THE EFFECTS OF ACUTE AND CHRONIC UP-REGULATION OF PYRUVATE DEHYDROGENASE ON MYOCARDIAL METABOLISM

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Introduction: Pyruvate dehydrogenase (PDH) is a key metabolic enzyme in the regulation of substrate utilisation in the heart. Dichloroacetate (DCA) is a potent activator of PDH activity but its effects are not limited to PDH flux alone. DCA is proposed to alter both fatty acid oxidation and glucose metabolism and storage in addition to its impact on PDH, however, these alterations are poorly understood in the heart [1]. Alterations in PDH activity have been observed in many cardiovascular-related diseases, such as diabetes and left ventricular hypertrophy, suggesting that metabolism plays a role in disease onset and progression [2]. Further clarification of the impact of increased PDH flux on cardiac metabolism using DCA may provide key mechanistic information about the role of PDH in substrate utilisation with the potential to be transferred therapeutically to various cardiovascular diseases. Therefore, in this work, the effects of both acute and chronic DCA treatment on cardiac metabolism were assessed *in vivo* and in the perfused heart.

Methods:

Up-regulation of PDH flux in vivo: Up-regulation of PDH flux was assessed *in vivo* in rats using hyperpolarized [1-^{13}C]pyruvate. Approximately 1 ml of 80 mM [1-^{13}C]-labelled sodium pyruvate was injected into an anaesthetised rat via a tail vein cannula. Individual cardiac-localised ^{13}C spectra were acquired every second over a period of 60s and incorporation of the [^{13}C]label into bicarbonate was used as an *in vivo* measure of PDH flux. Acute PDH up-regulation was assessed in rats (n = 5; 300-350 g) immediately following a bolus infusion of DCA (1.5 ml, 30 mg ml^{-1}). Control rats (n = 5; 300-350 g) received an injection with [1-^{13}C]-labelled sodium pyruvate but in the absence of an infusion with DCA. Chronic PDH up-regulation was assessed in rats (n = 5; 300-350 g) who were treated with DCA (0.75 g l^{-1}) given *ad libitum* in drinking water for 5 weeks and PDH assessed as described above after 5 weeks of treatment.

Acute and Chronic Up-Regulation of PDH Flux in the Perfused Heart: The acute effect of DCA on glycolytic flux was assessed in control hearts (400-450 g; n = 6) perfused with [^{3}H]glucose via the Langendorff method in the presence of 1 mM DCA with no prior DCA treatment. Glycolytic flux was also assessed, with no DCA in the perfusion buffer, in hearts rapidly excised from control rats (n = 7; 400-450 g) and rats chronically treated with DCA for 5 weeks (n = 7; 400-450 g).

Results: PDH flux was significantly enhanced *in vivo* following both an acute infusion (0.006±0.001 s^{-1} vs. 0.014±0.001 s^{-1} Control vs. DCA; $p<0.01$) and chronic treatment with DCA for 5 weeks (Fig 1; 0.006±0.001 vs. 0.011±0.001 Control vs. DCA; $p < 0.05$). In accordance with this, preliminary data suggests a reduction in levels of the PDH regulatory enzymes PDK1 and PDK4 in chronically treated hearts. Glycolytic flux and lactate production were significantly decreased in the perfused heart following both acute (Fig 1; $p < 0.05$) and chronic (Fig 1; $p < 0.05$) PDH up-regulation. This was accompanied by a decrease in GLUT4 levels in chronically treated hearts obtained from preliminary analysis.

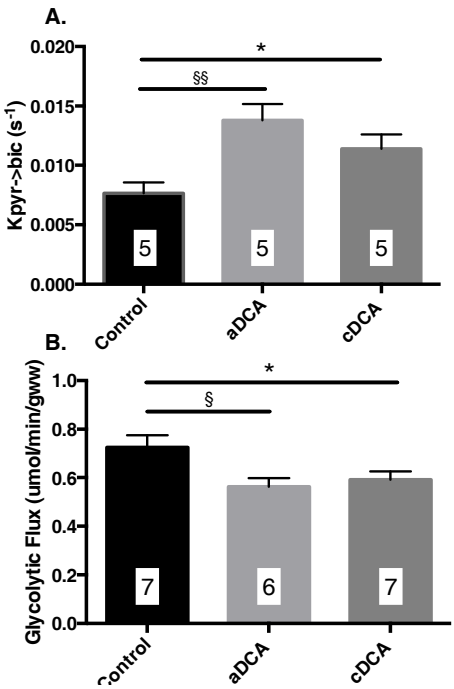

Figure 1 The Effects of Acute and Chronic DCA Treatment on Cardiac Metabolism. A. *In Vivo* Pyruvate Flux following Acute (aDCA) and Chronic (cDCA) DCA Treatment. B. The Effects of Acute and Chronic DCA Treatment on Glycolytic Flux in the Perfused Heart.

Discussion: Both acute and chronic DCA treatment act to up-regulate PDH flux *in vivo* promoting glucose oxidation, however, this is accompanied by an unexpected reduction of glycolytic flux and lactate production. This suggests that either glucose is fully oxidized and therefore metabolized more efficiently or alternatively, enhanced glucose oxidation leads to feedback inhibition of glycolysis. Further assessment of the effect of PDH up-regulation on fatty acid oxidation and the intermediates within the TCA cycle will aid further clarification of the role of PDH in substrate utilisation.

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