Endothelial NO Synthase Target of Aldosterone

Johann Bauersachs, Daniela Fraccarollo

The mineralocorticoid hormone aldosterone plays a pivotal role in sodium resorption and potassium excretion and, consequently, in fluid balance and blood pressure homeostasis. Current studies indicate that mineralocorticoid receptors are present not only in epithelial cells, and aldosterone also acts on nonepithelial tissues, including the heart, blood vessels, and brain. In the vascular system, mineralocorticoid receptors and the enzyme 11β-hydroxy-steroid dehydrogenase type 2, which confers aldosterone specificity to the mineralocorticoid receptor, could be localized in endothelial and vascular smooth muscle cells. Other than the mineralocorticoid receptor, steroidogenic enzymes necessary to synthesize aldosterone are also expressed in extra-adrenal tissues, including the vascular wall, which, although still controversial, may be consistent with de novo aldosterone production acting in an autocrine/paracrine fashion.

Aldosterone induces oxidative stress in vascular cells through NADPH oxidase activation, which plays a central role for endothelial dysfunction and atherosclerotic vascular disease. Mineralocorticoid receptor blockade increased NO bioavailability and improved impaired endothelial function by decreasing oxidative stress in hypertension, atherosclerosis, and heart failure. In a rat model of heart failure, aldosterone antagonism reduced vascular superoxide anion formation, and in combination with an angiotensin-converting enzyme inhibitor, increased the expression of the endothelial NO synthase (eNOS) and restored the attenuated NO-mediated relaxation.

In the current issue of Hypertension, Nagata et al report that treatment of cultured human endothelial cells with aldosterone results in the enhanced generation of reactive oxygen species through activation of NADPH oxidase, mainly via translocation of the subunit p47phox to the membrane. Most importantly, the authors demonstrated that aldosterone exerts an inhibitory effect on eNOS activity, via tetrahydrobiopterin (BH4) oxidation and protein phosphatase 2A activation, providing new important insights into the mechanisms underlying aldosterone-induced vascular damage. Aldosterone reduced vascular endothelial growth factor–induced eNOS phosphorylation at Ser1177 and intracellular cGMP concentration but did not alter Akt Ser473 phosphorylation levels. Pretreatment with the selective mineralocorticoid receptor blocker eplerenone or the protein phosphatase inhibitor okadaic acid normalized eNOS phosphorylation. Addition of BH4 overexpression of the rate-limiting BH4 generating enzyme GTP cyclohydrolase 1, or p47phox knockdown reversed the inhibitory effect of aldosterone on NO formation (Figure). In addition, the eNOS dimer:monomer ratio was reduced in aldosterone-treated endothelial cells, which was reversed by aldosterone antagonism or cotreatment with BH4. The authors concluded that aldosterone downregulated eNOS function through BH4 oxidation and uncoupling of eNOS.

Accumulating data suggest that an important mechanism underlying endothelial dysfunction is eNOS uncoupling, a condition that leads to eNOS-mediated superoxide anion production instead of NO, possibly resulting from a mismatch between eNOS and its cofactor BH4 (reviewed in Reference 7). The uncoupling of eNOS has been linked to the failure of the enzyme to form dimers. However, it is important to note that changes in the dimer:monomer ratio are not directly related to eNOS uncoupling, because the oxidase activity of the monomers is limited, and the dimeric form is more active and able to generate superoxide. To provide a clear evidence for eNOS uncoupling by aldosterone in the present study, the superoxide anion production attributable to uncoupled eNOS should have been analyzed by quantifying reduction of superoxide anion formation in the presence of an eNOS inhibitor. Whether aldosterone induces downregulation of eNOS activity also in vivo by eNOS uncoupling remains to be supported by appropriate experimental studies. However, evidence of vascular uncoupling of eNOS has been demonstrated in hypercholesterolemia, diabetes, and deoxycorticosterone acetate-salt–induced hypertension. Although also in hamsters with idioopathic cardiomyopathy, eNOS uncoupling seemed to contribute to vascular superoxide formation; in rats with chronic heart failure after myocardial infarction, the persistent vascular superoxide formation after endothelial denudation argues against a significant contribution of uncoupled eNOS.

As in the study by Nagata et al, reduced eNOS activity in aldosterone-treated endothelial cells was associated also with protein phosphatase 2A activation, site-specific dephosphorylation of eNOS at Ser1177 may represent an important mechanism modulating eNOS enzyme activity and NO bioavailability in the vasculature by aldosterone. Recent data show that mineralocorticoid receptor blockade improves endothelial dysfunction and oxidative stress by normalization of reduced eNOS phosphorylation at Ser1177 and enhanced eNOS-derived NO bioavailability early after experimental myocardial infarction. Furthermore, in rats with chronic heart failure after large myocardial infarction, eplerenone especially in combination with an angiotensin-converting enzyme inhibitor increased myocardial eNOS phosphorylation at Ser1177.

Of interest, aldosterone has been reported to induce vasodilation by stimulating endothelial NO release through rapid nongenomic effects. However, these data are still controversial. At least in the present study by Nagata et al, production of...
reactive oxygen species was not detected within 2 hours of aldosterone exposure, suggesting that aldosterone effects were mainly mediated via a genomic mechanism.

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Disclosures
None.

References
8. Bendall JK, Alp NJ, Warrick N, Cai S, Adlam D, Rockett K, Yokoyama M, Kawashima S, Channon KM. Stoichiometric relationships between endothelial tetrahydrobiopterin, endothelial NO synthase (eNOS) activity, and eNOS coupling in vivo: insights from transgenic mice with endothelial-targeted GTP cyclohydrolase 1 (GCH1), prevent eNOS uncoupling. Moreover, dephosphorylation of eNOS Ser1177 by protein phosphatase 2A (PP2A) leads to a loss of NO formation by aldosterone. The selective mineralocorticoid receptor (MR) blocker eplerenone or phosphatase inhibition by okadaic acid prevents these effects. VEGF indicates vascular endothelial growth factor; siRNA, small interfering RNA.

Proposed mechanisms in the study by Nagata et al. of interactions among aldosterone, eNOS, and superoxide anion (O$_2^-$) underlying aldosterone-induced oxidative stress and reduced NO bioavailability in the endothelium. Aldosterone increases O$_2^-$ generation by NADPH oxidase via p47phox, resulting in NO scavenging by reactive oxygen species. Oxidation of tetrahydrobiopterin (BH4) by increased reactive oxygen species, such as peroxynitrite (OONO$^-$) to dihydrobiopterin (BH2) and biopterin (B), reduces BH4 levels and promotes eNOS uncoupling, that is, eNOS-mediated O$_2^-$ production instead of NO. As a result, activation of soluble guanylate cyclase (sGC) and subsequent cGMP generation is reduced. Supplementation with BH4 or overexpression of the rate-limiting enzyme for BH4 synthesis, GTP cyclohydrolase 1 (GCH1), prevent eNOS uncoupling. Moreover, dephosphorylation of eNOS Ser1177 by protein phosphatase 2A (PP2A) leads to a loss of NO formation by aldosterone. The selective mineralocorticoid receptor (MR) blocker eplerenone or phosphatase inhibition by okadaic acid prevents these effects. VEGF indicates vascular endothelial growth factor; siRNA, small interfering RNA.
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