

# MOLECULAR MECHANISM OF THE INHIBITORY EFFECT OF ALDOSTERONE ON ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVITY

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Although the pro-inflammatory and pro-fibrotic actions of aldosterone on the vasculature have been reported, the effects and molecular mechanisms of aldosterone on endothelial function are yet to be determined. We investigated how aldosterone regulates endothelial nitric oxide synthase (eNOS) function in human umbilical vein endothelial cells (HUVECs). HUVECs were incubated for 16 hrs with aldosterone  $10^{-7}$  mol/L. The concentration of reactive oxygen species (ROS) was estimated by measuring DCF chemiluminescence. Signal transduction was estimated by Western immunoblots. Realtime RT-PCR was performed to measure expression of transcripts of endogenous GTP cyclohydrolase-1 (GCH1) and components of NAD(P)H oxidase. In order to eliminate the possible effect of the glucocorticoid receptor (GR), and to emphasize the role of mineralocorticoid receptor (MR), we used GR siRNA and knocked down GR expression in several experiments. NO output was estimated by intracellular cGMP concentration. ROS production increased significantly in aldosterone-treated HUVEC, but was abolished by pre-treatment with eplerenone. Transcripts of p47<sup>phox</sup> were increased by aldosterone treatment. Vascular-Endothelial-Growth-Factor-induced eNOS Ser 1177 but not Akt Ser 473 phosphorylation levels were reduced significantly by pretreatment with aldosterone. Pretreatment with either eplerenone or okadaic acid restored phosphorylation levels of eNOS Ser 1177 in aldosterone-treated cells, suggesting that protein phosphatase (PP) 2A was upregulated by aldosterone via MR. The decrease in NO output caused by aldosterone pretreatment was reversed significantly by either 5,6,7,8-tetrahydrobiopterin (BH<sub>4</sub>), GCH1 overexpression, or p47<sup>phox</sup> knockdown. These results suggest that aldosterone inhibits eNOS function through bimodal mechanisms of BH<sub>4</sub> deficiency and PP2A activation.