compared to wild-type mice (P<0.05), reactive oxygen species generation by 93% (P<0.001) and monocyte/macrophage infiltration by 160% (P<0.001), and raised plasma ET-1 by 130% (P<0.05). EPO had no effect on wild-type mice. Exercise training prevented all of the above effects of EPO as well as ET-1 (P<0.05). EPO-induced SBP rise and adverse vascular effects are dependent on the pre-existing level of ET-1 expression. Exercise training prevented EPO-induced BP rise and adverse vascular effects in part by inhibiting ET-1 overexpression-induced oxidative stress, inflammation and immune activation.


Endothelin-1-induced oxidative stress and inflammatory cell infiltration contribute to high-fat diet induced-atherosclerosis and aneurysm formation in apolipoprotein E knockout mice

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Endothelin (ET)-1 promotes reactive oxygen species (ROS) production and inflammation in the vasculature. ET-1 has been implicated in the pathogenesis of atherosclerosis in both human and animal models. Abdominal aorta aneurysms (AAA) occur in association with atherosclerosis. We hypothesized that ET-1-induced ROS and inflammation contribute to the development of atherosclerosis and increase occurrence of AAA in high-fat diet (HFD)-fed apolipoprotein E knockout (ApoE−/−) mice. Eight-week-old male transgenic mice overexpressing ET-1 in the endothelium (eET-1, ApoE−/−, eET-1/ApoE−/−) and wild-type mice were fed HFD for 8 weeks. eET-1/ApoE−/− mice presented 2-fold and 4-fold more atherosclerotic lesions in aortic sinus and ascending aorta, respectively, compared to ApoE−/− mice (P<0.05). Aortic aneurysms were observed at suprarenal level in 6 of 15 eET-1/ApoE−/− compared to none of 15 ApoE−/− mice (P<0.05). ET-1 overexpression increased ROS production >2.6-fold in peri-vascular fat (PVAT), media and plaques of ApoE−/− mice (P<0.05). ET-1 overexpression increased monocyte/macrophage infiltration 5- and 8-fold in PVAT and media of ApoE−/− mice, respectively (P<0.05). CD4+ T cell infiltration was observed with greater frequency in PVAT (3/6) and plaques (5/6) in ascending aorta of eET-1/ApoE−/− compared to ApoE−/− (1/6) mice (P<0.05). Spleen pro-inflammatory Ly-6Chi monocytes were 65% higher in ApoE−/− compared to wild-type mice (P<0.05), which was further increase by 26% in eET-1/ApoE−/− mice (P<0.05). Stretching and fragmentation of elastin fibers at suprarenal level were detected only in eET-1/ApoE−/− mice. These results suggest that ET-1 promotes development of atherosclerotic lesions and AAA by increasing oxidative stress, monocyte/macrophage and T cell infiltration. ET-1-induced alteration in elastin fibers may play an important role in AAA development.


Flow regulation of inner medullary collecting duct endothelin-1 production

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Collecting duct (CD)-derived endothelin-1 (ET-1) inhibits Na and water reabsorption; its deficiency causes marked hypertension. CD ET-1 synthesis is enhanced by extracellular fluid volume expansion. In cultured cortical CD (CCD) cells, increased flow (as would occur in tubule fluid during volume expansion) stimulates ET-1 production; this is ENaC-dependent. Since the inner medullary CD (IMCD) is the major site of renal ET-1 synthesis, we examined the effect of flow on IMCD ET-1 production. Mouse IMCD3 cells were subjected to static conditions or flow (2 dyn/cm² for 2 h), followed by determination of ET-1 mRNA. Flow increased ET-1 mRNA by 2.2-fold. Absence of perfusate Ca prevented the flow response. BAPTA, W-7, calmidazolium, KN-93, calphostin C and cyclosporin A reduced the ET-1 flow response, indicating that PKC and Ca/calmodulin/calmodulin kinase/calcineurin pathways are essential for the flow response. Amiloride or benzamil did not affect the ET-1 response to flow. Increasing perfusate osmolality to 450 mOsm with NaCl, mannitol or urea elicited a marked flow response (4.4-fold increase in ET-1 mRNA). Removal of cilia with chloral hydrate reduced the flow response, while flow failed to stimulate ET-1 mRNA in mIMCD3 cells deficient in polycystin-2. These data suggest that IMCD ET-1 synthesis is stimulated by tubule fluid flow via increased solute delivery and possibly cilia deformation, which in turn activates PKC- and Ca-dependent pathways. We propose that Na delivery stimulates CCD ET-1, which, in turn, inhibits CCD Na reabsorption, while solute and water delivery stimulate IMCD ET-1, which, in turn, inhibits IMCD water and urea reabsorption.


Ubiquitin modification plays an important role in ET-1-dependent endothelin type B receptor trafficking

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Two types of endothelin receptors, ETAR and ETBR, are internalized upon ET-1 stimulation, but their fates are different after stimulation despite of their sequence homology. To get insights into the mechanisms for different fates of these receptors, we examined stimulation- induced ubiquitination of the receptors. After ET-1 stimulation, ETBR was ubiquitinated, whereas ETAR was not. The mutant ETBR receptor in which all lysine residues in C-terminal (C-tail) were replaced by arginine was not ubiquitinated. After ET-1 treatment, the amount of cell surface ETBR decreased rapidly, but that of ETBR mutant was virtually unchanged. In addition, the level of ERK phosphorylation and Ca2+ response was enhanced in mutant ETBR-expressing cells compared to those in wild type ETBR-expressing cells following ET-1 stimulation. There are 8 lysine residues in ETBR C-tail for probable ubiquitination: 3 lysines before and 5 lysines after palmitoylation site (PS). The mutant in which 5 lysine residues after PS were replaced with arginine was not ubiquitinated upon ET-1 stimulation, whereas the mutant in which 3 lysine residues before that site were replaced with arginine was ubiquitinated. ETBR mutants in which either one of 5 lysine residues after PA was left unreplaced were ubiquitinated and internalized following ET-1 stimulation. These results indicate that ubiquitination of either one of lysine residues in ETBR C-tail is sufficient for ET-dependent internalization.