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Brain metabolism under different anesthetic conditions using hyperpolarized [1-¹³C]pyruvate and [2-¹³C]pyruvate

Małgorzata Marja ska^a, Alexander A. Shestov^b, Dinesh K. Deelchand^a, Emily Kittelson^a, and Pierre-Gilles Henry^a

^aCenter for Magnetic Resonance Research and Department of Radiology, University of Minnesota, 2021 6th ST SE, Minneapolis, Minnesota 55455, United States

^bDepartment of Radiology, University of Pennsylvania, 423 Guardian Drive, Philadelphia, PA 19104, United States

Abstract

Carbon-13 NMR spectroscopy (¹³C MRS) offers the unique capability to measure brain metabolic rates *in vivo*. Hyperpolarized ¹³C reduces the time required to assess brain metabolism from hours to minutes compared to conventional ¹³C MRS. This study investigates metabolism of hyperpolarized [1-¹³C]pyruvate and [2-¹³C]pyruvate in the rat brain *in vivo* under various anesthetics: pentobarbital, isoflurane, α-chloralose, and morphine. The apparent metabolic rate from pyruvate to lactate modeled using time courses obtained after injection of hyperpolarized [1-¹³C]pyruvate was significantly greater for isoflurane than for all other anesthetic conditions, and significantly greater for morphine than for α-chloralose. The apparent metabolic rate from pyruvate to bicarbonate was significantly greater for morphine than for all other anesthetic conditions, and significantly lower for pentobarbital than for α-chloralose. Results show that relative TCA rates determined from hyperpolarized ¹³C data are consistent with TCA cycle rates previously measured using conventional ¹³C MRS under similar anesthetic conditions, and that using morphine for sedation greatly improves detection of downstream metabolic products compared to other anesthetics.

Keywords

hyperpolarized ¹³C MRS; brain metabolism; lactate; bicarbonate

Introduction

Conventional ¹³C NMR spectroscopy offers the unique capability to measure metabolic fluxes noninvasively *in vivo*. However, measurement of metabolic rates requires long data acquisition times (> 1 h) and large amounts of highly enriched ¹³C-labelled substrates¹.

Hyperpolarization using dynamic nuclear polarization (DNP) yields dramatic ¹³C signal enhancement and reduces the time required to assess brain metabolism from hours to

minutes². Nonetheless, relatively few studies have been performed in the intact, healthy brain using substrates hyperpolarized with DNP²⁻⁸. This is due in part to the relatively slow uptake of hyperpolarized substrates in the healthy brain due to the blood-brain-barrier.

Assessment of metabolic activity using hyperpolarized ¹³C relies on the ability to measure the conversion of the hyperpolarized substrate into metabolic products. When using hyperpolarized [1-¹³C]pyruvate, carbon from pyruvate enters the TCA cycle by conversion to acetyl Co-A and hyperpolarized CO₂ (in equilibrium with bicarbonate). Therefore, in principle, the time course of hyperpolarized bicarbonate directly reflects the TCA cycle activity² since in the brain nearly all acetyl Co-A enters the TCA cycle. However, the extent to which hyperpolarized bicarbonate data can yield a quantitative estimate of the TCA cycle rate in the brain has not been validated.

Similarly, the use of hyperpolarized [2-¹³C]pyruvate and [1-¹³C]acetate generates metabolic hyperpolarized products 2-oxoglutarate (2OG) and glutamate^{6,7}. Pyruvate and acetate might provide different information since pyruvate is primarily metabolized by neurons, while acetate is metabolized by glia. No previous study has examined the effect of different anesthetics on the detection of metabolic products from hyperpolarized [2-¹³C]pyruvate.

In this study, we investigated metabolism in the rat brain *in vivo* under different levels of brain activity, as measured by the TCA cycle rate in previous experiments with conventional ¹³C MRS¹. The measurements were performed following injection of hyperpolarized [1-¹³C]pyruvate and [2-¹³C]pyruvate, and various levels of brain energy metabolism were achieved using different anesthetics.

Experimental

Samples

Samples of pure [1-¹³C]pyruvic acid (Cambridge Isotope Laboratories, Andover, MA) and pure [2-¹³C]pyruvic acid (Isotec, Miamisburg, OH) mixed with 15 mM trityl radical OX63 were hyperpolarized using a HyperSense DNP system (Oxford Instruments, UK) for 60 min and 90 min, respectively. [1-¹³C]pyruvate and [2-¹³C]pyruvate samples were then dissolved in TRIS buffer, NaOH and Na₂EDTA solution, to produce 4 mL of hyperpolarized solutions at a concentration of ~35 mM and ~105 mM, respectively, and a pH of 7. For varied concentrations study, [1-¹³C]pyruvate samples of 21.2 mM, 36.6 mM, 72.3 mM were also used.

Animals

All animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Male Sprague-Dawley rats (n = 26; weight = 249 ± 23 g, mean ± SD; Charles River Laboratories) were intubated and ventilated with a 70%:30% N₂O:O₂ mixture and 1.8% isoflurane. Body temperature was maintained at 37°C using a heating pad with warm water circulation. One femoral vein and both femoral arteries were cannulated for infusion of hyperpolarized substances, blood pressure monitoring, and blood sampling, respectively. If isoflurane was used for the entire experiment, the isoflurane level

was set to 1.5% after completion of surgery. If another anesthetic was used, the isoflurane was discontinued after the surgery and replaced by intravenous administration of either pentobarbital (low dose: bolus - 30 mg/kg, then 30 mg/kg/h; high dose: bolus - 70 mg/kg, then 70 mg/kg/h), α -chloralose (bolus - 50 mg/kg, then 25.4 mg/kg/h), or morphine sulfate and pancuronium mixture (bolus - 50 mg/kg morphine sulfate and 1.0 mg/kg pancuronium, then 35.2 mg/kg/h in a molar ratio of 3:1) with a syringe pump (Model '11' Plus, Harvard Apparatus, Holliston, MA). In such cases, at least one hour elapsed between switch in anesthetics and injection of hyperpolarized ^{13}C -pyruvate, which is sufficient to achieve a new metabolic steady-state^{9,10}. Ventilation was continued with a mixture of 30% O_2 and 70% N_2O with all four anesthetics. Animals were placed in a home-built holder, and the head position was fixed using ear rods and a bite-bar. Blood gases were measured every 20 minutes and ventilation rate was adjusted to maintain stable physiological conditions. The respiration rate was on average 26 breaths per minute for all anesthetics except for morphine for which it was 40 breaths per minute.

Following MR adjustments, animals were injected intravenously with hyperpolarized samples. Fourteen animals were injected with hyperpolarized $[1-^{13}\text{C}]$ pyruvate samples (~2.2 mL, 35 mM), and seven with hyperpolarized $[2-^{13}\text{C}]$ pyruvate samples (~2.2 mL, 105 mM). Additional five animals under pancuronium/morphine anesthesia were each injected with three samples of varied concentrations of hyperpolarized $[1-^{13}\text{C}]$ pyruvate (~2.2 mL, 21.2 mM, 36.6 mM, 72.3 mM). The order in which three different concentration samples were injected was randomized and at least 70 minutes elapsed between each injection. Injections started about 18 s after the dissolution, lasted for ~6 s and did not affect the physiology of the animals.

In Vivo Spectroscopy

All ^{13}C spectra were acquired on a 9.4-T, 31-cm horizontal bore magnet (MagneX Scientific, Oxford, UK) interfaced with an Agilent Direct Drive console (Agilent, Santa Clara, CA, USA). The radiofrequency coil assembly consisted of an inner ^{13}C linearly polarized surface coil (12 mm diameter) and a ^1H quadrature surface coil (two loops of 14 mm diameter). A sealed external reference sphere of 4.1 mm diameter containing the 99% enriched $[^{13}\text{C}]$ -formic acid (Aldrich Chemical Co., Milwaukee, WI) was attached in the center of the inner carbon coil.

Spectra (30,000 complex points, spectral width 50 kHz) were acquired using a pulse-acquire sequence with a 10 μs , 43° flip angle square pulse at the coil center (on average 18° in the brain area detected by the coil), and ^1H decoupling during acquisition. $[1-^{13}\text{C}]$ pyruvate data were acquired with $T_R = 1.5$ s and 128 scans, and $[2-^{13}\text{C}]$ pyruvate data were acquired with $T_R = 1$ s and 128 scans.

Modeling

A two-pool linear model was used to fit the hyperpolarized ^{13}C time courses of $[1-^{13}\text{C}]$ lactate and ^{13}C -bicarbonate obtained following $[1-^{13}\text{C}]$ pyruvate injection to determine the apparent metabolic rates from pyruvate to lactate (rate constant $k_{P,L}$) and from pyruvate to bicarbonate ($k_{P,B}$):

$$\frac{dLac}{dt} = k_{P-L} * Pyr - \frac{1}{T_{1Lac}} * Lac \quad (1)$$

$$\frac{dHCO_3^-}{dt} = k_{P-B} * Pyr - \frac{1}{T_{1HCO_3^-}} * HCO_3^- \quad (2)$$

where Pyr , Lac , and HCO_3^- are peak integrals of hyperpolarized pyruvate, lactate and bicarbonate, respectively, normalized by the averaged integral of formate (external reference); t is time; and T_{1Lac} and $T_{1HCO_3^-}$ are spin-lattice relaxation times for lactate and

bicarbonate, respectively. The rate constants k_{P-L} and k_{P-B} reflect the combined effect of transport to the brain through the blood-brain-barrier, transport into cells and cell organelles, and lactate dehydrogenase (LDH) and pyruvate dehydrogenase (PDH) complex activities in cytosol and mitochondria, respectively. Note that k_{P-L} and k_{P-B} are “apparent” rate constants, but the actual reaction rates may vary with time.

The model assumes that the contribution of brain pyruvate to the total pyruvate is negligible compared to plasma pyruvate. Simulation of pyruvate transport with K_M , V_{max} and K_d using previously published values¹¹ shows that the concentration of brain ^{13}C pyruvate is ~100 times smaller than plasma ^{13}C -pyruvate signal (not shown). Assuming that 10% of the MR signal detected by the surface coil comes from the blood (based on the rat's head anatomy and the sensitivity profile of the surface coil), the contribution of brain ^{13}C pyruvate to the total ^{13}C -pyruvate signal would be only ~10%. Similarly, the model assumes that all detected lactate signal comes from the brain. This is justified by the lack of detection of hyperpolarized ^{13}C lactate in the blood using an implanted arterial coil¹². Finally, constant concentrations of lactate and bicarbonate, and $T_{1Lac} = T_{1HCO_3^-} = T_1$ were assumed. Any loss

of ^{13}C bicarbonate or lactate (e.g., through dilution of unlabeled molecules) was incorporated into the apparent T_1 . The normalized hyperpolarized ^{13}C pyruvate time courses were used as an input function to fit lactate and bicarbonate time courses. Three parameters were fitted, k_{P-L} , k_{P-B} , and T_1 .

Similarly, equation 1 was used to fit the hyperpolarized ^{13}C time courses of $[2-^{13}C]$ lactate obtained following $[2-^{13}C]$ pyruvate injection to determine the apparent metabolic rates from pyruvate to lactate (rate constant k_{P-L}).

The system of linear differential equations was solved with a Runge-Kutta 4th order procedure. Least-squares minimization was performed with BFGS or Simplex algorithms. The errors for the fitted parameters were estimated using Monte Carlo simulations with experimental noise levels. All numerical procedures were carried out in Matlab (The MathWorks, Inc., Natick, MA, USA).

Statistical Analysis

Statistical analysis was conducted using SAS Software for Windows (version 9.1, SAS Institute, Cary, NC, USA). For $[1-^{13}\text{C}]$ pyruvate data, one-way analysis of variance (ANOVA) was used to compare rate constants and T_1 for each anesthetic condition. For $[2-^{13}\text{C}]$ pyruvate data, unpaired, two-tailed Student's t-tests were used to compare rate constants and T_1 for each anesthetic condition.

Results

After injection of hyperpolarized $[1-^{13}\text{C}]$ pyruvate, resonances corresponding to $[1-^{13}\text{C}]$ pyruvate, $[1-^{13}\text{C}]$ pyruvate hydrate, $[1-^{13}\text{C}]$ lactate and ^{13}C -bicarbonate were observed in the rat brain *in vivo* under all anesthetic conditions. Representative time courses of bicarbonate signal with different anesthetics are shown in Figure 1A. The maximum bicarbonate signal was observed ~22 s after beginning of the injection for all anesthetics. In addition, the $^{13}\text{CO}_2$ signal was observed only in the morphine group (Figure 1B). A two-pool linear model was used to simultaneously fit the time courses of the hyperpolarized $[1-^{13}\text{C}]$ lactate and ^{13}C -bicarbonate (Figure 1C) and determine the values of the apparent unidirectional rate constants and T_1 (Figure 3). The k_{P-L} ranged from 0.007 (α -chloralose) to 0.019 (isoflurane) and was significantly greater for isoflurane than for all other anesthetic conditions ($p < 0.0001$). Additionally, k_{P-L} was significantly greater for morphine than for α -chloralose ($p = 0.0064$). However, no significant difference between morphine and pentobarbital, or between α -chloralose and pentobarbital was measured. The k_{P-B} ranged from 0.0014 (pentobarbital) to 0.0050 (morphine) and was significantly greater for morphine than for all other anesthetic conditions ($p < 0.0001$). The k_{P-B} was significantly lower for pentobarbital than for α -chloralose ($p = 0.04$), but there was no significant difference between isoflurane and pentobarbital, or isoflurane and α -chloralose. T_1 was significantly shorter for α -chloralose than for pentobarbital ($p = 0.0037$) and isoflurane ($p < 0.0001$), but not significantly different than that obtained for morphine.

After injection of hyperpolarized $[2-^{13}\text{C}]$ pyruvate, resonances corresponding to $[2-^{13}\text{C}]$ pyruvate, $[2-^{13}\text{C}]$ pyruvate hydrate, and $[2-^{13}\text{C}]$ lactate were observed in the rat brain *in vivo* under isoflurane and morphine (Figure 2A). In addition to these metabolites, $[5-^{13}\text{C}]$ 2OG and $[1-^{13}\text{C}]$ citrate were also observed in the rat brain *in vivo* under morphine (Figure 2A). *In vivo* time courses of ^{13}C metabolites, pyruvate, lactate, and 2OG, were measured with a temporal resolution of 1 s in the rat brain under morphine (Figure 2B). The time courses of the hyperpolarized $[2-^{13}\text{C}]$ lactate were fitted (Figure 2C) to determine the values of the apparent unidirectional rate constants and T_1 (Figure 3). The k_{P-L} was significantly higher for isoflurane than for morphine ($p = 0.02$), and T_1 was significantly lower for isoflurane than for morphine ($p = 0.001$). Additionally, the k_{P-L} for isoflurane and morphine obtained from fitting the time courses of the hyperpolarized $[1-^{13}\text{C}]$ lactate and $[2-^{13}\text{C}]$ lactate were not significantly different.

Discussion

Results obtained with hyperpolarized $[1-^{13}\text{C}]$ pyruvate show that the conversion rate of pyruvate to bicarbonate is consistent with previously reported values of TCA cycle rates

under different anesthetics. The lowest rate was detected for pentobarbital, which is known to strongly depress brain metabolism^{10,13}. The highest rate was detected for morphine, and intermediate values were obtained for the other anesthetics, isoflurane and α -chloralose. Relative $k_{P,B}$ values are consistent with previous studies with conventional ^{13}C , which showed that the TCA cycle rate is approximately two times higher with morphine than with α -chloralose ($V_{\text{TCA}} = 1.01 \pm 0.23 \mu\text{mol/min/g}$ vs. $V_{\text{TCA}} = 0.53 \pm 0.08 \mu\text{mol/min/g}$ ⁹; and $V_{\text{TCA}} = 1.09 \mu\text{mol/min/g}$ vs. $V_{\text{TCA}} = 0.56 \mu\text{mol/min/g}$ ¹⁴). In addition, the faster metabolic rate under morphine greatly improved the measurement of the hyperpolarized CO_2 signal in the brain, which was barely detectable with other anesthetics.

In contrast to bicarbonate, the conversion rate of pyruvate to lactate did not change significantly with different anesthetics. The lowest rate was detected for α -chloralose and pentobarbital, and the highest for isoflurane. This is consistent with known effect of isoflurane, which greatly increases lactate concentration in the brain¹⁵. The use of isoflurane or similar anesthetics is therefore advantageous for lactate detection. The fit for the lactate curve is good, but not perfect, suggesting that some of the assumptions made in the modeling may not be fulfilled. For example, we assumed that no lactate is generated in the blood, based on the lack of lactate using an implanted arterial coil, but there may still be a small amount of lactate generated below the detection threshold. Nonetheless, the modeling approach used here works reasonably well for this application and has the advantage of simplicity.

As with $[1-^{13}\text{C}]$ pyruvate, results obtained with hyperpolarized $[2-^{13}\text{C}]$ pyruvate in the brain show that morphine greatly improves detection of downstream metabolic products, in this case 2OG and citrate. There was no overlap between 2OG and the infused substrate as in the case of hyperpolarized $[1-^{13}\text{C}]$ acetate⁶. The resonance at 182 ppm, referred to as 2OG in the present work, was identified previously as either $[5-^{13}\text{C}]$ glutamate⁷ or $[5-^{13}\text{C}]$ 2OG⁶ since they both resonate at the same frequency as confirmed by our high-resolution, liquid-state NMR experiments. Assignment of 2OG was confirmed with a polarization transfer experiment after injection of hyperpolarized $[1,2-^{13}\text{C}_2]$ acetate⁶, but the definite assignment of the 182 ppm resonance when using other hyperpolarized substrates (especially substrates primarily metabolized by neurons, such as pyruvate) remains to be confirmed experimentally. Surprisingly, the 2OG resonance was much higher than the citrate resonance, whereas the concentration of citrate is generally thought to be 2-fold higher than 2OG. This possibly reflects a contribution of $[5-^{13}\text{C}]$ glutamate to the 182 ppm resonance when using a neuronal substrate such as pyruvate. The assignment of $[1-^{13}\text{C}]$ citrate is based on the chemical shift previously reported in the perfused heart¹⁶.

The $k_{P,L}$ values obtained through the modeling of $[1-^{13}\text{C}]$ lactate and $[2-^{13}\text{C}]$ lactate time courses were not significantly different for the same anesthetic. The difference in $k_{P,L}$ between anesthetics was consistent for $[1-^{13}\text{C}]$ lactate and $[2-^{13}\text{C}]$ lactate data. Additionally, the effective T_1 were significantly different for $[1-^{13}\text{C}]$ lactate and $[2-^{13}\text{C}]$ lactate data as expected.

We report detection of 2OG under morphine, but not isoflurane. Although another study reported detection of a resonance at 182 ppm under isoflurane⁷, that study differed from ours

in a number of ways: 1. the data was averaged from two injections improving signal-to-noise ratio (SNR); 2. the magnetic field strength was different: 3 T vs. 9.4 T in our study, and faster relaxation of hyperpolarized ^{13}C spins at higher field could explain signal loss; 3. the diameter of the ^{13}C detection coil was different (28 mm vs. 12 mm in our study); 4. the amount of injected hyperpolarized pyruvate was 1.7 times higher (1.56 mmol/kg body weight vs. 0.9 mmol/kg body weight on average in the present study). All these factors could explain the higher sensitivity. The 1.7 times higher amount of injected pyruvate probably accounts for no more than 20% improvement in SNR, since it relies primarily on the nonsaturable component of pyruvate transport (passive diffusion)⁴. Consistent with this, separate experiments using $[1-^{13}\text{C}]$ pyruvate showed only a 15–20% improvement in SNR in lactate and bicarbonate when increasing the amount of injected pyruvate from 0.35 to 0.68 mmol/kg (Figure 4). Considering the low signal of 2OG and citrate, higher doses of pyruvate were used for $[2-^{13}\text{C}]$ pyruvate studies than for $[1-^{13}\text{C}]$ pyruvate to have a better chance of observing these signals.

Nitrous oxide has analgesic properties in humans and other species and has been used in conjunction with other volatile anesthetics as balancing agent¹⁷. With the use of N_2O , it is possible to reduce the administered dose of the primary (volatile agent) and thus minimize its side effects^{18,19}. The use of N_2O in conjunction with isoflurane has led to the higher and less variable values of blood pressure and heart rate and thus the more stable physiological status than isoflurane alone when used in mice²⁰. In addition, previous studies which investigated TCA rates under different anesthesia with conventional ^{13}C MRS used $\text{O}_2/\text{N}_2\text{O}$ mixture for ventilation^{9,13,21}. Since as a sole agent, N_2O is incapable of producing general anesthesia²² and not affecting the entropy of EEG²³, it is expected to have a small effect on brain metabolism. In addition, differences we observed between groups are most likely not due to N_2O since it was used in all animals.

A number of limitations related to anesthesia must be acknowledged. First, in this study as in many studies investigating the influence of anesthesia on brain metabolism, male Sprague-Dawley rats were used. However, different strains can respond differently to anesthetics and analgesics²⁴. Second, the use of any anesthesia can have long term effects especially with repeated exposure²⁵. Third, while using morphine results in faster metabolism, it could be problematic for long-term (survival) studies due to the high risk of dependency and is better suited for non-survival studies. In addition, high doses of morphine may be problematic with free-breathing animals due to potential respiratory arrest (as opposed to artificial ventilation in the present study). Finally, anesthetics can affect the dilation of the blood vessels which influences the cerebral blood flow²⁶. This effect would affect mostly pyruvate signal.

One drawback of hyperpolarized ^{13}C is that it is difficult to obtain “absolute” values for metabolic rates. Most hyperpolarized ^{13}C papers in the brain report signal intensity of the metabolic product, without attempting to determine metabolic rates. In studies that have attempted to determine metabolic rates^{27–29} including the present study, the reported rates are apparent rate constants of conversion of ^{13}C labelled substrate (in s^{-1}) from which absolute metabolic rates cannot be easily derived. Nonetheless, such “relative” conversion

rates can be valuable to compare metabolic rates in different conditions, for example a control vs. disease group, with the ability to measure these rates in just few minutes.

Conclusion

In conclusion, we show that the rate of conversion of hyperpolarized [1-¹³C]pyruvate to bicarbonate reflects TCA cycle activity, and therefore constitutes a good indicator of brain energy production. In addition, we show that using morphine as an anesthetic greatly improves detection of downstream metabolic products. Therefore, the use of morphine could be of great benefit for hyperpolarized brain ¹³C studies in animal models.

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List of abbreviations:

DNP	dynamic nuclear polarization
LDH	lactate dehydrogenase
PDH	pyruvate dehydrogenase

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Figure 1.

Hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ studies. (A) Single-shot *in vivo* ^1H decoupled ^{13}C spectra acquired 15 s after injection of hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ solution in two representative rats under isoflurane and morphine. Spectra are shown with 10-Hz line-broadening and scaled to the external reference. Pulse-acquire, 43° pulse at the center of the coil. (B) Representative *in vivo* time courses of bicarbonate measured in four rats after injection of hyperpolarized $[1-^{13}\text{C}]\text{pyruvate}$ solution into femoral vein under different anesthetic conditions with the radiofrequency coil placed over the rat head. The integral of bicarbonate was scaled by averaged integral of external reference (formic acid). Pulse-acquire, ^1H decoupling during acquisition, 43° pulse at the center of the coil, $T_R = 1.5$ s, number of experiments ($N_{\text{EX}} = 68$). (C) *In vivo* hyperpolarized ^{13}C time courses of lactate and bicarbonate measured in one representative animal under morphine fitted with a two-pool model. pyr: pyruvate; lac: lactate; ext ref: external reference

Figure 2.

Hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ studies. (A) Single-shot *in vivo* ^1H decoupled ^{13}C spectra acquired in two representative rats after injection of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ solution into femoral vein under isoflurane and morphine at the same time point after beginning of injection. Spectra are shown with 10-Hz line-broadening and scaled to the external reference (formic acid). Pulse-acquire, 43° pulse at the center of the coil. (B) Representative *in vivo* time courses of $[2-^{13}\text{C}]\text{pyruvate}$, $[2-^{13}\text{C}]\text{lactate}$, and $[5-^{13}\text{C}]\text{2-oxoglutarate}$ measured after injection of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ solution into femoral vein with the radiofrequency coil placed over the rat head. Pulse-acquire, ^1H decoupling during acquisition, 43° pulse at the center of the coil, $T_R = 1$ s, $N_{EX} = 90$. (C) *In vivo* hyperpolarized ^{13}C time courses of lactate measured in one representative animal under morphine fitted with a two-pool model. 2OG: 2-oxoglutarate

Figure 3.

Kinetic rate constants and effective T_1 measured under different anesthesia. Data are shown as means and standard deviations. (A) $[1-^{13}\text{C}]$ pyruvate studies using pentobarbital (5 animals, 11 injections), isoflurane (3 animals, 6 injections), α -chloralose (3 animals, 10 injections), and morphine (3 animals, 11 injections). * - different at 0.05 significance level. (B) $[1-^{13}\text{C}]$ pyruvate studies using isoflurane (4 animals, 7 injections), and morphine (5 animals, 8 injections).

Figure 4.

The influence of injected amount of hyperpolarized $[1-^{13}\text{C}]$ pyruvate on the lactate and bicarbonate signal observed with the surface coil placed over the rat head. Data points are shown with mean and standard deviations from five animals. Pulse-acquire, ^1H decoupling during acquisition, 43° pulse at the center of the coil, $T_R = 1.5$ s, $N_{\text{EX}} = 128$.