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Acute effect of mineralocorticoid receptor antagonism on vascular function in healthy older adults

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ABSTRACT

Mineralocorticoid receptor (MR) activation by aldosterone may regulate vascular function in health or contribute to vascular dysfunction in cardiovascular disease. Whether the effects are beneficial or detrimental to vascular function appear to be dependent on the integrity of the vascular endothelium and whether the responses are short-term or chronic. Acute modulation of MR activation has resulted in conflicting outcomes on vascular function in young healthy adults. Little is known about the vascular role of aldosterone and MR activation in healthy human aging. The primary objective of this study was to examine whether acute inhibition of MR by the selective antagonist eplerenone, influences vascular function in healthy older adults. We performed a randomized, double-blind, placebo-controlled crossover study in 22 adults (61 ± 1 years; mean ± SE, 53–79 years) who were free from overt clinical cardiovascular disease. We measured brachial artery flow-mediated endothelium-dependent dilation and endothelium-independent dilation to sublingual nitroglycerin (0.4 mg) following eplerenone (100 mg/dose, 2 doses, 24 h between doses) or placebo. In response to acute MR antagonism, flow-mediated dilation decreased by 19% (from 6.9 ± 0.5 to 5.6 ± 0.6%, $P = 0.02$; placebo vs. eplerenone). Endothelial nitric oxide synthase (eNOS) activity also decreased following MR antagonism based on the ratio of phosphorylated $eNOS^{Ser1177}$ to total eNOS (1.53 ± 0.08 vs. 1.29 ± 0.06, $P = 0.02$). Nitroglycerin-induced dilation and blood pressure were unaffected (nitroglycerin-induced dilation: 21.9 ± 1.9 vs. 21.0 ± 1.5%, $P = 0.5$ and systolic/diastolic blood pressure: 135/77 ± 4/2 vs. 134/77 ± 4/2 mmHg, $P \geq 0.6$). In conclusion, acute MR antagonism impairs vascular endothelial function in healthy older adults without influencing vascular smooth muscle responsiveness to exogenous nitric oxide or blood pressure.

1. Introduction

The mineralocorticoid receptor (MR) is an important regulator of blood pressure by modifying renal sodium retention (Williams, 2005). In addition to the classical role of MR in renal epithelial cells, substantial new data support the presence of functional MR in human vascular endothelial and smooth muscle cells and animal studies reveal a role for vascular MR in regulating vessel function in vivo (Barrett Mueller et al., 2015; Caprio et al., 2008; Jaffe and Mendelsohn, 2005; McCurley et al., 2012). Activation of extra-renal MRs has been shown to lead to direct vascular effects that may contribute to normal vascular function in health and also to vascular dysfunction in cardiovascular disease (reviewed in (McCurley and Jaffe, 2012)). Both aldosterone and cortisol can activate the MR but vascular tissues likely respond to aldosterone due to the expression and function of the cortisol-inactivating enzyme 11-beta-hydroxysteroid dehydrogenase type 2 (11βHSD2), expressed in vascular endothelial and smooth muscle cells (Caprio et al., 2008; Jaffe and Mendelsohn, 2005; McCurley et al., 2012).

Data on the acute vascular effects of MR activation by aldosterone in humans are conflicting (McCurley and Jaffe, 2012; Toda et al., 2013). The discrepant findings may be explained by the health or disease state of the population under study due to the presence or absence of vascular damage and pre-existing vascular oxidative stress and inflammation. Differences in study design might also be contributing to the

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divergent results including the vascular bed under investigation, physiological versus pathological doses of aldosterone administration, and concomitant use of other drugs. The effect of MR activation also depends on whether the responses under investigation are short-term or chronic. One approach to study the role of aldosterone activation of MR in vascular function is to experimentally increase aldosterone levels (e.g., aldosterone infusion or fludrocortisone-induced aldosterone excess). However, results based on acute elevations in aldosterone, in the absence of a physiological stimulus to induce these elevations, may not represent what normally occurs under physiological conditions. A second experimental approach is to inhibit MR activation using an MR antagonist. This may be more physiologically relevant because it permits determination of the tonic influence of MR activation on vascular function.

Endothelial dysfunction is one of the earliest signs of atherosclerosis and is characterized by impairment in endothelium-dependent vasodilation (Davignon and Ganz, 2004). Healthy endothelial cells produce vasodilators which diffuse to medial smooth muscle cells and activate signaling pathways that induce smooth muscle cells to relax thereby resulting in vasodilation (Davignon and Ganz, 2004). Nitric oxide is a major mediator of endothelial-dependent vasorelaxation that is produced by healthy endothelial cells when the enzyme endothelial nitric oxide synthase (eNOS) is activated by phosphorylation at the serine residue 1177 (Ser1177) (Reviewed in (Huang, 2003; Landmesser and Drexler, 2007)). In the setting of cardiovascular disease, there are extensive data demonstrating that MR contributes to endothelial dysfunction (Abiose et al., 2004; Farquharson and Struthers, 2000; Keidar et al., 2004; Rajagopalan et al., 2002; Sartorio et al., 2007; Thai et al., 2006). However, in young healthy adults, the results are inconsistent with some studies demonstrating that aldosterone activation of MR promotes endothelium-dependent nitric oxide-mediated vasodilation (Nietlispach et al., 2007), while other studies demonstrate that MR activation impairs endothelium-dependent dilation (Farquharson and Struthers, 2002) or has no effect (Schmidt et al., 2003).

Little is known about the role of vascular MR activation in healthy human aging. We have recently demonstrated that chronic (1 month) administration of an MR antagonist did not significantly change flow-mediated vasodilation but rather influenced endothelial function in an adiposity-dependent manner in healthy older adults (Hwang et al., 2013b). The acute vascular effects of MR antagonism in healthy older adults, have never been explored. Therefore, we conducted a randomized double-blind placebo-controlled crossover study using short-term oral administration of the selective MR antagonist eplerenone (2 doses, 100 mg/dose) in older men and women free from overt clinical cardiovascular disease. The primary objective of this study was to investigate whether inhibition of MR activation acutely influences vascular function in healthy human aging. Endothelial dysfunction may be due to decreased nitric oxide production or to increased nitric oxide degradation due to the presence of oxidative stress (Vita, 2011). Endothelial dysfunction also leads to vascular inflammation that contributes to cardiovascular disease (Vita, 2011). Thus, to gain preliminary mechanistic insight, we also examined the effect of acute MR antagonism on the levels of blood markers of oxidative stress and inflammation and on the expression levels of endothelial cell proteins that are known to influence vascular function.

2. Methods

2.1. Subjects

Twenty-two older adults (8 men and 14 women), 53 to 79 years of age, were enrolled in this study. Subject recruitment was completed by using flyers and by advertising in newspapers and on the radio. Research volunteers who met one or more of the following criteria were excluded from study participation: age <50 years, evidence of diabetes, cardiovascular, liver or renal disease, use of antihypertensive or vasoactive medications or hormone replacement therapy, use of antioxidant supplements, use of tobacco products, history of alcohol abuse, recent hospitalization, weight loss or gain greater than 5 lb in the prior 3 months, participation in exercise training >30 min ≥3 times per week and for female volunteers, pre- or peri-menopausal status. To reduce the risk of hyperkalemia that is associated with eplerenone use, subjects were also excluded from the study if their baseline serum potassium was greater than 5.5 mmol/L, serum creatinine was greater than 1.6 mg/dL, or creatinine clearance was less than 30 mL/min.

Based on these exclusion criteria, the enrolled subjects were sedentary, non-smokers and were free from overt cardiovascular and other clinical diseases (e.g., diabetes, liver and renal disease) as assessed by medical history, physical examination, resting ECG, urinalysis, blood chemistries and hematological evaluation. All subjects demonstrated normal ECG and blood pressure responses to a graded exercise test on a treadmill. The exercise testing protocol has previously been described (Christou et al., 2005). Briefly, after a 6–10 min warm-up, subjects walked at a comfortable speed that corresponded to 70 to 80% of their age-predicted maximal heart rate. The treadmill grade was increased 2.5% every two minutes until volitional exhaustion.

This study was carried out in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Institutional Review Boards of the University of Florida, Texas A&M University, and Scott & White Health System. The purpose, nature, and risks of the study were explained to the volunteers and their written informed consent was obtained prior to participation. No subjects withdrew from the intervention after randomization.

2.2. Study design and general experimental procedures

The current investigation followed a randomized double-blind placebo-controlled crossover design using the MR antagonist eplerenone. Eplerenone and placebo (Consolidated Midland Corporation, Brewster, New York) were administered orally. Based on the 3–6 h elimination half-life of eplerenone (Ravis et al., 2005), at least 1 week washout was allowed between eplerenone and placebo. We chose to use eplerenone due to its higher selectivity for MR and fewer side effects compared to spironolactone, the other FDA-approved MR antagonist. We selected to use a dose of 100 mg for the following reasons: 1) this dose was reported to be more effective at inhibiting the MR than 25 or 50 mg but equally effective to 200 mg eplerenone (White et al., 2003); 2) it was associated with similar side effects to placebo including similar serum potassium levels (White et al., 2003); and 3) it has previously been administered without titration (Cook et al., 2003; Ravis et al., 2005).

Following oral administration of 100 mg eplerenone, peak plasma concentration is reached within approximately 2 h and is maintained for several hours (Ravis et al., 2005). In order to study vascular function during peak eplerenone levels, we scheduled the endothelial function test 3 h after eplerenone administration. However, we decided to administer one more dose of 100 mg of eplerenone the day prior to data collection to ensure that there was adequate time for potential upregulation or downregulation of endothelial cell protein expression levels in response to eplerenone. The timing of the doses was based on previous in vitro and animal studies which demonstrated that within 24 h of aldosterone administration, endothelial cell protein levels of NADPH oxidase (a major source of superoxide anions), nitrotyrosine (a marker of oxidative stress), eNOS and phosphorylated eNOS ($eNOS^{Ser1177}$), and superoxide dismutase (SOD; an endogenous antioxidant enzyme) changed and that these changes were inhibited by MR antagonism (Gromotowicz et al., 2011; Nagata et al., 2006; Taye and Morawietz, 2011).

Therefore, in the current study a single dose of eplerenone (100 mg) was administered at 7 am on the day prior to data collection followed