Using Hyperpolarized [1-^{13}C]Pyruvate as a Dynamic Marker for Intracellular pH

M. A. Schroeder[1], H. J. Atherton[1], L. C. Heather[1], M. A. Cole[1], K. Clarke[1], G. K. Radda[1], and D. J. Tyler[1]
[1]Physiology, University of Oxford, Oxford, Oxfordshire, United Kingdom

Introduction

As alterations to both intracellular and extracellular pH are characteristic processes in myocardial ischaemia, the ability to dynamically monitor pH in the heart may have important clinical implications. Recently, Gallagher *et al* demonstrated dynamic imaging of extracellular pH in living animals following the infusion of hyperpolarized bicarbonate and subsequent MR detection of the ratio of $H^{13}CO_3^-$ and $^{13}CO_2$ [1], utilizing the rapid, pH dependant, carbonic anhydrase (CA)-mediated equilibrium between the two species in the blood. This study has been performed to assess the validity of using hyperpolarized [1-^{13}C]pyruvate as a marker of intracellular pH in the isolated perfused heart. Previous work in this system has confirmed that both the $H^{13}CO_3^-$ and $^{13}CO_2$ peaks derived from [1-^{13}C]pyruvate metabolism are detectable with high temporal resolution [2]. However, activity of CA in cardiac myocytes is much lower than in the blood [3] and the time course of intracellular $H^{13}CO_3^-/^{13}CO_2$ equilibration is not clear. Here we have correlated intracellular pH measurements made in healthy hearts using hyperpolarized [1-^{13}C]pyruvate, with measurements made using ^{31}P MR spectroscopy (MRS). Additionally, we have compared the kinetics of pH equilibration in healthy hearts and ischaemic hearts, a state in which intracellular acidosis is expected.

Methods

[1-^{13}C]pyruvate was hyperpolarized in a HyperSense system (Oxford Instruments, UK) by the method of Ardenkjaer-Larsen [4]. Five rat hearts were perfused in the Langendorff mode and placed in the bore of an 11.7 T vertical bore MR scanner. Hyperpolarized [1-^{13}C]pyruvate was infused into the heart while it was functioning normally and spectra were acquired with 1s temporal resolution. A fully relaxed ^{31}P spectrum was then acquired over a 10 minute period from the healthy myocardium and intracellular pH was calculated based on the resonance frequency of the P_i peak. A 10 minute period of no-flow ischaemia was then initiated, followed by a second dose of the same hyperpolarized tracer, infused immediately upon reperfusion. Cardiac ^{13}C MR spectra were analyzed using the AMARES algorithm[5] and pH was calculated by substituting the quantified areas of the $H^{13}CO_3^-$ and $^{13}CO_2$ peaks into the Henderson-Hasselbach equation. pH measurements made using ^{13}C MR were fit to a first-order exponential equation to ascertain the equilibration time of the $H^{13}CO_3^-/^{13}CO_2$ ratio and measurements made in individual hearts were compared to pH measurements made using ^{31}P MRS. In the ischaemic hearts, pH measurements made using ^{13}C MR were fit to a bi-exponential equation to model both ^{13}C equilibration and recovery from acidosis.

Results

pH measured using the $H^{13}CO_3^-/^{13}CO_2$ equilibrium was observed to equilibrate to a value of 7.07 ± 0.02 with a time constant of 3 s (Figure 1, dark line). In a heart-by-heart comparison, the mean pH following pyruvate infusion showed close agreement with measurements of intracellular pH made using ^{31}P MRS (Figure 2). Upon reperfusion following ischaemia, the intracellular pH measured using hyperpolarized ^{13}C MR showed dramatically different equilibration kinetics than in the healthy hearts (Figure 1, light line). When fit to an equation with bi-exponential form, the pH equilibrated with a fast time constant of 3 s, identical to that in the healthy hearts, and a slow time constant of 60s, assumed to be due to pH recovery following ischaemia.

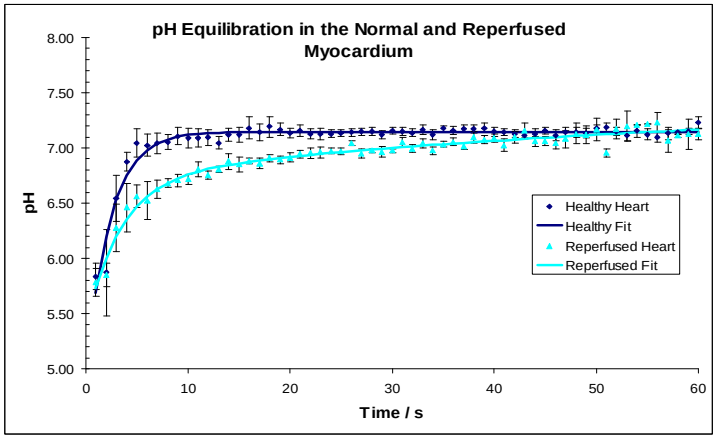

Figure 1 *pH equilibration in the normal and reperfused myocardium, averaged across all 5 hearts, following infusion of hyperpolarized [1-^{13}C]pyruvate. Data are expressed ± SEM.*

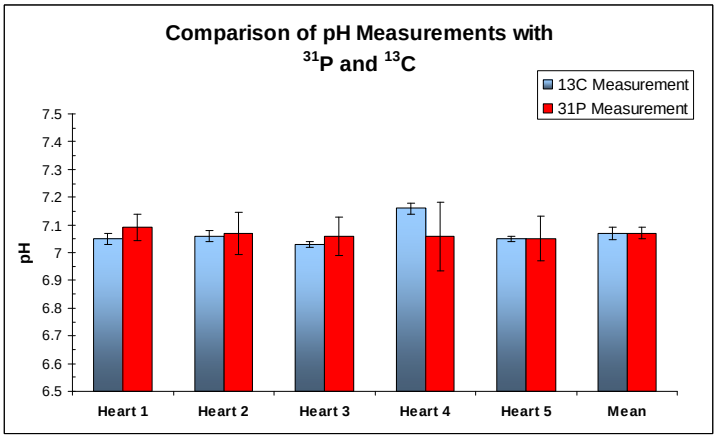

Figure 2 *Comparison of intracellular pH values, as determined by ^{31}P and ^{13}C. ^{13}C measurements were made following pyruvate arrival at the heart. Individual heart data are expressed ±SEM.*

Discussion

Comparison between hyperpolarized ^{13}C MR results and validated ^{31}P MRS measurements would suggest that at physiological pH values, hyperpolarized [1-^{13}C]pyruvate offered an accurate measure of intracellular pH approximately 15 s after pyruvate infusion. Additionally, this study has indicated that post-ischaemia $H^{13}CO_3^-$ and $^{13}CO_2$ equilibrate according to dramatically different kinetics than in healthy hearts. In healthy hearts, the pH equilibration time most likely reflected the level of intracellular cardiac CA activity. In reperfused hearts two simultaneous recovery processes were observed: pH equilibration via CA and recovery from acidosis. By mathematically correcting for the pH equilibration process, it seems feasible that hyperpolarized ^{13}C MR may be useful for the dynamic measurement of intracellular pH with a sub-second temporal resolution. This information could be useful in monitoring alterations to cardiac metabolism and ion exchange processes that occur due to ischaemic heart disease. In the future this study will be extended over a wider pH range, to confirm that the [1-^{13}C]pyruvate-derived $H^{13}CO_3^-/^{13}CO_2$ ratio does purely reflect intracellular pH and excludes contributions from extracellular pH that may occur due to low intracellular cardiac CA activity and rapid cellular CO_2 efflux.

References

1. Gallagher, F.A., et al., Nature, 2008. **453**(7197): p. 940-3.
2. Merritt, M.E., et al., PNAS USA, 2007. **104**(50): p. 19773-7.
3. Leem, C.H. & R.D. Vaughan-Jones, J Physiol, 1998. **509 (Pt 2)**: p471-85.
4. Ardenkjaer-Larsen, J.H., et al., PNAS USA, 2003. **100**(18): p. 10158-63.
5. Naressi, A., et al., Comput Biol Med, 2001. **31**(4): p. 269-86.
6. Schroeder, M.A., et al., PNAS USA, 2008. **105**(33): p. 12051-6.

Acknowledgements - This study was supported by the Medical Research Council, the British Heart Foundation and GE Healthcare.