The toxicity of indoxyl sulfate to endothelial progenitor cells is rescued by niacin
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Background: Uremic toxin, indoxyl sulfate (IS), may impair proliferation and function of endothelial cells (ET) as well as endothelial progenitor cells (EPC). Nicotinic acid (niacin), a lipid-lowering drug, has antioxidant effect. Methods & results: EPC were isolated from healthy subjects and incubated with 1 mM IS. IS decreased the viability of EPC by 34%, and was restored by 1 mM niacin. IS did not induce apoptosis of EPC, but increased autophagy and senescence of EPC, which were all restored by adding niacin. The ability of migration and tube formation of EPC were 50% inhibited by IS. Niacin restored the migration of EPC by 40%, but not tube formation. IS significantly increased ROS and heme oxygenase-1 expression, and decreased the expression of eNOS and VEGF. All these adverse effects of IS were antagonized by niacin. Conclusion: Niacin had beneficial effects on ET in uremic patients, in addition to lipid-lowering effect, through its restoration of EPC function.


Endothelin-1 does not alter macrophage phenotype
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ET-1 and inflammation are involved in the pathogenesis of hypertension. Macrophages, which are recruited to the inflamed vasculature, express both endothelin-A and endothelin-B receptors. Macrophages can also produce and release ET-1 but the precise effects of ET-1 on macrophage phenotype are unclear. Bone marrow derived macrophages (prepared from C57BL6 mice) were stimulated for 24 h with ET-1 (10–1000 pg/ml), LPS/INFγ (100 ng/ml and 10 ng/ml) and IL4/IL13 (10 ng/ml). Cytokines (TNFα, IL6 and IL10) and ET-1 were measured by ELISA and qRT-PCR and mRNA for iNOS, MCP-1, mannose phages and does not induce an alternative activation. This suggests that ET-1 is not pro-inflammatory to macrophages, but ET-1 did not. In addition, ET-1 pre-treatment and co-stimulation did not affect the response to LPS. In vitro ET-1 does not activate macrophages to alter phenotype and also does not have a co-stimulating effect with factors known to stimulate classical or alternative activation. The current paradigm of G protein coupled receptor (GPCR) signalling involves transactivation of protein tyrosine kinase receptors. We utilised human vascular smooth muscle cells (VSMC) to address the question if a GPCR, the endothelin receptor, could transactivate a serine/threonine kinase receptor, specifically the TGF-β receptor, TβR1. Signalling molecules were assessed by Western blotting and proteoglycan synthesis by 35S-sulphate and 35S-Met/Cys incorporation and molecular size by SDS-PAGE. Endothelin-1 treatment led to a time and concentration dependent increase in cystolic phosphomimad2C which was blocked by the mixed endothelin receptor antagonist bosentan and the TβR1 antagonist SB431542. Endothelin-1 treatment led to a time-dependent increase in nuclear phosphomimad2C. Endothelin-1 stimulated proteoglycan synthesis was partially blocked by SB431542 and completely inhibited by bosentan. The effect of endothelin to stimulate an increase in glycosaminoglycan size on biglycan was also blocked in a concentration-dependent manner by SB431542. These data extend the current paradigm of GPCR signalling to include the transactivation of
the serine kinase receptor for TGF-β (TβRI). This response will be considered in the context of responses to endothelin-1 and the options for therapeutically targeting endothelin-1 broadened to include downstream signalling otherwise associated with TGF-β receptor activation.


Structure of the precursor of salmon, Oncorhynchus keta, endothelins and phylogenetic analysis
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Abstracts

Endothelin (ET)-related receptors homologous to mammalian receptors have been predicted to exist in this species. Here we cloned cDNAs encoding preproendothelins (PPETs) from the intestinal cDNA library. Salmon ETs cDNAs encode 200 amino acids, including a 20-amino-acid putative signal sequence, as well as mature ETs, big ETs, and ET-like sequences. This sequences together with other published PPET sequences were used to analyze the phylogenetic relationship among all ET family genes.


Decreased MYPT-1 phosphorylation at Thr696 and Cdc42 protein expression are associated with decreased contractile responses to ET-1 in corpora cavernosa and internal pudendal artery from Goto-Kakizaki diabetic rats
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Endothelin-1 (ET-1) plays a crucial role in the development of erectile dysfunction (ED). The Goto-Kakizaki (GK) rat is a non-obese type 2 diabetes mellitus model, which displays ED and increased ET-1 plasma levels. The present study tested the hypothesis that GK rats display increased corpora cavernosa (CC) and internal pudendal artery (IPA) contractions to ET-1 as a contributing mechanism for ED. GK rats demonstrated impaired erectile function represented by decreased CC and IPA reactivity to ET-1. Whereas decreased phosphorylation of MYPT-1 and Cdc42 protein expression may account for decreased ET-1 responses, it indicates that ED in GK rats is not associated with augmented CC and IPA reactivity to ET-1.


Generation of Edn2-iCre transgenic mice
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Endothelin-2 (ET-2) is a potent vasoconstrictive peptide. Though similar to ET-1, recent studies suggest that ET-2 may act through distinct pathways, necessitating deeper study. ET-2 may play a role in heart failure, inflammation, macular degeneration, and cancer metastasis. It is transiently expressed and tightly regulated in the periovulatory ovary, where it may aid ovulation by inducing constriction of the follicular wall. Here, we present a transgenic mouse line that expresses iCre (codon-improved Cre recombinase) under the regulation of the promoter of the endothelin-2 (edn2) gene, which was developed as a novel model for characterizing the expression of ET-2 and for conditional deletion of genes in cells where ET-2 is expressed. A vector was generated containing iCre, a polyadenylation signal sequence, and an frt-neomycin-resistance-frt cassette. Two homologous regions of the edn2 gene flanking the ATG start codon were isolated from a BAC (bacterial artificial chromosome) clone and inserted upstream and downstream of the iCre-pA-FNF cassette. Homologous recombination was used to re-insert the cassette into the BAC plasmid. Following purification, the plasmid was inserted into fertilized eggs of C57BL/6 mice through pronuclear injection, and resulting eggs were implanted into pseudopregnant mice. Five Edn2-iCre transgenic containing lines of mice were established, and one line was bred with ROSA26 reporter. Offspring were used to localize iCre-expressing cells through X-gal staining. Characterization of the staining pattern revealed that iCre was expressed in granulosa cells of ovulatory follicles, cardiomyocytes, the pituitary, and the liver. We expect this novel mouse model to be a useful tool for future studies on the role of ET-2.


The calcitonin gene-related peptide (CGRP) plays beneficial roles in myocardial ischemia elicited by endothelin-1
Satoshi Homma, Satoshi Sakai, Ken-ichi Yanagi, Yumi Miyauchi, Kazutaka Aonuma, Takashi Miyauchi

Purpose: In addition to the adrenergic and cholinergic nerves, the cardiovascular tissues are also innervated by several peptidergic neurons that mediate nonadrenergic noncholinergic (NANC) functions. Among such neuropeptides, CGRP is released from capsaicin-sensitive sensory neurons in peripheral organs. CGRP is known as...