Selective Excitation of [^{13}C]Bicarbonate Following Injection of Hyperpolarized [1-^{13}C]Pyruvate Allows for Enhanced Signal

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Introduction

Hyperpolarized (HP) [1-^{13}C]pyruvate has been shown to be metabolized to [^{13}C]bicarbonate and other products both *in vivo* and in isolated perfused hearts. The appearance of a [^{13}C]bicarbonate signal following an injection of hyperpolarized [1-^{13}C]pyruvate reflects the activity of pyruvate dehydrogenase (PDH) in heart.[1] In some metabolic circumstances, the [^{13}C]bicarbonate signal is low and consequently the time-dependent evolution of this signal is difficult to characterize.[2] Unlike traditional NMR spectroscopy, hyperpolarized substances lose magnetization with time due to two effects, application of a series of RF readout pulses to observe the signal as well as T_1 relaxation. However, frequency selective RF readout pulses could be used to detect [^{13}C]bicarbonate without concomitant destruction of the "upstream" HP pyruvate signal. This in effect would conserve HP [1-^{13}C]pyruvate for a longer period of time and increase the amount of HP [^{13}C]bicarbonate detected on each scan.

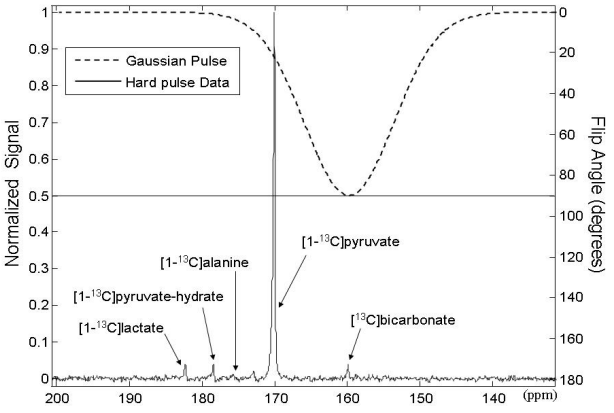

Figure 1: ^{13}C spectrum obtained with a single hard 90-degree pulse following injection of hyperpolarized [1-^{13}C]pyruvate. Bloch simulation (dotted line) of the Gaussian excitation profile. The selective pulse was calibrated to 90-degree excitation (right axis) centered on the bicarbonate peak.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee. After general anesthesia, mouse hearts were rapidly excised and immediately perfused in Langendorff mode with Krebs-Henseleit bicarbonate buffer containing 5 mM glucose. Hearts were placed in the center of an 8 mm NMR tube positioned in a RF coil tuned to ^{13}C in a Varian VNMRS 14.1 Tesla system. An 8 mL bolus of 4 mM hyperpolarized [1-^{13}C]pyruvate was injected over a 25 s period directly above the aorta. ^{13}C spectra were collected using a 90-degree flip angle repeated every 5 s. In a separate experiment, the process was repeated using a series of 90-degree Gaussian selective pulses centered on the [^{13}C]bicarbonate peak at 160 ppm. Selective pulses were constructed using the standard "gauss" pulse shape included in the Varian VnmrJ software. Hyperpolarized samples were prepared using an Oxford HyperSense DNP system.

Results

The metabolic products of [1-^{13}C]pyruvate, [1-^{13}C]lactate, [1-^{13}C]alanine, [1-^{13}C]pyruvate-hydrate, and [^{13}C]bicarbonate were all detected in hearts when using a series of non-selective 90-degree square pulses for observation but the SNR was poor for all metabolites (~2-14) other than pyruvate (Figure 1). The [^{13}C]bicarbonate signal was detectible in only three spectra collected at 10 s, 15 s and 20 s. However, when a 90-degree Gaussian centered on the bicarbonate peak was used for readout, the SNR in the [^{13}C]bicarbonate signal improved 8-fold and the signal was detected in 14 spectra (t=10-80 s). The [1-^{13}C]pyruvate signal also displayed ~3-fold signal enhancement compared to the square pulse spectra and its peak intensity maximum shifted to a later time point.

Conclusions

The evolution of HP [^{13}C]bicarbonate derived from [1-^{13}C]pyruvate was observed in perfused mouse hearts with higher SNR when using a series of 90-degree frequency-selective Gaussian excitation pulses than when using standard 90-degree hard-pulses for excitation. The frequency selective pulse allows greater amounts of polarization to be sustained through the metabolic processes thereby maintaining polarization in [^{13}C]bicarbonate for a longer time period. The increase in pyruvate signal seen when using the frequency-selective pulse centered at bicarbonate reflects the small flip angle, ~20 degrees, experienced at the pyruvate frequency. The HP pyruvate that is not destroyed by the RF pulsing remains in the coil and accumulates, generating a larger signal than the experimental protocol using a 90-degree pulse. Simulations show that the optimal flip angle using hard pulses depends upon delivery rate of the pyruvate and the repetition time as well as the rate of metabolic flux for each metabolite. While the pyruvate signal is improved, the ratio of bicarbonate to pyruvate has increased 2.5-fold, demonstrating that a selective pulse will generate greater intensity for an individual metabolite than the use of a hard-pulse with a smaller flip angle. Thus, each individual metabolite may be optimized with shaped pulses. Although $^{13}CO_2$ was not detected in these experiments, it should be possible to use a selective pulse to enhance the HP-CO_2 signal and thereby allow a measure pH in vivo.

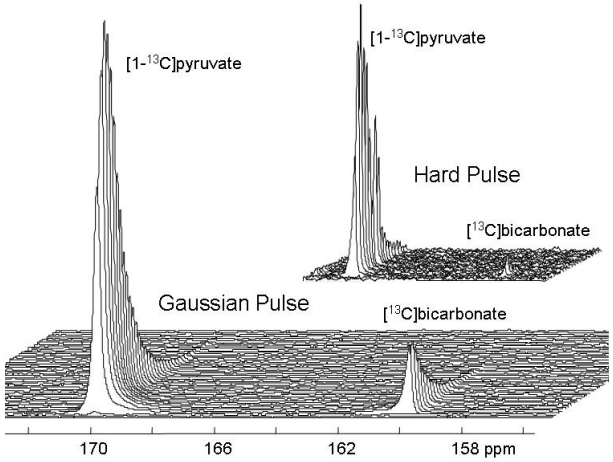

Figure 2: Stacked plots of the ^{13}C amplitudes following excitation with a 90-degree Gaussian compared to 90-degree Hard Pulse (inset) taken every 5 seconds. Only [1-^{13}C]pyruvate at 171 ppm and [^{13}C]bicarbonate at 160 ppm are shown in the two plots.

REFERENCES

[1] Merritt ME, Harrison C, Storey CJ, Jeffrey FMH, Sherry AD, Malloy CR. Hyperpolarized 13C allows a direct measure of flux through a single enzyme-catalyzed step by NMR. Proceedings of the National Academy of Sciences of the United States of America 2007;104(50):19773-19777.

[2] Merritt ME, Harrison C, Storey CJ, Sherry AD, Malloy CR. Inhibition of carbohydrate oxidation during the first minute of reperfusion after brief ischemia: NMR detection of hyperpolarized $^{13}CO_2$ and $H^{13}CO_3^-$. Magnetic Resonance in Medicine 2008;60(5):1029-1036.