Probing energy metabolism in the ischemic rat heart with hyperpolarized 13C MRS

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Background

The application of MR methods to follow the biological conversion of hyperpolarized ^{13}C -labelled metabolites provides a non-invasive means to study metabolism in real time. The high metabolic activity of the heart makes this technique particularly suited to detect the changes in cardiac metabolism known to result from heart failure or ischemic injury [1,2]. Energy metabolism in ischemic rat hearts has been interrogated with hyperpolarized ^{13}C-labelled pyruvate *ex vivo* [3,4], and its hyperpolarized metabolites have been imaged in the ischemic pig heart *in vivo* [5]. In both, cases an increase in the conversion to labelled lactate vs. bicarbonate is observed, reflecting the expected decrease in pyruvate oxidation. As a widely-used laboratory animal, the rat provides an attractive platform for cardiac metabolic studies, benefiting from well-established disease models and an extensive body of work on its physiology and metabolism.

Methods

Animal Model. Male Wistar rats (n=8, 262 ±14g) were anesthetized with isoflurane and intubated. Catheters were installed in the femoral arteries for blood sampling and invasive blood pressure measurement and in a femoral vein for infusion. Myocardial ischemia was effected via occlusion with a snare installed by passing a suture underneath the left coronary artery. The two ends of the suture were then threaded through a small piece of tubing, to form the snare around the left coronary artery. The reversible occlusion was created by tightening the snare and holding it in place with a clip for 15 minutes (no occlusion in control experiments) and hyperpolarized sodium [1-^{13}C]pyruvate was infused as soon as possible afterward. At the end of the experiment, the heart was stained with Evans blue to assess the extent of the ischemic area.

Polarization. 12 x 10µl glassy beads of a frozen 2.7M sodium [1-^{13}C]pyruvate and 50mM TEMPOL in HOD and glycerol (18% v/v) were polarized with microwave irradiation (197.25 GHz, 55mW) for ~2hr at 1.0K in a custom-built 7T polarizer. The sample was rapidly dissolved in 6mL of preheated D_2O and transferred automatically to a phase separator / infusion pump in the magnet bore [6], where its polarization was ~20%, and 1.7mL was infused IV by remote control.

MR acquisition. Scanning was formed in a 9.4T 31cm horizontal bore magnet with a VNMRS console (Varian). A surface coil with a single loop for ^{1}H and two overlapping loops in quadrature for ^{13}C was positioned over the heart. Breathing motion and blood pressure signals were used for respiratory and cardiac gating. Coil placement was confirmed with ^{1}H anatomical images. Following the hyperpolarized ^{13}C infusion, dynamic series of 40 single pulse ^{13}C spectra were acquired (gated, TR ≈ 3s) using a 30° BIR4 pulse centered at ~175 ppm.

Data analysis. Spectral peaks of metabolites were quantified by fitting using Bayes (Washington University). Areas under curve (AUC) for metabolite hyperpolarized signal time courses were calculated using the spectral signal amplitude and the variable interval between the gated acquisitions. Ratios of ^{13}C-bicarbonate-to-[1-^{13}C]lactate AUCs acquired before and after the ischemic occlusion were compared. Student's *t*-test was used to assess statistical significance.

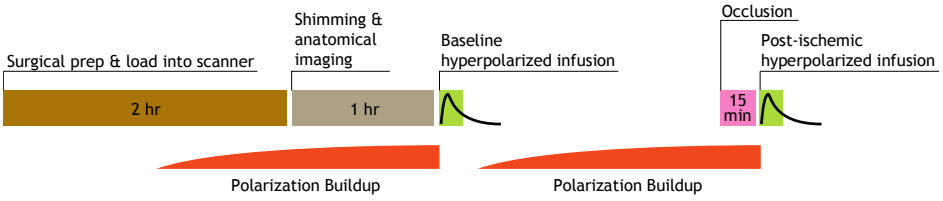

Figure 1. Timeline of hyperpolarized myocardial ischemia experiment

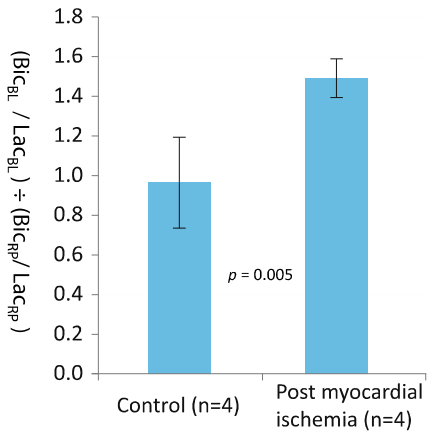

Figure 2. Change in the hyperpolarized bicarbonate-to-lactate ratio before and after 15 min occlusion of the left coronary artery. BL: baseline, RP: reperfusion

Results and Discussion

The conversion of [1-^{13}C]pyruvate to its metabolites [1-^{13}C]lactate, [1-^{13}C]alanine and ^{13}C -bicarbonate was detected before and after myocardial ischemia. Quantitation of the peak areas revealed a significant reduction in the bicarbonate-to-lactate ratio, 1.49 ± 0.10 fold lower, compared to 0.96 ± 0.23 in control experiments ($p < 0.01$), consistent with a shift away from oxidative metabolism and to anaerobic, glycolytic metabolism. While the bicarbonate-to-lactate ratio varied between animals, its variation between consecutive infusions of hyperpolarized [1-^{13}C]pyruvate was smaller and more consistent. The mean delay between the removal of the occlusion and the start of the infusion was 105 s (range: 80-120 s); greater differences may be observed in earlier reperfusion.

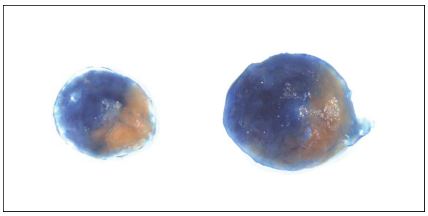

Figure 3. Evans blue-stained heart slices showing extent of unstained ischemic region

Conclusion

This study demonstrates the feasibility of using hyperpolarized ^{13}C MRS to detect metabolic changes in rat myocardial metabolism *in vivo* after a brief ischemic episode, which provides the opportunity to investigate changes in energy metabolism with other probes. Additionally, the functional effects of other interventions, such as metabolism-modulating drugs, can be investigated.

References

[1] J.R. Ussher *et al.*, Heart Metab, 32, 9 (2006). [2] H. Ashrafian *et al.*, Circulation, 116, 434 (2007). [3] M.E. Merritt *et al.*, Magn Reson Med, 60, 1029 (2008). [4] D.R. Ball *et al.*, NMR Biomed, (2013). [5] A.Z. Lau *et al.*, Magn Reson Med, 69, 1063 (2013). [6] A. Comment *et al.*, Concept Magn Reson B, 31B, 255 (2007).