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Effects of isoflurane anesthesia on hyperpolarized ^{13}C metabolic measurements in rat brain

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Abstract

Purpose—Commonly used anesthetic agents such as isoflurane are known to be potent cerebral vasodilators, with reported dose-dependent increase in cerebral blood flow and cerebral blood volume. Despite the widespread use of isoflurane in hyperpolarized ^{13}C pre-clinical research studies, a quantitative assessment of its effect on metabolic measurements is limited. This work investigates the effect of isoflurane anesthesia dose on hyperpolarized ^{13}C MR metabolic measurements in rat brain for $[1-^{13}\text{C}]$ pyruvate and 2-keto $[1-^{13}\text{C}]$ isocaproate.

Methods—Dynamic 2D and 3D spiral chemical shift imaging was used to acquire metabolic images of rat brain as well as kidney and liver following bolus injections of hyperpolarized $[1-^{13}\text{C}]$ pyruvate or 2-keto $[1-^{13}\text{C}]$ isocaproate. The impact of a ‘low dose’ vs. a ‘high dose’ of isoflurane on cerebral metabolite levels and apparent conversion rates was examined.

Results—The cerebral substrate signal levels, and hence the metabolite-to-substrate ratios and apparent conversion rates, were found to depend markedly on isoflurane dose, while signal levels of metabolic products and their ratios, e.g. bicarbonate/lactate, were largely insensitive to isoflurane levels. No obvious dependence on isoflurane was observed in kidney or liver for pyruvate.

Conclusion—This study highlights the importance of careful attention to the effects of anesthesia on the metabolic measures for hyperpolarized ^{13}C metabolic imaging in brain.

Keywords

hyperpolarized ^{13}C ; isoflurane; anesthesia; brain; metabolite ratios; metabolic imaging

Introduction

Hyperpolarized ^{13}C magnetic resonance spectroscopic imaging is a powerful tool for in vivo measurement of metabolism and has shown great promise in several applications including tumor diagnosis and treatment monitoring [1–9]. Although transport across the blood brain barrier (BBB) within the short T_1 relaxation times of the hyperpolarized agents is a limiting factor for neurological applications, recent studies have demonstrated hyperpolarized ^{13}C brain imaging with a number of substrates. These include pyruvate [6–12], ethyl pyruvate [13], ketoisocaproate [14], acetate [15], dehydroascorbic acid [16], diethyl succinate [17] and dimethylethanol [18]. The most commonly used agent, $[1-^{13}\text{C}]$ pyruvate (Pyr) has been useful in the assessment of brain tumor metabolism by monitoring its conversion to lactate (Lac) and bicarbonate (Bic), allowing the differentiation of tumor from normal tissue in an animal model [6–9].

Despite the ubiquitous use of anesthetic agents in hyperpolarized ^{13}C pre-clinical research studies, a quantitative assessment of their effect on these metabolic measurements is limited [19]. Isoflurane, which is the most widely used anesthetic agent, is known to be a potent cerebral vasodilator. A number of studies have reported a dose-dependent increase in cerebral blood flow (CBF) and cerebral blood volume (CBV) with isoflurane as well as heterogeneous changes in CBF distribution and glucose utilization in several species [20–26]. These modulations could potentially impact the transport and uptake of the ^{13}C labeled substrate into brain tissue and its metabolic conversion.

This work aims to investigate the effect of isoflurane anesthesia dose on hyperpolarized ^{13}C MR metabolic measurements in rat brain for $[1-^{13}\text{C}]$ pyruvate and 2-keto $[1-^{13}\text{C}]$ isocaproate (KIC). Using dynamic chemical shift imaging (CSI), the impact of a ‘low dose’ vs. a ‘high dose’ of isoflurane on cerebral metabolite levels and apparent conversion rates was examined. As a complement to the brain study, experiments were also performed with pyruvate to examine the influence of isoflurane dose on metabolic measurements in kidney and liver.

Methods

The samples to be polarized consisted of either 25 μL of a mixture of 14-M $[1-^{13}\text{C}]$ pyruvic acid (Sigma-Aldrich, USA) and 15-mM Ox063 trityl radical, or 32 μL of a mixture of 8-M $[1-^{13}\text{C}]$ ketoisocaproic acid (converted from corresponding sodium salt, Cambridge Isotope Laboratories, USA) and 11-mM Ox063 trityl radical. In both cases 3 μL of a 1:50 dilution of Dotarem (Guerbet, France) were added just prior to polarization. The samples were polarized via dynamic nuclear polarization using a HyperSense system (Oxford Instruments Molecular Biotools, Oxford, UK), for 1–1.5 h each, to achieve liquid-state polarization at dissolution of approximately 25% for pyruvate and 15% for KIC. The polarized samples were dissolved in a solution of 80-mM NaOH mixed with 40-mM TRIS buffer, 50-mM NaCl and 0.1 g/L EDTA- Na_2 , leading to an 80-mM solution of the hyperpolarized substrate with a pH of about 7.5.

Healthy male Wistar rats (278 ± 59 g body weight, $n=13$) were injected with 2.6–3.2 mL of the hyperpolarized solution (target dose = 1 mmol/kg body weight) through a tail vein catheter at a rate of approximately 0.25 mL/s. The time from dissolution to start of injection was approximately 18 s.

The rats were anesthetized initially with 2.5% isoflurane in oxygen (1.5 L/min) for tail vein catheterization. Respiration, rectal temperature, heart rate and oxygen saturation were monitored throughout the experiments. The oxygen flow rate was kept constant at 1.5 L/min while the isoflurane level was adjusted to a “low” or “high” dose. The low dose was

approximately 0.75 – 1.5% isoflurane in oxygen, targeting a respiration rate of about 80–85 breaths/min and the high dose was 2 – 3.25% targeting a respiration rate of about 50–55 breaths/min. Each animal received multiple (2–4) injections of the hyperpolarized substrate, approximately 1.5 h apart, alternating between low and high doses. Before each ^{13}C injection, isoflurane was slowly adjusted to the next target dose, and maintained at the target for at least 10 min and up to an hour before the injection. The first ^{13}C injection was performed at low isoflurane dose for approximately half of the rats and at high isoflurane dose for the others. All animal procedures were approved by the SRI Institutional Animal Care and Use Committee.

For the brain experiments, 7 of the rats received 4 injections each of hyperpolarized Pyr (n=3, rat ID P1-P3) or KIC (n=4, rat ID K1-K4), 2 injections each at the low and high isoflurane doses. To assess variability among injections, 3 control rats (ID C1-C3) received 3 Pyr injections each while maintaining a constant isoflurane dose of 1.75–2.5% targeting a respiration rate of about 60 breaths/min throughout the experiment. For the body (kidney and liver) experiments, 3 rats (ID P4-P6) received 2 injections each of Pyr, alternately at low and high isoflurane dose.

All experiments were performed on a clinical 3T Signa MR scanner (GE Healthcare, Waukesha, WI), using a high-performance insert gradient coil operating at maximum amplitude of 500 mT/m with a slew rate of 1865 mT/m/ms [9] and custom-built dual-tuned ($^1\text{H}/^{13}\text{C}$) quadrature transmit-receive RF coils, operating at 127.9 MHz and 32.2 MHz, respectively. The brain imaging experiments used a rat brain RF coil (diameter=50 mm, length=60 mm), while the body imaging studies used a bigger rat body RF coil (diameter=80 mm, length=90 mm). The transmit ^{13}C RF power was calibrated using a reference phantom, containing an 8-M solution of ^{13}C -urea, which was placed on top of the animal and removed before the first ^{13}C injection.

Single-shot fast spin-echo (FSE) ^1H MR images in the axial, sagittal and coronal planes with nominal in-plane resolution of 0.47 mm and 2-mm slice thickness were acquired as anatomical references for prescribing the ^{13}C CSI experiments. Additional ^1H MR images matching the ^{13}C CSI prescriptions were also acquired for overlay of the ^{13}C metabolic maps. Dual-echo FSE images (0.25-mm in-plane resolution, 1-mm slice thickness, 58 slices, echo time TE1/TE2 = 11.3/56.7 ms, repetition time (TR) = 5000 ms, echo train length=8, NEX=2) were used for the brain 2D experiments, while 3D spoiled gradient-recalled echo (SPGR) images (0.625-mm in-plane resolution, 1.25-mm slice thickness, 96 slices, TE/TR=2.1/8.9 ms, NEX=2) were used for the body 3D experiments.

Brain 2D ^{13}C CSI

The single-shot 2D spiral CSI sequence described in [12] was used for dynamic metabolic imaging of the brain. Imaging parameters were: FOV=43.5 mm, nominal resolution=2.7 mm, 10-mm slice thickness, TR=3 s, TE=3 ms. Sixteen time points were acquired with a delay of 3 s from the start of the injection, using a variable flip angle scheme [27],

$\theta_i = \tan^{-1} \left(1 / \sqrt{16-i} \right)$ for the 16 excitations. The data for control rats was taken from the previously published study [12], with imaging starting at 9 s after start of injection, while all other parameters (including hardware, setup and imaging) were the same as described here. In one case (rat K1), a single time-point image was acquired at 25 s after KIC injection, instead of dynamic imaging, to achieve higher leucine (Leu) signal-to-noise ratio (SNR). This acquisition used a 90° excitation and all other parameters were the same as the dynamic brain imaging protocol.

Body 3D ^{13}C CSI

Dynamic 3D data were acquired from a volume covering both kidney and liver using the 3D spiral CSI sequence described in [28]. Imaging parameters were: FOV=80×80×60 mm³, nominal resolution=5×5×5 mm³, 12 z-phase encoding steps, 4-cm slab excitation. The faster gradient insert coil allowed the total acquisition time per volume (T_{acq}) to be reduced to 1.6 s by using a single-shot spiral trajectory instead of the 3 spatial interleaves used in [28] for the same resolution. A constant flip angle of 5.625° was used for each excitation. Twenty-four time points were acquired, with a sampling interval of 2.5 s, starting with the Pyr injection.

The data were reconstructed similarly as the 2D and 3D spiral CSI cases described in [12,28]. Metabolic maps were calculated by integrating the signal around each peak in absorption mode, using a width of ± 18 Hz for the brain 2D data and ± 24 Hz for the kidney/liver 3D data. The metabolic images were corrected for differences in the solid-state polarization level of each sample, and in the time delay from dissolution to start of injection assuming a T_1 of 60 s in solution. Regions of interest (ROIs) were drawn manually for each organ and for vasculature to calculate the time-resolved signal intensities for the metabolites. Apparent Pyr-to-Lac rate constants (K_{pl}) were estimated from the signal time-courses after RF correction [29]. Metabolite ratios in the ROIs were calculated from the time-averaged metabolic maps.

Statistical significance was assessed using a paired Student's t-test to compare the low isoflurane data with the high isoflurane data. In cases where two measurements were taken at each isoflurane level, both measurements were included in the data. For the control rats, a paired t-test was done for the first and third injections.

Results

Representative time-averaged ^{13}C metabolic maps superimposed onto ^1H MR images in Fig. 1a show the metabolic products of [$1\text{-}^{13}\text{C}$]Lac and ^{13}C -bicarbonate (Bic) observed after hyperpolarized [$1\text{-}^{13}\text{C}$]Pyr injection. The conversion of Pyr to Lac takes place in the cytosol via the enzyme lactate dehydrogenase (LDH). Pyruvate is also converted to acetyl-coenzyme A (acetyl-CoA) in the mitochondria through pyruvate dehydrogenase (PDH), releasing the ^{13}C label as $^{13}\text{CO}_2$, which is in equilibrium with ^{13}C -Bic. [$1\text{-}^{13}\text{C}$]alanine (Ala), which is generated from pyruvate through alanine aminotransferase (ALT), was detected in the muscle tissues of the jaw and tongue but not in the brain, consistent with the low ALT activity in rat brain compared to LDH activity [12,30].

The ^{13}C maps in Fig. 1a demonstrate greatly elevated Pyr in brain (red ROI) and vasculature (black ROI) at the high isoflurane level compared to the low isoflurane level, consistent with the vasodilation effect, whereas lactate and bicarbonate remain unchanged. This effect was observed over a repeated set of measurements in each animal. The Pyr and Lac images shown here are the average of all 16 time-points acquired, whereas the Bic images were the average of time-points 5–16 to increase SNR as the first 4 time-points had little Bic signal. While the metabolite signal intensities can have some variation due to fluctuations in polarization levels, the commonly used metrics of metabolite-to-substrate ratios and apparent rate constants are independent of polarization. The ratio maps in Fig. 1b calculated from the images in Fig. 1a reflect the large change in Pyr, with the Lac/Pyr and Bic/Pyr ratios being considerably lower at the high isoflurane level.

The Pyr and Lac time-courses from an ROI in the brain are plotted in Fig. 2 along with Pyr time-courses from a vasculature ROI (ROIs shown in Fig. 1). The Bic time-courses are not shown due to insufficient SNR of the individual time-points. Similar to the metabolic maps,

the time-courses show increased Pyr signal at the high isoflurane level, without any distinct changes in the shape of the dynamic time-curve. The brain Lac signal level was similar for all 4 injections, resulting in very different apparent K_{pl} estimated from the time-courses, with an average of $0.025 \pm 0.003 \text{ s}^{-1}$ (mean $\pm$ std, 6 measurements in 3 rats) at the low isoflurane vs. $0.016 \pm 0.002 \text{ s}^{-1}$ at the high level ($p=0.0001$).

Figure 3 summarizes the time-averaged Pyr, Lac and Bic signal levels along with the corresponding Lac/Pyr, Bic/Lac and Bic/Lac ratios in the brain ROIs for 6 rats. For the rats P1-P3, the Pyr signal varied appreciably with isoflurane level (mean $\pm$ std: low = 5.99 ± 1.36 , high = 10.28 ± 2.99 , $p=0.0031$), while the Lac and Bic signals did not change over multiple injections in one animal (Lac: low = 3.37 ± 0.82 , high = 3.54 ± 1.15 , $p=0.5526$; Bic: low = 0.40 ± 0.11 , high = 0.41 ± 0.08 , $p=0.7711$). Thus, the ratios of the metabolic products to the substrate, i.e. Lac/Pyr and Bic/Pyr, varied significantly with isoflurane dose. The average Lac/Pyr ratio decreased from 0.56 ± 0.04 at the low isoflurane to 0.34 ± 0.04 at the high level ($p<0.0001$), with a percent change of 39.1 ± 1.8 from low to high isoflurane. The Bic/Pyr ratios show a similar trend as Lac/Pyr ratios, and the average Bic/Pyr ratio decreased from 0.07 ± 0.01 at the low isoflurane level to 0.04 ± 0.008 at high isoflurane ($p<0.0001$), with a percent change of 41.1 ± 5.2 from low to high isoflurane. As the Bic and Lac signals were similar despite the large Pyr variation, the ratio of products, Bic/Lac, did not vary with isoflurane and was robust to the vasodilation effect on the substrate (low = 0.12 ± 0.04 , high = 0.12 ± 0.03 , $p=0.9167$).

For the control rats (C1-C3) that had the isoflurane and respiration levels maintained constant throughout the experiment, the Pyr as well as the Lac and Bic signals did not differ between injections (Pyr: inj1 = 12.92 ± 2.46 , inj3 = 11.74 ± 2.64 , $p=0.3457$; Lac: inj1 = 7.35 ± 2.00 , inj3 = 6.54 ± 2.00 , $p=0.0736$; Bic: inj1 = 0.85 ± 0.08 , inj3 = 0.87 ± 0.06 , $p=0.7397$). Therefore, the ratios were also similar (Lac/Pyr: inj1 = 0.57 ± 0.06 , inj3 = 0.55 ± 0.06 , $p=0.1567$; Bic/Pyr: inj1 = 0.052 ± 0.004 , inj3 = 0.055 ± 0.007 , $p=0.1732$; Bic/Lac: inj1 = 0.11 ± 0.02 , inj3 = 0.12 ± 0.03 , $p=0.0846$). The isoflurane level for the control rats was closer to the high dose than the low dose. However, that data, which was taken from a previous study [12], started imaging at 9 s after Pyr injection for greater Lac and Bic SNR while this study used a 3 s delay to better capture the Pyr bolus. Hence, the metabolite signals and the ratios are higher for the control rats than the values for the high dose of the rats P1-P3.

Figure 4 depicts the images and ratio maps for hyperpolarized $[1-^{13}\text{C}]\text{KIC}$ and its conversion to Leu in the brain. KIC is metabolized to Leu by the branched chain aminotransferase (BCAT) enzyme, with the concomitant conversion of glutamate to alpha-ketoglutarate. BCAT enzyme activity is upregulated in some tumors [31] and is also linked to glutamate-glutamine cycling between neurons and glia in the brain [32]. The ^{13}C images here are from a single time-point acquisition at 25 s post KIC injection to achieve higher Leu SNR (rat K1). These images as well as the KIC time-courses (Fig. 5) from another rat demonstrate elevated KIC signal intensity at the high isoflurane level, indicating that the effect is not specific to Pyr. Leu time-courses are not shown in Fig. 5 due to insufficient SNR. The KIC and Leu signals averaged from a brain ROI and the corresponding Leu/KIC ratios are presented in Fig. 6. The high Leu signal for rat K1 is due to the single time-point acquisition while the other data are from dynamic acquisitions averaged over the time-course. There was a strong modulation of the KIC signal by isoflurane (low = 8.31 ± 2.62 , high = 13.86 ± 5.33 , $p<0.0001$), whereas the Leu signal was similar at both isoflurane levels (low = 0.98 ± 0.65 , high = 0.98 ± 0.69 , $p=0.3842$). Consequently, the Leu/KIC ratios also vary considerably with different isoflurane levels (low = 0.11 ± 0.04 , high = 0.06 ± 0.02 , $p=0.0032$), with a percent change of 36.7 ± 9.92 from low to high isoflurane. The statistical analysis above used a paired t-test by excluding the 3rd injection on K2.

Discussion

Hurd et. al. showed in [11] that the hyperpolarized Pyr signal observed in the brain region arises largely from the CBV, as the conversion of Pyr to Lac in the brain is significantly faster than the transport of Pyr across the blood-brain barrier. Hence, any Pyr taken up by the brain tissue is converted quickly and observed as its metabolic products, whereas nearly all the Pyr observed is in the CBV. Therefore, the higher Pyr signal observed in the brain ROI with high isoflurane is consistent with the increase in CBV due to isoflurane.

The increased Pyr in CBV does not result in an increase in the metabolic products, as reflected by the similar Lac and Bic signals. Given the fast metabolic conversion of Pyr in brain, that would indicate that the transport of Pyr through the BBB is likely similar at the different isoflurane levels. Alternatively, it may be possible that there are changes in Pyr transport/uptake. However, that would imply a compensatory change in metabolic conversion for both Lac and Bic in order to maintain similar product signal levels at the different isoflurane levels as observed in this study. In either case, the strong modulation of the Lac/Pyr and Bic/Pyr ratios is present. The ratio of metabolic products, i.e. Bic/Lac did not vary with isoflurane level, and provides a metric that may be immune to the vasodilation effect on the substrate as well as to the differences in polarization levels.

The modulation of the substrate signal by isoflurane was observed for both Pyr and KIC. For both substrates, the transport across the BBB occurs via a saturable carrier-mediated transport as well as a non-saturable diffusion transport. Pardridge [33] reported the kinetic parameters of BBB transport, with maximum velocity of transport $V_{\max}$ and non-saturable transport constant K_d as 88 nmol/min/g and 0.034 ml/min/g respectively for Pyr (and 91 nmol/min/g and 0.019 ml/min/g respectively for Lac as comparison). The active transport is expected to saturate at the doses used here and the diffusive transport depends on substrate concentration. Conn et al [34] reported a $V_{\max}$ of 71 nmol/min/g and K_d of 0.019 ml/min/g for KIC. The effect of isoflurane on other substrates may depend on their specific BBB transport and metabolic conversion rates.

While isoflurane acts as a cerebral vasodilator, enhancing CBF and CBV and hence the ^{13}C substrate signal, the response may be different for other anesthetic agents. For example, propofol, pentobarbital and alpha-chloralose have been reported to show lower CBF than isoflurane, as well as lower CBV reported for propofol and pentobarbital [20–23]. Halothane also shows a vasodilation effect similar to or greater than isoflurane, but with a different regional distribution of the changes in CBF compared to isoflurane [25].

In contrast to the striking variation observed in brain, there was no obvious dependence on isoflurane observed in kidney or liver. The time-averaged Pyr and Lac signal levels and the Lac/Pyr ratios from three rats in Fig. 7 demonstrate no significant difference at the two isoflurane levels (Liver: $p=0.2813$ for Pyr, $p=0.2896$ for Lac; $p=0.6017$ for Lac/Pyr ; Kidney: $p=0.4398$ for Pyr, $p=0.3029$ for Lac; $p=0.1165$ for Lac/Pyr). The apparent K_{pl} estimated from the time-courses was $0.013 \pm 0.001 \text{ s}^{-1}$ at both isoflurane doses for kidney ($p=0.7608$), and $0.028 \pm 0.005 \text{ s}^{-1}$ vs. $0.029 \pm 0.004 \text{ s}^{-1}$ for liver for low and high isoflurane doses respectively ($p=0.4950$). Previous studies have reported no change in renal or total hepatic blood flow at low (~1% vol) isoflurane concentration, compared to controls anesthetized with alpha-chloralose, but an approximately 15% decrease in blood flow in both regions at high (~2.4% vol) isoflurane concentration for rats [26]. In comparison, a more than 2-fold increase in CBF has been reported in some brain structures at high isoflurane compared to low isoflurane [20,21]. Without the BBB as a rate-limiting step in Pyr transport and uptake from blood to tissue, the Pyr levels in blood and tissue may change similarly and could be expected to produce a corresponding change in Lac. However, if the

Pyr-to-Lac conversion is saturated at the Pyr dose used here, a small decrease in Pyr levels may not lead to lower Lac signal.

A wide range of isoflurane levels was used in this study to quantify the effect on ^{13}C measurements. While an animal can normally be kept stable at a much tighter range of anesthesia during an experiment session, the large variation used here illustrates the need to consider the impact of isoflurane dose when comparing metabolite-to-substrate ratios and rate constants across multiple time-points or different studies for hyperpolarized metabolic imaging in brain. The long duration of anesthesia may also have an effect on the physiological state.

Conclusion

The cerebral substrate signal levels, and hence metabolite-to-substrate ratios and apparent rate constants acquired for metabolic imaging of hyperpolarized Pyr and KIC can depend markedly on anesthetic dose in rats anesthetized with isoflurane. In comparison, the signal levels of the metabolic products and their ratios were, within measurement accuracy, largely independent of isoflurane levels. This study highlights the importance of careful attention to the effects of anesthesia on the metabolic measures for hyperpolarized ^{13}C metabolic imaging.

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References

1. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, Deberardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR. Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. *Neoplasia*. 2011; 13:81–97. [PubMed: 21403835]
2. Brindle KM, Bohndiek SE, Gallagher FA, Kettunen MI. Tumor imaging using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Magn Reson Med*. 2011; 66:505–519. [PubMed: 21661043]
3. Golman K, Zandt RI, Lerche M, Pehrson R, Ardenkjaer-Larsen JH. Metabolic imaging by hyperpolarized ^{13}C magnetic resonance imaging for in vivo tumor diagnosis. *Cancer Research*. 2006; 66:10855–10860. [PubMed: 17108122]
4. Day SE, Kettunen MI, Gallagher FA, Hu D-E, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen J-H, Brindle K. Detecting tumor response to treatment using hyperpolarized ^{13}C magnetic resonance imaging and spectroscopy. *Nature Medicine*. 2007; 13:1382–1387.
5. Ross BD, Bhattacharya P, Wagner S, Tran T, Sailasuta N. Hyperpolarized MR imaging: neurologic applications of hyperpolarized metabolism. *Am J Neuroradiol*. 2010; 31:24–33. [PubMed: 19875468]
6. Park I, Larson PEZ, Zierhut ML, Hu S, Bok R, Ozawa T, Kurhanewicz J, Vigneron DB, VandenBerg SR, James CD, Nelson SJ. Hyperpolarized ^{13}C magnetic resonance metabolic imaging: application to brain tumors. *Neuro-oncology*. 2010; 12:133–144. [PubMed: 20150380]
7. Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris D, Matsumoto S, Koretsky AP, Brindle M. Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized $[1-^{13}\text{C}]$ pyruvate and ^{13}C magnetic resonance spectroscopic imaging. *Magn Reson Med*. 2011; 65:557–563. [PubMed: 21264939]
8. Park I, Bok R, Ozawa T, Phillips JJ, James CD, Vigneron DB, Ronen SM, Nelson SJ. Detection of early response to temozolomide treatment in brain tumors using hyperpolarized ^{13}C MR metabolic imaging. *J Magn Reson Imaging*. 2011; 33:1284–90. [PubMed: 21590996]

9. Park JM, Josan S, Jang T, Merchant M, Yen Y-F, Hurd RE, Recht L, Spielman DM, Mayer D. Metabolite kinetics in C6 rat glioma model using magnetic resonance spectroscopic imaging of hyperpolarized [1-¹³C]pyruvate. *Magn Reson Med*. 2012;1002/mrm.24181
10. Marja ska M, Iltis I, Shestov AA, Deelchand DK, Nelson C, U urbil K, Henry P-G. In vivo ¹³C spectroscopy in the rat brain using hyperpolarized [1-¹³C]pyruvate and [2-¹³C]pyruvate. *J Magn Reson*. 2010; 206:210–218. [PubMed: 20685141]
11. Hurd RE, Yen Y-F, Tropp J, Pfefferbaum A, Spielman D, Mayer D. Cerebral dynamics and metabolism of hyperpolarized [1-¹³C]pyruvate using time-resolved MR spectroscopic imaging. *Journal of cerebral blood flow and metabolism*. 2010; 30:1734–1741. [PubMed: 20588318]
12. Mayer D, Yen Y, Takahashi A, Josan S, Tropp J, Rutt BK, Hurd RE, Spielman DM, Pfefferbaum A. Dynamic and High-Resolution Metabolic Imaging of Hyperpolarized [1-¹³C]-Pyruvate in the Rat Brain Using a High-Performance Gradient Insert. *Magn Reson Med*. 2011; 65:1228–1233. [PubMed: 21500253]
13. Hurd RE, Yen Y-F, Mayer D, Chen A, Wilson D, Kohler S, Bok R, Vigneron D, Kurhanewicz J, Tropp J, Spielman D, Pfefferbaum A. Metabolic imaging in the anesthetized rat brain using hyperpolarized [1-¹³C] pyruvate and [1-¹³C] ethyl pyruvate. *Magn Reson Med*. 2010; 63:1137–1143. [PubMed: 20432284]
14. Butt SA, Sogaard LV, Magnusson PO, Lauritzen MH, Laustsen C, Akeson P, Ardenkjaer-Larsen JH. Imaging cerebral 2-ketoisocaproate metabolism with hyperpolarized ¹³C Magnetic Resonance Spectroscopic Imaging. *Journal of Cerebral Blood Flow and Metabolism*. 2012;1038/jcbfm.2012.34
15. Mishkovsky M, Comment A, Gruetter R. In vivo detection of brain Krebs cycle intermediate by hyperpolarized MR. *Proc ISMRM*. 2011:660.
16. Keshari KR, Kurhanewicz J, Bok R, Larson PEZ, Vigneron DB, Wilson DM. Hyperpolarized ¹³C dehydroascorbate as an endogenous redox sensor for in vivo metabolic imaging. *PNAS*. 2011; 108:18606–18611. [PubMed: 22042839]
17. Bhattacharya P, Zacharias NM, Sailasuta N, Chan HR, Perman WH, Epstein AL, Ross BD. Imaging TCA cycle metabolism in rat brain by hyperpolarization. *Proc ISMRM*. 2012:4410.
18. Grant AK, Vinogradov E, Wang X, Lenkinski RE, Alsop DC. Perfusion imaging with a freely diffusible hyperpolarized contrast agent. *Magn Reson Med*. 2011; 66:746–755. [PubMed: 21432901]
19. Marjanska M, Shestov A, Henry P-G. Brain metabolism under different anesthesia using hyperpolarized [1-¹³C]-pyruvate. *Proc ISMRM*. 2012:1692.
20. Todd MM, Weeks J. Comparative effects of propofol, pentobarbital, and isoflurane on cerebral blood flow and blood volume. *Journal of Neurosurgical Anesthesiology*. 1996; 8:296–303. [PubMed: 8884627]
21. Maekawa T, Tommasino C, Shapiro HM, Keifer-Goodman J, Kohlenberger RW. Local cerebral blood flow and glucose utilization during isoflurane anesthesia in rat. *Anesthesiology*. 1986; 65:144–151. [PubMed: 3740503]
22. Hendrich KS, Kochanek PM, Melick JA, Schiding JK, Statler KD, Williams DS, Marion DW, Ho C. Cerebral Perfusion During Anesthesia With Fentanyl, Isoflurane, or Pentobarbital in Normal Rats Studied by Arterial Spin-Labeled MRI. *Magn Reson Med*. 2001; 46:202–206. [PubMed: 11443729]
23. Lenz C, Rebel A, van Ackern K, Kuschinsky W, Waschke KF. Local cerebral blood flow, glucose utilization and flow-metabolism coupling during sevoflurane vs isoflurane anesthesia in rats. *Anesthesiology*. 1998; 89:1480–1489. [PubMed: 9856723]
24. Chi OZ, Anwar M, Sinha AK, Wei HM, Klein SL, Weiss HR. Effects of isoflurane on transport across the blood brain barrier. *Anesthesiology*. 1992; 76:426–431. [PubMed: 1539855]
25. Hansen T, Warner D, Todd M, Vust L, Trawick D. Distribution of cerebral blood flow during halothane vs isoflurane anesthesia in rats. *Anesthesiology*. 1998; 69:332–337. [PubMed: 3415014]
26. Conzen PF, Vollmar B, Habazettl H, Frink EJ, Peter K, Messmer K. Systemic and Regional Hemodynamics of Isoflurane and Sevoflurane in Rats. *Anesthesia & Analgesia*. 1992; 74:79–88. [PubMed: 1734802]

27. Zhao L, Mulker R, Tseng CH, Williamson D, Patz S, Kraft R, Walsworth RL, Jolesz FA, Albert MS. Gradient echo imaging considerations for hyperpolarized ^{129}Xe MR. *J Magn Reson B*. 1996; 113:179–183.
28. Josan S, Spielman D, Yen Y-F, Hurd RE, Pfefferbaum A, Mayer D. Fast volumetric imaging of ethanol metabolism in the rat liver with hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *NMR Biomed*. 2012;10.1002/nbm.2762
29. Josan S, Hurd RE, Kerr AB, Yen Y-F, Larson PE, Pfefferbaum A, Spielman D, Mayer D. Effects of RF excitation scheme on signal-to-noise-ratio and apparent rate constant estimation in dynamic volumetric imaging of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate. *Proc ISMRM*. 2011:3528.
30. Erakovic V, Zupan G, Varljen J, Laginja J, Simonic A. Altered activities of rat brain metabolic enzymes in electroconvulsive shock-induced seizures. *Epilepsia*. 2001; 42:181–189. [PubMed: 11240587]
31. Karlsson M, Jensen PR, Zandt R, Gisselsson A, Hansson G, Duus J, Meier S, Lerche MH. Imaging of branched chain amino acid metabolism in tumors with hyperpolarized ^{13}C ketoisocaproate. *Intl J Cancer*. 2010; 127:729–736.
32. Hutson S. Structure and function of branched chain aminotransferases. *Prog Nucleic Acid Res Mol Biol*. 2001; 70:175–206. [PubMed: 11642362]
33. Pardridge WM. Brain Metabolism: A Perspective From the Blood-Brain Barrier. *Physiol Rev*. 1983; 63:1481–1535. [PubMed: 6361813]
34. Conn AR, Fell DI, Steele RD. Characterization of α -keto acid transport across blood-brain barrier in rats. *Am J Physiol*. 1983; 245:E253–260. [PubMed: 6614164]

Figure 1.

Representative ^{13}C metabolic maps of a slice through the brain from 4 injections in one rat. Pyruvate was elevated at the high isoflurane level due to the vasodilation effect, while no change was observed in lactate and bicarbonate. Thus, the ratio maps showed lower values at high isoflurane. The spurious bicarbonate signal outside the brain region is likely from pyruvate signal contamination due to off-resonance as the B_0 homogeneity was optimized only over the brain.

Figure 2.

Pyruvate time-courses from ROIs in the brain and in vasculature show the pronounced modulation of pyruvate by different isoflurane levels. Lactate signal levels over the time-course in the brain ROI did not change with isoflurane.

Figure 3.

Time-averaged signal intensities and corresponding metabolite ratios from a brain ROI for 6 rats. The strong variation of Pyr with isoflurane (rats P1-P3) leads to significant variation in the Lac/Pyr and Bic/Pyr ratios while the ratio of products, Bic/Lac, was relatively insensitive to the isoflurane changes. For the control rats (C1-C3) with constant isoflurane throughout, the metabolite signal intensities did not differ over multiple injections.

Figure 4. Representative ^{13}C metabolic maps of KIC and Leu from a slice through the brain illustrates the changes in KIC signal with isoflurane dose while Leu signal is consistent for all 4 injections, resulting in a noticeable variation of the Leu/KIC ratios.

Figure 5. KIC time-courses from the brain and vasculature ROIs show the modulation of KIC signal by different isoflurane levels.

Figure 6.

Time-averaged signal intensities and corresponding metabolite ratios from a brain ROI for 4 rats. The strong difference in the KIC signal at the two isoflurane levels leads to significant variation in the Leu/KIC ratios.

Figure 7. Time-averaged signal intensities and metabolite ratios from ROIs in the kidney and liver for 3 rats. In contrast to the brain results, the metabolite signal intensities and corresponding ratios did not show a detectable dependence on the isoflurane level.