Published in final edited form as:
J Cardiovasc Pharmacol. 2009 December ; 54(6): 465–467. doi:10.1097/FJC.0b013e3181c37ddc.

Rapid Aldosterone Signaling and Vascular Reactivity: Relax or Don't Do It

Jane A. Leopold, M.D.
Brigham and Women's Hospital and Harvard Medical School, Boston, MA

The identification of functional mineralocorticoid receptors in blood vessels has led to the acknowledgement that aldosterone modulates vascular reactivity directly via receptor-mediated mechanisms and exerts pleiotropic effects on the vessel wall beyond regulating sodium and water homeostasis.[1] Landmark clinical trials of mineralocorticoid receptor blockade in patients with congestive heart failure or myocardial infarction demonstrated a mortality benefit that was attributed, in part, to improved vascular function although this was not studied directly in either trial.[2–4] This hypothesis is, however, supported by studies of patients with congestive heart failure that were treated with the mineralocorticoid receptor antagonist spironolactone; treatment with spironolactone was associated with a nearly 2-fold increase in endothelium-dependent vasodilation.[5–7] Similar studies performed in patients with hypertension and confirmed hyperaldosteronism revealed that 3 months of treatment with spironolactone restored endothelium-dependent vasodilation independently of any change in blood pressure.[8] Interestingly, these studies reported no observed difference in endothelium-independent vasodilation between patients and control subjects and one study found an enhanced vasoconstrictor response following infusion of the nitric oxide synthase (NOS) inhibitor *N*-monomethyl-L-arginine in hyperaldosteronemic subjects.[5–7] Taken together, these findings indicate that endothelial dysfunction with a preserved or enhanced vascular smooth muscle cell contractile response is the predominant vascular phenotype associated with chronic hyperaldosteronism.

Mechanistically, this (mal)adaptive vascular phenotype results from continuous transcription of mineralocorticoid receptor-responsive genes and signaling pathway activation. This, in turn, initiates both molecular and biochemical remodeling of the vasculature. The resultant vascular milieu favors vasoconstriction and is characterized by increased expression of endothelin, angiotensin converting enzyme, and angiotensin II receptors;[9] diminished nitric oxide (NO) generation and limited bioavailable NO;[10,11] increased reactive oxygen species (ROS) production and decreased antioxidant capacity;[11] and impaired vasodilatory capabilities owing to oxidative post-translational modification of soluble guanylyl cyclase and decreased expression of calcium-activated potassium channels.[12,13]

Thus, while there is agreement that chronic hyperaldosteronism induces vascular dysfunction the acute effect(s) of aldosterone on vascular reactivity remain controversial. This controversy has been fueled by conflicting results obtained from clinical studies that examined the effect of an acute aldosterone infusion on vascular reactivity in healthy subjects. Three studies reported different findings; one study found a vascular response consistent with endothelial dysfunction while results from the other two other studies showed either no effect or vasodilation in response to the aldosterone infusion.[14–16] A number of theories have been advanced to explain these discrepant findings including the health status and adrenergic state

Corresponding author: Jane A. Leopold, M.D., Brigham and Women's Hospital, 77 Avenue Louis Pasteur, NRB 0630K, Boston, MA 02115, Phone: (617) 525-4846, Fax: (617) 525-4803, jleopold@partners.org.

of the individuals that participated in these studies, the differences in dose and duration of aldosterone infusion, and/or sodium and potassium electrolyte shifts.[16] Despite these plausible theories, no consensus opinion has been reached to date with respect to the acute effects of aldosterone on vascular reactivity.

It is also difficult to predict the vascular response to an acute aldosterone exposure based solely on existing *in vitro* and *ex vivo* experimental evidence due to contradictory results, especially when examining the relationship between aldosterone and NO production. For example, it has been reported that aldosterone alone either has no immediate effect on vascular endothelial cell NO production, increases eNOS activity directly, or only enhances ATP-stimulated NO generation via increased Ser1179 phosphorylation and activation of the endothelial isoform of NOS (eNOS) when cells have been pretreated with aldosterone for 5 minutes.[17,18] Similar reports emerged when these studies were translated to *ex vivo* aortic ring experiments. One study found that an acute exposure to aldosterone had no effect on preconstricted vascular tone but enhanced acetylcholine-mediated vasodilation while the other demonstrated that aldosterone attenuated phenylephrine-mediated vasoconstriction.[17,18] Regardless of these findings, denudation of the endothelium or co-incubation with NOS inhibitors resulted in a loss of the observed vasodilation thereby implicating the endothelium and endothelium-derived NO in the acute vascular response to aldosterone. Findings with respect to the acute effect of aldosterone on vascular smooth muscle cells have been more consistent. Here, it has been shown that aldosterone induces vascular smooth muscle cell contraction by activation of phospholipase C, calcium mobilization, and by increasing ROS production by NADPH oxidase.[19,20]

In this issue, Heylen *et al.* provide greater insight into the acute effects of aldosterone on vascular reactivity by utilizing an elegant experimental platform that allowed the investigators to isolate endothelium- and vascular smooth muscle cell-dependent responses to aldosterone in an intact vessel and determine the relative contribution of each to the net change in vascular tone.[21] By infusing aldosterone intraluminally to target the endothelium, or adding aldosterone to the water bath to target vascular smooth muscle cells, they found that a 30 minute exposure to (patho)physiological concentrations of aldosterone elicited a vasodilator response that was dependent upon mineralocorticoid receptor activation and NO generation. Intraluminal infusion of aldosterone led to a greater duration of vascular relaxation compared to the short-lived vasodilation observed with extraluminal aldosterone and this short-lived response was shown to result from an increase in vascular smooth muscle ROS production. To determine if aldosterone was able to induce a similar vasodilator response once endothelial dysfunction was already established, arterioles were isolated from a rat model of chronic NOS inhibition. In these vessels, aldosterone had no effect on vascular reactivity. Thus, Heylen *et al.* found that an acute exposure to aldosterone stimulates concomitant endothelial vasodilator and smooth muscle cell vasoconstrictor signals and that the predominant effect on vascular tone is dependent upon the integrity of the endothelium.

In these studies, the authors offer *ex vivo* experimental confirmation of the opposing effects of rapid aldosterone signaling in the vascular endothelium and smooth muscle cells to regulate vascular relaxation. Conceptually, this paradigm is not new and has been suggested previously as an explanation for impaired vascular reactivity following a brief exposure to aldosterone when endothelial dysfunction exists.[22] This study also confirms prior work that links aldosterone to NO generation and endothelium-dependent vasodilation and implicates increased vascular smooth muscle cell ROS generation as a predominant mechanism to explain the opposing vasoconstrictor response. While increased ROS were detected during extraluminal administration of aldosterone, the authors downplay the significance of this finding. This observation is of interest when it is recognized that vascular smooth muscle cells express aldosterone synthase and generate aldosterone, although the cellular signals that

stimulate synthesis and release are incompletely known.[23,24] This suggests further that transient intravascular generation of aldosterone may increase ROS production and induce a vasoconstrictor response to oppose or augment any vascular tone regulatory stimulus elaborated by the endothelium.

Instead, the authors speculate that the vasoconstrictor response results from increased ROS that react with NO to form peroxynitrite and decrease bioavailable NO. While this is an attractive hypothesis, it is quite likely only a small part of the mechanism by which increased ROS influences vascular reactivity following an acute aldosterone exposure. In addition to peroxynitrite formation, ROS may mediate NO levels by oxidizing tetrahydrobiopterin or decreasing stores of the reducing equivalent NADPH;[25,26] when these eNOS cofactors are depleted, eNOS will uncouple and release superoxide in preference to NO. Superoxide is also converted to hydrogen peroxide which has been shown to increase Akt and eNOS activation.[27,28] When this occurs and cofactors are not replete, eNOS phosphorylation at Ser1177 increases eNOS-derived superoxide generation significantly.[29] Elevated levels of ROS may also decrease NO levels by stimulating protein phosphatase 2A to dephosphorylate and deactivate eNOS.[30] It should also be noted that the effect of acute aldosterone exposure on key ROS-sensitive vascular smooth muscle cell vasodilatory signaling molecules has not yet been studied. In the setting of chronic endothelial dysfunction, clinical studies have demonstrated that spironolactone restores endothelial function and vascular reactivity;[6] however, it remains unknown if spironolactone has any effect on the vasodilator response to an acute aldosterone exposure. Unfortunately, the authors did not provide any direct measures of NO or ROS signaling in these studies to determine if differences in levels of these molecules paralleled observed changes in vascular reactivity nor did they explore the effects of aldosterone antagonism on vasoreactivity to an acute aldosterone exposure in their model of chronic endothelial dysfunction. Instead they have laid the groundwork and provided us with a robust experimental system to begin to make these determinations.

Acknowledgments

This work was supported in part by NIH grant HL081110.

References

1. Booth RE, Johnson JP, Stockand JD. Aldosterone. Adv Physiol Educ 2002;26:8–20. [PubMed: 11850323]
2. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003;348:1309–1321. [PubMed: 12668699]
3. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709–717. [PubMed: 10471456]
4. Struthers AD. Impact of aldosterone on vascular pathophysiology. Congest Heart Fail 2002;8:18–22. [PubMed: 11821624]
5. Farquharson CA, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. Circulation 2000;101:594–597. [PubMed: 10673249]
6. Macdonald JE, Kennedy N, Struthers AD. Effects of spironolactone on endothelial function, vascular angiotensin converting enzyme activity, and other prognostic markers in patients with mild heart failure already taking optimal treatment. Heart 2004;90:765–770. [PubMed: 15201246]
7. Abiose AK, Mansoor GA, Barry M, Soucier R, Nair CK, Hager D. Effect of spironolactone on endothelial function in patients with congestive heart failure on conventional medical therapy. Am J Cardiol 2004;93:1564–1566. [PubMed: 15194040]

8. Nishizaka MK, Zaman MA, Green SA, Renfroe KY, Calhoun DA. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. Circulation 2004;109:2857–2861. [PubMed: 15173035]
9. Cachofeiro V, Miana M, de Las Heras N, Martin-Fernandez B, Ballesteros S, Fernandez-Tresguerres J, Lahera V. Aldosterone and the vascular system. J Steroid Biochem Mol Biol 2008;109:331–335. [PubMed: 18400490]
10. Hashikabe Y, Suzuki K, Jojima T, Uchida K, Hattori Y. Aldosterone impairs vascular endothelial cell function. J Cardiovasc Pharmacol 2006;47:609–613. [PubMed: 16680076]
11. Leopold JA, Dam A, Maron BA, Scribner AW, Liao R, Handy DE, Stanton RC, Pitt B, Loscalzo J. Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity. Nat Med 2007;13:189–197. [PubMed: 17273168]
12. Maron BA, Zhang YY, Handy DE, Beuve A, Tang SS, Loscalzo J, Leopold JA. Aldosterone increases oxidant stress to impair guanylyl cyclase activity by cysteinyl thiol oxidation in vascular smooth muscle cells. J Biol Chem 2009;284:7665–7672. [PubMed: 19141618]
13. Ambroisine ML, Favre J, Oliviero P, Rodriguez C, Gao J, Thuillez C, Samuel JL, Richard V, Delcayre C. Aldosterone-induced coronary dysfunction in transgenic mice involves the calcium-activated potassium (BKCa) channels of vascular smooth muscle cells. Circulation 2007;116:2435–2443. [PubMed: 17984374]
14. Farquharson CA, Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. Clin Sci (Lond) 2002;103:425–431. [PubMed: 12241543]
15. Nietlispach F, Julius B, Schindler R, Bernheim A, Binkert C, Kiowski W, Brunner-La Rocca HP. Influence of acute and chronic mineralocorticoid excess on endothelial function in healthy men. Hypertension 2007;50:82–88. [PubMed: 17502494]
16. Schmidt BM, Oehmer S, Delles C, Bratke R, Schneider MP, Klingbeil A, Fleischmann EH, Schmieder RE. Rapid nongenomic effects of aldosterone on human forearm vasculature. Hypertension 2003;42:156–160. [PubMed: 12860833]
17. Mutoh A, Isshiki M, Fujita T. Aldosterone enhances ligand-stimulated nitric oxide production in endothelial cells. Hypertens Res 2008;31:1811–1820. [PubMed: 18971560]
18. Liu SL, Schmuck S, Chorazcyzewski JZ, Gros R, Feldman RD. Aldosterone regulates vascular reactivity: short-term effects mediated by phosphatidylinositol 3-kinase-dependent nitric oxide synthase activation. Circulation 2003;108:2400–2406. [PubMed: 14557368]
19. Callera GE, Montezano AC, Yogi A, Tostes RC, He Y, Schiffrin EL, Touyz RM. c-Src-dependent nongenomic signaling responses to aldosterone are increased in vascular myocytes from spontaneously hypertensive rats. Hypertension 2005;46:1032–1038. [PubMed: 16157790]
20. Callera GE, Touyz RM, Tostes RC, Yogi A, He Y, Malkinson S, Schiffrin EL. Aldosterone activates vascular p38MAP kinase and NADPH oxidase via c-Src. Hypertension 2005;45:773–779. [PubMed: 15699470]
21. Heylen E, Huang A, Sun D, Kaley G. Nitric oxide-mediated dilation of arterioles to intraluminal administration of aldosterone. J Cardiovasc Pharm.
22. Schmidt BM, Sammer U, Fleischmann I, Schlaich M, Delles C, Schmieder RE. Rapid nongenomic effects of aldosterone on the renal vasculature in humans. Hypertension 2006;47:650–655. [PubMed: 16520409]
23. Hatakeyama H, Miyamori I, Fujita T, Takeda Y, Takeda R, Yamamoto H. Vascular aldosterone. Biosynthesis and a link to angiotensin II-induced hypertrophy of vascular smooth muscle cells. J Biol Chem 1994;269:24316–24320. [PubMed: 7929089]
24. Takeda R, Hatakeyama H, Takeda Y, Iki K, Miyamori I, Sheng WP, Yamamoto H, Blair IA. Aldosterone biosynthesis and action in vascular cells. Steroids 1995;60:120–124. [PubMed: 7792796]
25. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. J Biol Chem 2003;278:22546–22554. [PubMed: 12692136]

26. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003;111:1201–1209. [PubMed: 12697739]

27. Thomas SR, Schulz E, Keaney JF Jr. Hydrogen peroxide restrains endothelium-derived nitric oxide bioactivity -- role for iron-dependent oxidative stress. Free Radic Biol Med 2006;41:681–688. [PubMed: 16864001]

28. Dossumbekova A, Berdyshev EV, Gorshkova I, Shao Z, Li C, Long P, Joshi A, Natarajan V, Vanden Hoek TL. Akt activates NOS3 and separately restores barrier integrity in H2O2-stressed human cardiac microvascular endothelium. Am J Physiol Heart Circ Physiol 2008;295:H2417–2426. [PubMed: 18931031]

29. Chen CA, Druhan LJ, Varadharaj S, Chen YR, Zweier JL. Phosphorylation of endothelial nitric-oxide synthase regulates superoxide generation from the enzyme. J Biol Chem 2008;283:27038–27047. [PubMed: 18622039]

30. Nagata D, Takahashi M, Sawai K, Tagami T, Usui T, Shimatsu A, Hirata Y, Naruse M. Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. Hypertension 2006;48:165–171. [PubMed: 16754797]