAGATTGAT	TCCAGTTAAAGGCAGCGTCT
MMP9	CACTGTAACTGGGGGCAACT	CACTTCTTGTCAGCGTCGAA
MCP-1	CAGTTAATGCCCCACTCACC	TTCCTTATTGGGGTCAGCAC
RANTES	ATATGGCTCGGACACCACTC	CCACTTCTTCTCTGGGTTGG
18S	AAGTCCCTGCCCGTTTGTACACA	GATCCGAGGGCCTCACTAAAC

and 5 are expressed in human vascular tissue and appear to be dysregulated in pathological conditions [8–10]. Increased activation of Nox1, Nox2 and Nox5 has been demonstrated in hypertension-associated cardiovascular damage, whereas Nox4 activation has been associated with both beneficial [11,12] and injurious effects [13].

A key role for Nox1 in redox signaling in angiotensin II (AngII)-dependent models of hypertension has been demonstrated [14]. Nox1 has also been shown to be linked to AngII and aldosterone signaling in vascular cells [15–17]. However, the relationship between aldosterone and Nox1 in the context of vascular remodeling in hypertension is incompletely defined.

The stroke-prone spontaneously hypertensive rat (SHRSP) represents a robust model of human hypertension with end-organ damage. In this model, upregulation of Nox1 has been observed [18] and inhibition of aldosterone signaling is associated with beneficial results [19]. It has yet to be determined whether aldosterone mediates its effects through activation of Noxs. Here we hypothesise that in SHRSP rats, aldosterone promotes vascular damage through mechanisms involving Nox1.

2. Methods

2.1. Experimental animals

Animal experiments were performed in accordance with the United Kingdom Animals Scientific Procedures Act 1986 and ARRIVE Guidelines [20] and approved by the institutional ethics review committee. Male WKY and SHRSP rats aged at 16–18 weeks were used for experimentation. In additional studies rats were treated with canrenoic acid (CA) (10 mg/kg/day, in drinking water) from weaning until 18 weeks old. Male Nox1 knockout mice (Nox1-/y) aged 10–12 weeks were infused with aldosterone (3×10^{-4} mol/L/Kg/day) for 4 weeks by osmotic minipumps (model 2004, Alzet, Cupertino, CA) implanted under isoflurane (3% induction; 1.5% maintenance) anaesthesia. Animals were euthanized by exsanguination following cardiac puncture with immediate dissection of tissues that were rinsed, snap-frozen in liquid nitrogen and stored at −80 °C or fixed in formalin for preparation of histological analysis.

2.2. Blood pressure measurement

Mean arterial pressure was measured in WKY and SHRSP rats using tail-cuff plethysmography. Rats were placed in isolation chambers on a heated platform, and blood pressure measurements were obtained. Before recording measurements, animals were trained for 5 consecutive

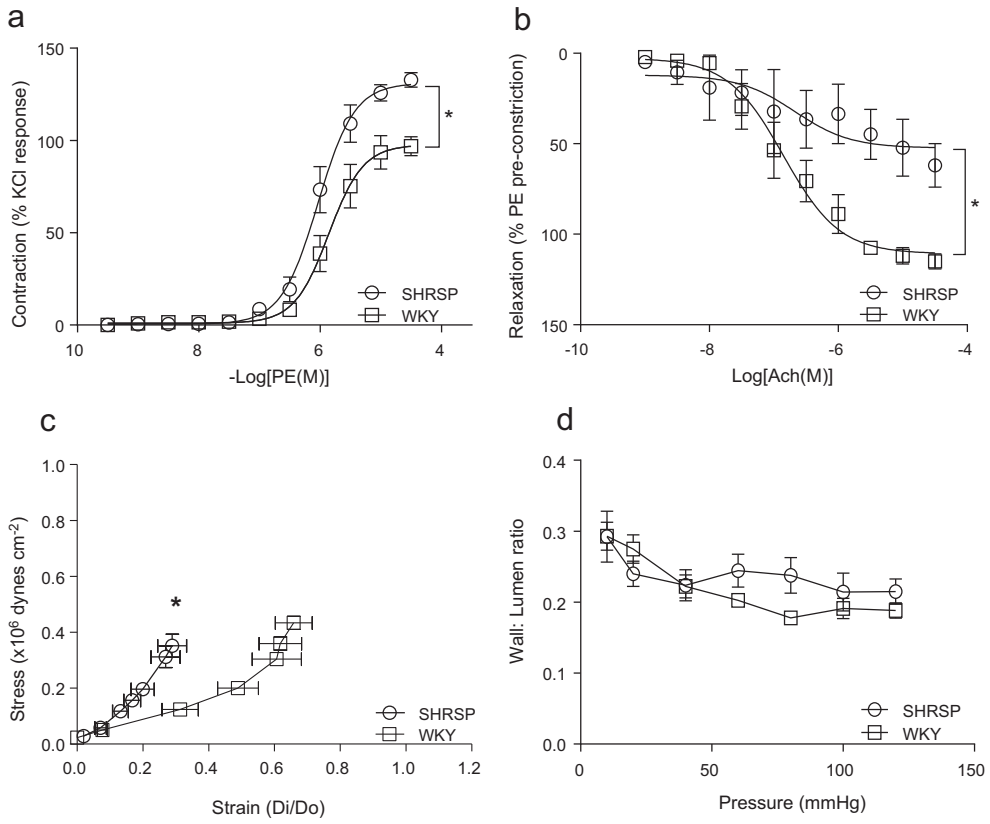

Fig. 1. Assessment of vascular contraction to phenylephrine (PE) (a) and endothelial-dependent relaxation to acetylcholine (Ach) assessed by wire myography (b). Vascular stress-strain curve (c) and wall to lumen ratio (d) assessed by pressure myography in WKY and SHRSP rats. Curves represent the mean ± SEM ($n = 8$–15 per group). *$P < 0.05$ vs WKY.