The Effect of Hyperpolarized [1-13C]Pyruvate Concentration on Metabolism in the Perfused Heart

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Introduction: Hyperpolarization of ^{13}C labelled pyruvate, via Dynamic Nuclear Polarization (DNP), is a useful tool for the assessment of cardiac metabolism in the perfused rat heart[1]. [1-^{13}C]Pyruvate can provide an accurate measurement of flux through the key regulatory enzyme, pyruvate dehydrogenase (PDH), as well as a measure of intracellular pH[2]. In addition, [2-^{13}C]pyruvate can provide a direct assessment of real time metabolism in the TCA cycle[3]. So far, the majority of hyperpolarized pyruvate studies in the perfused heart have used concentrations of pyruvate (2-2.5 mM) that are relatively high when compared with the normal physiological level of ~60 μM[4]. To ensure the correct interpretation of data obtained from such experiments, it is vital to understand the effect of this high concentration on downstream metabolism. Therefore, the aim of this work was to investigate the effect of [1-^{13}C]pyruvate concentration on the production of the downstream metabolites, lactate, alanine and bicarbonate, in the perfused rat heart.

Methods:

Sample preparation: Aliquots (40 mg) of [1-^{13}C]pyruvic acid (Sigma, UK) were mixed with the trityl radical OXO63, 15 mM (GE Healthcare, Amersham, UK) and a trace amount of Dotarem (Guerbet, France) and placed in a HyperSense hyperpolarizer (Oxford Instruments, Abingdon, UK). The samples were polarized at a frequency of 94.160 GHz for one hour and the maximum solid-state polarization level and time constant of polarization enhancement was recorded.

Heart perfusion: Hearts from 4 male Wistar rats (Harlan, UK) were perfused in the Langendorff mode with Krebs-Henseleit buffer containing 10 mM glucose and oxygenated with 95% O_2/5% CO_2. The hearts were placed in the bore of a vertical 11.7 T MRI system (Bruker-Biospin, Germany) for spectral assessment.

Spectroscopy: Following polarization, the pyruvic acid samples were dissolved with 6 ml of a NaOH/EDTA/Tris buffer, to neutralise the pH, and subsequently added to varying amounts of Krebs-Henseleit buffer to yield a concentration of 0.15 mM, 0.3 mM, 0.625 mM, 1.25 mM or 2.5 mM hyperpolarized sodium pyruvate. This solution was then delivered to the heart over 120 seconds and a series of 120 carbon spectra were acquired using a simple pulse-acquire spectroscopy sequence (TR = 1 s, FA = 30°, BW = 180 ppm, 8192 pts). The resultant spectra were quantified using the AMARES algorithm in jMRUI[5] and the peak area for each metabolite was plotted as a function of the infused pyruvate concentration.

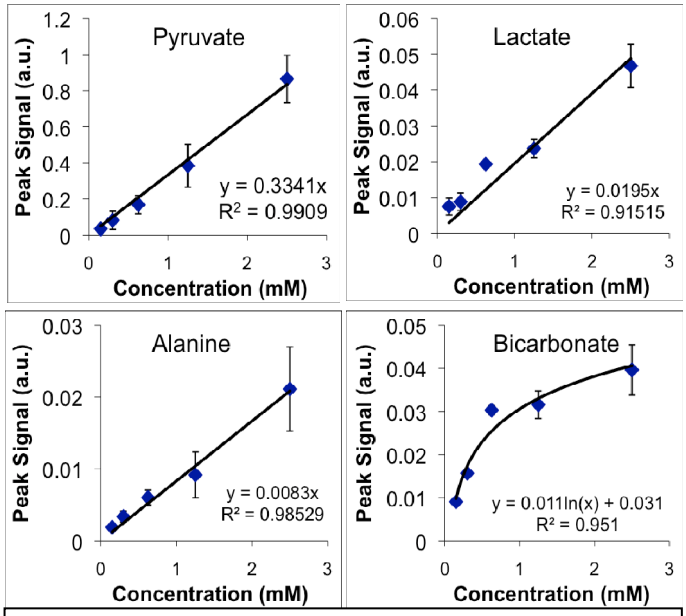

Figure 1: The variation of peak signal amplitude as a function of infused pyruvate concentration for pyruvate and its downstream metabolic products, lactate, alanine and bicarbonate.

Results and Discussion: The SNR of all acquired spectra was sufficient to allow for accurate quantification, even at the lowest concentration investigated (0.15 mM). Figure 1 shows the variation of peak metabolite signal as a function of the infused pyruvate concentration for both pyruvate and the downstream metabolites. As expected, the observed pyruvate signal showed a linear relationship with the infused pyruvate concentration. This linear relationship was also seen for lactate and alanine, demonstrating that the uptake of pyruvate was not limited, even at the highest concentration of 2.5 mM. This would also suggest that neither lactate dehydrogenase nor alanine aminotransferase were saturated at the 2-2.5 mM concentrations previously used. However, the bicarbonate signal demonstrates a Michaelis-Menton type response to increasing pyruvate concentration, reaching a plateau above 0.625 mM. This would indicate that flux through PDH was saturated at the higher concentrations of pyruvate.

Conclusion: The presented data suggest that an infused pyruvate concentration of 0.625 mM provides the optimal concentration for the study of pyruvate metabolism in the perfused rat heart, maximising the signal available from bicarbonate whilst minimising the metabolic consequences of infusing high concentrations of pyruvate. Further work is being conducting to investigate the effects of [2-^{13}C]pyruvate concentration on the down stream metabolic products, acetylcarnitine, citrate and glutamate.

References: [1] Merritt, M. *et al*, PNAS 104(50) p19773-77 2007, [2] Schroeder, M.A. *et al* Cardio. Res 86(1) p82-91 2010 [3] Schroeder, M.A. *et al* FASEB J 23(8) p2529-38 2009 [4]Atherton, H. J. *et al*, NMR in Biomedicine, 25 Aug 2010 [5] Naressi, A. *et al*, MAGMA 12 p 141-52 2001

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