A novel 13C MRS-based marker of pyruvate cycling in perfused mouse liver using [2-13C] pyruvate and 13C MRS

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Introduction

The pathways by which pyruvate is recycled from the TCA cycle (PEPCK and malic enzyme) have received recent attention. Despite being in principle a futile cycle (i.e. resulting in only a net waste of ATP), flux through pyruvate recycling pathways have been shown to correlate with essential physiological processes such as glucose-stimulated insulin release from pancreatic cell lines[1]. Current methods to measure pyruvate cycling rely on the introduction of uniformly labeled 13C-tracers and isotopomer analysis of the ^{13}C-^{13}C couplings in glutamate and/or glucose. Here we propose a simpler marker of pyruvate cycling based on the introduction of [2-^{13}C] pyruvate and the subsequent appearance of ^{13}C-label in the C3 position of pyruvate which can only occur via label scrambling in the TCA cycle followed by recycling back to pyruvate. As pyruvate is consumed quite rapidly in the liver, the ^{13}C-labeling in lactate (a stable direct conversion product of pyruvate) is used as surrogate.

Methods

Experiments were performed on a Bruker 11.7T (500MHz proton resonance frequency) wide bore NMR spectrometer using a 20mm TXO probe tuned for ^{13}C and ^{31}P observation. 10-20 week old, non-fasted C57/Bl6 mice were anesthetized (Nembtual, 50mg/kg IP) during the light cycle and, following a portal vein cannulation, livers were excised and placed in a custom 20mm NMR tube as part of the apparatus shown in Figure 1[2]. ATP levels, measured via ^{31}P NMR spectroscopy, were used to monitor hepatic viability and were unchanged (<5%) for the duration of the study. Livers were perfused for 90 min. with an initial dose of 7mM [2-^{13}C] pyruvate plus one of the following treatments: (1) PEPCK inhibitor (3-MPA, 3-mercapto-picolinic acid, 250µ), (2) Malic enzyme inhibitor (TA, tartronic acid, 10mM), (3) PEPCK inhibitor + Malic enzyme inhibitor, and (4) Control vehicle (buffer). After the perfusion, livers were freeze-clamped and homogenized in perchloric acid. The extract was then centrifuged and the supernatant was neutralized, freeze dried, re-suspended in D_2O, and analyzed by ^{13}C NMR in a 3mm broad-band probe.

Results/Discussion

Figure 2 displays a ^{13}C-NMR spectrum (natural abundance subtracted) from a control perfused liver and the 13C NMR spectrum of the corresponding perchloric acid extract. As pyruvate is quickly converted to the more stable lactate in liver, the C3/C2 ^{13}C-labeling ratio in lactate reflects the same ratio in pyruvate and provides a marker of the amount of pyruvate which has been recycled. In looking at Figure 3 we see that as expected, inhibition of PEPCK and malic enzyme each reduce the C3/C2 ratio in lactate and simultaneous inhibition of both PEPCK and malic enzyme further reduces this ratio to levels near the LOQ for this method which is ~0.03. While the spectrum from the whole perfused liver did not have suitable SNR to accurately quantitate the Lac C3 resonance, longer perfusion times or the use of larger livers may allow this measurement to be made without the need for extraction.

Conclusion

The perfused liver NMR technique along with [2-^{13}C] pyruvate have been employed to develop a novel marker of pyruvate cycling which was reduced upon treatment with inhibitors of each pyruvate cycling pathway. This method is more suitable for drug discovery research as it is quicker than established methods and can potentially be translated to whole tissues and possibly in vivo.

References

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2. Cohen, SM. In Research in Perfused Liver: Clinical and Basic Applications. Ballet, F. and Thurman, RG. Eds. John Libbey: London, 1991, Chapter 4.

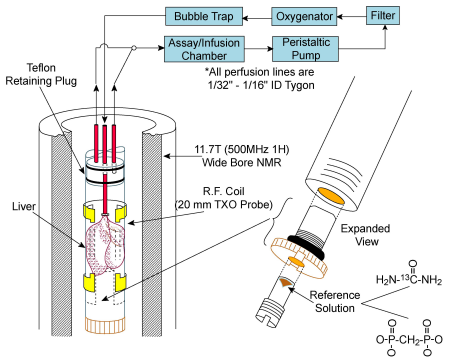

Figure 1: Essential Components of the perfused liver apparatus.

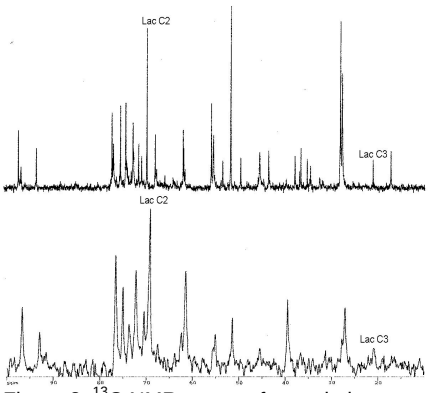

Figure 2: ^{13}C NMR spectra from whole perfused liver (bottom) and liver extract (top)

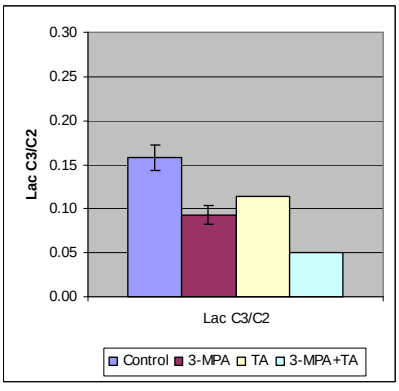

Figure 3: Effect of PEPCK and Malic enzyme inhibition on Lac C3/C2 ^{13}C-labeling ratio