Left ventricular remodelling in heart failure: the role of oxidative myofilament protein modifications, stress and female hormones

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2013-2014

Investigation of the possible role of stress and female hormones in the development of cardiomyocyte dysfunction

Background: The relationship between stress, female hormones and cardiovascular function from different aspects was suggested previously. However, the intensity and the duration of the stress seem to be important. Limited data are available about the effects of acute stress and influence of female hormones on cardiomyocyte contractile function. Identification of stress-induced protein alterations, the effects of stress and sex hormones on cardiomyocyte function could help to better understand the role of these factors during the process of stress-related left ventricular remodelling.
Aim: To assess how ovarian-derived sex hormones (in particular progesterone) modify the effects of single acute stress on the mechanical and biochemical properties of left ventricular cardiomyocytes in the rat.
Methods: Non-ovariectomized (control, n=8) and ovariectomized (OVX, n=8) female rats (Croatian collaboration) were kept under normal conditions or were exposed to stress (control-S, n=8 and OVX-S, n=8). Serum progesterone levels were measured using a chemiluminescent immunoassay. Left ventricular myocardial samples were used for isometric force measurements and protein analysis. Ca^{2+}-dependent active force (F_{active}), Ca^{2+}-independent passive force ($F_{passive}$), and Ca^{2+}-sensitivity of force production were determined in single, mechanically isolated, permeabilized cardiomyocytes. Stress- and ovariectomy-induced alterations in myofilament proteins (myosin-binding protein C [MyBP-C], troponin I [TnI], and titin) were analyzed by sodium dodecyl sulfate gel electrophoresis using protein and phosphoprotein stainings.
Results: Serum progesterone levels were significantly increased in stressed rats (control-S, 35.6±4.8 ng/mL and OVX-S, 21.9±4.0 ng/mL) compared to control (10±2.9 ng/mL) and OVX (2.8±0.5ng/mL) groups. F_{active} was higher in the OVX groups (OVX, 25.9±3.4 kN/m^2 and OVX-S, 26.3±3.0 kN/m^2) than in control groups (control, 16.4±1.2 kN/m^2 and control-S, 14.4±0.9 kN/m^2). Regarding the potential molecular mechanisms, F_{active} correlated with MyBP-C phosphorylation, while myofilament Ca^{2+}-sensitivity inversely correlated with serum progesterone levels when the mean values were plotted for all animal groups. $F_{passive}$ was unaffected by any treatment.
Conclusion: Stress increases ovary-independent synthesis and release of progesterone, which may regulate Ca^{2+}-sensitivity of force production in left ventricular cardiomyocytes. Stress and female hormones differently alter Ca^{2+}-dependent cardiomyocyte contractile force production, which may have pathophysiological importance during stress conditions affecting postmenopausal women.

An *in extenso* article related to this topic was published in the *Croatian Medical Journal*. The research was conducted in collaboration with the Department of Medical Biology and Genetics, J. J. Strossmayer University of Osijek, Faculty of Medicine Osijek.

2014-2015

1. Investigation of the functional and biochemical effects of the myeloperoxidase enzyme (MPO) on human permeabilized cardiomyocytes

Background and aims: The myeloperoxidase (MPO) enzyme, a member of the heme-peroxidase superfamily, was shown to be one of the most important contributors to damage cardiac macromolecules by formation of reactive oxidants and diffusible reactive oxygen species (ROS) upon ischaemia-reperfusion injury and inflammation. However, limited data are available about the direct effects of MPO-derived ROS on the contractility of human myocardial cells. Therefore we set out to characterize the mechanical effects of myeloperoxidase (MPO) in isolated left ventricular human cardiomyocytes. Oxidative myofilament protein modifications (sulfhydryl (SH) group oxidation and carbonylation) induced by the peroxidase and chlorinating activities of MPO were additionally identified. The specificity of the MPO-evoked functional alterations was tested with an MPO inhibitor (MPO-I) and the antioxidant amino acid methionine (Met).
Results: The combined application of MPO and its substrate, hydrogen peroxide (H_2O_2), largely reduced the active force (F_{active}), increased the passive force ($F_{passive}$) and decreased the Ca^{2+} sensitivity of force production (pCa_{50}) in permeabilized cardiomyocytes. H_2O_2 alone had significantly smaller effects on F_{active} and $F_{passive}$ and did not alter pCa_{50}. The MPO-I blocked both the peroxidase and chlorinating activities, while Met selectively inhibited the chlorinating activity of MPO. All of the MPO-induced functional effects could be prevented by the MPO-I and Met. Both H_2O_2 alone and MPO+H_2O_2 reduced the SH content of actin and increased the carbonylation of actin and myosin-binding protein C to the same extent. Neither the SH-oxidation nor the carbonylation of the giant sarcomeric protein titin was affected by these treatments.
Conclusions: MPO activation induces a cardiomyocyte dysfunction by affecting Ca^{2+}-regulated active and Ca^{2+}-independent passive force production and myofilament Ca^{2+} sensitivity, independently of protein SH oxidation and carbonylation. The MPO-induced deleterious functional alterations can be prevented by the MPO-I and Met. Inhibition of MPO may be a promising therapeutic target to limit myocardial contractile dysfunction during inflammation.

The results of this research have been published as an original research article in the *Free Radical Biology and Medicine*.

2. Altered myocardial force generation in end-stage human heart failure

Aims: This study aimed to elucidate the molecular background of increased Ca^{2+} sensitivity of force production in cardiomyocytes of end-stage human heart failure.
Methods and Results: Ca^{2+}-activated isometric force and the cross-bridge specific rate of force redevelopment (k_{tr}) were determined in Triton-skinned myocytes from end-stage failing and non-failing donor hearts. Measurements (control: pH 7.2, 0 mM inorganic phosphate (P_i)) were performed under test conditions that probed either the Ca^{2+}-regulatory function of the thin filaments (pH 6.5), the kinetics of the actin-myosin cross-bridge cycle (10 mM Pi), or both (pH 6.5, 10 mM P_i). The control maximal Ca^{2+}-activated force (F_{active}) and k_{trmax} did not differ between failing and non-failing myocytes. At submaximal [Ca^{2+}], however, both force and k_{tr} were higher in failing than in donor myocytes. The difference in the Ca^{2+} sensitivities of force production was preserved when the thin filament regulatory function was perturbed by acidosis (pH 6.5) but was abolished by cross-bridge modulation (i.e. by P_i) both at pH 7.2

and at pH 6.5. P_i induced a larger reduction in force but a smaller increase in k_{tr} in the failing myocytes than in the non-failing myocytes at submaximal [Ca^{2+}].
Conclusion: The enhanced P_i sensitivity of the actin-myosin interaction suggests that the P_i release step of the actin-myosin cross-bridge cycle is modified during end-stage human heart failure. This might be of functional importance when P_i accumulates (e.g. during cardiac ischaemia). Moreover, this alteration can influence cardiac energetics and the clinical efficacy of sarcomere targeted agents in human heart failure.

The results of this research have been published as an original research article in the *ESC Heart Failure*.

2015-2016

Circulating ACE2 activity correlates with cardiovascular disease development

Background: It was shown recently that Angiotensin Converting Enzyme activity is limited by endogenous inhibition in vivo, highlighting the importance of Angiotensin II elimination (ACE2).
Aims: Potential contribution of the ACE2 to cardiovascular disease progression was addressed.
Methods: Serum ACE2 activities were measured in different clinical states (healthy, n=45; hypertensive, n=239; heart failure (HF) with reduced ejection fraction (HFrEF), n=141 and HF with preserved ejection fraction (HFpEF), n=47).
Results: ACE2 activity was significantly higher in hypertensive patients (24.8±0.8 U/ml) than that in healthy volunteers (16.2±0.8 U/ml, P=0.01). ACE2 activity further increased in HFrEF patients (43.9±2.1 U/ml, P=0.001) but not in HFpEF patients (24.6±1.9 U/ml) when compared to hypertensive patients. Serum ACE2 activity negatively correlated with left ventricular systolic function in HFrEF, but not in hypertensive, HFpEF or healthy populations. Serum ACE2 activity had a fair diagnostic value to differentiate HFpEF from HFrEF patients in this study.
Conclusions: Serum ACE2 activity correlates with cardiovascular disease development: it increases when hypertension develops and further increases when the cardiovascular disease further progresses to systolic dysfunction, suggesting that ACE2 metabolism plays a role in these processes. In contrast, serum ACE2 activity does not change when hypertension progresses to HFpEF, suggesting a different pathomechanism for HFpEF and proposing a biomarker based identification of these HF forms.

The results of this research have been published as an original research article in the *Journal of the Renin-Angiotensin-Aldosterone System (in press).*

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