Detection of lactate dehydrogenase reaction *in vivo* using carbon-13 magnetization transfer

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Introduction

Recently, carbon-13 magnetic resonance spectroscopy has been applied to studying enzymology *in vivo* [1]. As a result, the cerebral aspartate transaminase reaction has been detected and quantified by saturating the carbonyl carbon resonances of α-ketoglutarate and oxaloacetate. Here we report *in vivo* detection of cerebral lactate dehydrogenase reaction in rats treated with bicuculline using carbon-13 magnetization transfer (CMT).

Methods

Male adult Sprague-Dawley rats (180-200 g, n = 4) were anaesthetized with halothane. Both femoral veins were cannulated for the intravenous infusion of [2-^{13}C]glucose and administration of bicuculline or saline, and one artery was cannulated for intermittent sampling of blood for the measurement of plasma gases and glucose concentrations. After surgery, halothane was reduced to 1%. All experiments were performed on a Bruker 11.7 T AVANCE spectrometer interfaced to an 89 mm i.d. vertical-bore magnet. Two concentric surface coils (^{13}C with 10.8 mm i.d./4.3 mm width, ^{1}H with 23.6 mm i.d./5.4 mm width) were used. The coils were positioned approximately 0-1 mm posterior to bregma. After adjustment of NMR parameters, 99% enriched [2-^{13}C]-glucose (20 % wt/vol) was infused i.v. at 8 ml/hr over 5 minutes, followed by a continuous infusion at a lower rate which was adjusted over a small range to maintain plasma glucose concentration at 22.4 ± 3.1 mM. Before bicuculline injection, 1.0 ml blood was withdrawn 3 min after the beginning of glucose infusion to limit the peak rise in arterial blood pressure during bicuculline-induced seizure. Bicuculline (1 mg/kg) was injected 6 min after the start of [2-^{13}C]glucose infusion. Additional bicuculline (0.5 mg/kg per dose) was administrated to maintain elevated brain lactate level during data acquisition. For the CMT experiment, a 1-ms AHP pulse was used for 90^{o} excitation. WALTZ-4 with a 400 μs nominal 90^{o} rectangular pulse was used for ^{1}H decoupling. Broadband ^{1}H→^{13}C NOE was generated using a train of non-selective hard pulses with a nominal flip angle of 180^{o} spaced at 100 ms apart. When saturation transfer spectra were acquired, ^{13}C RF saturation of the carbonyl carbon (C2) of pyruvate at 207.9 ppm was performed with nominal γB_{1sat} = 315 Hz. When control spectra were acquired, the saturating pulse was placed at an equal spectral distance from the observed spin but on the opposite side of pyruvate C2. The saturated and control spectra were interleaved using the following scheme: (control-saturated-saturated-control)$_{NS/4}$. TR = 10 s, NS =128. The ^{13}C carrier frequency was centered around lactate C2 at 69.33 ppm.

Results

The initial bicuculline treatment led to a rapid increase in mean arterial blood pressure from 144-174 mm Hg (baseline) to 159-243 mm Hg and a rapid decrease in mean heart rate from 383-469 BPM (baseline) to 253-420 BPM. Both mean arterial blood pressure and heart rate returned to the baseline values ~4 min after bicuculline administration. Blood gasses were maintained within normal physiology limits (PCO_2 = 35-45 mm Hg, PO_2> 100 mm Hg) with few exceptions. Blood pH was 7.13±0.05. Body temperature was maintained at ~37.5 ^{o}C. Fig. 1(a) shows the CMT effect due to rapid exchange between pyruvate and lactate. A 30 Hz exponential line broadening was applied. At the end of the study, the saturation transfer experiment was repeated 15-min after intravenous injection of KCl. The postmortem results are shown in Fig. 1(b) where no CMT effect was detected due to postmortem depletion of pyruvate. All experimental and processing parameters in Fig 1(b) were kept the same as those in Fig. 1(a). The difference spectra showed significant intensity change in lactate C2 at 69.33 ppm in live rats only. The possible contribution from the non-specific off-resonance magnetization transfer effect presumably due to a small, immobilized lactate pool was investigated by shifting the frequency of the pyruvate saturation pulse by ±100 kHz while keeping the frequency of the continuous irradiation pulse in the control scans unchanged. No significant non-specific off-resonance ^{13}C magnetization transfer effect was detected for either glucose or lactate in the difference spectra (Fig. 2).

Discussion

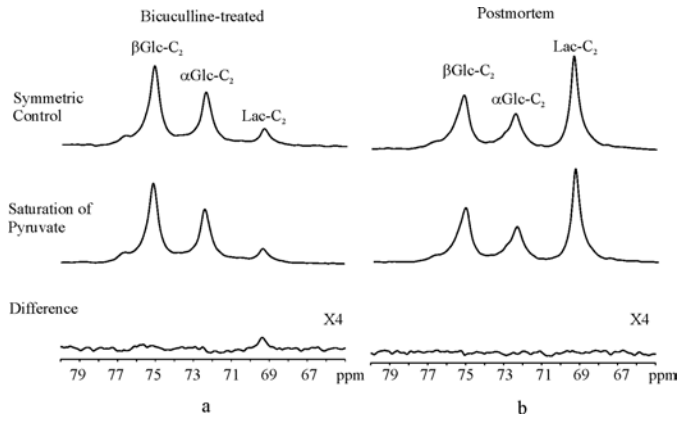

Fig. 1. ^{13}C spectra (summed from 4 rats) showing the CMT effect due to rapid exchange between pyruvate and lactate. Upper: control; middle: saturation of pyruvate; lower: difference.

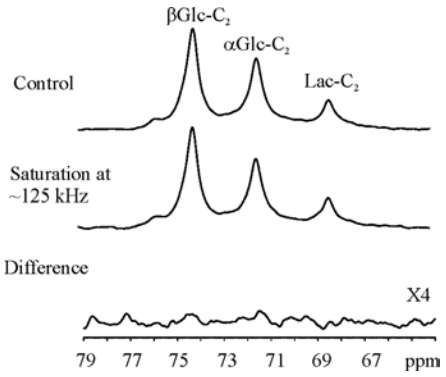

Fig. 2. Spectra showing no significant nonspecific off-resonance ^{13}C magnetization transfer effect.

The methyl proton of lactate at 1.32 ppm has been shown to be affected by off-resonance RF irradiation presumably resulted from a small pool of immobilized lactate [2]. Strong evidence has shown that the nonspecific off-resonance magnetization transfer effect of the methyl protons of lactate is, at least partially, via protein-mediated magnetic coupling to water [3]. Since protons are saturated by the broadband NOE and WALTZ decoupling pulses used in measuring the CMT effect, protein-mediated magnetic coupling between lactate and water protons was quenched by proton saturation. The negative result observed in Fig. 2 indicates that the nonspecific off-resonance MT effect at the pyruvate C2 frequency due to immobilized lactate contributes negligibly to the observed CMT effect originated from lactate dehydrogenase-catalyzed fast exchange between pyruvate and lactate. The positive result in Fig 1(a) demonstrates that the CMT effect of lactate dehydrogenase reaction is measurable *in vivo* after raising the lactate level by intravenous infusion of bicuculline. Lactate is known to be significantly elevated in a variety of physiological and pathological conditions (e.g., in the brain of panic disorder patients, in exercised muscle and in various cancerous tissues). A non-invasive MRS method for *in vivo* detection of lactate dehydrogenase reaction should be very useful. In particular, the activity and isoenzyme composition of lactate dehydrogenase is known to be altered dramatically in cancerous tissues. Therefore, the magnetization transfer effect of the lactate dehydogenase reaction may be used as a valuable marker for cancer accessible to noninvasive MRS techniques.

References

1. Shen, Magn Med Reson 54:1321 (2005). 2. de Graaf et al, Magn Reson Med 41:1136 (1999). 3. Swanson, J Magn Reson 135:248 (1998).