Brain metabolism under different anesthesia using hyperpolarized [1-^{13}C]-pyruvate

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Introduction

The low sensitivity of ^{13}C spectroscopy can be enhanced using hyperpolarization techniques such as dynamic nuclear polarization (DNP) (1). Detection of [1-^{13}C]pyruvate and its metabolic products including bicarbonate have been reported in the brain (2,3).

In this work, we investigate the ability to detect bicarbonate under different anesthesia and modeling of that signal to determine values of rate constants of bicarbonate and lactate production.

Methods

A mixture of [1-^{13}C]pyruvic acid and OX63 trityl radical was hyperpolarized by DNP (Hypersense, UK) for 90 min in a field strength of 3.35 T at approximately 1.4 K. The sample was then dissolved in 40 mM TRIS buffer, 40 mM NaOH and 0.32 mM Na_2EDTA solution to produce 4 mL of hyperpolarized solution at a concentration of ~35 mM. *In vivo* experiments were performed using a 9.4-T/31-cm bore magnet equipped with a Varian Direct Drive spectrometer. Four fasted male Sprague-Dawley rats were injected intravenously with approximately 2.2 mL of hyperpolarized [1-^{13}C]pyruvate under alpha-chloralose, pancuronium and morphine, isoflurane and pentobarbital anesthesia. The number of injections for each anesthesia is reported in Table 1.

In vivo decoupled-^{13}C NMR spectra were acquired using a coil assembly consisting of a ^{1}H quadrature surface coil (two loops of 14 mm diameter) and an inner ^{13}C linearly polarized surface coil (12 mm diameter). Time courses were acquired with pulse-acquire with a pulse of 43° at the center of the coil.

The model included apparent unidirectional flux from pyruvate to lactate ($k_{pyr\text{-}lac}$) and the apparent unidirectional flux from pyruvate to bicarbonate ($k_{pyr-HCO_3}$) (4) as it has been shown with physiologically-based model (2) that bicarbonate is produced mostly in the brain. Identical T_1 was assumed for lactate, and bicarbonate. The pyruvate signal was used as input function. Three parameters were fitted: $k_{pyr\text{-}lac}$, $k_{pyr-HCO_3}$, T_1. One-way analysis of variance (ANOVA) was used to compare rate constants and T_1 for each anesthesia using SAS Software for Windows.

Results

The bicarbonate signal was observed in all animals. Figure 1 shows representative time courses of bicarbonate signals scaled by maximum pyruvate signal for different anesthesia. Figure 2 shows representative experimental time courses fitted with a two compartment model. Table 1 reports the apparent rate constants for conversion of pyruvate to lactate and for conversion of pyruvate to bicarbonate. The $k_{pyr\text{-}lac}$, $k_{pyr-HCO_3}$, and T_1 are statistically different between different anesthesia with p values of 0.018, 0.0022, 0.01 respectively. $K_{pyr\text{-}lac}$ was significantly greater for isoflurane than that observed with pancuronium/morphine while $k_{pyr-HCO_3}$ was significantly lower for pentobarbital than all other tested anesthesia. T_1 was significantly greater for pentobarbital than alpha-chloralose.

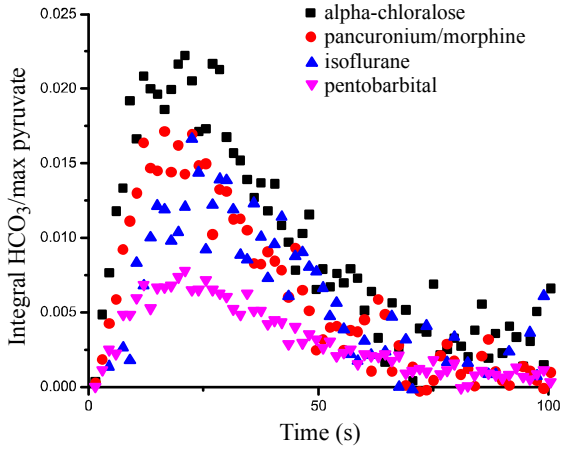

Figure 1. *In vivo* time courses of bicarbonate measured in four rats after injection of hyperpolarized [1-^{13}C]pyruvate into femoral vein under different anesthesia. T_R = 1.5 s.

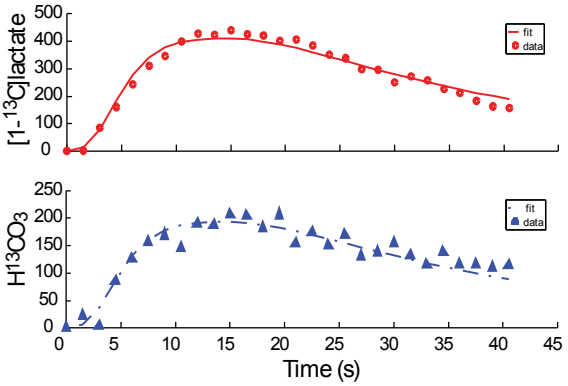

Figure 2. Experimental time courses fitted with a two compartment model.

Discussion and Conclusions

The rate of conversion of pyruvate to bicarbonate was sensitive to anesthesia. The lowest rate was observed for pentobarbital, which is known to strongly depress brain metabolism. In contrast, the rate of conversion from pyruvate to lactate was relatively insensitive to anesthesia. These results suggest that bicarbonate production better reflects downregulated brain energy metabolism than lactate dehydrogenase activity.

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References: 1. Abragam and Goldman, *Rep Prog Phys* 1978. **2.** Marjanska *et al.*, *J Magn Reson Med* 2010. **3.** Hurd *et al.*, *Magn Reson Med* 2010. **4.** Wiesinger et al., *Proc. Intl. Magn. Reson. Med.* **18**, 3282 (2010).

Table 1. Kinetic rate constant measured under different anesthesia in one animal per anesthesia.

anesthesia	# injections	$k_{pyr\text{-}lac}$	$k_{pyr-HCO_3}$	T_1 (s)
alpha-chloralose	5	0.0163 ± 0.0014	0.0045 ± 0.0009	17 ± 2
pancuronium/morphine	4	0.0125 ± 0.0018	0.0049 ± 0.0013	19 ± 1
isoflurane	3	0.0179 ± 0.0039	0.0042 ± 0.0015	20 ± 2
pentobarbital	4	0.0139 ± 0.0006	0.0015 ± 0.0012	23 ± 2