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The toxicity of indoxyl sulfate to endothelial progenitor cells is rescued by niacin

Kwan-Dun Wu, Yu-Chin Huang, VinCent Wu

Department of Internal Medicine, National Taiwan University, Taipei, Taiwan
E-mail address: kdwu@ntuh.gov.tw (K.-D. Wu)

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 autophage 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.

doi:10.1016/j.lfs.2013.12.135

Endothelin-1 does not alter macrophage phenotype

Rebecca C. Moorhouse[a], Neeraj Dhaun[a,b], David J. Webb[a], David C. Kluth[b]

[a]*Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK*
[b]*Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK*
E-mail address: r.moorhouse@sms.ed.ac.uk (R.C. Moorhouse)

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 C57/BL6 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, IL 6 and IL 10) and ET-1 were measured by ELISA and qRT-PCR and mRNA for iNOS, MCP-1, mannose receptor and Arginase 1 by qRT-PCR. Whereas LPS/INFγ increased production of TNFα, IL6 and IL10 and upregulated markers of classical activation (iNOS and MCP-1) ET-1 did not. IL4/IL13 stimulation upregulated markers of alternative activation (mannose receptor and arginase 1) 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. This suggests that ET-1 is not pro-inflammatory to macrophages and does not induce an alternative activation.

		Untreated controls	ET-l (pg/ml)		LPS (100 ng/ml) & INFγ (10 ng/ml)	IL4 & IL13 (10 ng/ml)
			10	1000		
Concentration (pg/ml)	TNFα	210	231	216	2530	234
	1L6	39	39	29	548	27
	1L10	113	123	121	914	139

N = 4 repeated in triplicate, *P < 0.001 compared to untreated control.

doi:10.1016/j.lfs.2013.12.136

Endothelin-1 activates extracellular signal-regulated kinases 1 and 2 through transactivation of platelet-derived growth factor receptor in skeletal muscle cells

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

Department of Cellular Pharmacology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
E-mail address: thigashi@med.hokudai.ac.jp (T. Higashi)

Background: Endothelin (ET) system plays a critical role in the development of insulin resistance and type 2 diabetes. However, the molecular mechanism for ET receptor (ETR) signaling activated by ET-1 in skeletal muscle remains to be determined. The purpose of this study was to determine the signaling molecules involved in ET-1-stimulated phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in rat skeletal muscle (L6) cells. Methods: Change in the phosphorylation level of ERK1/2 was analyzed by Western blot experiment. Dominant negative dynamin (K44A) along with monomeric strawberry red fluorescent protein (RFP) was overexpressed in L6 cells using adenovirus-mediated gene transfer. Infection efficiency of the adenovirus was determined by fluorescence of RFP with flow cytometry. Results: ET-1 induced concentration-dependent phosphorylation of ERK1/2 in L6 cells. The phosphorylation of ERK1/2 was abolished by BQ-123 (a selective ETAR antagonist), YM-254890 (a Gαq/11 protein inhibitor), PD98059 (a mitogen-activated protein kinase/ERK kinase 1/2 inhibitor), and AG370 (a platelet-derived growth factor receptor (PDGFR) kinase inhibitor). The ERK1/2 phosphorylation in response to ET-1 was inhibited by overexpression of dominant negative dynamin (K44A), which blocks clathrin-mediated endocytosis of cell surface receptor. Conclusions: Gq/11 protein-coupled ETAR is involved in the ET-1-induced phosphorylation of ERK1/2 in L6 cells. The ETAR-mediated phosphorylation of ERK1/2 is dependent on transactivation of PDGFR, which requires dynamin-dependent receptor internalization.

doi:10.1016/j.lfs.2013.12.137

Endothelin receptor transactivates the TGFβ receptor to stimulate proteoglycan synthesis in human vascular smooth muscle cells

Danielle Kamato, Narin Osman, Peter J. Little

Discipline of Pharmacy, School of Medical Science and Diabetes Complications Lab, Exercise and Disease Program, Health Innovations Research Institute, RMIT University, Melbourne, Australia
E-mail address: danielle.kamato@rmit.edu.au (D. Kamato)

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, TβRI. 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 cytosolic phosphoSmad2C which was blocked by the mixed endothelin receptor antagonist bosentan and the TβRI antagonist SB431542. Endothelin-1 treatment led to a time-dependent increase in nuclear phosphoSmad2C. 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