Gluco-CEST of Cerebral Glucose Metabolism: Correlation with 31P MRS

Kai-Hsiang Chuang[1], Fatima A Nasrallah[1], Guilhem Pages[2], Philip William Kuchel[2], and Xavier Golay[3]

[1]MRI Group, Singapore Bioimaging Consortium, Singapore, Singapore, Singapore, [2]Mechanistic Systems-biology NMR Group, Singapore Bioimaging Consortium, Singapore, Singapore, Singapore, [3]Institute of Neurology, University College of London, United Kingdom

Introduction

Isotopically labeled 2DG has been used as a surrogate marker of glucose uptake and metabolism *in vivo* (1). ^{13}C-2DG has been used to monitor the uptake of 2DG as well as its metabolism into ^{13}C-2DG6P (2). In addition, 2DG6P can be measured by ^{31}P NMR (3), thus avoiding the use of an expensive ^{13}C-labeled molecule. CEST provides a novel approach to detect glucose and glycogen by measuring the protons that exchange between water and the hydroxyl groups of the carbohydrate(s) (4). We demonstrated that CEST-based MRI could also detect the exchange of the hydroxyl protons between 2DG or 2DG6P, and water. This enabled mapping the relative rates of cerebral glucose uptake in the rodent brain. To understand further the characteristics of gluco-CEST we compared the detected CEST effect with the 2DG6P signal, measured by ^{31}P MRS under various physiological conditions.

Methods

Experiments on rats were approved by the institutional animal care and use committee (BMSI, Singapore). Male Wistar rats (240-350 g) were fasted for 24 h before imaging. They were anaesthetized with isoflurane, intubated orally, and mechanically ventilated. Arterial blood gases, glucose, and end-tidal CO_2 were monitored; while the rectal temperature was maintained at 37°C by a feedback-controlled air-heater. 0.5 g kg^{-1} of 2DG (Sigma, Singapore) was injected via the tail vein in a bolus, followed by repeated scanning for a least 1 h to record uptake curves. Three levels of isoflurane (1.0%, 1.5% and 2.0% v/v) were used to study the effects of suppression of cerebral metabolism. Experiments under hypercapnia (1.5% v/v CO_2) and 1.0% isoflurane were performed to explore the influence of increased cerebral blood flow.

The MRI scanning was conducted on a 31-cm horizontal-bore Varian 9.4 T magnet (Agilent, Inc, USA). Single-shot spin-echo echo-planar imaging was used to acquire a slice crossing the somatosensory area of the brain with thickness = 2 mm in a 64 × 32 matrix and FOV = 32 × 32 mm^2. A train of saturation pulses of 1.5 μT and 58 ms duration was applied at 33 different frequency offsets spanning ± 4 ppm from the water frequency. To calibrate for $\mathbf{B}_0$ shifting, the WASSR method (5) was used with 0.1 μT pulses and offset frequencies within ± 1ppm from the water resonance. The total scanning time was 10.2 min per CEST experiment and 1.2 min for the WASSR experiment, making a temporal resolution of ~11.5 min.

Data were processed using custom-written software in Matlab (Mathworks, USA). The z-spectra were calculated by interpolation with a cubic spline and the magnetization transfer asymmetry was calculated by subtraction of the signal intensity at the negative frequency offset from the positive one. The intensity of the CEST image was calculated as the integral of the spectral intensity within ± 0.25 ppm at 1 ppm, incorporating the spectral envelope of most of the exchangeable protons from the hydroxyl groups of the saccharides. The ^{31}P MR spectra were acquired by using a double-tuned surface coil (Rapid Biomed, Germany) with a non-selective pulse and TR = 20 s. The signal was averaged for 11 min to match the timing of the CEST experiment. The data were processed using TopSpin (Bruker, Karlsruhe, Germany). 2DG6P was quantified by comparing its signal intensity ratio with that of γ-ATP. Cerebral pH was measured by using the chemical shift of Pi and/or 2DG6P with respect to PCr (6).

Results and Discussion

Gluco-CEST showed a markedly enhanced signal within 20 min under 1.0% isoflurane, slightly lower at 1.5% and largely depressed under 2.0% isoflurane (Fig.1). The 2DG6P levels were comparable under 1.0% and 1.5% isoflurane. The 2DG6P intensity gradually increased after 2DG injection, peaked at ~33 min and slowly decreased over the next 40 min. Under 2.0% isoflurane, the 2DG6P accumulated much more slowly, indicating reduced cerebral metabolism. Under hypercapnia (Fig.2), gluco-CEST had a similar time course but slightly lower amplitude. The 2DG6P measured by ^{31}P NMR was comparable under normal and hypercapnic conditions.

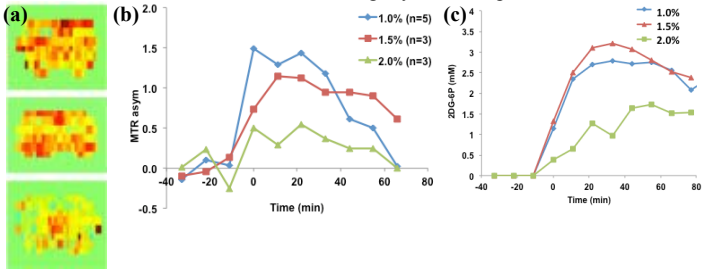

Fig.1 *(a) Gluco-CEST images under 1.0%, 1.5% and 2.0% isoflurane (top to bottom); (b) CEST MTRasym and (c) 2DG-6P signal normalized to γ-ATP under different level.*

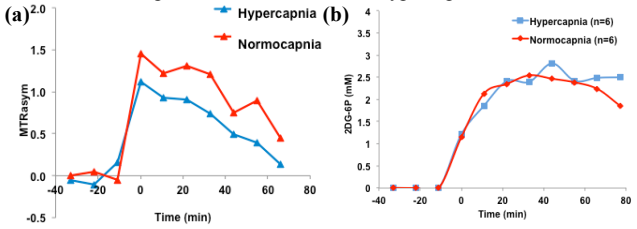

Fig.2 *(a) CEST MTRasym and (b) 2DG6P levels under hypercapnia (blue) and normocapnia (red) and under 1.0% isoflurane.*

A similar CEST signal at 1.0% and 1.5% isoflurane suggested that gluco-CEST might reflect similar cerebral metabolic rate at both concentrations of isoflurane. However, different from the ^{31}P MR measurements, the earlier peak and faster decay indicated a larger proportion of the CEST signal might be dominated by exchange with 2DG rather than 2DG6P. Higher cerebral blood flow under hypercapnia did not appear to increase the delivery of 2DG. Although both isoflurane and hypercapnia could change the pH, which is known to affect the CEST signal intensity, the intracellular pH measured by ^{31}P was similar under all the physiological conditions studied. The blood glucose concentration measured by blood sampling didn't show difference so the slightly reduced CEST signal under hypercapnia might be due to extracellular acidosis and attenuated CEST signal of intracellular 2DG.

References

1. Sokoloff L (1981) Eur. Neurol. 20:137-145. 2. Kotyk JJ, et al (1989) J. Neurochem. 53:1620-1628. 3. Deuel RK, et al (1985) Science 228:1329-1331. 4. van Zijl PCM, et al (2007) PNAS 104:4359-4364. 5. Kim M, et al (2009) MRM 61:1441-1450. 6. Kintner DB, et al (2000) Neurochem. Res. 25:1385-1396.