

A Method for Measuring Cerebral Glucose Metabolism In Vivo by ^{13}C -NMR Spectroscopy

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Current methods for estimating the rate of cerebral glucose utilization (CMR_{glc}) typically measure metabolic activity for 40 min or longer subsequent to administration of [^{13}C]glucose, 2-[^{14}C]deoxyglucose, or 2-[^{18}F]deoxyglucose. We report preliminary findings on estimating CMR_{glc} for a period of 15 min by use of 2-[6- ^{13}C]deoxyglucose. After a 24-hr fast, rats were anesthetized, infused with [1- ^{13}C]glucose for 50 min, and injected with 2-[6- ^{13}C]deoxyglucose (500 mg/kg). During the subsequent 12.95 min the estimated value of CMR_{glc} was 0.6 ± 0.4 micromol/min/g (mean \pm SD, $N = 7$), in agreement with values reported for anesthetized rats studied with the 2-[^{14}C]deoxyglucose method and other ^{13}C -NMR methods that measure CMR_{glc} . In rats injected with bicuculline methiodide (a known stimulant of CMR_{glc}), CMR_{glc} increased by more than 75% during 12.95 min following injection of bicuculline (Wilcoxon signed rank test, $P = 0.042$, $N = 8$). Magn Reson Med 48:1063–1067, 2002. © 2002 Wiley-Liss, Inc.

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Glucose is the primary substrate for the brain's energy metabolism, and the rate of cerebral glucose utilization (CMR_{glc}) is closely correlated with neuronal activity (1). Current methods that employ NMR spectroscopy allow measurement of CMR_{glc} over a period of 45 min, which is required for the isotopic enrichment to reach steady state (2,3). Because cerebral functions are likely to change in minutes, or even seconds, measurement of cerebral glucose metabolism over shorter time periods is desirable and would allow a finer determination of "functional activation" of the brain evoked by specific stimuli. The present study may be considered a first step toward that goal.

The autoradiographic 2-[^{14}C]deoxyglucose (2-[^{14}C]DG) method for estimation of local CMR_{glc} (4) and its adaptation for use in man with 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and positron emission tomography (PET) (5) exploited the property of 2-deoxyglucose to be phosphor-

ylated by hexokinase to 2-deoxyglucose-6-phosphate, which was then trapped in the brain cells. However, because autoradiography and PET cannot distinguish between the isotopic enrichments of the precursor and product pools, these methods require relatively long experimental periods to allow clearance of the unmetabolized precursor, i.e., 2-[^{14}C]DG or 2-[^{18}F]FDG, from the tissues.

NMR spectroscopy has proved to be capable of detecting and distinguishing between 2-[6- ^{13}C]deoxyglucose and its phosphorylated product 2-[6- ^{13}C]deoxyglucose-6-phosphate in conscious rats (6). Nontracer doses of 2-deoxyglucose were subsequently used to estimate rates of cerebral glucose metabolism in mice (7) and rats (8) from chemical analyses done postmortem. The present study was designed to develop a technique that uses NMR spectroscopy and nontracer doses of 2-[6- ^{13}C]deoxyglucose to estimate CMR_{glc} in vivo in less than 15 min.

THEORY

The rate of glucose phosphorylation in brain is equal to the rate of cerebral glucose utilization, i.e., CMR_{glc} . The rates of phosphorylation of glucose and of 2-deoxyglucose ($\text{CMR}_{2\text{DG}}$) at any time (t) are related by the following equation

$$\text{CMR}_{\text{glc}}(t) = \text{CMR}_{2\text{DG}}(t) \cdot [\text{glucose}(t)] / (\text{K}_p \cdot [2\text{-deoxyglucose}(t)]) \quad [1]$$

which can be derived from Michaelis-Menten enzyme kinetics modified for the interactions of competing substrates (9). K_p is the so-called phosphorylation coefficient, and is equal to $V_{\text{max}}^* K_m / V_{\text{max}} K_m^*$, where V_{max}^* and V_{max} are the maximal velocities, and K_m^* and K_m are the Michaelis-Menten constants of hexokinase for 2-deoxyglucose and glucose, respectively (4,10). The phosphorylation coefficient is the factor that allows computation of glucose phosphorylation rates from measured rates of deoxyglucose phosphorylation, and is necessary for the quantitative determination of the absolute value of CMR_{glc} . However, it is not needed for comparison of relative values. The phosphorylation coefficient has been determined in rat brain in vivo and found to equal 0.22 (10).

Like the previously described autoradiographic 2-deoxyglucose method (4), this technique takes advantage of the ability of hexokinase in brain to phosphorylate 2-deoxyglucose (2DG) to 2-deoxyglucose-6-phosphate, a product that is not metabolized further and accumulates within

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the cells. The toxic effects of 2-deoxyglucose are not immediate, and occur when the levels of 2-deoxyglucose-6-phosphate rise to levels that block glucose-6-phosphatase activity (11). We assume that CMR_{2DG} can be determined from the rate of appearance of 2-deoxyglucose-6-phosphate, and that the toxic effects become manifest when the slope begins to fall at later times. Equation [1] assumes negligible glucose-6-phosphatase activity, which has been shown to be very low in rat brain (12,13)—so low that there is no significant loss of products of 2DG phosphorylation from the brain for at least 45 min after administration of 2DG (14).

METHODS

All procedures were performed with the approval of the Institutional Animal Care and Use Committee at the University of Texas Medical Branch at Galveston. Male Sprague-Dawley rats (weighing approximately 380 g), which had been deprived of food (but not water) for 24 hr, were anesthetized with isoflurane (3.0% for induction) in a mixture of 30% O₂ and 70% N₂, intubated, and mechanically ventilated. The isoflurane (0.5–1.0%) anesthesia was maintained throughout the subsequent surgical and NMR procedures. Polyethylene (PE50) catheters were inserted into one femoral artery and one femoral or tail vein. The arterial catheter was used to monitor arterial blood pressure, pulse, hematocrit, and plasma glucose concentration. The venous catheter was used for infusion of [1-¹³C]glucose and bicuculline. To catheterize the internal carotid artery, a 2-cm oblique incision was made on the right ventrolateral side of the neck to expose the common carotid (CCA), internal carotid (ICA), and external carotid (ECA) arteries, and the CCA and ECA were ligated. The pterygopalatine artery was then isolated and ligated to prevent incorrect insertion of the catheter (PE50), which was threaded through an incision in the CCA into the ICA. The surgical wound was then closed with the PE50 catheter affixed to the rat's body.

The rats were placed on a Plexiglas cradle in the bore of the Varian Inova 4.7 NMR spectrometer with a 16-mm surface coil (Doty Scientific, Columbia, SC) dual tuned to ¹H and ¹³C, which was placed on the surface of the head. An NMR standard containing dimethylsulfoxide (DMSO) was placed on the other side of the coil on top of the rat's head. The rat's core temperature was monitored by rectal thermometer and maintained either by a jacket of circulating warm water or by warm air blown into the bore of the magnet.

A bolus (170 mg/kg) of D-[1-¹³C]glucose (Cambridge Isotopes, Inc., Andover, MA) was injected into the femoral or tail vein and followed immediately by a constant infusion (initial flow rate, 10 μL/min) of 25% (wt/vol) D-[1-¹³C]glucose solution. Scout images were taken to verify the positioning of the surface coil. A direct detection pulse sequence was then used to observe ¹³C, with Waltz decoupling and acquisition time of 0.22 s, spectral width of 13000 Hz, and a recycle delay of 1.4 s. Transients were collected every 4.97 min for a period of about 30 min, until the ¹³C content of cerebral glucose became stable. NMR transients were collected every 38.9 s for the next 20 min, during which time a bolus of 99% enriched 2-[6-¹³C]deoxyglucose (500 mg/kg; Cambridge Isotopes, Inc.,

Andover, MA) was administered into the internal carotid artery. In rats given bicuculline, a 1.2 mg/kg bolus of bicuculline methiodide (Sigma, St. Louis, MO) was injected intravenously an average of 6.7 min later. At the end of the experiment the rats were killed by decapitation while they were still anesthetized. Arterial blood glucose concentration was measured just prior to administration of 2-[6-¹³C]deoxyglucose to ensure moderate hyperglycemia (200–300 mg/dL), and again at the end of the procedure.

Blood samples were centrifuged, and the supernatant plasma fractions were stored at –80°C, until further processing. At a later time, the pH of the plasma samples was adjusted to 7.0 with KOH and 3.6% perchloric acid, and then centrifuged for 15 min at 13000 × *g*. The supernatant fraction was retained, adjusted to pH 7.0 with KOH, and centrifuged again. The supernatant of this centrifugation was then dried under a vacuum (model A290; Savant Instruments, Farmingdale, NY), dissolved in 1 ml of 99% D₂O (to replace exchangeable hydrogen atoms with deuterium), centrifuged, and dried under vacuum. High-resolution NMR spectra were carried out in a Bruker 600 MHz NMR spectrometer.

CMR_{glc} was estimated from NMR signals of carbons C1 of glucose, C6 of 2-deoxyglucose, and C6 of 2-deoxyglucose-6-phosphate (illustrated in Figs. 1 and 2). The ¹³C-NMR signal from the DMSO was used to quantify the ¹³C content after calibration with a known concentration of [1-¹³C]glucose and 2-[6-¹³C]deoxyglucose, and consideration of the effects of nuclear Overhauser enhancement, partial saturation, and coil loading. For the computation of $CMR_{glc}(t)$ by Eq. [1], two time points were used, in which the latter time point was the sum of 20 NMR free-induction decays (FIDs) (each approximately 38.9 s) acquired over a total of 12.95 min, and the former time point was the sum of a variable number of FIDs, acquired over an average of 7.56 min. The rate of phosphorylation CMR_{2DG} for each experiment was obtained from the difference in concentration of 2-[6-¹³C]deoxyglucose-6-phosphate at the two time points, divided by the time interval. The rate of cerebral glucose metabolism CMR_{glc} at each time point was estimated from CMR_{2DG} and from the concentrations of glucose and 2-deoxyglucose during the time interval represented by that time point, according to Eq. [1]. The concentration of glucose was obtained from the cerebral concentration of [1-¹³C]glucose divided by the fractional enrichment of the C1 carbon of glucose in plasma. We assumed that the fractional enrichment of glucose in plasma equaled the fractional enrichment of glucose in brain at the time our measurements were made.

The NMR spectra were zero-filled to 4K and processed with a line-broadening of 20 Hz. Analysis of the NMR spectra was accomplished with the software package NUTS (NMR Utility Transform Software; Acorn NMR, Inc., Livermore, CA). Statistical calculations were performed with Minitab Statistical Software, release 12 (Minitab Inc., State College, PA). Values are reported as means ± SD. For statistical tests, *P* < 0.05 was considered significant.

RESULTS

In the control group, CMR_{glc} was 0.6 ± 0.4 micromol/min/g (*N* = 7) during the 12.95 min immediately following

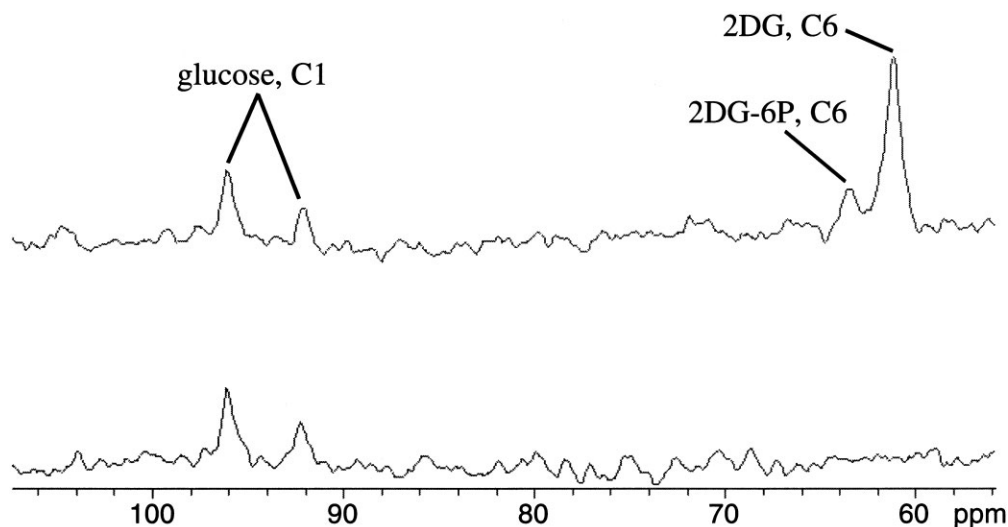


FIG. 1. Stacked plot of spectra of a rat from the control group. The lower spectrum was acquired during 12.95 min prior to injection of 2-[6- ^{13}C]deoxyglucose, and shows the resonances of C1-*beta* and C1-*alpha* isomers of [1- ^{13}C]glucose (96.6 ppm and 92.7 ppm, respectively). The upper spectrum was acquired during the 12.95 min subsequent to injection of 2-[6- ^{13}C]deoxyglucose, and shows the additional resonances of 2-[6- ^{13}C]deoxyglucose (61.2 ppm) and 2-[6- ^{13}C]deoxyglucose-6-phosphate (63.4 ppm). Chemical shift offsets are reported relative to tetramethylsilane.

injection of 2-[6- ^{13}C]deoxyglucose. Cerebral glucose concentrations decreased during this period, from 13 ± 3 mM to 12 ± 3 mM (paired *t*-test, $P = 0.045$).

Because of signal-to-noise ratio (SNR) limitations (6.7 min of acquisition; see below), we were not able to measure the concentration of 2-deoxyglucose-6-phosphate with sufficient

precision at the time of bicuculline injection. Within these limitations, we estimate that in the bicuculline-treated group, CMR_{glc} was 0.4 ± 0.3 micromol/min/g ($N = 8$) prior to injection of the bicuculline, which is not significantly different from the control group (*t*-test, $P = 0.32$). CMR_{glc} increased to 1 ± 0.7 micromol/min/g during 12.95 min following the

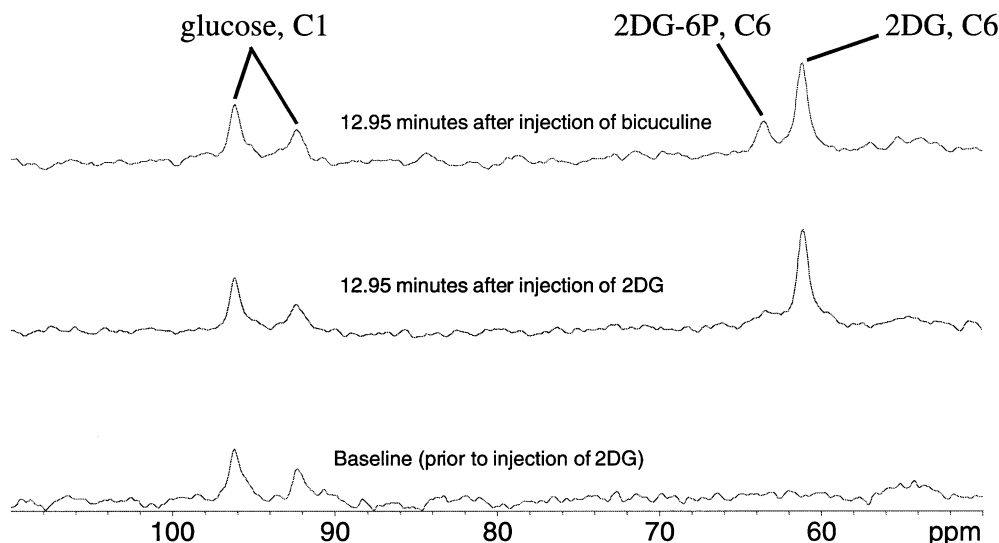


FIG. 2. Stacked plot of spectra from a bicuculline-treated rat. The bottommost spectrum was acquired during 6.48 min prior to injection of 2-[6- ^{13}C]deoxyglucose (^{13}C -2DG). The middle spectrum was acquired during 12.95 min, from the start of the injection of ^{13}C -2DG until just prior to the injection of bicuculline. The uppermost spectrum was acquired during 12.95 min, beginning with the start of the bicuculline injection. The vertical scale has been normalized to the number of FIDs summed in each spectrum. Using the middle and the uppermost spectra, we illustrate the estimation of the rates of phosphorylation of ^{13}C -2DG and of glucose. The concentration of [6- ^{13}C]2-deoxyglucose-6-phosphate increased from 3 mM in the former spectrum to 4 mM in the latter spectrum, yielding a rate $\text{CMR}_{2\text{DG}}$ of phosphorylation of 2-[6- ^{13}C]deoxyglucose equal to $0.08 \mu\text{mol}/\text{min}/\text{mL}$ or $0.06 \text{ micromol}/\text{min}/\text{g}$ (after multiplying by the cerebral water content of $0.77 \text{ ml}/\text{g}$ (19)). Concentrations of [1- ^{13}C]glucose and of 2-[6- ^{13}C]deoxyglucose were 8 mM and 8 mM, respectively, in the period prior to bicuculline injection, and 15 and 10 mM, respectively, in the period subsequent to bicuculline injection. The fractional enrichment of carbon C1 of glucose in plasma was 57%, yielding estimates of cerebral glucose concentration (14, 26 mM) and CMR_{glc} (0.5, 0.7 micromol/min/g) prior to and subsequent to bicuculline injection, respectively.

injection of bicuculline (paired *t*-test, $P = 0.018$), more than twice the value prior to bicuculline injection. If we neglect any accumulation of 2-deoxyglucose-6-phosphate that occurred up to the time of bicuculline injection, and thus overestimate CMR_{2DG} as well as CMR_{glc} , we find that CMR_{glc} increased from 0.7 ± 0.6 micromol/min/g (prior to injection) to 1.5 ± 0.8 micromol/min/g (12.95 min after injection).

The estimates of CMR_{glc} prior to bicuculline injection were calculated using Eq. [1], under the assumption that the average CMR_{2DG} for the 12.95-min interval is the value at the beginning of that interval. Clearly this is an approximation, and would tend to overestimate CMR_{2DG} prior to injection of bicuculline and thus would underestimate the increase of CMR_{glc} caused by bicuculline. Regardless of the value of CMR_{2DG} , the effect of bicuculline on CMR_{glc} , as assessed by the ratio of CMR_{glc} after bicuculline to CMR_{glc} prior to bicuculline, was 3.0 ± 1.9 (95% confidence interval: (1.4, 4.6)). Nonparametric analysis (Wilcoxon signed rank test) indicated that the estimated median of the ratio was 2.5, with a lower bound of 1.75 ($P = 0.042$). We note that by Eq. [1], this ratio equals [glucose]/[2-deoxyglucose] as measured after administration of bicuculline, divided by [glucose]/[2-deoxyglucose] as measured prior to bicuculline. Therefore, the effect of bicuculline on CMR_{glc} can be estimated from the [1- ^{13}C]glucose and the 2-[6- ^{13}C]deoxyglucose resonances, independent of CMR_{2DG} or K_p . The assessment of the relative increase in CMR_{glc} using these latter resonances takes advantage of their greater SNRs, and may provide a means of circumventing the poor SNR of the 2-[6- ^{13}C]deoxyglucose-6-phosphate resonance in future applications.

Cerebral glucose concentration rose from 11 ± 2 mM immediately prior to the injection of bicuculline to 16 ± 5 mM after 12.95 min (paired *t*-test, $P = 0.007$). Since we administered two drugs in succession (2-[6- ^{13}C]deoxyglucose followed by bicuculline), we examined the possibility that the effect of bicuculline on CMR_{glc} derived simply from the time course of effects of the prior administration of 2-[6- ^{13}C]deoxyglucose. There was no significant difference in cerebral glucose concentrations between the control and the bicuculline-treated group immediately prior to the injection of 2-[6- ^{13}C]deoxyglucose (*t*-test, $P = 0.54$) or during the 6.48 min subsequent to injection of 2-[6- ^{13}C]deoxyglucose in controls and just prior to injection of bicuculline in the treatment group (*t*-test, $P = 0.12$). Observing that bicuculline was injected an average of 6.7 min after injection of 2-[6- ^{13}C]deoxyglucose in the bicuculline-treated group, we compared measurements from the 12.95-min period immediately following injection of bicuculline in the latter group with measurements from the 6.48–19.43-min period after injection of 2-[6- ^{13}C]deoxyglucose in the control group. The value of CMR_{glc} in the control group did not change significantly from the beginning to the end of the 12.95-min period (increasing from 0.5 ± 0.4 micromol/min/g to 0.7 ± 0.6 micromol/min/g, paired-*t*-test, $P = 0.247$), whereas the cerebral glucose concentrations tended to decrease (15 ± 5 mM vs. 11 ± 2 mM; paired-*t*-test, $P = 0.089$).

DISCUSSION AND CONCLUSIONS

Previous studies demonstrated the feasibility of detecting 2-[6- ^{13}C]deoxyglucose and 2-[6- ^{13}C]deoxyglucose-6-phos-

phate in vivo (6), but stopped short of estimating CMR_{glc} . The present studies show that it is possible to quantify the rate of cerebral glucose utilization from measurements of the rate of phosphorylation of 2-[6- ^{13}C]deoxyglucose by NMR spectroscopy. We succeeded in estimating CMR_{glc} in less than 15 min in anesthetized control rats. In anesthetized rats administered bicuculline, a GABA_A receptor antagonist that is known to increase CMR_{glc} (15), SNR limitations prevented precise measurement of CMR_{glc} using the concentration of 2-deoxyglucose-6-phosphate. However, from changes in the concentrations of glucose and 2-deoxyglucose, we could estimate the relative increase in CMR_{glc} during the 13-min time period following bicuculline administration. Both groups had been made hyperglycemic by prior intravenous infusion of [1- ^{13}C]glucose (which is used to estimate the isotopic enrichment of cerebral glucose). The relatively rapid measurement of CMR_{glc} during a period of less than 15 min may facilitate the assessment of acute responses of CMR_{glc} to stimuli, provided the limitations in SNR can be overcome.

CMR_{glc} was 0.6 ± 0.4 micromol/min/g in the quiescent, anesthetized rat, and increased by more than 75% in animals treated with bicuculline. The estimate of CMR_{glc} obtained with this method agrees well with the value of approximately 0.60 micromol/min/g measured over a 45-min period with the 2-[^{14}C]deoxyglucose method in rats under thiopental anesthesia (4) and in hyperglycemic rats (16). It is also in agreement with the value of 0.79 ± 0.21 micromol/min/g obtained from estimates of the contribution of CMR_{glc} to oxidative metabolism in rat brain during enflurane anesthesia by ^{13}C -NMR spectroscopy (17).

The variability in our estimates of CMR_{glc} is higher than that found in those previous studies, and we offer the following explanations. The SNR of 2-[6- ^{13}C]deoxyglucose-6-phosphate (Figs. 1 and 2) and the brevity (12.95 min) with which we estimate the rate of CMR_{2DG} limits the precision with which we can estimate CMR_{glc} . However, in the control animals we observed approximately equal contributions to the variability of our estimate of CMR_{glc} (in Eq. [1]) from CMR_{2DG} and from [glucose]/[2-deoxyglucose] (data not shown), suggesting that the physiological response to 2-[6- ^{13}C]deoxyglucose is an important source of variability. (Reducing the dose of 2-[6- ^{13}C]deoxyglucose may alleviate this problem.) Additionally, the lack of a steady state during estimation of CMR_{glc} , although necessary to permit measurements in short time periods, does contribute to the inter-animal variability of the estimates. It is also possible that endogenous fluctuations in the rate of glucose phosphorylation may contribute to the enhancement of variability compared to measurements averaged over longer periods of time.

Miller et al. (18) found that after intravenous injections in rats of 2-deoxyglucose at doses of 250 mg/kg and higher, the concentration of cerebral glucose temporarily decreased by 50%, but then returned to its preinjection level 10 min after the injection. We also observed a tendency for decreased cerebral glucose concentration in control rats during the 12.95 min after injection of 2-deoxyglucose, but the decreases did not achieve statistical significance. Inasmuch as our experiments were performed on 24-hr fasted rats, in which liver glycogen stores are markedly reduced, the glycogenolytic response to 2-deoxyglucose would be expected to be blunted. The constant infusion of

[1-¹³C]glucose also would dampen the changes in glucose concentration that could be detected by our method.

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