

### **Myocardial ischemia-reperfusion induces upregulation of contractile endothelin ET<sub>B</sub> receptor in rat coronary arteries**

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**Objective** We have previously shown increased expression of endothelin ET<sub>B</sub> receptors in coronary arteries of ischemic heart disease patients. ET<sub>B</sub> receptors are normally primarily situated in the vascular endothelial cells mediating vasodilation, whereas only a limited part are situated in the vascular smooth muscle cells mediating vasoconstriction. This study aims to examine whether heart ischemia-reperfusion leads to upregulation of contractile ET<sub>B</sub> receptors in the smooth muscle layer of the coronary arteries and to investigate the signaling pathways involved in the putative ET<sub>B</sub> receptor upregulation.

**Methods and Results** Thirteen Sprague-Dawley male rats (body weight 260-410 g) were anaesthetized with Hypnorm-Midazolam and subjected to 15 min occlusion of left anterior descending coronary artery (LAD) followed by 22 h of reperfusion. The contractile response to the specific ET<sub>B</sub> receptor agonist Sarafotoxin 6c (S6c) (1 pM to 30 nM) was investigated after cumulative additions in a sensitive wire-myograph. LAD segments, situated in the post-ischemic area displayed significantly augmented ET<sub>B</sub> receptor mediated vasoconstriction (68±3% of maximal contraction, n=7) compared to coronary arteries from the non-ischemic area (23±6 % of maximal contraction, n=8) and sham operated rats (22±5% of maximal contraction, n=5). Increased density of ET<sub>B</sub> receptors localized in the vascular smooth muscle layer was confirmed by immunohistochemistry in LAD segments from the post-ischemic area compared to the non-ischemic segments.

Interestingly, a rapid ET<sub>B</sub> receptor upregulation also appeared when coronary arteries from healthy non-operated rats were incubated for a short-term (7 h) in a physiological saline solution. We used this in-vitro model to study the signaling pathway involved in the ET<sub>B</sub> receptor upregulation. Incubation (7 h) of the coronary arteries in the presence of either the transcriptional inhibitor (Actinomycin D, 4µM) or the selective mitogen-activated protein kinase kinase (MEK1/2) inhibitor (U0126, 10 µM) significantly attenuated the S6c mediated response compared to vehicle. Immunohistochemical staining displayed enhanced phosphorylation of ERK1/2 after 4 h of incubation and demonstrated ET<sub>B</sub> receptors in the vascular smooth muscle cells after 7h of incubation.

**Conclusion** The results demonstrate that heart ischemia-reperfusion in rats induces an upregulation of contractile ET<sub>B</sub> receptors in the vascular smooth muscle cells in coronary arteries in the post-ischemic area. This study suggests that the upregulation of the ET<sub>B</sub> receptors depends on a transcriptional upregulation and involves the MEK/ERK type of MAPK.