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Research Report

Aldosterone-induced oxidative stress and inflammation in the brain are mediated by the endothelial cell mineralocorticoid receptor

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ABSTRACT

Elevated aldosterone levels, which promote cerebral vascular oxidative stress, inflammation, and endothelial dysfunction, may increase stroke risk, independent of blood pressure and other risk factors. The main target receptor of aldosterone, the mineralocorticoid receptor (MR), is expressed in many cell types, including endothelial cells. Endothelial cell dysfunction is thought to be an initiating step contributing to cardiovascular disease and stroke; however the importance of MR expressed on endothelial cells in the brain is unknown. Here we have examined whether endothelial cell MR mediates cerebral vascular oxidative stress and brain inflammation during aldosterone excess. In male mice, aldosterone (0.72 mg/kg/day, 14 days) caused a small increase (~14 mmHg) in blood pressure. The MR blocker spironolactone (25 mg/kg/d, ip) abolished this increase, whereas endothelial cell MR-deficiency had no effect. Aldosterone increased superoxide production capacity in cerebral arteries, and also mRNA expression of the pro-inflammatory cytokines chemokine (C–C motif) ligand 7 (CCL7), CCL8 and interleukin (IL)-1β in the brain. These increases were prevented by both spironolactone treatment and endothelial cell MR-deficiency; whereas IL-1β expression was blocked by spironolactone only. Endothelial cell MR mediates aldosterone-induced increases in cerebrovascular superoxide levels and chemokine expression in the brain, but not blood pressure or brain IL-1β. Endothelial cell-targeted MR antagonism may represent a novel approach to treat cerebrovascular disease and stroke, particularly during conditions of aldosterone excess.

Abbreviations: BW, body weight; CCL2, chemokine (C–C motif) ligand 2; CCL7, chemokine (C–C motif) ligand 7; CCL8, chemokine (C–C motif) ligand 8; CCL12, chemokine (C–C motif) ligand 12; CCR2, chemokine (C–C motif) receptor 2; DOCA, deoxycorticosterone acetate; (IL)-1β, interleukin-1β; (IL)-6, interleukin-6; KW, kidney weight; MR, mineralocorticoid receptor; PdB, phorbol-12; 13-dibutyrate; SHR-SP stroke-prone spontaneously hypertensive rat; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α

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1. Introduction

Aldosterone is a steroid hormone synthesized from cholesterol in the adrenal cortex. Following a reduction in blood pressure, angiotensin II stimulates aldosterone release from the adrenal gland. Aldosterone acts on the mineralocorticoid receptor (MR) expressed on renal epithelial cells to promote sodium and water reabsorption, and thus restore blood pressure. However, a chronically elevated plasma level of aldosterone is an independent cardiovascular risk factor, and high aldosterone levels (e.g. due to an adrenal adenoma) are associated with increased risk of stroke (Vyssoulis et al., 2010). Consistent with this, elevated systemic aldosterone causes oxidative stress and endothelial dysfunction in the cerebral circulation (Chrissobolis et al., 2014), and other vascular beds (Blanco-Rivero et al., 2005; Kasal et al., 2012; Leibovitz et al., 2009; Leopold et al., 2007; Tasatargil et al., 2009; Virdis et al., 2002).

Many studies on MR function have focused on transporting epithelia, such as in the distal convoluted tubule and collecting duct of the kidney. However recent experimental evidence suggests that MR is expressed in a wide variety of cells, including non-epithelial tissues such as inflammatory cells, cardiomyocytes, vascular endothelial cells (including in humans (Caprio et al., 2008)) and smooth muscle cells. Furthermore, MR are highly expressed in many brain regions (Dinh et al., 2012; Hawkins et al., 2012), including in cerebral vessels (Rigsby et al., 2007a).

Endothelial cell dysfunction, a hallmark of most cardiovascular diseases including stroke, typically involves oxidative stress and inflammation, and is thought to be a key initiating step in cardiovascular disease development. Chronic administration with aldosterone causes cerebral vascular oxidative stress (Chrissobolis et al., 2014), and endothelial cell MRs contribute to cardiac inflammation and systemic artery endothelial dysfunction (Rickard et al., 2014), however no study has yet investigated whether the endothelial cell MR might contribute to early pathological changes in the cerebral circulation and brain. Inflammation of the brain is associated with multiple models of hypertension (Harrison et al., 2012) including deoxycorticosterone acetate (DOCA)/salt (mineralocorticoid-dependent) hypertension (Rodrigues and Granger, 2012; Sriramula et al., 2015) and can contribute to neurodegenerative conditions including Alzheimer's disease (Saavedra, 2012). Thus, the aim of this study was to test the hypothesis that aldosterone-induced increases in cerebral vascular oxidative stress and brain inflammation are endothelial cell MR-dependent. For these studies we compared the effects of endothelial cell MR deletion with those of the MR inhibitor spironolactone, which attenuates cerebral vascular remodeling during hypertension (Rigsby et al., 2007b, 2011), and is protective during cerebral ischemia (Oyamada et al., 2008).

2. Results

2.1. Blood pressure and kidney weights

2.1.1. Wild-type and EC-MRKO mice

Baseline blood pressure was similar in EC-MRKO vs WT mice (Table 1). Vehicle treatment was without effect, whereas aldosterone increased blood pressure in WT mice (Table 1). Similar effects on blood pressure were found in EC-MRKO mice, where vehicle treatment had no effect and aldosterone increased blood pressure. There was a marked increase in kidney weight:body weight ratio in aldosterone- vs vehicle-treated WT mice. Kidney weight:body weight ratio was similar in aldosterone- and vehicle-treated EC-MRKO mice (Table 1).

2.1.2. Control and spironolactone-treated mice

Baseline blood pressure was similar in mice assigned to control and spironolactone treatment groups (Table 1). Vehicle treatment had no effect on blood pressure, whereas aldosterone increased blood pressure by ~14 mmHg. In mice pre-treated with spironolactone, neither vehicle nor aldosterone had any effect on blood pressure. The aldosterone-induced increase in kidney weight:body weight ratio was also attenuated by spironolactone treatment (Table 1).

Table 1 – Mouse characteristics.

	Baseline SBP (mmHg)	Treatment subgroup	ΔSBP (mmHg) Week 1	ΔSBP (mmHg) Week 2	KW:BW (mg:g)
WT	119±3 (25)	Vehicle	0±3 (12)	−2±2 (12)	12.0±0.4 (11)
		Aldosterone	8±3 (13)*	9±3 (12)**	14.6±0.6 (13)**
EC-MR KO	114±2 (26)	Vehicle	0±3 (13)	2±4 (11)	11.9±0.3 (13)
		Aldosterone	8±3 (13)*	9±4 (9)	12.1±0.3 (14)
C57	125±2 (25)	Vehicle	7±2 (14)	10±2 (10)	13.6±0.3 (17)
		Aldosterone	14±3 (11)*	14±2 (11)	17.2±0.9 (17)***
Spironolactone	128±3 (10)	Vehicle	5±6 (4)	2±5 (5)	14.3±0.8 (5)
		Aldosterone	4±2 (4)	4±2 (5)	15.6±0.5 (5)

Baseline blood pressures, kidney weight (KW): body weight (BW) ratio, and changes in systolic blood pressure (SBP) in response to vehicle and aldosterone treatment (number in brackets indicates n value).

* $P<0.05$ vs corresponding vehicle.

** $P<0.01$ vs corresponding vehicle.

*** $P<0.001$ vs corresponding vehicle.