Topic 22 – Heart failure, cardiomyopathy – C

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0232

A new role of the brain natriuretic peptide in the heart: modulation of cardiac precursor 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 both neonatal and adult mice, BNP injection increased the number of newly formed cardiomyocytes (neonatal: + 23%, p= 0.009 and adult: +68%, p= 0.005) and the number of CPCs (neonatal: + 142%, p= 0.002 and adult: +134%, p= 0.04). BrdU injection to neonatal BNP 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 (n=6-7 mice per group). 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 modulates the fate of CPCs via NPR-B binding and that long term BNP treatment is correlated *in vitro* and *in vivo* 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.

0330

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 (CEC). 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 (Ca^{2+}) 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). Interestingly, the expression of the p11 partner Annexin A2 was significantly increased (1,52±0.32 vs. 0,53±0.14, P<0.05) suggesting improved p11 stability. At the CEC level, stimulation of the 5-HT4R pathway by prucalopride did not exert an effect on all CEC parameters evaluated in both PMI and Sham myocytes. Nevertheless, induction of p11 expression by Brain-Derived Neuron Factor treatment in freshly isolated healthy myocytes showed a remarkable increase in Ca2+ transient amplitude (0,27±0,02 vs. 0,18±0,01, P<0.05) and basal Ca2+level (0,67±0,01 vs. 0,64±0,01, P<0.05) following prucalopride stimulation compared to untreated cells.

Conclusion: Cardiac expression of p11 seems to play a role in the modulation of the response to 5-HT4R pathway in both failing and non-failing hearts.

0018

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 α-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 markers, 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 dilated 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.

0271

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 ablate ShcA specifically in the cardiovascular system from early embryonic development. Conditionally mutant mice developed signs of severe dilated 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-disk and M-