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The natriuretic peptide/guanylyl cyclase-A system functions as a stress-responsive regulator of angiogenesis in mice

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Background

Cardiac atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) modulate blood pressure and volume by activation of the receptor guanylyl cyclase-A (GC-A) and subsequent intracellular cGMP formation. Here we report what we believe to be a novel function of these peptides as paracrine regulators of vascular regeneration.

Results

In mice with systemic deletion of the *GC-A* gene, vascular regeneration in response to critical hindlimb ischemia was severely impaired. Similar attenuation of ischemic angiogenesis and arteriogenesis was observed in mice with conditional, endothelial cell-restricted *GC-A* deletion (termed here EC GC-A KO mice). In contrast, smooth muscle cell-restricted *GC-A* ablation did not affect ischemic neovascularization. Immunohistochemistry and RT-PCR analyses of mRNA obtained from laser-microdissected cells revealed BNP expression in activated satellite cells within the ischemic muscle, suggesting that local BNP elicits these protective endothelial effects. To verify these results, we studied BNP mRNA expression in cultured C2C12 cells, a mouse myoblast cell line. Quantitative real time RT-PCR demonstrated that BNP mRNA was strongly expressed in C2C12 cells and was induced by hypoxia. Taken together these in vivo/in vitro data suggest that expression of BNP induced in activated satellite cells of the ischemic muscle stimulates angiogenesis via endothelial GC-A.

Cardiac hypertrophy and angiogenesis are coordinately regulated during physiological or adaptive cardiac growth. ANP and especially BNP are among the earliest and most sensitive stress-responsive "fetal genes" induced in cardiomyocytes in response to pressure overload. To test whether this cardiac induction of NPs participates in the regulation of coronary angiogenesis, EC GC-A KO mice and respective control littermates where subjected to transverse aortic constriction (TAC). The induced pressure gradient (~50 mmHg) and the load-provoked increases in left ventricular mass were similar in *EC GC-A KO* mice and controls. Left ventricular systolic function was not altered, as fractional shortening and ejection fraction remained optimal. However, in contrast to the control mice, EC GC-A KO mice developed significant left ventricular diastolic dysfunction. Histological analyses showed that hearts from EC GC-A KO mice exhibit mild fibrosis, as assessed by sirius red staining. Assessment of capillary and

endothelial content by isolectin stainings revealed a significant decrease in EC GC-A KO compared to control hearts. These observations indicate that endothelial GC-A activation participates in angiogenesis during the early compensatory phase of pathologic hypertrophy.

Lastly, the functional responses to ANP and BNP were studied in spontaneously immortalized, well characterized microvascular endothelial cells from rat epididymal fat pad capillaries (RFPEC). BNP increased intracellular cGMP content and activated cGMP-dependent protein kinase I as well as the phosphorylation of vasodilator-stimulated phosphoprotein and p38 MAPK. BNP stimulated proliferation, migration and angiogenic sprouting of RFPEC with a similar concentration-dependency. These pro-angiogenic responses were largely suppressed in the presence of Rp-8-pCPT-cGMPs, a specific PKG I inhibitor. ANP, but not c-ANP-(4–23), a ligand for the NP clearance receptor (NPR-C), exerted similar effects than BNP. Notably, the maximal NP-induced pro-angiogenic effects were as strong as those evoked by vascular endothelial growth factor (VEGF-A, 50 ng/ml).

Conclusion

We conclude that BNP, produced by activated satellite cells within ischemic skeletal muscle or by cardiomyocytes in response to pressure-load, regulates the regeneration of neighboring endothelia via GC-A. This paracrine communication might be critically involved in coordinating muscle regeneration/hypertrophy and angiogenesis.

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