Creatine kinase, myofilament stiffness and cardiomyocyte mechanics

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Pulmonary artery hypertension (PAH) is a complex disease with interactions between the pulmonary vasculature (PV) and the right ventricle (RV). PAH is caused by increased constriction of the PV. This increases vascular resistance and hence RV afterload, leading to RV failure, which is the most common cause of death in PAH.

We have investigated RV contractile dysfunction in an animal model of PAH (Fowler *et al.*, 2015). Experiments were conducted with local ethical approval and in accordance with UK Home Office and European Parliament Directive 2010/63/EU guidelines on the use of animals in research. PAH and RV failure were induced in rats by a single IP injection (60 mg/kg) of monocrotaline (FAIL) and compared to saline-injected animals (CON). FAIL rats were humanely killed upon showing external signs of heart failure. CON rats were killed on equivalent days.

In anesthetized animals, *in vivo* right end ventricular diastolic pressure-volume relationships (EDPVR) were measured by Millar catheterization of the RV. We observed significantly steeper EDPVR in FAIL rats indicating reduced compliance and greater resistance to ventricular filling, hence *in vivo* diastolic dysfunction. This was not linked to increased fibrosis, therefore changes in the properties of single ventricular myocytes were investigated. Intact single myocytes were attached to glass fibres and stretched to record the end diastolic force-length relationships (EDFLR). The slope of the EDFLR was significantly steeper in RV myocytes from FAIL rats, in accord with the steeper EDPVR in vivo.

It was observed that the RV myocytes from FAIL rats had significantly shorter diastolic sarcomere lengths compared to CON but that diastolic intracellular Ca^{2+} (measured with Fura-2) or myofilament Ca^{2+} sensitivity was not different. Sarcomere length was increased by application of an intracellular Ca^{2+}-buffer (BAPTA-AM) or by myofilament cross-bridge inhibition (40 mM BDM). The sarcomere lengthening effect of BDM was significantly greater in FAIL than CON, suggesting a cross-bridge based, Ca^{2+}-independent mechanism.

A decrease in the local ATP:ADP can lead to rigor like cross-bridge formation. Creatine kinase, bound to the myofilaments, is known to maintain ATP:ADP but to be decreased in heart failure. Western blot analysis showed a significant reduction in protein levels of creatine kinase in the RV of FAIL rats. Furthermore, when single myocytes were skinned by application of saponin and exogenous creatine kinase applied the diastolic sarcomere length of RV myocytes from FAIL rats was significantly increased, while in CON myocytes, inhibition of creatine kinase shortened sarcomere length. We suggest that decreased creatine kinase expression leads to diastolic dysfunction, *via* local reduction in ATP:ADP ratio and thus to Ca^{2+}-independent force production and diastolic sarcomere shortening.

Currently, there is no cure for PAH and it is acknowledged that novel treatments are needed. Current therapy targets the PV with no specific RV treatments in current guidelines. The importance of RV failure in patient survival indicates the heart is a potential therapeutic target. It has been proposed that established treatments for left ventricular failure may be beneficial to the failing RV in PAH (*e.g.* Handoko *et al.*, 2010). We tested whether and how the β_1-adrenoceptor blocker, metoprolol, improves RV function in our animal model of PAH.

When PAH was established, rats were orally given metoprolol (10 mg/kg/day, BB) or placebo (FAIL). Compared with FAIL, BB significantly improved median survival from 23 to 31 days post-monocrotaline. BB improved *in vivo* cardiac function, including a decrease in EDPVR. BB treatment also improved mRNA and protein levels of creatine kinase. β_1-adrenoceptor blockade and cardiac energetics may be potential 'novel' therapeutic targets for RV failure in PAH.

Fowler ED, Benoist D, Drinkhill MJ, Stones R, Helmes M, Wust RC, Stienen GJ, Steele DS & White E (2015). Decreased creatine kinase is linked to diastolic dysfunction in rats with right heart failure induced by pulmonary artery hypertension. *J Mol Cell Cardiol* **86**, 1-8.

Handoko ML, de Man FS, Allaart CP, Paulus WJ, Westerhof N & Vonk-Noordegraaf A (2010). Perspectives on novel therapeutic strategies for right heart failure in pulmonary arterial hypertension: lessons from the left heart. *Eur Respir Rev* **19**, 72-82.

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