Endothelin-1 increases superoxide production in human coronary artery bypass grafts

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Aims- Endothelin-1 (ET-1) has been shown to increase endothelial superoxide (O_2^-) production in experimental animal models and ET receptor blockade improves endothelial function in patients with atherosclerosis.The aim of this study was to elucidate whether ET-1 increases O_2^- production in human vessels, if this effect is receptor dependent and which source of O_2^- is predominantly involved.

Methods- Segments of internal mammary artery (IMA) and saphenous vein (SV) were harvested from 90 patients undergoing elective coronary artery bypass graft surgery. Paired vessel rings were incubated in the presence and absence of ET-1 (10^{-10} M) for 45 min and analysed for O_2^- production using lucigenin-enhanced chemiluminescence. In subgroups, additional rings were analysed after incubation with ET-1 and either the ET_A receptor antagonist BQ123 (10^{-6}M) alone, or in combination with the ET_B receptor antagonist BQ788 (10^{-6}M; dual BQ). In order to investigate through which enzymatic source ET-1 mediates its effects paired IMA segments were incubated with inhibitors of known sources of O_2^-. BH4, an essential cofactor for eNOS, were measured, using HPLC, both in a subset of IMA, SV and HUVEC in the presence and absence of ET-1 and lung and aorta from ET-1 transgenic mice.

Results- ET-1 increased O_2^- production in both IMA (2.6±1.48 vs. 1.43±0.79 relative light units/s/mg tissue (RLU); n=33; p<0.0001) and SV (1.38±0.85 vs. 1.09±0.62 RLU; n=24; p=0.013). ET-1-induced O_2^- production was significantly higher in IMA than in SV (p<0.001). The increase in O_2^- production induced by ET-1 in IMA was inhibited by co-incubation with dual BQ (p<0.05; n=15) and BQ123 (p<0.05; n=17). Only tiron, a superoxide scavenger and DPI, an inhibitor of flavin dependent enzymes could significantly block ET-1-induced O_2^- production. ET-1 exposure did not affect levels BH4, in human vessels, HUVEC and in the lung or aorta of transgenic mice overexpressing ET-1 in the endothelium.

Conclusion- ET-1 increases O_2^- production in both IMA and SV from patients with CAD via a receptor-dependent pathway involving the ET_A receptor and NADPH oxidase.