Effect of Epinephrine on Metabolism of HP [1-^{13}C]pyruvate in Low-flow Myocardial Ischemia

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Introduction

Adrenergic agents are widely used to simultaneously increase oxygen demand and mechanical performance of the heart. This intervention is used for assessing two conditions: flow-limiting coronary artery disease, or confirming viable (also termed hibernating) myocardium prior to revascularization. However, the effects of adrenergic stimulation on mechanical function reflect both metabolic and nonmetabolic factors. The use of hyperpolarized (HP) substrates directly probes the metabolic consequences of adrenergic stimulation rather than indirectly assessing metabolism based on mechanical function. In this study, we tested whether metabolism of HP [1-^{13}C]pyruvate is sensitive to adrenergic stimulation, low flow ischemia, or both.

Experimental Methods

[1-^{13}C]pyruvic acid was polarized in a HyperSense using standard methods. Hearts excised from male Sprague-Dawley rats were placed in an 18-mm NMR tube attached to a water-jacketed glass perfusion apparatus bubbled continuously with 95:5 O_2/CO_2 and placed inside the bore of a 9.4T vertical-bore magnet. Hearts were studied using standard Langendorff methods at 37°C in four groups:

1) normal perfusion pressure, 100 cm H_2O;
2) normal perfusion with epinephrine;
3) low perfusion pressure (LPP), 25 cm H_2O;
4) low perfusion pressure with epinephrine.

All hearts were initially perfused at 100 cm H_2O and supplied with Krebs-Henseleit buffer containing 0.75% bovine serum albumin, 0.4 mM non-labeled free fatty acid, 5.5 mM [U-^{13}C]glucose, 1 mM [3-^{13}C]pyruvate, 0.1 mM [3-^{13}C]lactate. Then the perfusion pressure was reduced to 25-cm H_2O for the ischemia group after 30 min. In the stimulated hearts (groups 2 and 4), epinephrine (1 μM) was added to the perfusate at 50 min of perfusion. All hearts were monitored continuously by ^{31}P NMR. After 1 h, HP[1-^{13}C]pyruvate was injected directly above the heart using a catheter committedly and ^{13}C acquisition was initiated. Hearts were freeze-clamped for later analysis. All ^{13}C NMR spectra were acquired with decoupling using 20° pulses, 1-s acquisition time, and 1-s delay time. FIDs were zero-filled before Fourier transformation and relative peak areas in the phased spectra were measured by integration. The area of each metabolite resonance was normalized by the total area of all metabolite resonances and plotted as a function of time. The heart tissues were extracted with perchloric acid and analyzed by high resolution ^{1}H NMR (600 MHz).

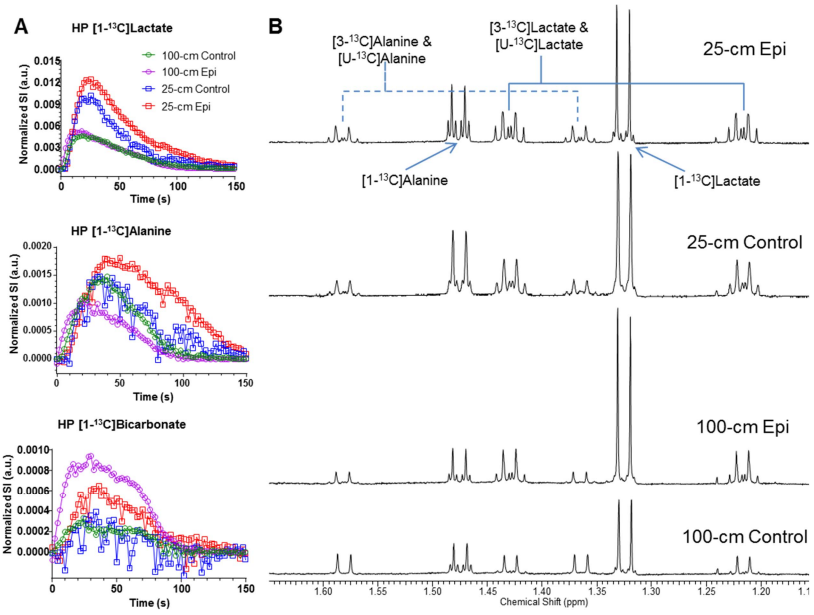

Figure. (A) Representative plots of normalized ^{13}C intensities versus time of HP [1-^{13}C]lactate, [1-^{13}C]alanine, and [^{13}C]bicarbonate of hearts perfused at 100-cm or 25-cm H_2O pressure, with or without epinephrine stimulation. (B) Representative high resolution ^{1}H NMR of tissue extracts from hearts perfused under normal or low pressure, with or without epinephrine.

Results and Discussion

Baseline heart rate was 280 ± 9 bpm and coronary flow was 17.4 ± 1.7 mL/min. Under normal perfusion pressure, epinephrine increased heart rate (+23%) and coronary flow (+11%) as expected. Also as expected, LPP reduced HR (-67%) and coronary flow (-82%). In the setting of LPP, epinephrine increased HR (+60%) but did not alter coronary flow. ^{31}P NMR spectroscopy confirmed significant myocardial ischemia under LPP conditions, demonstrated by a marked increase in [inorganic phosphate]/[ATP]. As shown in the Figure, epinephrine caused an increase in HP [^{13}C]bicarbonate appearance under normal perfusion pressure conditions, from 1.2 ± 0.4% to 3.1 ± 1.4% of total signal ($p<0.04$), as would be expected for increased flux through pyruvate dehydrogenase. There was a trend for increased lactate appearance in LPP hearts, but this effect did not reach statistical significance. Epinephrine had no effect on the rate of bicarbonate production under LPP. In the ^{1}H NMR spectra, the satellites are due to [3-^{13}C]alanine, [3-^{13}C]lactate, [U-^{13}C]alanine and [U-^{13}C]lactate. Uniformly ^{13}C-labeled lactate and alanine were derived from perfusate [U-^{13}C]glucose. Epinephrine stimulated glycogenolysis (note the increase in ^{12}C lactate) under normal perfusion pressure conditions but not during ischemia. Unexpectedly, epinephrine stimulated incorporation of HP[1-^{13}C]pyruvate to enter the alanine but not lactate pool (see the 3-bond ^{13}C-^{1}H coupling).

Conclusions

During normal perfusion conditions, epinephrine increased glycolysis to lactate, glycogenolysis to lactate, and production of HP bicarbonate from [1-^{13}C]pyruvate. During reduced coronary flow, epinephrine had little effect on the HP signals after HP[1-^{13}C]pyruvate, likely because coronary flow and therefore oxygen delivery could not increase in response to epinephrine. Exogenous pyruvate is preferentially converted to alanine rather than lactate during ischemia after epinephrine, indicating compartmentation of HP pyruvate metabolism in ischemic heart tissue.

References

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