Malate-Aspartate Shuttle Reversal Allows for Lactate Concentration Increases Upon Rapid Changes in ^{13}C Pyruvate Concentration

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Introduction

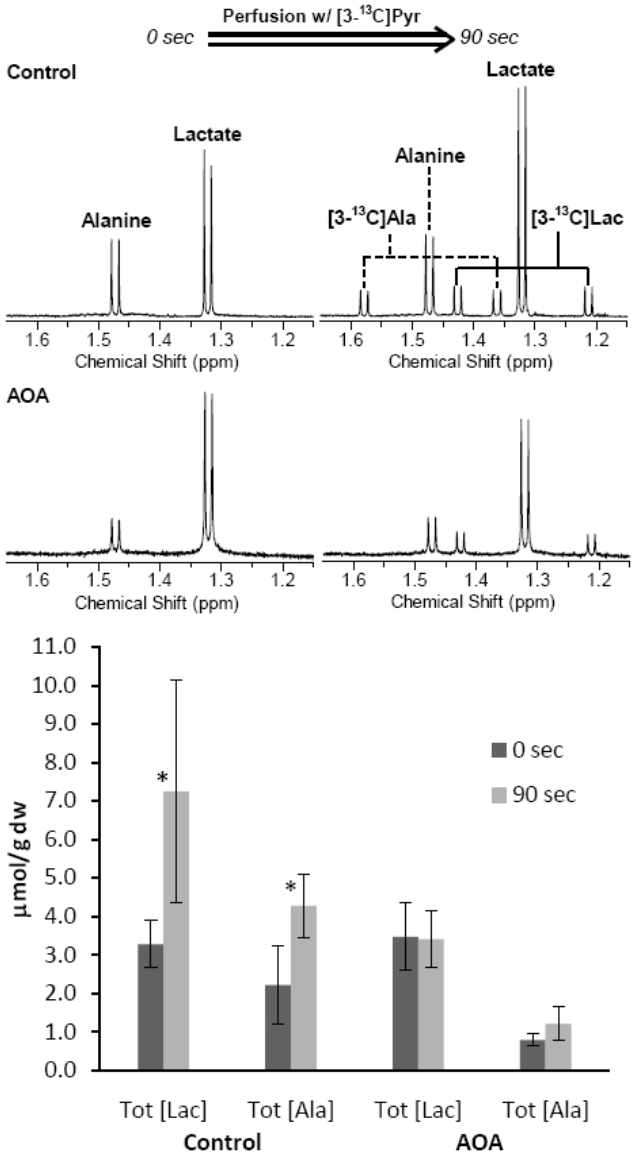
The appearance of hyperpolarized [1-^{13}C]lactate may provide diagnostic information in metastatic cancer or the ischemic myocardium after injection of hyperpolarized (HP) [1-^{13}C]pyruvate. The amount of [1-^{13}C]lactate in tissue is of course the product: (fractional enrichment) * [lactate]. This simple relationship means that the appearance of HP [1-^{13}C]lactate, neglecting T_1 effects, will be sensitive to both the size of the exchanging lactate pool and the rate of entry of ^{13}C into the lactate pool. The sizes of the lactate and alanine pools, in turn, are sensitive to the [pyruvate] because of the high activity of lactate dehydrogenase and alanine aminotransferase in most tissues. Since the time course of a typical HP ^{13}C study is limited to about 120 seconds because of T_1 effects, it is not always clear whether the HP ^{13}C lactate (or alanine) signal has a contribution from changing pool size, exchange of isotope into the pool, or some combination. This information is essential for proper kinetic models designed to analyze HP ^{13}C data. The purpose of this study was to test whether pool sizes change significantly within 90 seconds after exposure to low concentrations of pyruvate in a highly metabolically active model.

Methods

Hearts were excised from anesthetized Sprague-Dawley rats (300-350 g) and perfused using standard Langendorff methods with Krebs-Henseleit medium. Two experiments were examined: 1) 'Bolus': glucose (Glc, 5 mM) alone followed by Glc + 2 mM [3-^{13}C]Pyr, 2) 'Aminotransferase Inhibition': Glc + aminooxyacetate (AOA, 0.5 mM) followed by Glc + AOA + 2 mM [3-^{13}C]Pyr. Four groups were studied (n=5 in each group): *Group 1*, perfused with Glc alone for 30 min; *Groups 2*, perfused with Glc for 30 min and then switched to [3-^{13}C]Pyr for 90 seconds; *Group 3,* perfused with Glc plus AOA for 30 min; *Groups 4*, perfused with Glc plus AOA for 30 min and then switched to Glc plus AOA plus [3-^{13}C]Pyr for 90 seconds. At the end of the perfusion period hearts were freeze-clamped. ^{1}H NMR spectra were obtained of the perchloric acid extracts (dissolved in $^{2}H_2O$ and spiked with an internal standard, 2,2-dimethyl-2-silapentane-5-sulfonic acid, DSS). The concentrations of ^{13}C and ^{12}C alanine and lactate were determined using Chenomx Software and analysis of the ^{13}C satellites in the ^{1}H NMR spectrum.

Results

Switching perfusion from unlabeled substrates to [3-^{13}C]Pyr, changes the ^{1}H spectrum of the methyl group of alanine and lactate from the typical doublet to one that also contains a doublet of doublets due to the larger $^{1}J_{C\text{-}H}$ coupling (see figure to the right). In controls, lactate and alanine pool nearly double over 90 seconds. In the presence of AOA, no change in pool sizes was observed for either lactate or alanine over the same 90 second period.



Panels from top to bottom: ^{1}H Spectra of lactate and alanine before and after perfusion with [3-^{13}C]pyruvate, concentration of total lactate and alanine. * $P < 0.05$ vs 0 sec.

Conclusions

The control experiment closely models a typical *in vivo* study, a bolus administration or rapid increase in concentration of HP [1-^{13}C]pyruvate at the tissue of interest. Small but significant increases in substrate pool sizes were observed under these conditions. During the inhibition of aminotransferases by AOA, enzymes also involved in the malate-aspartate shuttle, an increase in [alanine] was not observed, but, unexpectedly, [lactate] did not increase either. Alanine production was inhibited due to aminotransferase inhibition, but lactate production was inhibited due to the lack of NADH replenishment from the malate-aspartate shuttle. These results support a reversal of the malate-aspartate shuttle to provide NADH for the production of lactate following a rapid increase in [pyruvate].