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.


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

Pierre Paradis, Muhammad O.R. Mian, Tili Barhoumi, Asia Rehman, Melissa W. Li, Koren K. Mann, Ernesto L. Schiffrin

4Lady Davis Institute for Medical Research, McGill University, Montreal, PQ, Canada
3Department of Oncology, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada
2Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada

E-mail address: pierre.paradis@mcgill.ca (P. Paradis)

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 perivascular 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.


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

Koji Terada, Takahiro Horinouchi, Tsunehito Higashi, Prabha Nepal, Mika Horiguchi, Chizuru Hatate, Akimasa Hoshi, Yosuke Mai, Soichi Miwa

Hokkaido University, Japan

E-mail address: terada@med.hokudai.ac.jp (K. Terada)

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.


Flow regulation of inner medullary collecting duct endothelin-1 production

Meghana M. Pandit, Donald E. Kohan

Division of Nephrology, University of Utah Health Sciences Center, USA

E-mail address: meghana.pandit@utah.edu (M.M. Pandit)