Propionate increases Hyperpolarized $H^{13}CO_3^-$ Signal in Perfused Mouse Hearts

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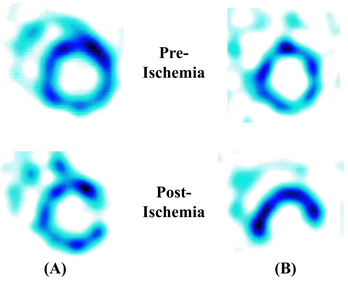
ABSTRACT

Background: As early as 2008, MR imaging of [1-^{13}C]pyruvate and its metabolites, including bicarbonate, in post-ischemic pig hearts was reported (1). Since the method does not use ionizing radiation, there is widespread interest in applications in other fields including oncology. In the heart, pyruvate is oxidized to acetyl-CoA and CO_2. Oxidation of hyperpolarized (HP) [1-^{13}C]pyruvate to HP [^{13}C]bicarbonate is reduced in injured myocardium, and the presence of preserved flux through pyruvate dehydrogenase (PDH) may identify viable myocardium. However, oxidation of alternative substrates normally present in the blood also reduces the appearance of HP [^{13}C]bicarbonate even in healthy myocardium. Propionate, a short-chain three-carbon fatty acid normally present in the blood, is known to activate PDH, and it is under study as a nutritional therapy for heart failure. The efficacy of propionate for restoring PDH flux in hearts supplied with high concentrations of glucose and fatty acids was studied using ^{13}C NMR isotopomer analysis paired with experiments using HP [1-^{13}C]pyruvate. ^{13}C NMR is a standard method for measuring fluxes in metabolic pathways.

INTRODUCTION

^{13}C NMR methods have been used to investigate metabolism since the early 1970s. Compared to other isotope tracer methods, ^{13}C NMR provides investigators unparalleled resolution. Nevertheless, the low sensitivity of the ^{13}C nucleus remains a problem, limiting its use in magnetic resonance spectroscopy (MRS) imaging (2). Recently, Dynamic Nuclear Polarization (DNP) technology has allowed investigators to increase the sensitivity of the ^{13}C nucleus up to > 10^4 fold through a process known as hyperpolarization (HP) (2). Increased sensitivity allows for the application of HP in a wide number of fiends lincluding oncology, and cardiology (1, 2).

[13C]Bicarbonate Images from Golman, et al. MRM, 2008 (1)



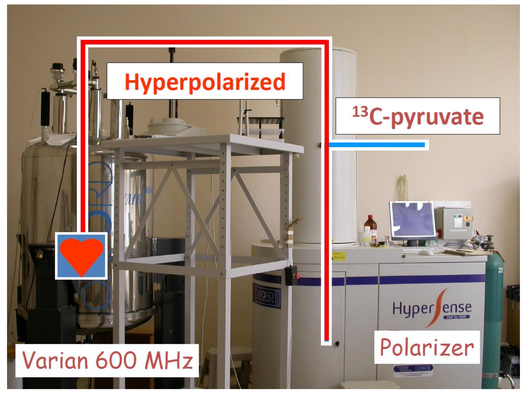
(A) Hearts experiencing 15-minutes of ischemia. CKMB negative; no infarction.

(B) Hearts experiencing 45-minutes of ischemia. CKMB positive; infarction.

As shown above, differences in MR $H^{13}CO_3^-$ signal were detected before and after induced ischemia/infarction in pig hearts perfused with [1-^{13}C]pyruvate by Golman, et al. (1). The $H^{13}CO_3^-$ signal responds to remote ischemia for up to two hours post ischemic event, therefore HP $H^{13}CO_3^-$ MRS presents possible clinical utility in differentiating remote angina pectoris from non-cardiac chest pain (1). Nonetheless, since PDH activity is responsive to competing substrates, a need to target the [1-13C]pyruvate to the PDH complex for metabolism into $^{13}CO_2$ is clear (3).

In this study, we perfused C57/bl6 mouse hearts with HP [1-^{13}C]pyruvate and other ^{13}C labeled substrates in the presence and absence of propionate. NMR spectra were recorded using a cryogenically-cooled probe on both whole hearts and dried tissue extracts. Propionate has two effects on TCA cycle flux. Propionate enters the TCA cycle as an anaplerotic substrate at the level of succinyl-CoA. Propionate also serves to activate the pyruvate dehydrogenase (PDH) complex (4). Activation of the PDH complex increases $^{13}CO_2$ production by the PDH complex, due to rapid enzymatic conversion, $^{13}CO_2$ detected as $H^{13}CO_3^-$ during HP ^{13}C NMR.

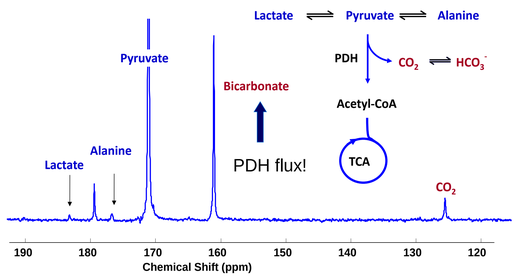
MATERIALS AND METHODS



Perfusion and Animal Prep Protocol (IACUC approved): Hearts from *ad libitum* fed C57/bl6 mice were excised and placed in ice cold perfusion buffer. Aortas were cannulated and perfused in a non-circulating Langendorff mode, where Krebs-Henseleit perfusion buffer flows retrogradely down the cannulated aorta (5). Aortic valve closure ensures that the coronary arteries receive pressured flow of nutrient buffer (5). Continuous bubbling of a mixture of O_2 and CO_2 ensured pH maintenance at 7.4, and oxygenation of the mouse myocardium. The buffer was maintained at 37°C to simulate physiological temperature.

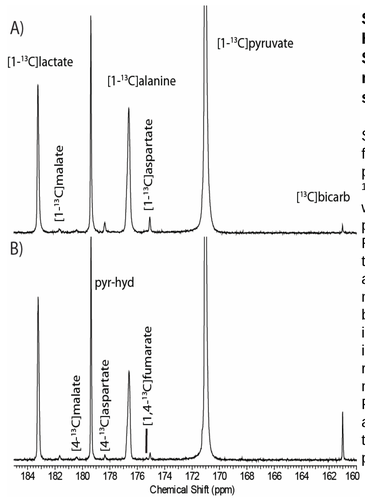
Perfusion Conditions: Substrates provided to mouse hearts in group A (spectra labeled A) were: 8.25 mM unlabeled glucose, 2mM [U-^{13}C]acetate; the group contained four hearts. After perfusion conditions reached steady-state, HP [1-^{13}C]pyruvate was added. Hearts belonging to group B (spectra labeled B) were treated with identical conditions, except 2 mM of unlabeled propionate was added to the perfusate.

Hyperpolarization Experiments: HP was carried out in an Oxford HyperSense polarizer. NMR spectra of working hearts were obtained using a 14.1 T Bruker Avance 3 HD console. The perfusion apparatus containing a still beating mouse heart was placed in the NMR bore. A ^{13}C optimized cryogenically-cooled probe was used to increase the sensitivity of the NMR magnet. To our knowledge, this is the first application of cooled-probe technology to hyperpolarization experiments. Perfusion apparatuses were quickly removed after spectra acquisition. Mouse hearts were freeze-clamped immediately to stop further metabolism.



^{13}C NMR of Heart Tissue Extracts: Perchloric acid tissue extract, along with the use of a cryogenically-cooled probe, allows for high-resolution NMR of tissues. Analysis of isotope isomers (isotopomers) present in solution can help elucidate underlying tissue metabolism. Isotopomer analysis uses glutamate carbon resonances to describe the metabolic activities of tissues. J-coupling between adjacent ^{13}C nuclei present in the mixture of isotopomers produces characteristic multiplets. These multiplets are used to describe the complex population of isotopomers that led to their detection.

RESULTS

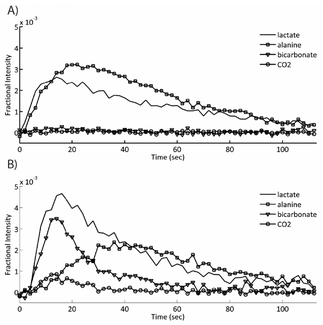


Summed ^{13}C Hyperpolarization Spectra (from 25 most intense spectra):

Summed HP spectra from mouse hearts perfused with HP [1-^{13}C]pyruvate and both with (B) and without propionate (A). Resonances represent the compound that they are labeled. Note the marked increase in the bicarbonate resonance intensity in (B). This increase is presumably results from propionate mediated activation of PDH flux, and increased anaplerosis which leads to increased $^{13}CO_2$ production.

Effects of propionate on HP [^{13}C]bicarbonate signal: Propionate has a net activating effect on pyruvate dehydrogenase (PDH) flux. Pyruvate dehydrogenase catalyzes the conversion of pyruvate to acetyl-CoA and CO_2. CO_2 is rapidly converted to bicarbonate via carbonic anhydrase. Perfusion with HP [1-^{13}C]pyruvate produces HP $^{13}CO_2$ (and subsequently $H^{13}CO_3^-$) upon flux through the PDH complex (5). One way to represent the hyperpolarization data is fractional intensity (NMR signal) vs. time. The area under this curve represents the total signal intensity in a given amount of time. In propionate perfused hearts, integrated [^{13}C]bicarbonate intensity increased about 37-fold compared to hearts perfused without propionate.

13C HP fractional intensity by time plots:
X-axis: time, Y-axis: fractional intensity. Taking the area under the curve allows one to compute total intensity for a given metabolite. HP [^{13}C]bicarb intensity increased 37-fold in the presence of propionate in (B) compared to hearts perfused without propionate (A).



Effects of propionate on mouse heart metabolism: At steady state, the equilibrium of the transamination reaction of α-ketoglutarate (AKG) to glutamate (Glu) allows analysis of the glutamate resonance to determine the labeling status of AKG. The main source of ^{13}C isotope was [U-^{13}C]acetate. Acetate labels carbons 4 and 5 of AKG. At steady-state, this label will appear in carbons 1 through 3 of AKG. Perfusion with [U-^{12}C]propionate provides an anaplerotic influx of unlabeled carbon. Propionate enters the TCA cycle via conversion to succinyl-CoA. Succinyl-CoA is converted to oxaloacetate, which provides the first three carbon atoms of AKG. The *a priori* effects of such perfusion conditions on Glu isotope content is an increase in unlabeled atoms in carbon positions 1, 2 and 3 in propionate-perfused hearts. In propionate-perfused hearts, there was an increase in intensity of the C4 doublet caused by J-coupling between carbons 4 and 5 (C4D45), compared to the quartet (C4Q) produced by J-coupling between carbons 3, 4 and 5. Isotopomer analysis confirmed a significant increase in anaplerosis (y) in propionate-perfused hearts compared to hearts treated without propionate (y = 0.25 ± 0.02 with propionate vs. y = 0.12 ± 0.01 without propionate).

RESULTS

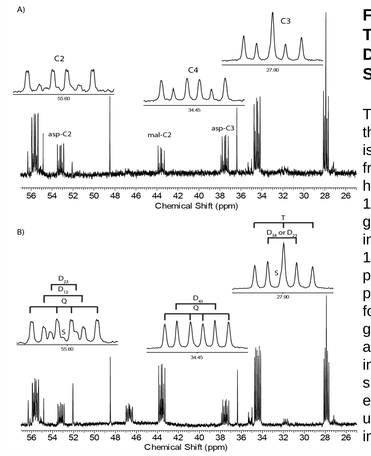
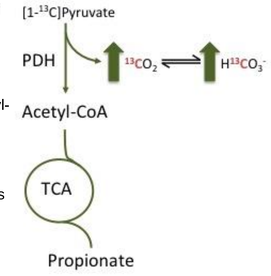


Figure 3: Heart Tissue Extract ^{1}H Decoupled ^{13}C NMR Spectra:

These spectra represent the populations of isotopomers generated from perfusion of mouse hearts with [U-13C]acetate, unlabeled glucose, and a short injection of HP [1-13C]pyruvate (A- without propionate, B – with propionate) . Multiplets for the resonances of glutamate C2, C3 and C4 are enlarged. Note increased D45, on spectrum B as would be expected given unlabeled propionate influx into the TCA cycle.

CONCLUSION

Schematic representation of propionate's effects on PDH flux:



[1-^{13}C]Pyruvate enters the TCA cycle through conversion to Acetyl-CoA by the pyruvate dehydrogenase complex. This produces $^{13}CO_2$ which is rapidly converted to $H^{13}CO_3^-$ by carbonic anhydrase. $H^{13}CO_3^-$ is detected as a peak at around 161 ppm. Propionate enters the TCA cycle through conversion to Succinyl-CoA.

As in the rat, pyruvate flux through the PDH complex in mouse hearts is responsive to substrate competition(3). In the mouse, perfusion of hearts with acetate in the absence of propionate led to markedly lower production of HP $H^{13}CO_3^-$. Our results show that propionate perfusion avidly increases anaplerosis of the TCA cycle. Propionate also acts to increase bicarbonate production, as demonstrated by our hyperpolarization experiments. As hyperpolarization signal is proportional to (fractional enrichment) · (polarization) · (concentration), any effects that increase concentration of the substrate will influence the sensitivity of future NMR and MR imaging applications. Propionyl-L-carnitine, a metabolite of propionate has been shown to have cardioprotective effects in humans (7). Given interest in using HP $H^{13}CO_3^-$ imaging to non-invasively diagnose various ischemic heart diseases, co-infusion of propionyl-L-carnitine and HP [1-13C]pyruvate could be a viable strategy to increase the sensitivity of HP $H^{13}CO_3^-$ imaging (1).

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