A new role of the brain natriuretic peptide in the heart: modulation of cardiac progenitor cell proliferation and differentiation.

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The actual role of the brain natriuretic peptide (BNP) in the heart remains elusive despite its reported protective effect in ischemic animal hearts. Because recently BNP was shown to control the proliferation and differentiation of murine embryonic stem cells, we asked in this study whether BNP could influence the proliferation and differentiation of cardiac progenitor cells (CPC) in vitro and in vivo. We first identified a c-kit+ Sca-1+ cell population present in neonatal and adult hearts which expressed the NPR-A and NPR-B receptors. In vitro, these cells proliferated and in presence of BNP differentiated into CPCs (c-kit+) Sca-1- Nkx2.5+ and into mature cardiomyocytes. In parallel, BNP was injected to newborn and adult healthy mice (n=6 mice per group). In the hearts of neonatal and adult mice, BNP injection increased the number of newly formed cardiomyocytes (neonatal: +23%, p=0.009 and adult: +65%, p=0.005) and the number of CPCs (neonatal: +142%, p=0.002 and adult: +134%, p=0.04). BrdU injection in neonatal CPC treated mice demonstrated that BNP stimulated CPC proliferation. In anticipation that BNP might be used as a therapeutic agent, we injected BNP into mice undergoing myocardial infarction (mice 7-8 week old). Higher numbers of Nkx2.5+ cells were detected in both the infarcted (+38%, p<0.03) and non infarcted areas (+69%, p=0.02) of BNP treated hearts one week after surgery. Finally, by isolating neonatal cardiac cells from the hearts of NPR-A or NPR-B deficient mice, we demonstrated that BNP stimulates the fate of CPCs via NPR-B binding and that long term BNP treatment is correlated in vitro and in vivo and with decreased Protein Kinase G activity. Our results highlight a new key role for BNP in the control of CPC proliferation and/or differentiation. This new function of BNP should be evaluated in therapies aimed to induce cardiac cell regeneration and should reopen the debate about the therapeutic use of BNP for patients suffering from heart diseases.

Cardiac p11 expression is related to 5-HT4 receptor pathway in failing and non-failing rat left ventricular cardiomyocytes

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Aim: Heart failure is the inability to maintain a sufficient cardiac output necessary to meet metabolic demand. Cellular compensatory mechanisms, such as the serotonin 4 receptor (5-HT4R) pathway, take place to improve the defective cardiac excitation-contraction coupling (CCEC). However, little is known about the regulation of this pathway. Our objective was to investigate the potential involvement of the 5-HT4R partner p11 in the activation of the pathway during heart failure.

Methods and results: Wistar rats underwent ligation of the left coronary artery to mimic infarction. Control Sham-operated animals underwent the same surgery without ligation. Seven weeks post myocardial infarction (PMI) hearts were collected and/or enzymatically digested in order to perform biochemical studies or to assess intracellular calcium (Ca2+) handling at the single cell level. p11 mRNA expression in the left posterior wall was significantly increased at 7 weeks PMI compared to Sham (272±40 vs.141±27 A.U, P<0.05). However, p11 protein levels in 7 weeks PMI myocytes showed a trend toward a decrease compared to sham (0.16±0.02 vs. 0.25±0.09), inter-}

0232
Role of epac signalling in doxorubicin-induced cardiotoxicity

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The canonical mechanisms underlying doxorubicin (Dox)-induced cardiotoxicity involve Reactive Oxygen Species production, DNA intercalation and topoisomerase II inhibition which trigger DNA damage, oxidative stress and alteration of calcium homeostasis leading to myocyte death and heart dysfunction. However, alternative mechanisms with prior or concomitant dox induced alteration of signalling pathways are emerging. β-adrenergic signalling and especially Epac (exchange protein directly activated by cAMP) could be worth investigating as Epac activate small G proteins Rac1 and Rho A known to be implicated in dox-induced cardiotoxicity. We investigated the time/dose-dependent Dox effect in in vitro (neonatal rat cardiomyocytes) and in vivo on a mice models on 1/ the transcriptional activity of cardiac remodelling markers (ANF, SRE and SkM o-actin) 2/ Epac’s downstream effectors (small G proteins expression, MEF-2,NFAT) and 3/ Epac’s role in dox-induced DNA damage. In vitro, Dox treatment resulted in an alteration of Epac signalling through inhibition of stress and remodelling makers, a modulation of Epac’s downstream effectors and a direct effect of Dox on Epac1 and Epac2 expression. Moreover, the protein level of DNA damage marker (H2AX pS139) was modulated by Epac1 and 2 inhibitors and by Epac2 inhibitor which suggest a new protective pathway through Epac1 specific inhibition. In vivo, echocardiography of Dox-treated mice (3 iv injections, 12mg/kg total dose) showed a delayed cardiomyopathy from 15 weeks. At the molecular level, we observed a statistically significant Epac1, Epac2, Rho A and Rac1 expression modulation between 6 and 20 weeks suggesting a time-dependent regulation of Epac signalling in Dox-induced cardiotoxicity. Our results indicate for the first time an integrated time-course of Dox induced-alteration in Epac signalling, and a potential role of Epac in myocyte death induced by Dox.

0018
Conditional ablation of ShcA induces heart failure through dysfunctional neuregulin and dystrophin signaling

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ShcA is an adaptor protein that binds to tyrosine kinase receptors. Its germ line deletion is embryonic lethal with abnormal cardiovascular system formation. We used the Cre-LoxP technology and the smooth muscle protein-22 (Sm22) cre transgene, to ablare ShcA specifically in the cardiovascular system from early embryonic development. Conditionally mutant mice developed signs of severe diluted cardiomyopathy, myocardial infarctions, and premature death. No evidence of a vascular contribution to the phenotype was observed. Histological analysis of the heart reveals aberrant intercalated z-disc and M-
effects of gevokizumab, a potent modulator of IL-1β suggest a therapeutic benefit of the IL-1β treatment of I/R induced LV dysfunction.

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Gevokizumab, an IL1-beta modulating antibody exerts promising cardioprotective effects against ischemia-reperfusion injury in diabetic rats.

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Aims: Enhanced myocardial interleukin-1 beta (IL1-β) production is involved in ischemia/reperfusion (I/R) induced left ventricular (LV) dysfunction. We tested neutralization of IL-1β as a potential therapeutic target for the treatment of I/R induced LV dysfunction.

Methods: We assessed in diabetic (Goto Kakizaki, GK) rats the preventive effects of gevokizumab, a potent modulator of IL-1β, administered once a week, started 4 weeks prior to a 20 min of transient ischemia induced by left coronary artery occlusion and continued 90 days after I/R, on LV remodeling/function (echocardiography) 7 and 90 days after I/R, LV hemodynamics (LV catheterization), LV tissue perfusion (MRI) and LV collagen density (image analysis) were assessed 90 days after I/R.

Results: I/R induced early LV expansion followed by late LV dilatation, associated with LV dysfunction as well as after 90 days, reduced LV tissue perfusion and LV collagen accumulation. Gevokizumab limited both early LV expansion as well as late LV dilatation, associated with an improved LV function. Ninety days after I/R, gevokizumab improved both LV systolic and LV diastolic functions, illustrated by the increase in LV end-systolic pressure volume relation, and the reductions in LV end-diastolic pressure and LV end-diastolic pressure volume relation. Moreover, long-term gevokizumab moderately increased LV tissue perfusion and significantly reduced LV collagen density.

Conclusions: Our results, obtained using a clinically relevant model of I/R, suggest a therapeutic benefit of the IL-1β modulating antibody, gevokizumab, in myocardial I/R injury.

LVDD: left ventricular diastolic diameters; LVFS:LV fractional shortening; LVESPVR and LVEDPVR: LV end-diastolic and end-systolic pressure volume relations. *:p<0.05 vs GK; † p<0.05 vs GK I/R

0190

Early and delayed IL-1 beta antibody gevokizumab treatments prevent cardiac remodeling and reverse coronary endothelial dysfunction following myocardial infarction injury in Goto Kakizaki rats

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Aims: Cardiac interleukin-1 beta (IL1-β) production is enhanced acutely after myocardial infarction and is involved in myocardial damages. We tested if early and delayed IL1-β modulations by IL-1β antibody, gevokizumab, prevent left ventricular (LV) remodeling and endothelial dysfunction induced by LV ischemia/reperfusion (I/R) in diabetic rats.

Methods: Gevokizumab (Gevo; 10 mg/kg) was administered 1 hour (early) or 7 days (delayed) following reperfusion, after a 20 min of transient ischemia induced by LV artery occlusion and continued every week for 90 days. Delayed perindopril (1 mg/kg) was used as a positive control. LV remodeling and function were assessed (Echocardiography) at 7 and 90 days.

Results: At 7 days, early Gevo limited the early LV expansion and reduction of FS induced by I/R. At 90 days both of early and delayed Gevo as well as perindopril limited in a similar manner, the LV late dilatation, the reduction of FS and LV systolic and diastolic dysfunction induced by I/R. At 90 days, GK coronary endothelium-dependent relaxation to acetylcholine was impaired by I/R (59±13 vs.17±4%, p<0.05). Early, delayed Gevo and perindopril restored the (86±4, 92±2 and 98±1% respectively; p<0.05 vs GK+I/R) coronary relaxation to acetylcholine. Early, delayed Gevo and perindopril significantly reduced collagen density and leukocytes infiltration at 90 days.

Conclusions: In a clinically relevant model of acute myocardial infarction, the IL-1β antibody gevokizumab started early or late after myocardial reperfusion exerts immediate and late cardiovascular protection.