investigate the contribution of the cAMP-binding protein Epac (Exchange protein directly activated by cAMP) in the regulation of the contractile properties of rat ventricular cardiac myocytes. We report that both PKA and Epac increased cardiac sarcomere contraction but through opposite mechanisms. Differently from PKA, selective Epac activation by the cAMP analog 8-pCPT reduced Ca2+ transient amplitude and increased cell shortening in intact cardiomyocytes as well as myofilament Ca2+ sensitivity in permeabilized cardiomyocytes. Moreover, ventricular myocytes, which were infected in vivo with a constitutively active form of Epac, showed enhanced myofilament Ca2+ sensitivity compared to control cells infected with GFP alone. At the molecular level, Epac increased phosphorylation of two key sarcomeric proteins, cardiac Troponin I (cTnI) and cardiac Myosin Binding Protein-C (cMyBP-C). The effects of Epac activation on myofilament Ca2+ sensitivity and on cTnI and cMyBP-C phosphorylation were independent of PKA, and were blocked by protein kinase C (PKC) and Ca2+ + calmodulin kinase II (CaMKII) inhibitors. Altogether these findings identify Epac as a new regulator of myofilament function.

**J003**

**HEMIN PREVENTS IN STENT RESTENOSIS IN RAT AND RABBIT MODELS: HEME OXYGENASE-1 AS A NEW THERAPEUTIC TARGET TO PREVENT RESTENOSIS**

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Recent reviews have concluded that although drug eluting stent (DES) are efficient in reducing in stent restenosis, their use does not have a significant effect on overall long-term survival as compared with bare metal stent. DES is associated with delayed vascular wall healing and endothelial function restoration, which mandates longer-term dual antiplatelet therapy. Recent studies demonstrated that the microsatellite polymorphism in the promoter of heme oxygenase-1 (HO-1) gene is related to angiographic restenosis. HO-1 is a rate-limiting enzyme in heme degradation; leading to the generation of free iron, biliverdin, and carbon monoxide (CO). HO-1 is recognized to offer protection in many cardiovascular disorders. We aim to assess the potential protective effect of hemin, a potent HO-1 inducer, in the development of ISR in both rat and hypercholesterolemic rabbit.

In a rat model of aorta stenting and rabbit iliac stenting, chronic treatment with hemin (50 mg/kg/48h/ip) reduced neointima growth (-30% and -50% in hemin-treated rats and rabbits respectively), and most importantly stent struts remained covered, contrarily to the use of sirolimus eluting stent. Analysis of the cells facing the arterial lumen (electron microscopy) revealed an ultra-structure similar to endothelial cells and the expression of CD31 (immunogold labeling). Endothelial coverage was similar in hemin-treated rats and greater in hemin-treated rabbits when compared to their control groups. Analysis of protein expression, in rats, revealed that hemin, limited the early inflammatory, apoptotic and proliferative cellular events common to ISR. More particularly, hemin treatment was associated with a decrease activity of key regulators of smooth muscle cell migration and proliferation, p42/44, RhoA and an increase of the expression of both cyclin dependent kinase inhibitors, p21 and p27kip1. This beneficial effect of hemin was abolished in presence of SnPP, an inhibitor of HO-1. Finally, CORM-3, a specific carbon monoxide donor, limited ISR.

In conclusion, hemin reduced neointima growth without compromising re-endothelialization of the stented arteries. HO-1 plays important role in limiting neointima growth, at least through the production of CO, and can be regarded as a new therapeutic target to prevent ISR.

**J004**

**DARBEPOETIN-α PROTECTS HEART AGAINST ISCHEMIA-REPERFUSION: ROLE OF BCL-2 FAMILY PROTEINS**

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Heart can usually survive a short period of ischemia, but when this period is too long, damages of the cardiac tissue became irreversible and are possibly exacerbated by blood reperfusion. Loss of cardiomyocytes via apoptosis is believed to contribute to the continuous decline of ventricular function described in heart failure. Limiting these deleterious responses is of major importance in cardiac surgery and for the treatment of coronary thrombosis. The purpose of this study was to assess the short and long term cardioprotective effects of the long lasting effect erythropoietin analogue darbepoetin-α (DA) in a myocardial ischemia-reperfusion model in rat and ; to investigate the signaling pathway through which DA potentially limits apoptosis of cardiomyocytes.

Rat were subjected to 40 min left coronary artery ligation followed by 3h, 72 h or 4 weeks reperfusion and they received either DA (3 or 30 µg/kg) or vehicle i.v. prior ischemia. Left ventricle (LV) function was assessed by echocardiography prior surgery and after reperfusion. Hearts were collected for histological analysis, protein analysis and reactive oxygen species (ROS) production.

In DA3 and DA30 72hrs groups, both LV shortening fraction and LV ejection fraction were higher vs. control (P<0.05), matching with histological analysis revealing a relative LV infarct size 72h post ischemia of 40 ± 5% in control vs. 27 ± 3 and 17 ± 2% in DA3 and DA30, respectively. DA treatment lowered ROS production, the activity of caspase 3 in 3h and 72h reperfusion groups, and activated the JAK2/Akt signaling pathway and then increased both phosphorylated Bad and GSK3β proteins. This was consistent with the decrease of Bad-Bcl-X expression in DA30 group, suggesting an increased level of Bcl-X protein. Similar results were obtained in DA-treated rats reperfused 4 weeks; in which cardiac fibrosis was significantly lower than that in control group.

DA pre-treatment limited in a dose dependent manner the early and late I/R-induced heart injury in rat. Anti-apoptotic effects mediated through the activation the survival kinase Akt that regulates the Bcl-2 family proteins and activates GSK3β is central in the DA cardioprotective mechanism.