Determination of cerebral glucose transport and metabolic kinetics by dynamic MR spectroscopy

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Johns Hopkins University Medical School, Departments of Radiology and Anesthesiology and Critical Care, Baltimore 21205; National Institutes of Health, In Vivo Nuclear Magnetic Resonance Research Center, Biomedical Engineering and Instrumentation Program, National Center for Research Resources, Bethesda 20892; Food and Drug Administration, Center for Drug Evaluation and Research, Office of Research Resources, Division of Clinical Pharmacology, Rockville, Maryland 20850; and Resonance Magnetique des Systemes Biologiques, Unité Mixte de Recherche 5536, Centre National de la Recherche Scientifique, Université Victor Segalen 2, F-33076 Bordeaux Cedex, France

Van Zijl, P. C. M., D. Davis, S. M. Eleff, C. T. W. Moonen, R. J. Parker, and J. M. Strong. Determination of cerebral glucose transport and metabolic kinetics by dynamic MR spectroscopy. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1216–E1227, 1997.—A new in vivo nuclear magnetic resonance (NMR) spectroscopy method is introduced that dynamically measures cerebral utilization of magnetically labeled [1-13C]glucose from the change in total brain glucose signals on infusion. Kinetic equations are derived using a four-compartment model incorporating glucose transport and phosphorylation. Brain extract data show that the glucose 6-phosphate concentration is negligible relative to glucose, simplifying the kinetics to three compartments and allowing direct determination of the glucose-utilization half-life time (t = ln2/(k + k)) from the time dependence of the NMR signal. Results on isofluorane (n = 5) and halothane (n = 7)-anesthetized cats give a hyperglycemic t = 5.10 ± 0.11 min⁻¹ (SE). Using Michaelis-Menten kinetics and an assumed half-saturation constant Kᵢ = 5 ± 1 mM, we determined a maximal transport rate Tₘₐₓ = 0.83 ± 0.19 µmol·g⁻¹·min⁻¹, a cerebral metabolic rate of glucose CMRGlc = 0.22 ± 0.03 µmol·g⁻¹·min⁻¹, and a normoglycemic cerebral influx rate CIRGlc = 0.37 ± 0.05 µmol·g⁻¹·min⁻¹. Possible extension of this approach to positron emission tomography and proton NMR is discussed.

[13C]glucose utilization; brain; Michaelis-Menten kinetics; cat; nuclear magnetic resonance spectroscopy

GLUCOSE IS THE PRIMARY fuel for energy metabolism in normal brain, and the availability of noninvasive in vivo methods for the elucidation of its transport and metabolic kinetics should be important in the study of a multitude of brain disorders (17, 21). An ideal method for measuring cerebral rates of influx (CIRGlc) and metabolism (CMRGlc) should allow direct monitoring of the separate processes of tissue uptake and utilization of glucose under physiological conditions. At present, the most sensitive in vivo approach is the use of radiolabels, which can be applied in tracer amounts (9, 10, 14, 23, 26, 27). However, the interpretation of these measurements is complicated by the fact that the sum of all radiolabels, which basically includes all metabolic products, is measured (13). This problem has been addressed by using glucose derivatives that are trapped after phosphorylation, e.g., deoxyglucose analogs (14, 26, 27), but these compounds may differ in their transport and utilization properties with respect to glucose. This difference is generally accounted for empirically by the use of so-called lumped constants in positron emission tomography (PET) (7, 22). It has been suggested that the kinetic equations for deoxyglucose may need to account for the potentially reversible character of saturable phosphorylation (14), but irreversibility is generally used as a reasonable assumption.

An alternative approach that can be applied to study glucose utilization without the need for a lumped constant is the nuclear magnetic resonance (NMR) study of nonradioactive magnetically labeled glucose, e.g., the naturally occurring isotopomer [1-13C]glucose (3, 4, 11, 30). In principle, NMR can study both the uptake (influx) and metabolism of [1-13C]glucose (11, 18, 30), because different metabolites labeled in different carbon positions generally result in different NMR spectral frequencies (chemical shifts). This specific labeling has been studied by 13C NMR in animals and humans (2, 4, 11). Indirect determination of transport kinetics was recently also achieved by applying equilibrium Michaelis-Menten kinetics to the experimentally measured ratios of plasma and tissue glucose (11). However, NMR is an insensitive method with several inherent problems that interfere with optimum use of its enormous potential. For instance, the time necessary to acquire a 13C NMR spectrum is much longer than the typical time constant for glucose uptake and phosphorylation, thereby prohibiting direct kinetic determination of the rates of glucose influx and utilization. The sensitivity of 13C NMR can be improved by
detecting the proton nuclei coupled to the labeled carbon nuclei (8, 24), but until recently this approach prohibited detection of \([^{13}\text{C}]\)glucose due to its overlap with the dominant water resonance (108 M proton concentration due to 2 protons/water molecule vs. millimolar concentration for the glucose). Therefore, this \([^{1-13}\text{C}]\)glucose transport step has not yet been studied dynamically by \(^{13}\text{C}\) NMR, and assumptions for this part of the metabolic process have to be made when formation of \([^{13}\text{C}]\)glutamate (18) and \([^{13}\text{C}]\)lactate (25) is studied. We have recently developed proton NMR methods that can simultaneously detect all metabolites, even when they are resonating at the same frequency as water (29), and we apply this method here to dynamically study uptake and metabolism of \([^{1-13}\text{C}]\)glucose in healthy cat brain. This approach is the closest possible NMR analog of a PET experiment, since we can limit the detected signal to consist of glucose and phosphorylated glucose only. NMR is not able to use fluorodeoxyglucose due to its toxicity when used in the amounts required for NMR (5).

**Glossary**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>(C_p, C_{p}^t, C_{p}^t)</td>
<td>Unlabeled ((^{12}\text{C})), labeled ((^{13}\text{C})), and total plasma glucose concentrations (mM)</td>
</tr>
<tr>
<td>(C_{e}, C_{e}^t, C_{e}^t)</td>
<td>Unlabeled, labeled, and total tissue glucose concentrations (mM)</td>
</tr>
<tr>
<td>(C_{M}, C_{M}^t, C_{M}^t)</td>
<td>Unlabeled, labeled, and total phosphorylated glucose concentrations in brain tissue (mM)</td>
</tr>
<tr>
<td>(C_i)</td>
<td>Sum of phosphorylated and tissue glucose concentrations (mM)</td>
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<tr>
<td>(C_{Glc})</td>
<td>Total concentration of glucose (mM): (C_{Glc} = C_p + C_e + C_M)</td>
</tr>
<tr>
<td>(C_{blood})</td>
<td>Arterial blood glucose concentration (mg/dl)</td>
</tr>
<tr>
<td>(C_{X,eq})</td>
<td>Concentrations at equilibrium</td>
</tr>
<tr>
<td>(CMRG_{Glc})</td>
<td>Cerebral metabolic rate of glucose ((\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}))</td>
</tr>
<tr>
<td>(CIR_{Glc})</td>
<td>Cerebral influx rate of glucose ((\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}))</td>
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<tr>
<td>(f_{vss})</td>
<td>Vascular volume fraction</td>
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<tr>
<td>(GC-MS)</td>
<td>Gas chromatography-mass spectroscopy</td>
</tr>
<tr>
<td>(Hct)</td>
<td>Hematocrit</td>
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<tr>
<td>(HMQC)</td>
<td>Heteronuclear multiple quantum coherence</td>
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<tr>
<td>(K_{1})</td>
<td>Michaelis-Menten half-saturation constant (mM) for glucose transport</td>
</tr>
<tr>
<td>(K_{hase})</td>
<td>Michaelis-Menten half-saturation constant for glucose phosphorylation (hexokinase-catalyzed = hase)</td>
</tr>
<tr>
<td>(k_1)</td>
<td>Rate constant for glucose influx in the brain ((\text{min}^{-1}))</td>
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<tr>
<td>(k_2)</td>
<td>Rate constant for glucose efflux from the brain ((\text{min}^{-1}))</td>
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<tr>
<td>(k_3)</td>
<td>Rate constant for glucose phosphorylation in brain tissue ((\text{min}^{-1}))</td>
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<tr>
<td>(k_4)</td>
<td>Rate constant for hydrolysis (dephosphorylation) of glucose phosphate ((\text{min}^{-1}))</td>
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<tr>
<td>(k_5)</td>
<td>Rate constant for metabolism of glucose phosphate ((\text{min}^{-1}))</td>
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<tr>
<td>(MRS)</td>
<td>Magnetic resonance spectroscopy</td>
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<tr>
<td>(MRI)</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>(NMR)</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>(PET)</td>
<td>Positron emission tomography</td>
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<tr>
<td>(T_{max})</td>
<td>Michaelis-Menten maximum transport rate of glucose ((\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}))</td>
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<tr>
<td>(T_0)</td>
<td>Time necessary for labeled glucose to reach brain after start of infusion (min)</td>
</tr>
<tr>
<td>(t_0)</td>
<td>Half-life time for glucose utilization ([n/2/(k_5 + k_3)])</td>
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<tr>
<td>(V_d)</td>
<td>Whole brain water volume (ml/g)</td>
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**Materials and Methods**

Metabolic model. The model presented here is based on Sokoloff’s \([^{14}\text{C}]\)deoxyglucose model (14, 27) developed for autoradiography. It is extended to include the specific assumptions necessary for our NMR experiments. For instance, although tracer kinetics do not apply, it is assumed that first-order kinetics are still valid because the observed processes are unsaturable. Second, \([^{1-13}\text{C}]\)glucose is a naturally occurring isotopomer of glucose, and it is generally assumed that the kinetic constants are the same as for the nonmagnetic isotopomer \([^{12}\text{C}]\)glucose, thereby avoiding the need for a lumped constant. We would like to point out that this assumption is not trivial, because atoms of different weight and different size react equivalently chemically, but may do so at a different rate. This effect is actually used by archaeologists to study evolution from the behavior of carbon isotopes during photosynthesis (for a review of this literature see Ref. 28). However, although this effect is significant (a few percent) for \(\text{CO}_2\) fixation, it is expected to be negligible for the glucose transport and metabolism steps studied here. The reasons are that the bond to the isotope is not broken and that the change from \(^{12}\text{C}\) to \(^{13}\text{C}\) should not influence the size \((M = 181)\) and shape of the glucose unit significantly for an enzyme to distinguish between the two isotopes. Thus the general assumption in NMR and PET that isotopic labeling of a single carbon atom does not influence the enzyme kinetics of transport and metabolism is very plausible.

To describe the experiment completely, the model needs four compartments (Fig. 1), namely a blood compartment, a compartment for glucose in tissue, a compartment for phosphorylated glucose and lactate, and a compartment for CO2 fixation. The compartments and fluxes in the model are pictured in Fig. 1. The model is based on the net rates of glucose transport and metabolism, and the fluxes between the different compartments are determined by the known concentrations of glucose, phosphorylated glucose, and CO2. The fluxes between the compartments are determined by the known concentrations of glucose, phosphorylated glucose, and CO2. The fluxes between the compartments are determined by the known concentrations of glucose, phosphorylated glucose, and CO2. The fluxes between the compartments are determined by the known concentrations of glucose, phosphorylated glucose, and CO2. The fluxes between the compartments are determined by the known concentrations of glucose, phosphorylated glucose, and CO2.
phosphorylated glucose in tissue, and a compartment for metabo-
lolic products synthesized after phosphorylation. The bound-
ary between the first two compartments consists of the cap-
illary and cell membranes (BBB). Following Sokoloff et al. (26, 27), the capillary glucose concentration will be approxi-
mated by the arterial plasma glucose concentration. With the
assumption of rapid equilibration of glucose over the cell
membrane, the next compartment is introduced, described by the single
compartment model. The bound-
ary is the hexokinase-catalyzed phosphorylation of
glucose. Because the [13C]glucose utilization is not saturable,
it is not necessary to include the effect of hydrolysis of
phosphorylated glucose back to glucose, and it is accurate to
assume a zero rate constant $k_5$ for this step. To account for
efflux of phosphorylated glucose into different products, a
fourth compartment is introduced, described by the single
rate constant $k_5$. This approach is supported by data from
direct 13C NMR spectroscopy at equilibrium (2, 4), which
show that the concentrations of any intermediates between
total [1-13C]glucose and [4-13C]glutamate are negligible, indicat-
ing rapid conversion to each next step. Thus consecutive
metabolism after phosphorylated glucose in healthy brain
can be described by a single rate constant $k_5$ to [4-13C]gluta-
mate. In this respect, it is important to notice that it is
indifferent for our model if the step to the first product after
phosphorylation is rate determining or if the last step
(conversion to glutamate) is.

Following Sokoloff et al. (27), the concentrations of glucose in
blood plasma and tissue are denoted by $C_P$ and $C_E$, whereas
the concentration of phosphorylated glucose is denoted by $C_M$. The
total tissue glucose concentration is $C_i$. All compartments contain normal
and 13C-enriched compounds, whereby enrichment is denoted by
an asterisk (*). Care has to be taken in deriving the rate
equations, since the rates are determined by the total glucose
concentrations (labeled + unlabeled, e.g., $C_{i\text{eq}} = C_P + C_E$), whereas only changes in the labeled compounds are
measured by our proton-detected 13C NMR spectroscopy method.
To account for this, our equations are derived with the
assumption that the unlabeled glucose concentration remains
constant during the short experiment. We have con-
Firmed this assumption for the blood compartment using
GC-MS (see RESULTS). With the assumption that hyperglycemia
(20–30 mM glucose) does not alter the steady-state
transport and metabolism of glucose, and using the fact that the
concentration of unlabeled glucose is constant, the rates of
change of $C_{i\text{eq}}(t)$ and $C_{i\text{eq}}(t)$ are given by

$$
\frac{dC_{i\text{eq}}(t)}{dt} = k_1 C_{i\text{eq}}(t) - (k_2 + k_3)C_{i\text{eq}}(t)
$$

$$
\frac{dC_{i\text{eq}}(t)}{dt} = k_2 C_{i\text{eq}}(t) - (k_2 + k_3)C_{i\text{eq}}(t)
$$

The solution to Eq. 1 for the tissue concentration of labeled
and unlabeled glucose at time $T$ after introduction of [1-13C]glucose into the
blood is

$$
C_{i\text{eq}}(T) = C_{i\text{eq}}(0) e^{-k_3 T} \int_0^T C_{i\text{eq}}(t) e^{k_2 T} dt
$$

In our [1-13C]glucose infusion protocol, we rapidly (within
0.5–2 min) raise the total plasma glucose concentration
($C_{i\text{eq}} = C_P + C_E$) to the desired hyperglycemic level (20–30
mM) and keep it constant (at equilibrium) for the remainder
of the experiment. Thus the concentration of labeled glucose
can be taken constant at any point in time, and $C_E(t) = C_{i\text{eq}}$.
The integral in Eq. 3 can then be evaluated for any time $T$

$$
C_{i\text{eq}}(T) = \frac{k_1}{k_2 + k_3} C_{i\text{eq}}(0) \left[1 - e^{-k_3 T} \right]
$$

Analogously to Eqs. 1 and 3, the solution for the concentration
of phosphorylated glucose at any time point $T$ is given by

$$
C_{i\text{eq}}(T) = k_3 e^{-k_3 T} \int_0^T C_{i\text{eq}}(t) e^{k_2 T} dt
$$

Substitution of Eq. 4 into Eq. 5, evaluation of the integral and
rearrangement of the expression in terms of a common
denominator leads to the following result

$$
C_{i\text{eq}}(T) = \frac{k_1 k_3}{k_2 + k_3(k_2 + k_3 - k_2 k_3 k_5)} C_{i\text{eq}}(0) \left[1 - e^{-k_3 T} \right]
$$

Although most compounds detected by NMR have different
chemical shifts in the proton or carbon frequency range,
spectral resolution does not allow in vivo separation of the
signals of blood and tissue glucose and glucose 6-phosphate
(G-6-P). Thus the operational equations have to describe
the concentration $C_{i\text{eq}}$, which is the sum of the
contributions of labeled tissue glucose and G-6-P ($C_E$) and labeled plasma
glucose, corrected for the vascular-tissue volume fraction $f_{\text{vas}}$

$$
C_{i\text{eq}} = (1 - f_{\text{vas}})[C_{i\text{eq}}(T) + C_{i\text{eq}}(T)] + f_{\text{vas}} C_{i\text{eq}}(T)
$$

Finally, to describe the change of the labeled tissue plus
plasma glucose, $C_{i\text{eq}}$, as a function of time from the start of
infusion to equilibrium, the expressions at equilibrium
($dC_{i\text{eq}}/dt = dC_{i\text{eq}}/dt = 0$) have to be evaluated. Combination of
Eqs. 1, 2, and 7 leads to the following two expressions at
equilibrium

$$
C_{i\text{eq}} = \frac{k_1 (k_2 + k_3)}{k_2 (k_2 + k_3)} C_{i\text{eq}}
$$

$$
C_{i\text{eq}} = \frac{k_2 (k_2 + k_3)}{k_2 (k_2 + k_3)} C_{i\text{eq}}
$$

The complete expression describing the combined changes of
glucose in plasma and tissue plus G-6-P in tissue can be obtained from combining Eqs. 4, 6, 7, and 8

$$
C_{i\text{eq}} = \frac{C_{i\text{eq}}}{(k_2 + k_3)} \left[1 - (1 - f_{\text{vas}}) k_1 (k_2 + k_3) + f_{\text{vas}} k_1 (k_2 + k_3) \right]
$$

$$
\frac{dC_{i\text{eq}}}{dt} = \frac{(1 - f_{\text{vas}}) k_1 (k_2 + k_3) + f_{\text{vas}} k_1 (k_2 + k_3)}{1 - e^{-k_3 T - T_0}}
$$

$$
+ f_{\text{vas}} k_1 (k_2 + k_3) (1 - e^{-k_3 T - T_0})
$$

In this equation a correction time delay $T_0$ has been added to
adjust for the delay time between the start of the infusion and
the time that glucose reaches the brain, which basically is the
intercept of the experimental curve with the time axis.
Equation 9a shows that the experimental curve can be fitted
independently of knowledge of the actual plasma and tissue glucose concentrations by calibrating the signal intensities with respect to the signal intensity at equilibrium. The curve has to be fitted to four rate constants. The time intercept T_0 is well known from the experimental arterial plasma data and is not an additional variable. If the plasma contribution to the spectrum can be neglected (f_{vas} = 0), the curve represents the combined tissue glucose and has to be fitted to only three rate constants

\[ C^*_G(T) = \frac{C^*_{eq}}{(k_3 + k_2)(k_2 + k_5 - k_5)}[k_5(k_2 - k_3)[1 - e^{-(k_2 + k_5)(T - T_0) + k_5}(k_2 + k_5)[1 - e^{-(k_2 + k_5)(T - T_0)}]\] (9b)

Equations 9a and 9b are the exact operational equations to be used for fitting the experimental data to a four-compartment model. A quick check of their accuracy is provided by calculating the limit for equilibrium between inflow and metabolism: for long times T, all rate constants and vascular fractions drop out.

Determination of rate constants from the proton-detected 13C NMR spectra. To obtain the rate constants and T_0, the experimental glucose uptake and phosphorylation curve has to be fitted to Eqs. 9a or 9b. Fitting of a single experimental curve to three or four rate constants may lead to a large variance in the results. This variance can be reduced if additional restrictions and/or reasonable starting values are available for the variables. To simplify the problem, it would be very convenient to know the concentration of phosphorylated glucose to pyruvate do not have protons connected to a labeled carbon nucleus. However, it should still be shown that we detect [1-13C]glucose only and not any other metabolites. As mentioned, most compounds detected by NMR spectroscopy have different chemical shifts in the proton or carbon frequency range and can be detected separately (30). Although spectral resolution does not allow in vivo separation of the signals of blood and tissue [1-13C]glucose and [1-13C]G-6-P, the total glucose signals (4.6 and 5.4 ppm) can be separated from all other brain metabolic glucose products that are present in sufficient concentration during our 30- to 40-min experiment (Fig. 2). First of all, the metabolic products in the subsequent series of reactions from phosphorylated glucose to pyruvate do not have protons connected to 13C and are therefore not detected. Second, [3-13C]pyruvate has the proton resonance at 2.4 ppm and its 13C resonance at 28.5 ppm. The first experimental (Fig. 2) proton signal appearing in addition to the total glucose resonances in healthy brain is at 2.4 ppm and could in
principle be [3-13C]pyruvate (15) or [4-13C]glutamate-glutamine (24, 29). As mentioned above, combined proton and carbon studies have shown the first detectable product to be [4-13C]glutamate (2, 4, 8, 29). Thus our experiment measures total labeled glucose: C(13)Glc(T).

Two cases can now be distinguished, one in which data interpretation is straightforward and one requiring an additional assumption. If the vascular contribution is negligible, spectral intensities can be scaled with respect to the equilibrium spectral intensities and the resulting exponential can be fitted to Eq. 12, allowing direct determination of the tissue glucose half-life time without additional assumptions.

\[ t_{1/2} = \frac{\ln 2}{k_2 + k_3} \]  

(13)

If the vascular contribution is not negligible, the experimental NMR signal intensities represent C(13)Glc(T) in Eq. 10, and to obtain the corresponding C(13)P(T) in each point, the data have to be corrected. This can be done by using Eq. 10 to calculate C(13)Glc(eq) from the equilibrium ratio of plasma and tissue glucose reported in the literature and the experimentally determined plasma level for labeled glucose C(13)P. After the C(13)Glc(T) curve is normalized with respect to C(13)Glc(eq), the correct C(13)P(T) values can be obtained using Eq. 10 and the experimental C(13)Glc(T) values. The final C(13)P(T) curve can then be fitted to Eq. 12.

RESULTS AND DISCUSSION. We will evaluate the influence of the vascular contribution on the final rate constants. The tissue-to-plasma glucose ratio at equilibrium has been determined for rats with the use of rapidly frozen brains (19, 22) and in humans with the use of [13C]NMR spectroscopy (11). In the following we neglect the phosphorylated glucose contribution in the notation, as warranted by the experimental results. The animal studies, which report plasma glucose in millimolar units, show a concentration dependence of C(13)P,eq/C(13)E,eq in the literature and the experimentally determined glucose half-life time without additional assumptions.

The solution for this equation is

\[ C(13)Glc(T) = k_1C(13)P,eqT \]  

(15)

For our case of negligible phosphorylation, we determine k1 from Eq. 11 and from the initial rate approximation and compare the results and accuracies for different vascular fractions and plasma-to-tissue ratios. In the general case including phosphorylation, determination of k3 by the initial rate approach reduces the situation to two equations (Eqs. 8b and 9b) and three unknowns, which may in some cases be fitted accurately.

At equilibrium, the rate constants are related to the glucose influx (CIR Glc) and utilization (CMR Glc) rates in micromoles per gram per minute by

\[ CIR Glc/V_d = k_1C(13)P,eq \]  

(16)

\[ CMR Glc/V_d = k_2C(13)P,eq - k_3C(13)P,eq \]  

(17)

These parameters, describing the facilitated influx of glucose and its metabolism, respectively, are equivalent to the quantities V2 and V4 used by Mason et al. (18) in their evaluation of glutatione production by the trichloroacetic acid cycle. Equation 17 also indicates that, in the present model at equilibrium, the effective glucose transport rate \( k_1C(13)P,eq - k_3C(13)P,eq \) is equal to the phosphorylation rate.

Data processing protocol. In our experiments, the NMR signal intensity of total [1-13C]glucose and the total arterial blood glucose concentration (glucose analyzer) are measured as a function of time. The data are processed as follows.

1) The plasma glucose concentrations before infusion (C(13)P = C(13)P0) and at the plateau phase (6–10 points) of the total glucose curve (C(13)P(eq) is measured from the experimentally determined total arterial blood glucose concentrations (C(13)P(eq) and hematocrits (Hct)

\[ C(13)P(eq)(mM) = C(13)P(eq)(mg/dl)/18.1\{1 - (Hct/100)\} \]  

(18)

in which 18.1 is the molar mass of [1-13C]glucose divided by 10 to correct for the I/d conversion. These concentrations are then used to determine the enriched plasma glucose concentration at each measured time point.

2) The enriched plasma glucose level C(13)P,eq and the rat plasma-to-tissue ratio of glucose C(13)P,eq/C(13)E,eq are used to calculate the enriched tissue glucose concentration at equilibrium.

3) The complete experimental NMR signal intensity curve is then calibrated in terms of C(13)Glc(T) in millimolar and in terms of C(13)P(T) in millimolar units, as described in Determination of rate constants from the proton-detected [13C]NMR spectra.

4) The time constant T50 is taken as the time that it takes for the arterial plasma levels to reach at least 65% specific activity. This determination of T50 is accurate, since the blood glucose rate is measured every 0.5 min in the arteries lateral and contralateral (alternatingly) to the (femoral) infusion vein. Thus assumption of similar plasma concentrations in the brain arteries and these arteries seems appropriate, since all are the result of a complete blood recirculation.

5) The rate constants and rates are determined as described in Determination of rate constants from the proton-detected [13C]NMR spectra.

In vivo NMR measurements. Experiments were performed on a 4.7 T GE CSI system equipped with shielded gradients (bore size 35 cm) of up to 0.19 T/m and corrected for additional small residual gradients by preemphasis. The so-called gradi-
ent-enhanced HMOC pulse sequence that was used has been described in detail elsewhere, and the parameters that were applied were exactly as described previously (29). In short, gradient lengths were 2 ms; postgradient delays of 300 μs were used for recovery. Additional gradients (G(αdd)) of 0.06 and 0.04 T/m were used in the z and x directions. In some cats we used combined coherence selection gradient ratios G1: G2: G3 = 3:5:0 and 2:2:1 in different gradient directions, which select for the same heteronuclear coherence pathway. In other cases we only used 3:5:0 in two or three gradient directions. Variation of these selection gradients does not influence our spectra (29). A two-step phase cycle was used in addition to gradient selection, resulting in proton spectra that are free of water (Fig. 2). It is important to notice that, since only one of two coherence pathways is selected, spectra generally cannot be phased and have to be processed as magnitude spectra.

The experimental protocol was as follows. After initial spin-echo imaging to determine the slice position and before the start of infusion, hardware and sequence performance were always tested on a 100 mM [1-13C]glucose phantom placed on top of the coil. For this test, slice selection was moved from the cat (below the coil) to the phantom. After the test, slice position and width and proton pulse widths were optimized on the cat brain and the slice was shimmed using the water spin echo. The initial carbon pulse width was calculated from the in vivo proton pulse width using the phantom proton-to-carbon pulse-width ratio. This initial value always proved to be close to correct. Radio frequency pulse widths generally did not vary more than 5–10% per cat experiment. Data were processed with a line broadening of 13 Hz. In different experiments, the number of scans acquired per spectrum varied from 64 to 128, with different total scan times ranging between 1 and 2.5 min. Before the start of the infusion, 10–12 reference spectra were acquired, which were later added and normalized to the intensity of the single spectra. This reference spectrum was subtracted from the data acquired during infusion (difference spectroscopy). The purpose of the high number of reference spectra is to reduce signal-to-noise losses upon subtraction.

Concentric surface coils (1H transmit/observe: 4 cm; 13C transmit: 6 or 7.5 cm) were used together with band-pass filtered selective inversion spoiling sequences (320 μs). The carbon pulses were 65–140 μs, depending on the load and available power. In most experiments no 13C decoupling was applied.

GC-MS analysis. Immediately after blood sampling, aliquots of 0.05 ml of arterial blood were mixed with 0.05 ml of internal standard d-[1-13C]glucose (5 mg/ml; 13C = 90%; Cambridge Isotopes, Woburn, MA) and immediately frozen at −70°C. Before GC-MS analysis, frozen blood samples were lyophilized and then derivatized by addition of 0.1 ml of N-trimethylsilyl imidazole (Pierce, Rockford, IL) and 0.1 ml of acetonitrile and heating in a sonicator (Branson Instruments, Danbury, CT) at 65°C for 30 min. Subsequently, four 1-μl aliquots of each sample were analyzed on a HP5890/HP5971A GC-MS ( Hewlett-Packard, Palo Alto, CA) equipped with a HP–1 Column (12 m × 0.2 mm × 0.33-μm film thickness). Injector and detector temperatures were 270 and 280°C, respectively. The carrier gas was helium at a constant flow of 0.44 ml/min. Oven temperature stepping was programmed at 6°C/min from 160 to 250°C. Selected ions were monitored at 435 for d-glucose, 436 for d-[1-13C]glucose, and 441 for d-[1,2,3,4,5,6-13C]glucose, with the multiplier set at 1,000 V above autotune values. From these data, d-glucose to ν-d-[1-13C]glucose isotope ratios were calculated. The standard curves obtained for d-glucose and d-[1,13C]glucose (99% 13C) were linear over the range of 0.5 to 4 mg/ml.

Animal preparation. Animals were handled in accordance with the standards established by the US Animal Welfare Acts, set forth by the National Institutes of Health (NIH) guidelines and guidelines of the Johns Hopkins University Animal Care and Use Committee. There were two groups of cats, namely those studied at NIH (cats 1–5) and those studied at Johns Hopkins University (cats 6–12), for which the anesthetic protocols were slightly different. Fasted cats (adults, about 3.0 kg, either sex) were initially anesthetized with ketamine (cats 1–5, 44 mg/kg ip) or pancuronium bromide (cats 6–12, iv) and placed under mechanical ventilation with 1% isoflurane in 30:70% O2–N2O (cats 1–5) or 0.5–1.5% halothane in oxygen-enriched air (cats 6–12). The tidal volume was adjusted to maintain PaCO2 between 30 and 40 and PaO2 > 100 mmHg. Immobilization was maintained with pancuronium bromide (0.2 mg·kg−1·h−1). The time between induction and the start of the infusion experiments was always at least 3 h, resulting in an anesthetic regimen of mainly isoflurane and halothane for the two groups, respectively. Arterial pH was maintained between 7.35 and 7.45, using sodium bicarbonate as needed. Heart rate and blood pressure were monitored through the femoral artery catheter. Blood gases were measured with Radiometer ABL electrodes and analyzer (Copenhagen, Denmark). Body temperature was controlled by a thermostat-controlled water-circulated heating pad. KCl for euthanasia was administered through the femoral vein catheter. The NMR coil was placed on the head, without tissue retraction.

For the glucose uptake experiments, the blood glucose level was rapidly increased from normal (~60–70 mg/dl = 3.3–3.9 mM) to hyperglycemic (>250 mg/dl = 13.8 mM) within 0.5–2 min and kept constant within ±10% for the remainder of the experiment (Fig. 2B). This was achieved using an initial bolus of 1.25 ml/kg) and a subsequent infusion of exponentially decreasing rate (from 1.25 ml·kg−1·min−1 in the first min to 0.11 ml·kg−1·min−1 at 25 min, after which the rate was not changed) of a solution of 0.75 M [1-13C]glucose in saline. [13C]glucose (99%) was obtained from Cambridge Isotopes. Blood glucose and lactate levels (YSI model 2300 Stat Glucose/Lactate Analyzer; Yellow Springs Instruments, Yellow Springs, OH) were monitored by extraction of 0.2 ml arterial blood every 0.5 min for the first 6 min of the experiment, every 2 min for the next 6 min, and every 5–10 min at later stages. Both remained stable within 10%. All cats studied responded similarly to this protocol. Extract studies. In vivo sampling of brain tissue was performed under anesthesia. Halothane and mechanical ventilation were continued during removal of cats (n = 3) from the magnet after completion of NMR experiments. The skin and muscle were incised and reflected using electrocautery and coagulation. The bone was removed as above, and bleeding was controlled using bone wax. The dura was carefully reflected with a forceps and scalpel to prevent bleeding. The superficial cortex with a spatula and rapidly frozen by pressing samples using tongs prepared by submersion in liquid nitrogen. The frozen tissue was wrapped in aluminum foil and frozen in liquid nitrogen until further processing. The animals were killed with potassium chloride injection. Extracts were ground to a fine powder using a mortar and pestle cooled by immersion in liquid nitrogen. The powder was homogenized with 6% perchloric acid using a Tekmar Tissumizer (Tekmar, Cincinnati, OH) for 3–5 min and centrifuged at 17,500 g for 15 min.
at 4°C. The supernatant was neutralized with 3 M K$_2$CO$_3$/1 M KOH, mixed with chelating resin (Sigma Chemical, St. Louis, MO) to remove paramagnetic metal ion contamination, and centrifuged once more. The supernatant was freeze-dried, the lyophilized brain extract was dissolved in D$_2$O and filtered through a column to remove paramagnetic metal ion contamination, and the pH was adjusted to 7. High resolution proton-detected [1-13C]glucose NMR spectra were recorded with a Bruker MSL-500 NMR spectrometer [repetition time: 2 or 7 s, 12 µsec (60°) pulse; sweep width 25 kHz; 32 K data blocks zero-filled to 64 K, 2 Hz exponential line broadening].

### RESULTS

Kinetic parameters. Figure 2A shows an example of proton-detected [1-13C]glucose difference spectra acquired before and during infusion. Figure 2B compares the normalized arterial glucose level input function (100% at equilibrium) in this cat to the normalized changes in total proton-detected [1-13C]glucose NMR signal. It is clear from the difference between the two curves that the NMR signal indeed represents brain tissue glucose input and not the arterial glucose input. These normalized curves were first used to determine the hyperglycemic rates $k_2 + k_3$ (Eq. 12) and $k_3$ (Eq. 11) for 12 cats, assuming negligible vascular glucose fraction (Table 1). Subsequently, the $k_2 + k_3$ values at vascular fractions of 3 and 5% were determined after the total-labeled glucose curves were corrected for these plasma fractions, using the procedure outlined in METHODS AND MATERIALS. The results in Table 1 show that, when normalized curves are fit, $k_2 + k_3$ is the same for all vascular fractions between 0 and 5%. The rate constant $k_2$ was determined in two different ways, namely from $k_2 + k_3$ using Eq. 11 and by using an initial rate approach (Fig. 2C) in which the tangent of the initial part of the fitted curve is determined experimentally. The data clearly indicate that much lower (incorrect) results are obtained for $k_3$ values determined by the initial rate approach when the curve is not corrected for the vascular fraction. This makes sense, since the largest errors in the curve intensity are made for small total glucose values, which are in the region where the initial rate fitting occurs. The $k_2$ values at 3 and 5% are equal within error, and the result of the initial-rate approach at these two fractions (Table 1: $k_3 = 0.055 ± 0.003$) agrees well within the SE with $k_3$ determined using the plasma-to-tissue glucose equilibrium ratio and Eq. 11 (Table 1: $0.052 ± 0.001$). It should be noticed that this agreement is not due to the fact that the plasma-to-tissue glucose equilibrium ratio is used to correct the curves, because very different $k_3$ values are found for the individual cats when the $k_3$ numbers determined from $k_2 + k_3$ and from the initial rates at $f_{vas} = 3−5\%$ (e.g., cats 2 and 3) are compared. To see whether the chosen plasma-to-tissue glucose ratio

### Table 1. Plasma glucose concentrations (from glucose analyzer) and hyperglycemic rate constants $k_2 + k_3$ and $k_3$ determined from individual cat data for different vascular fractions

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>C$_{t, eq}$ mM</th>
<th>C$_{p, eq}$ mM</th>
<th>C$_{t, eq}$</th>
<th>C$_{p, eq}$</th>
<th>k$<em>{2} + k</em>{3}$ min$^{-1}$</th>
<th>k$_{3}$ min$^{-1}$</th>
<th>k$_{3}$ From Initial Rate, min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_{t, eq}$</td>
<td>C$_{p, eq}$</td>
<td>C$_{t, eq}$</td>
<td>C$_{p, eq}$</td>
<td>f$_{vas}$ = 0%</td>
<td>f$_{vas}$ = 3%</td>
<td>f$_{vas}$ = 5%</td>
</tr>
<tr>
<td>1</td>
<td>17.0</td>
<td>3.1</td>
<td>20.1</td>
<td>0.139</td>
<td>0.139</td>
<td>0.139</td>
<td>0.0535</td>
</tr>
<tr>
<td>2</td>
<td>16.2</td>
<td>6.4</td>
<td>22.6</td>
<td>0.130</td>
<td>0.130</td>
<td>0.130</td>
<td>0.0500</td>
</tr>
<tr>
<td>3</td>
<td>20.1</td>
<td>3.9</td>
<td>24.0</td>
<td>0.122</td>
<td>0.123</td>
<td>0.123</td>
<td>0.0473</td>
</tr>
<tr>
<td>4</td>
<td>17.2</td>
<td>3.3</td>
<td>20.5</td>
<td>0.152</td>
<td>0.152</td>
<td>0.152</td>
<td>0.0585</td>
</tr>
<tr>
<td>5</td>
<td>14.8</td>
<td>4.1</td>
<td>18.9</td>
<td>0.134</td>
<td>0.134</td>
<td>0.134</td>
<td>0.0515</td>
</tr>
<tr>
<td>6</td>
<td>13.0</td>
<td>9.5</td>
<td>22.5</td>
<td>0.135</td>
<td>0.135</td>
<td>0.135</td>
<td>0.0519</td>
</tr>
<tr>
<td>7</td>
<td>17.6</td>
<td>4.5</td>
<td>22.1</td>
<td>0.159</td>
<td>0.159</td>
<td>0.159</td>
<td>0.0612</td>
</tr>
<tr>
<td>8</td>
<td>26.8</td>
<td>10.3</td>
<td>37.1</td>
<td>0.132</td>
<td>0.132</td>
<td>0.132</td>
<td>0.0508</td>
</tr>
<tr>
<td>9</td>
<td>20.4</td>
<td>5.8</td>
<td>26.2</td>
<td>0.126</td>
<td>0.126</td>
<td>0.126</td>
<td>0.0485</td>
</tr>
<tr>
<td>10</td>
<td>20.8</td>
<td>8.1</td>
<td>28.9</td>
<td>0.130</td>
<td>0.130</td>
<td>0.130</td>
<td>0.0500</td>
</tr>
<tr>
<td>11</td>
<td>23.6</td>
<td>6.6</td>
<td>30.2</td>
<td>0.140</td>
<td>0.140</td>
<td>0.140</td>
<td>0.0538</td>
</tr>
<tr>
<td>12</td>
<td>18.3</td>
<td>8.0</td>
<td>26.3</td>
<td>0.129</td>
<td>0.129</td>
<td>0.129</td>
<td>0.0496</td>
</tr>
<tr>
<td>Avg.</td>
<td>18.8</td>
<td>6.1</td>
<td>24.9</td>
<td>0.136</td>
<td>0.136</td>
<td>0.136</td>
<td>0.0522</td>
</tr>
<tr>
<td>SD</td>
<td>3.8</td>
<td>2.4</td>
<td>5.2</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
<td>0.0041</td>
</tr>
<tr>
<td>SE</td>
<td>1.0</td>
<td>0.7</td>
<td>1.5</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

†Calculated from the mean $k_2 + k_3$ at 3 different vascular fractions using Eq. 11 and C$_{p, eq}^{tot}/C_{E, eq}^{tot} = 2.6$. Avg., average.
influences the agreement between the initial rate and total-curve determination of \( k_1 \), we fitted our results at two other \( C_{P,eq}/C_{E,eq} \) ratios for a vascular fraction of 5%. The results (Table 2) show that the average \( k_1 \) from both approaches is again comparable.

Determination of unlabeled glucose levels in blood during the infusion protocol. To determine the plasma levels of nonenriched glucose during the infusion protocol, we used blood samples of two cats to perform mass spectroscopy. The results for both cats were similar, and Fig. 3 shows the data for one of these cats, in which blood glucose levels were ramped up quickly to \( \sim 9 \) mM and then more slowly increased to 16 mM (290 mg/dl). It is clear from this figure that the unlabeled \( ^{12}C \) glucose level remains stable within the error of the measurement, despite the hyperglycemic level of the total blood glucose. The correspondence between the glucose analyzer and the GC-MS data is good. It is interesting that the analyzer generally gives a 10% higher value than GC-MS, but this will not significantly influence the calculations performed in calibration of the normalized experimental NMR curve.

Extract studies. Figure 4, A and B, shows extract spectra taken in vivo and postmortem. The reliability of the in vivo study is confirmed by the low level of the \( [1-{ }^{13}C] \) lactate resonance at 21 ppm, which should not be confused with the acetate standard. Lactate is very high in the postmortem spectrum. Figure 4, C and D, shows close-ups for the \( [1-{ }^{13}C] \) glucose region, which contains resonances for \( \alpha \)- and \( \beta \)-isomers of \( [1-{ }^{13}C] \) glucose (at 93.11 and 96.95 ppm, respectively). It can be seen that the contribution of \( [1-{ }^{13}C]G-6-P \) to total labeled glucose is negligible in both spectra, confirming the validity of the simplified approach using three compartments. To confirm the correctness of this result, we also prepared a phantom with approximately equal amounts of \( [1-{ }^{13}C] \) glucose and \( [1-{ }^{13}C]G-6-P \) (Fig. 4E). This spectrum shows that the two resonances can indeed be separated. Finally, Fig. 4F shows the relevant glucose region for a heart extract. In this organ the \( [1-{ }^{13}C]G-6-P \) concentration is higher than in the brain and can be clearly distinguished.

DISCUSSION

The results in Table 1 show that dynamic \( ^{13}C \) NMR spectroscopy can directly provide the quantity \( k_2 + k_3 \) and thus the tissue glucose half-life time at hyperglycemia. It is important to realize that this parameter is independent of \( C_{P,eq}/C_{E,eq} \) (Tables 1 and 2) and can be obtained from a simple exponential curve fit of the normalized experimental signal intensities. Thus the accuracy of this parameter depends only on the assumptions made in deriving the rate equations and on the experimental accuracy of the signal intensities. The quality of the NMR data (e.g., Fig. 2) and the very small standard deviation of only 8% indeed indicate good experimental accuracy, and the assumptions made in deriving the rate equations have also been well tested. First of all, our experiments (Fig. 2) show that plasma glucose is already constant at hyperglycemia within 0.5 to 2 min, whereas the first spectrum (average over the first 84 s) shows only a small change with respect to control. Thus the derivation based on a constant total blood glucose level during the experiment is well founded. Second, our extract data show convincingly that the concentration of phosphorylated glucose is negligible compared with total glucose, thereby validating the use of the simplified Eqs. 10-12 for total glucose. Third, the GC-MS data indicate that the unlabeled glucose concentration is stable during the duration of our experiments in the cat, supporting the initial assumptions in Eqs. 1 and 2, in which the nonlabeled glucose does not contribute to the change in total glucose. Fourth, the results in Table 1 show that the determined hyperglycemic \( k_2 + k_3 \) values are independent of the vascular fraction and therefore can be determined accurately from the experimental data without any assumptions. However, to determine \( k_1 \) it is necessary to assume a certain plasma-to-tissue glucose ratio, whereas determination of CMR_{Glc} requires an additional assumption.

The values in Tables 1 and 2 indicate that the \( k_1 \) values determined by the initial rate approach at vascular fractions 3–5% are equal (within SD) to the ones obtained from \( k_2 + k_3 \) and \( C_{P,eq}/C_{E,eq} \) using Eq. 11. This confirms the accuracy of our model, especially the assumption of unidirectional metabolism. Because the initial rate approach is less accurate than the use of Eq. 11, we will use the \( k_1 \) values derived from \( k_2 + k_3 \) in our subsequent calculations.

It is clear from Tables 1–3 and from Eq. 11 that the choice of \( C_{P,eq}/C_{E,eq} \) will profoundly influence \( k_1 \). In addition, further analysis of the results...
CEREBRAL GLUCOSE UTILIZATION FROM DYNAMIC MRS

A. In vivo brain extract

B. Postmortem brain extract

C. In vivo brain extract

D. Postmortem brain extract

E. Phantom

F. In vitro heart extract
in terms of cerebral influx and utilization rates and the comparison of these rates with the literature are complicated by the fact that the rate constants \( k_1 \)–\( k_3 \) determined in dynamic uptake experiments are concentration dependent. This dependence is expressed in the relationship between Sokoloff’s constants and the Michaelis-Menten parameters for the individual rate constants \( k_1 \) and \( k_2 \)

\[
k_3 \text{ (min}^{-1}) = \frac{T_{\text{max}}/V_d}{C_P^{\text{tot}} + K_t} \quad \text{and} \quad k_2 \text{ (min}^{-1}) = \frac{T_{\text{max}}/V_d}{C_E^{\text{tot}} + K_t}
\]

in which \( T_{\text{max}} \) (in \( \mu mol \cdot g^{-1} \cdot min^{-1} \)) and \( K_t \) (in mM) are the Michaelis-Menten maximum glucose transport rate and half-saturation constant, respectively. Notice that all concentrations are in millimolar units, which is required based on Eq. 1, in which the use of equal units for the rate constants (min\(^{-1}\)) corresponds to equal

### Table 3. Rate constants and glucose influx and utilization rates at normoglycemia

<table>
<thead>
<tr>
<th>This Study</th>
<th>Radio-label(^b) NMR(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_2 + k_3 ) at ( C_{P,eq}^{\text{tot}} = 24.9 \text{ mM} )</td>
<td>0.136 ± 0.003 min(^{-1})</td>
</tr>
<tr>
<td>( C_{P,eq}^{\text{tot}} / C_{E,eq}^{\text{tot}} ) at ( C_{P,eq}^{\text{tot}} = 4 \text{ mM} )</td>
<td>2.6(^d) 3.6(^e) 4.0(^f) 4.0(^g)</td>
</tr>
<tr>
<td>( k_1 ), mM</td>
<td>5 ± 1(^\text{f}) 5 ± 1(^\text{f}) 4.0(^h) 3.9(^h)</td>
</tr>
<tr>
<td>( T_{\text{max}}, \mu mol \cdot g^{-1} \cdot min^{-1} )</td>
<td>1.04 ± 0.20 0.83 ± 0.19 1.00(^i) 1.16(^j)</td>
</tr>
<tr>
<td>( CMR_{\text{Glc}}, \mu mol \cdot g^{-1} \cdot min^{-1} )</td>
<td>0.218 ± 0.031 0.219 ± 0.034 0.30(^k) 0.35(^k)</td>
</tr>
<tr>
<td>( k_1 ), min(^{-1})</td>
<td>0.150 ± 0.013 0.120 ± 0.144 0.16 0.19</td>
</tr>
<tr>
<td>( k_2 ), min(^{-1})</td>
<td>0.207 ± 0.009 0.177 ± 0.011 0.26 0.31</td>
</tr>
<tr>
<td>( k_3 ), min(^{-1})</td>
<td>0.184 ± 0.025 0.256 ± 0.040 0.39 0.45</td>
</tr>
<tr>
<td>( k_2 + k_3 ), min(^{-1})</td>
<td>0.391 ± 0.035 0.433 ± 0.051 0.65 0.76</td>
</tr>
<tr>
<td>( t_c ), min</td>
<td>1.77 ± 0.18 1.60 ± 0.21 1.07 0.91</td>
</tr>
<tr>
<td>( CIR_{\text{Glc}}, \mu mol \cdot g^{-1} \cdot min^{-1} )</td>
<td>0.463 ± 0.042 0.370 ± 0.044 0.50 0.59</td>
</tr>
</tbody>
</table>

\(^a\)Maximum deviation based on SE in measured \( k_2 \) and \( k_3 \) value and assumed range for \( k_1 \). \(^b\)Average over literature on human studies published until 1991, reviewed by Gjedde (9). \(^c\)Based on 1 type of processing protocol. \(^d\)Assumed (see Data processing protocol). \(^e\)Calculated from literature. \(^f\)Estimated based on literature data. \(^g\)Reported value.

units for the concentrations. Combination of Eqs. 17 and 19 gives

\[
T_{\text{max}} / CMR_{\text{Glc}} = \frac{(C_{P,eq}^{\text{tot}}/V_d + K_t)(C_P^{\text{tot}}/C_E^{\text{tot}} + K_t)}{(C_{P,eq}^{\text{tot}}/C_E^{\text{tot}} - C_T^{\text{tot}})K_t}
\]

It is important to see the implication of this equation, namely that the \( T_{\text{max}} \)-to-CMR\(_{\text{Glc}} \) ratio, \( K_t \) or the \( C_P^{\text{tot}} \)-to-\( C_E^{\text{tot}} \) ratio is concentration dependent. It seems unlikely that glucose transport parameters will change in acute hyperglycemia, and it has been documented that hexokinase is saturated \( [K_{\text{base}}] = 0.067 \text{ mM} \) (Ref. 16) at a very low tissue glucose concentration, leading to a concentration-independent CMR\(_{\text{Glc}} \) (10, 16, 20). Thus it has to be concluded that the \( C_P^{\text{tot}} \)-to-\( C_E^{\text{tot}} \) ratio depends on the plasma glucose concentration. To determine all relevant parameters, we use the \( C_P^{\text{tot}} \)-to-\( C_E^{\text{tot}} \) ratio at normoglycemia \( (C_{P,eq}^{\text{tot}} = 4 \text{ mM}) \) and a \( K_t = 5 \pm 1 \text{ mM} \) to determine \( T_{\text{max}} \), CMR\(_{\text{Glc}} \) and the normoglycemic CIR\(_{\text{Glc}} \) from our experimentally determined \( k_2 \) and \( k_3 \) value at hyperglycemia. This can be achieved by calculating \( T_{\text{max}} / CMR_{\text{Glc}} \) from Eq. 20 at normoglycemia and using this value to determine \( C_P^{\text{tot}} / C_E^{\text{tot}} \) at hyperglycemia \( (C_{P,eq}^{\text{tot}} = 24.9 \text{ mM}) \). From this number, Eq. 19 can be used to determine \( T_{\text{max}} \) and Eq. 20 to subsequently determine CMR\(_{\text{Glc}} \). The influence of \( K_t \) and the normo-

---

**Fig. 4.** High-resolution \( ^{13} \text{C} \) spectra \((^{13} \text{C} \text{ frequency 125 MHz); 11.7 T Magnet}) of brain extracts collected in vivo (A, C) and postmortem (B, D). Spectra for a phantom with both \( [^{1-13} \text{C}] \text{ glucose-6-P} \) and \( [^{1-13} \text{C}] \text{ glucose} \) are shown in E. Spectrum of a heart extract (high-working heart preparation perfused with buffer containing \( [^{1-13} \text{C}] \text{ glucose, [U-}^{13} \text{C}] \text{ palmitate, ketone bodies, Krebs solution, blood, and insulin}) is given in F. Success of in vivo brain experiment is confirmed by low lactate level (resonance at 21 ppm). Standard is 1 mM acetate (at 24 ppm). Sample sizes and number of scans were 200 mg/ml tissue (20,000 scans), 1.6 g/ml tissue (10,000 scans), 1 mM (6,000 scans), and 2 g/ml (8,000 scans) for in vivo brain, postmortem brain, phantom, and heart, respectively. Data show negligible presence of \( [^{1-13} \text{C}] \text{ glucose-6-P} \) (resonances at 96.95 and 93.11 ppm for \( \beta \)-and \( \alpha \)-isomers, respectively) in brain and only a small contribution (<4%) in heart. GABA, \( \gamma \)-aminobutyric acid, glu, glutamate, glt, glutamine.
Glycemic tissue-to-plasma glucose ratio on this determination is depicted in Fig. 5, A and B. Our numbers (Table 3) compare well with recent compilations of gray matter radiolabel data by Gjedde (9) as well as with a recent compilation of static 13C and dynamic proton NMR transport studies from human NMR (12). To judge whether this is reasonable, we obtained MR images to determine the gray-to-white matter ratio in brain slice chosen for our cat studies. Figure 6 shows a coronal image with a typical slice position indicated. Using a voxel count of the gray and white matter regions, we determined that the gray-to-white matter ratio of the total area is ∼80:20%. Because white matter has about one-half the aerobic metabolic rate (9) of gray matter and because rates are expected to be lower in anesthetized animals, it can be concluded that there is good agreement between our metabolic and transport data in an area of predominantly gray matter in the anesthetized cat with data from literature on transport data in an area of predominantly gray matter and because rates are expected to be lower in anesthetized animals, it can be concluded that there is good agreement between our metabolic and transport data in an area of predominantly gray matter in the awake human. Actually, with the use of our results, some insight can be obtained into recent data obtained by Gruetter et al. (12), who gave two potential combinations of $K_t$, $T_{max}$, and $CMR_{Glucose}$ values, namely the ones in Table 3 and another combination, $K_t = 4.8$ mM, $T_{max} = 0.80$ μmol·g⁻¹·min⁻¹, and $CMR_{Glucose} = 0.32$ μmol·g⁻¹·min⁻¹. However, this second series leads to a $C_{eq}^{tot}/C_{eq}^{CE}$ ratio of 4.4 and therefore can be discounted. We would also like to point out that, based on Eq. 12, $T_{max}$ can be determined within a small range by measuring $C_{eq}^{tot}/C_{eq}^{CE}$ and $K_2 + K_3$ at hyperglycemia because the contribution of $K_t$ is small in the denominator. To obtain an experimental value for $K_t$, for more accurate determination of $CMR_{Glucose}$, the rate constants have to be studied as a function of concentration.

In summary, we have designed a new glucose-uptake NMR spectroscopy experiment to determine tissue glucose utilization half-life times from a simple exponential fit of the experimental signal intensities. It was demonstrated that this model simplification is allowed for the brain through MR extract studies, which showed a negligible concentration of phosphorylated glucose compared with glucose. One organ that should also be suitable for this approach is the heart, in which the phosphorylated glucose concentration is <4% of the total glucose concentration (Fig. 4F). The validity of this simplification for other tissues needs to be established using extract studies. The rate constants of brain glucose influx and utilization determined using this new approach are in the same order of magnitude as those obtained from previous PET and NMR studies, but the present approach is simpler. One important reason that the rates can be determined accurately is because the rate constants (not the rates) of transport and metabolism are slowed down at hyperglycemia. Actually, the principles established in this paper can also be applied to dynamic PET studies of radiolabeled [11C]- or [14C]glucose. These studies are normally complicated by the fact that labeled metabolic products are impossible to distinguish from unlabeled glucose, necessitating the use of glucose analogs such as fluorodeoxyglucose. With the use of hyperglycemia (unlabeled glucose hyperglycemic steady state), it should be possible to slow down glucose utilization so that the glucose uptake curve reaches equilibrium before appreciable amounts of the first metabolic product (glutamate) are formed. The use of natural radioactively labeled glucose analogs and the possibility to measure half-life times with a single exponential data fit should allow accurate localized pathology studies using PET. Because PET studies use tracer concentrations, the labeled and unlabeled blood glucose concentrations can easily be kept stable and all rate equations derived here apply. Similar arguments hold for proton NMR, but, although metabolic products should be distinguishable, these measurements are complicated by the fact that difference spectroscopy has to be performed and that present sensitivity may be too low to perform time-resolved imaging studies. However, technical progress in NMR is ever continuing, and it may be possible that some of these problems can be solved in the near future.

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