Simultaneous in Vivo Measurement of Lumped Constant and Rate Constants in Experimental Cerebral Ischemia Using F-18 FDG

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Lumped and transfer rate constants in ischemic brain tissue must be measured to estimate accurately cerebral glucose utilization by the deoxyglucose method. We studied the bilateral middle cerebral artery occlusion model in 17 cats, 5 with a 1-hour occlusion, 5 with a 4-hour occlusion, and 7 with a sham operation. The time course of cerebral tissue radioactivity (C*(t)) was monitored by external coincidence counting during a programmed infusion of [18F]-2-deoxy-2-fluoro-d-glucose ([18F]-2-FDG). Arterial plasma concentration (Cp*(t)) of the tracer was kept constant during the first 45 minutes. Rate constants were estimated from C*(t) and Cp*(t) by a nonlinear least-squares fitting routine. The lumped constant was estimated from the fit of the ratio of extraction fractions of glucose and [18F]-2-FDG by nonweighted, nonlinear least-squares fitting. In the 4-hour-occlusion model, the transfer constant k3* was 23% lower, k3* 39% lower, and the lumped constant 78% higher than in the sham-operated animals. In the 1-hour-occlusion model, k3* was 26% lower than in the sham-operated animals but the lumped constant was not significantly different. The rate of glucose utilization was significantly different in the 4-hour-occlusion model compared to the sham-operated animals (48% decrease, p<0.05) but was not significantly different in the 1-hour-occlusion model. (Stroke 1987;18:158-167)

The deoxyglucose method, originally introduced by Sokoloff et al., has been widely employed in animal experiments using the autoradiographic technique. The method requires the measurement of individual lumped and transfer rate constants, especially when applied to pathological conditions. Until now, the lumped and transfer rate constants have not been measured simultaneously in the same animal. In fact, these constants are measured very seldom because of the large series of animals required to reduce error in the estimated variables, especially when [14C]-2-deoxy-d-glucose ([14C]-2-DG) is used. The use of [18F]-2-deoxy-2-fluoro-d-glucose ([18F]-2-FDG) makes it possible to measure both the lumped and transfer rate constants simultaneously in the same animal by measuring the time course of brain tissue tracer concentration using external coincidence counting. We describe a method for simultaneous measurement of the lumped and transfer constants in the same animal in a middle cerebral artery (MCA) occlusion model.

Materials and Methods

Theory

Equation 34 from Sokoloff et al. relates the extraction fractions to the tissue content of a glucose analogue as

$$\frac{C_e^*}{E^*} = \frac{dC_e^*/dT}{R_1} + \left( k_{3}^* + k_{3}^* \right) e^{-\left( k_{2}^* + k_{3}^* \right) t} \int_{0}^{T} \frac{C_p}{C_p} e^{k_{2}^* + k_{3}^*} \, dt \frac{\lambda V_e K_m}{9V_m K_m^*},$$

where $C_p$, $C_e^*$, and $C_m$ are glucose concentration in the arterial plasma, arterial whole blood, and draining venous whole blood, respectively. $C_e^*(t)$, $C_m^*(t)$, and $C_p^*(t)$ are tracer concentration in the arterial plasma, arterial whole blood, and draining venous whole blood, respectively. Further, $E^*$ and $E$ equal $[C_e^*(t) - C_m^*(t)]/C^*_m$ and $(C_e^*-C_m^*)/C^*_m$, the cerebral extraction ratios for tracer and glucose, respectively. $C_e^*$ is the tracer concentration in the tissue precursor pool. It can be seen from the model equation (Equation 3 in Ref. 1) that $(K_2^* + K_3^*)$ is equal to the fractional turnover rate of tracer in the precursor pool and can be used to calculate the half-life of that pool. $(\lambda V_e K_m/(9V_m K_m^*))$ is the lumped constant and is composed of 6 separate constants. $\lambda$ is the steady-state hydrolysis of glucose-6-phosphate to free glucose and phosphate and is calculated as $1 - K_3 C_p/k_{3}^* C^*_m$. $C_m^*$ is the steady-state tissue concentration of phosphorylated glucose. $\lambda$ represents the ratio of distribution volumes for deoxyglucose and glucose in the tissue: $\lambda = [k_{2}^*/(k_{2}^* + k_{3}^*)]/$
\[ V_m'^*/V_m' = \frac{k_1/(k_2 + k_3)}{k_1'/k_3'} \] 
\[ V_m'^* = \frac{k_1^*/k_3^*}{(k_2^* + k_3^*)} \] 
\[ \text{Where } V_m' \text{ is the ratio of maximal velocities of phosphorylation of deoxyglucose and glucose by hexokinase. } K_m'^*/K_m'^* \text{ is the ratio of the Michaelis-Menten constants and represents kinetic properties of the enzymes. Hence, } V_m'^*/V_m' = \frac{k_1^*/k_3^*}{(k_2^* + k_3^*)} \text{ is equal to the ratio of the transfer constants and } R_i \text{ is the glucose utilization rate.}

Having the arterial plasma concentration of tracer analogue constant \[ [C_p'^*(t) = C_p'^*] \text{ allows analytical calculation of the integral. After rearrangement, the relation between arterial and venous concentration of the tracer can be expressed as a function of the lumped constant (LC) and the rate constants (k_1'^* to k_3'^*) as shown in Equation 1.}

\[ g(t) = \frac{C_p'^*[C_p'^*(t) - C_v'^*(t)]}{C_p'^*(t)(C_v'^* - C_p'^*)} + \left(1 - e^{-(k_2'^* + k_3'^*)t}\right) \times LC \]  
\[ (1) \]

Substituting \[ R_i = C_p'^*/LC [k_1'^*/(k_1'^* + k_3'^*)] \text{ (Phelps et al)} \]
into Equation 1 and rearranging gives Equation 2 (for full details see Kato et al).

\[ g(t) = \left[1 + (k_2'^*/k_3'^*) e^{-\alpha t + k_1'^*}\right] \frac{C_p'^*}{LC} \]  
\[ (2) \]

The operational equation for calculating glucose utilization (Rᵢ) using deoxyglucose, derived from the Sokoloff equation for constant C_p'^* permitting analytical calculation of integrals, is

\[ R_i = \frac{C_p'^*}{LC} \times \frac{C_p'^*(T) - k_1'^* e^{-\alpha t + k_1'^*} \left(1 - e^{-(k_2'^* + k_3'^*)T}\right)/(k_2'^* + k_3'^*)}{T - \left(1 - e^{-(k_2'^* + k_3'^*)T}\right)/(k_2'^* + k_3'^*)} \]  
\[ (3) \]

where \[ C_p'^*(T) \] represents the tissue concentration measured at time T. Substituting the above equation for \[ R_i = C_p'^*/LC [k_1'^*/(k_1'^* + k_3'^*)] \text{ (Phelps et al)} \]
into Equation 3 and solving for C_p'^*(T) gives

\[ C_p'^*(T) = \frac{C_p'^*}{k_2'^* + k_3'^*} \times \left[ k_3'^* T + \frac{k_2'^*}{k_2'^* + k_3'^*} \left(1 - e^{-(k_2'^* + k_3'^*)T}\right) \right] \]  
\[ (4) \]

Equation 4 should be corrected for efficiency of the measuring system. The extracranial radioactivity was also corrected for the system efficiency and linearly added to the signal from the brain tissue. It was assumed that most of the extracranial signal came from the blood pool. The addition of 2 sources of coincidence signals permitted derivation of Equation 5, which took into account the different sources of radioactivity observed with external coincidence counting:

\[ Hcts(T) = M \times \frac{k_3'^* C_p'^*}{k_2'^* + k_3'^*} \times \left[ k_3'^* T + \frac{k_2'^*}{k_2'^* + k_3'^*} \left(1 - e^{-\alpha t + k_1'^*}\right) \right] \times M \times VC \]  
\[ (5) \]

In this equation Hcts(T) represents the head counts detected by external coincidence counting at time T, VC is the extracranial radioactivity, and M is the calibration factor between the coincidence detector system and the well counter, used for blood sample radioactivity assay. Since M × VC can be considered constant when \[ C_p'^*(t) \] is maintained constant, it was possible to subtract the amount of radioactivity from the extracranial blood pool (M × VC) from Hcts. The estimation of M is described later. M × VC was assumed to be the radioactivity observed as the head count in the first 15 seconds or so after the start of injection (see Figure 2).

**Radiopharmaceuticals**

2-Deoxy-d-1-[3H]glucose (H-2-DG, specific activity 15 Ci/mmol) was obtained from Amersham Corp. and [18F]-2-deoxy-2-fluoro-d-glucose (F-2-FDG, specific activity 680 mCi/mmol) was prepared at the Medical Cyclotron Unit of the Montreal Neurological Institute by adapting methods described earlier.

**Preparation of Animals**

Seventeen cats of either sex, weighing between 3.6 and 4.2 kg, were fasted, except for water, for approximately 18 hours before the experiment. The animals were anesthetized by intraperitoneal injection of pentobarbital sodium, 40 mg/kg body wt. After tracheostomy, respiration was controlled by a Mark 6 Ventilator (Palm Springs, Calif.). Surgery was carried out under halothane anesthesia (1.0–1.5%). Two venous catheters (PE-50 and PE-90, Clay-Adams Inc.), required for infusion, were inserted into one branch of the right femoral vein and the main trunk of the right femoral vein. A large polyethylene tube (PE-90, Clay-Adams Inc.) was inserted into the main trunk of the right femoral artery. The tip of the femoral artery catheter was positioned in the common iliac artery. Cerebral venous blood was sampled through a small polyethylene tube (PE-50, Clay-Adams Inc.) inserted anteriorly 0.5 cm from the midline of the parietal region into the superior sagittal sinus to obtain mainly cerebral venous blood from the territories of the middle and anterior cerebral arteries. This tube was attached to...
the skull by cyanoacrylate glue and gel-foam. Both temporal muscles were resected at the margin of the zygomatic arches to minimize the count due to radioactivity from extracerebral structures. The skull over the MCA region was not removed to avoid surgical effects on the cerebral cortex.

Three groups of cats (sham-operated, 1-hour-occlusion, and 4-hour-occlusion) were studied. The three groups comprised 7, 5, and 5 cats, respectively. The geometrical limitations of coincidence measurements used in our experiments required the bilateral MCA model to get more uniform ischemic brain tissue in the field of view of our coincidence detector system (Figure 1). Using the transorbital approach, the horizontal portions of both MCA's were exposed under an operating microscope and occluded at the lateral margin of the optic nerve by Zen clips (Ohwa Tsusho Co., Ltd., Tokyo, Japan). The interval between left and right MCA occlusion was kept within 5 minutes. Special care was taken to avoid subarachnoid hemorrhage or surgical trauma to the cerebral cortex; if this occurred, animals were not included in the study. The dural defect was packed with gel-foam and cotton plugs to avoid leakage of cerebrospinal fluid. The sham-operated group went through the entire surgical procedure except for occlusion by Zen clips.

After finishing all surgical procedures, anesthesia was changed from halothane to i.v. ketamine hydrochloride (20 mg/kg body wt.) and gallamine (2 mg/kg body wt.) given at 50- to 60-minute intervals.

**Measurement of Miscellaneous Physiological and Biochemical Variables**

Arterial blood pressure was measured intermittently by a transducer (Trantec, Bently-Trantec Corp.). Arterial blood pH, Paco₂, and Pao₂ were measured with a blood gas analyzer (Instrumentation Laboratory System 1302). Hematocrit was determined in blood samples centrifuged in a 5412 (Eppendorf) centrifuge. Rectal temperature was maintained at approximately 37°C with a heating blanket. Physiological variables were kept at the following levels: mean arterial blood pressure, 120 ± 10 mm Hg; pH, 7.4 ± 0.05; Paco₂, 35 ± 2 mm Hg; Pao₂, 100 ± 10 mm Hg.

The concentration of glucose in the plasma was measured enzymatically using a YSI Model 23A glucose analyzer (Yellow Springs Instrument Co., Inc.). Arterial plasma H-2-DG concentration was determined by assaying H in a sample volume of 50 μl in a liquid scintillation counter (1215 Rackbeta Liquid Scintillation Counter, Wallacoy Turku, Finland). ²²F-2-FDG concentrations in arterial plasma, arterial whole blood, and cerebral venous whole blood were determined by assaying ²²F radioactivity in 50-μl samples using a NaI(Tl) well counter. Results were expressed per unit weight and corrected for radioactive decay from the start of tracer infusion.

**Detector System**

Head coincidence counts were detected by 2 diametrically opposed NaI(Tl) detectors (1/4 in. diameter) 19 cm apart, face to face. A 2-in.-thick lead collimator with a 7 × 20-mm rectangular opening was placed in front of each detector (Figure 1). Additional lead bricks, also 2 in. thick, were used to shield the detectors laterally against stray radiation from the animal's body. Coincidence signals from the detectors were measured in an energy window of 130–750 KeV by a counting system interfaced to a PDP-12 computer. Head counts were integrated over 30-second intervals during the first 10 minutes and then over 1-minute intervals for 45 minutes until the end of the continuous infusion of ²²F-2-FDG.

Tissue volume viewed by the detector pair corresponded to the parietal lobe where cerebral ischemia was produced by MCA occlusion. The 7 × 20-mm rectangular zones were marked on the exposed skull of the animals as follows: A perpendicular line was drawn from the most caudal and superior margin of the zygomatic arch to the midline of the vertex. Against this perpendicular line, a horizontal line was drawn 1 cm caudally and rostrally parallel to the zygomatic arch 2 cm above the superior margin of the zygomatic arch. The 7 × 20-mm rectangular zone was marked just above this horizontal line.

**Determination of Cross-Calibration Factor Between the Detector Pair and the Well Counter**

At the end of the infusion (47 minutes after the start of the infusion), the cat was killed with a solution of 10 ml KCl and 5 ml 1.0 M trichloroacetic acid to stop enzymic activity.

The whole brain tissue was excised according to the shape of the rectangular collimator, homogenized in a syringe for 2 minutes with an ultrasound tissue homogenizer (30,000 rpm) (Ultra-Torax, Janke and Kon-
Lumped and Rate Constant Measurements

Three groups of cats (sham-operated, 1-hour-occlusion, and 4-hour-occlusion) were used for determination of the lumped and rate constants. The femoral artery was used for the measurement of \( C_p \), \( C_p^* \), \( C_r \), and \( C_r^* \). The superior sagittal sinus was used for the measurement of \( C_s \) and \( C_s^* \). To determine the infusion schedule in a cat, 30 \( \mu \)Ci of \(^{18}\)F-2-DG dissolved in <1 ml of physiological saline was injected as a bolus into the femoral vein through a small polyethylene tube (PE-50). This bolus study was performed in every cat 2 hours after completion of all surgical procedures except for the clipping of the MCA’s. The sampling schedule for blood was 5, 19, 28, 35, 45, and 54 seconds and 1:6, 1:24, 1:44, 2:21, 3:32, 4:50, 6:31, 9:40, 12:53, 17:56, and 23:58 minutes:seconds and 28, 38, and 47 minutes. The plasma disappearance curve (impulse–response function) was fitted to the sum of two or three exponential terms using a nonlinear least-squares routine. The parameters of the impulse–response function were then used to determine the infusion schedule required to obtain a constant arterial plasma concentration of \(^{18}\)F-2-FDG for 45 minutes as described by Patlak and Pettigrew.9 Cats were injected through a femoral vein with 3–6 mCi of \(^{18}\)F-2-FDG dissolved in 6.0 ml of physiological saline according to the determined infusion schedule. In the original publication, Sokoloff et al1 described 1 \( \mu \)mol of deoxyglucose as the maximal permissible dose for adult rats weighing 325–450 g, or about 2.5 \( \mu \)mol/kg. In our experiments, we injected about 1.8 \( \mu \)mol of \(^{18}\)F-2-FDG per kilogram of total body weight, lower than that accepted by Sokoloff et al1 as maximal. The total amount injected was about 6.85 \( \mu \)mol during a 45-minute infusion. Assuming the total volume of blood in a cat to be about 10% of the body weight and a homogeneous distribution of \(^{18}\)F-2-FDG in the cat’s blood, the injected \(^{18}\)F-2-FDG gave a molar concentration of about 17.13 \( \mu \)M. This concentration is well below the \( K_m \) for \(^{18}\)F-2-FDG (\( K_m = 6.9 \pm 1.1 \) mM).10 Also note that the tracer was not injected as a bolus but infused during 45 minutes.

A bolus at time 0 was followed by a programmed infusion of 4.5 ml of the tracer solution. Programmed infusion of \(^{18}\)F-2-FDG was controlled by a microcomputer-controlled Harvard peristaltic pump (No. 52-1351). During infusion, arterial and cerebral venous blood samples were taken from the femoral artery and the superior sagittal sinus, respectively. To know the exact time of each sample and to reduce the complexities of time keeping, paired sampling was used. To synchronize arterial and cerebral venous sampling, all cerebral venous sampling times were adjusted to the arterial sampling times. The value of the cerebral venous blood concentration at the arterial sampling time, required in Equation 1, was estimated by cubic spline interpolation. The sampling schedule was as follows: 0:5, 1, 1.5, 2, 3, 4, 6, 8, 11, 14, 18, 22, 25, 27, 30, 32, 34, 36, 40, 42 minutes and 43:30 minutes:seconds, and 45 minutes. An aliquot of each blood sample was centrifuged immediately in an Eppendorf centrifuge for 1 minute at 12,000g. The blood and plasma samples were assayed for \(^{18}\)F-2-FDG, and the glucose concentrations in plasma were measured as described above.
Radioactivity in the MCA region was monitored by external coincidence detection and corrected for radioactive decay. Using Equation 5, the rate constants $k_1^*$, $k_2^*$, and $k_3^*$ were estimated from the time course of the tissue and the arterial plasma radioactivities by a nonlinear least-squares fitting program.

The lumped constant was determined from Equation 2, rewritten as

$$g(t) = (1 + Ce^{-k'p}) \times LC$$

with $C = k_1^*/k_2^*$ and $D = k_2^* + k_3^*$, by nonweighted, nonlinear least-squares fitting.

Cerebral Blood Flow Measurement

In some animals the degree of ischemia produced by occluding the MCA with a Zen clip was assessed by measuring cerebral blood flow (CBF) by the $^{133}$Xe washout technique. The area of the brain monitored was about the same as that in the $^{18}$F-2-FDG study. One mCi of $^{133}$Xe dissolved in 1 ml of physiological saline was rapidly injected into a catheter inserted in the lingual artery. Tissue radioactivity was recorded for 10-15 minutes after each injection. Brain radioactivity was measured sequentially with the same 2 detectors used for coincidence counting. Regional CBF was calculated from the $^{133}$Xe clearance curves according to a modification of the height-over-area method developed by Yamamoto et al, and based on the stochastic analysis of Zierler. Data were analyzed using a PDP-12 (DEC) computer. Arterial blood gas was analyzed in the arterial and venous blood pressure was measured immediately before the CBF measurements. $^{133}$Xe measurements were made over the parietal region where consistent and uniform ischemic tissue was produced in the MCA occlusion model in the cat.

Results

**Physiological variables.** Physiological variables of animals used in this study are given in Table 1. The parameters did not vary significantly between groups (sham-operated vs. 1-hour-occlusion, sham-operated vs. 4-hour-occlusion). Physiological variables were generally very stable within an experiment except for slight variation in plasma glucose levels. However, glucose variability was well within the levels shown by Savaki et al to be acceptable for the deoxyglucose model. The variability for plasma glucose shown in Table 1 comes mostly from animal-to-animal variation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood gas analysis</th>
<th>Mean blood pressure</th>
<th>Rectal temp.</th>
<th>$C_a^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{pH}$</td>
<td>$P_{aco_2}$ (mm Hg)</td>
<td>$P_{aco_2}$ (mm Hg)</td>
<td>Before occl. (mm Hg)</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>7.31 ± 0.08</td>
<td>34.4 ± 1.1</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>4-hour</td>
<td>5</td>
<td>7.32 ± 0.06</td>
<td>34.5 ± 3.1</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>1-hour</td>
<td>5</td>
<td>7.30 ± 0.07</td>
<td>33.4 ± 3.2</td>
<td>109 ± 11</td>
</tr>
</tbody>
</table>

Values given are mean ± SD.

*Arterial plasma glucose.

**Variation in arterial plasma concentration of tracer.** The arterial plasma concentration of $^{18}$F-2-FDG was maintained constant from 1 to 45 minutes. The coefficient of variation for arterial plasma $^{18}$F-2-FDG concentrations was 7.83 ± 1.79 (mean ± SD) (n = 7) in the sham-operated, 8.53 ± 1.58 (n = 5) in the 1-hour-occlusion, and 7.88 ± 1.60 (n = 5) in the 4-hour-occlusion groups. The coefficient of variation was defined as the magnitude of the standard deviation from the mean — in other words, the size of variation from the mean. The same principle was used by Rei-vich et al to describe to what extent a plateau in a constant infusion protocol was actually achieved.

**Kinetic constants.** Rate constants obtained for each animal and their group means are given in Tables 2 and 3. Only the difference in $k_2^*$ was significant (p < 0.05) between the sham-operated and the 1-hour-occlusion group (26% lower in the 1-hour-occlusion group). $k_1^*$ and $k_1^*$ in the 1-hour-occlusion group were 15 and 9% lower than those in the sham-operated group, but these differences were not significant. However, the sham-operated group and the 4-hour-occlusion group showed significant differences (p < 0.05) for $k_1^*$ and $k_1^*$ ($k_1^*$ and $k_1^*$ were 23 and 39% lower in the 4-hour-occlusion group). $k_1^*$ in the 4-hour-occlusion group was 17% lower than in the sham-operated group, but again, the difference was not significant. However, there is a significant difference (p < 0.05) in LC's between the sham-operated and the 4-hour-occlusion groups (78% higher in the 4-hour-occlusion group).

**Lumped constant.** The typical time courses of the arterial plasma, arterial whole blood, and venous whole blood concentrations of $^{18}$F-2-FDG obtained in LC determinations in the sham-operated and the 4-hour-occlusion groups are shown in Figure 3. The calculated function g(t) from these data is also shown for each group. In contrast to those in the 4-hour-occlusion group, arterial and venous whole blood curves seem to approach each other late in the experiment in the sham-operated group. This tendency seems to be more noticeable in the 4-hour-occlusion group. The extraction fractions in the sham-operated group are quite different from those in the 4-hour-occlusion group. LC was calculated using Equation 2. The value for each animal and the group means are given in Tables 2 and 3. LC in the 1-hour-occlusion group was 15% higher than in the sham-operated group, but this difference was not significant. However, there is a significant difference (p < 0.05) in LC's between the sham-operated and the 4-hour-occlusion groups (78% higher in the 4-hour-occlusion group).
Focal glucose utilization rate. Focal cerebral metabolic rate for glucose in the region monitored by external coincidence counting was calculated as \( R_c \), using Equation 3. Calculated \( R_c \) values are given in Tables 2 and 3. Focal \( R_c \) was not significantly different between the sham-operated and the 1-hour-occlusion groups (21% lower in the 1-hour-occlusion group). However, focal \( R_c \) was significantly different \((p < 0.05)\) when the sham-operated group was compared with the 4-hour-occlusion group (\( R_c \) was 48% lower in the 4-hour-occlusion group).

Cerebral blood flow. Regional CBF studies were done before and after bilateral MCA occlusion in some cases. After MCA occlusion, rCBF was reduced between 40 and 56%. There was no significant difference in reduction of rCBF between 1 and 4 hours after occlusion.

### Discussion

The simultaneous measurement of LC and rate constants in cats with bilateral MCA occlusion, using external coincidence counting, is described. MCA occlusion was chosen as an appropriate model for incomplete regional cerebral ischemia. External coincidence counting to measure brain radioactivity following the administration of a positron-emitting isotope has been described and used previously in animal experiments.

Although that portion of the cat brain monitored consisted of a mixture of gray and white matter, it consisted mostly of gray matter, which has much higher glucose utilization than white matter. Therefore, the tissue could be considered homogeneous with respect to the kinetics of \(^{18}F\)-2-FDG. Most of the extracerebral radioactivity was excluded by resection of the temporal muscles, but extracerebral radioactivity from the skull (particularly from the diploic vein) and the dura could not be excluded. However, we feel that appropriate correction for extracerebral radioactivity was made. The skull and dura were preserved to eliminate surgical effects on the cerebral cortex.

Rate constants for the glucose analogues \(^{18}F\)-2-FDG and \(^{13}C\)-2-DG in the human brain have usually been measured using i.v. bolus injections of the tracer. \(^{4,16,19,20}\) A bolus injection produces a sharp peak in the initial part of the \( C_t^* \) curve and leads to inaccuracies in the measurement of \( C_t^* \) as well as of the true tissue activity during the first pass of the bolus. To

### Table 2. Rate Constants and Lumped Constants in Sham-Operated Cats

<table>
<thead>
<tr>
<th>Variable</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1^* (\text{min}^{-1}) )</td>
<td>0.065</td>
<td>0.070</td>
<td>0.068</td>
<td>0.088</td>
<td>0.080</td>
<td>0.062</td>
<td>0.082</td>
<td>0.074 ± 0.004</td>
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<tr>
<td>( k_2^* (\text{min}^{-1}) )</td>
<td>0.108</td>
<td>0.134</td>
<td>0.133</td>
<td>0.180</td>
<td>0.131</td>
<td>0.111</td>
<td>0.149</td>
<td>0.135 ± 0.009</td>
</tr>
<tr>
<td>( k_3^* (\text{min}^{-1}) )</td>
<td>0.024</td>
<td>0.025</td>
<td>0.021</td>
<td>0.024</td>
<td>0.025</td>
<td>0.016</td>
<td>0.023</td>
<td>0.023 ± 0.001</td>
</tr>
<tr>
<td>Distribution volume†</td>
<td>0.492</td>
<td>0.440</td>
<td>0.442</td>
<td>0.432</td>
<td>0.513</td>
<td>0.488</td>
<td>0.476</td>
<td>0.469 ± 0.012</td>
</tr>
<tr>
<td>Half-life of precursor pool (min)‡</td>
<td>5.251</td>
<td>4.359</td>
<td>4.501</td>
<td>3.398</td>
<td>4.443</td>
<td>5.458</td>
<td>4.030</td>
<td>4.49 ± 0.27</td>
</tr>
<tr>
<td>LC§</td>
<td>0.429</td>
<td>0.451</td>
<td>0.376</td>
<td>0.319</td>
<td>0.419</td>
<td>0.398</td>
<td>0.330</td>
<td>0.389 ± 0.019</td>
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<tr>
<td>( R_c (\mu\text{mol}/100 \text{g/min}) )</td>
<td>30.70</td>
<td>20.9</td>
<td>24.5</td>
<td>39.2</td>
<td>31.4</td>
<td>31.2</td>
<td>31.6</td>
<td>31.1 ± 2.6</td>
</tr>
</tbody>
</table>

†Distribution volume = \( k_1^* / (k_2^* + k_3^*) \).
‡Half-life of precursor pool = \( \ln 2 / (k_2^* + k_3^*) \).
§Lumped constant.

### Table 3. Rate Constants and Lumped Constants in 1-Hour- and 4-Hour-Occlusion Cats†

<table>
<thead>
<tr>
<th>Variable</th>
<th>O11</th>
<th>O12</th>
<th>O13</th>
<th>O14</th>
<th>O15</th>
<th>Mean ± SEM</th>
<th>O41</th>
<th>O42</th>
<th>O43</th>
<th>O44</th>
<th>O45</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1^* (\text{min}^{-1}) )</td>
<td>0.039</td>
<td>0.058</td>
<td>0.070</td>
<td>0.083</td>
<td>0.065</td>
<td>0.063 ± 0.007</td>
<td>0.061</td>
<td>0.054</td>
<td>0.077</td>
<td>0.045</td>
<td>0.050</td>
<td>0.057 ± 0.005†</td>
</tr>
<tr>
<td>( k_2^* (\text{min}^{-1}) )</td>
<td>0.100</td>
<td>0.087</td>
<td>0.123</td>
<td>0.157</td>
<td>0.151</td>
<td>0.123 ± 0.014</td>
<td>0.102</td>
<td>0.105</td>
<td>0.176</td>
<td>0.081</td>
<td>0.100</td>
<td>0.112 ± 0.016</td>
</tr>
<tr>
<td>( k_3^* (\text{min}^{-1}) )</td>
<td>0.016</td>
<td>0.010</td>
<td>0.020</td>
<td>0.017</td>
<td>0.021</td>
<td>0.017 ± 0.002†</td>
<td>0.012</td>
<td>0.012</td>
<td>0.016</td>
<td>0.019</td>
<td>0.012</td>
<td>0.014 ± 0.001†</td>
</tr>
<tr>
<td>Distribution volume</td>
<td>0.335</td>
<td>0.599</td>
<td>0.492</td>
<td>0.477</td>
<td>0.376</td>
<td>0.456 ± 0.047</td>
<td>0.536</td>
<td>0.465</td>
<td>0.401</td>
<td>0.449</td>
<td>0.446</td>
<td>0.450 ± 0.022</td>
</tr>
<tr>
<td>Half-life of precursor pool</td>
<td>6.006</td>
<td>7.135</td>
<td>4.881</td>
<td>3.989</td>
<td>4.028</td>
<td>5.21 ± 0.61</td>
<td>6.096</td>
<td>5.947</td>
<td>3.611</td>
<td>6.946</td>
<td>6.240</td>
<td>5.77 ± 0.57†</td>
</tr>
<tr>
<td>LC</td>
<td>0.310</td>
<td>0.631</td>
<td>0.380</td>
<td>0.392</td>
<td>0.529</td>
<td>0.448 ± 0.058</td>
<td>0.605</td>
<td>0.619</td>
<td>0.557</td>
<td>0.877</td>
<td>0.811</td>
<td>0.694 ± 0.063†</td>
</tr>
<tr>
<td>( R_c (\mu\text{mol}/100 \text{g/min}) )</td>
<td>25.8</td>
<td>15.5</td>
<td>43.5</td>
<td>22.8</td>
<td>14.3</td>
<td>24.4 ± 5.3</td>
<td>13.4</td>
<td>16.5</td>
<td>30.1</td>
<td>12.5</td>
<td>8.1</td>
<td>16.1 ± 3.7†</td>
</tr>
</tbody>
</table>

†Significantly different from sham-operated animals \((p < 0.05)\).

Variables defined in Table 2.
FIGURE 3. Lines in graphs are added only as a visual aid. A) Arterial plasma, arterial whole blood, and cerebral venous blood (superior sagittal sinus) activity curves during 18F-2-FDG infusion in sham-operated cats. B) Calculated function g(t) and fitted curve for sham-operated cats. $\chi^2 = 0.190$, $v = 12$. C) Arterial plasma, arterial whole blood, and cerebral venous blood (superior sagittal sinus) activity curves during 18F-2-FDG infusion in cats undergoing 4-hour MCA occlusion. D) Calculated function g(t) and fitted curve for cats undergoing 4-hour MCA occlusion. $\chi^2 = 0.035$, $v = 17$.

overcome this problem, Kato et al.21 proposed measuring the rate constants using a steady plasma concentration during the initial 20 minutes. Since we were dealing with ischemic brain and wanted to estimate LC, the constant plasma level condition was prolonged up to 45 minutes to ensure complete blood/tissue equilibration.1

The effect of $k_4^*$ (the rate constant for dephosphorylation of 18F-2-FDG) was assumed to be negligible in the 45-minute study.1,14,16,22 It has been shown in this laboratory21 that the use of 3H-2-DG instead of 3H-2-FDG to obtain pulse-response functions does not produce significant error in the 18F-2-FDG infusion schedule.

Rate constants. Several studies of the 18F-2-FDG4,16,20,24,25 and the 1H-2-DG rate constants1 have recently been made in normal human subjects. The only animal studies reported are in albino rats1 and in dogs,21 both under normal physiological conditions. Kennedy et al.26 also reported $k_2^*$ and $k_3^*$ only in normal conscious monkeys.

The rate constants $k_1^*$, $k_2^*$, and $k_3^*$ describe the carrier-mediated transport of 18F-2-FDG from plasma to tissue, from tissue to plasma, and the phosphorylation by hexokinase, respectively. Any pathological condition that influences a biological system may change the rate constants. The main purpose of this work was to determine changes in the rate constants during the acute stage of cerebral ischemia. It should be pointed out that the method used in this work gives the rate constants for a mixture of gray and white matter in the volume of brain seen by the coincidence detectors. The use of unified rate constants is justified when rate constants in approximately the same volume of brain are compared. It should also be noted that tissue activity is heavily weighted toward gray matter. Essentially, we are comparing the rate constants (regardless of mixture) in a volume of brain seen by external detectors before (sham-operated animals) and after onset of ischemia. In evaluating reported changes in the rate constants, we must keep in mind the limitations of our experimental setup. Ginsberg et al.27 have reported the variation in size of the ischemic region in cats after MCA occlusion. Also, some contamination of ischemic tissue by normal brain tissue cannot be avoided when external coincidence counting is used.

Our results (see Tables 2 and 3) could be compared with those of Hawkins et al.,28 who reported measurements of the rate constants in chronic stroke patients, and to those of Heiss et al.,29 who reported average gray matter rate constants in ischemic tissue in stroke patients. Hawkins et al.28 found that the rate constants were generally lower in ischemic regions than in homologous regions of the contralateral hemisphere.
This pattern was found consistently in the more severely ischemic group (k_2* was 63% lower, k_3* 35% lower, and k_4* 48% lower in the ischemic region than in the contralateral hemisphere). In comparison, Heiss et al. reported that k_2* and k_3* were much more affected than k_4* (k_2* was 38% lower, k_3* 36% lower, but k_4* only 3.3% lower in ischemic tissue than in the contralateral hemisphere).

The concentration ratio of ^18F-2-FDG between tissue and plasma in the steady state is the distribution volume. The decrease in the distribution volume found in the present study compared with that in sham-operated animals (see Tables 2 and 3) is not significant in the 1- and 4-hour-occlusion groups. However, the increase in the half-life of the precursor pool is significant in the 4-hour-occlusion group (28%) when compared with that in sham-operated animals. These changes in the distribution volume and half-life of the precursor pool are similar to those reported by Hawkins et al. and Heiss et al.

A significant decrease in k_2* in the 1- and 4-hour-occlusion groups (Table 3) indicates the capability of the present method to detect early metabolic changes in experimental cerebral ischemia. The results reported here suggest that both k_2* and the distribution volume are relatively insensitive to changes in the early stage of cerebral ischemia. The insensitivity of k_2* may be due to the nature of the glucose transport system itself. Since glucose is transported to the brain by a saturable facilitated transport mechanism rather than by active transport, there is no energy required for transport. Our data indicate (see Tables 2 and 3 for k_2* and k_3*) that the glucose transport system might be preserved in the early stage of cerebral ischemia. Our results also suggest that glucose transport from brain tissue to plasma is less sensitive to changes occurring in the early stages of cerebral ischemia than is the glucose transport system from plasma to brain tissue.

The stability of the distribution volume (Tables 2 and 3) is due to the insensitivity of k_2* because k_3* is much smaller than k_2*. The increase in half-life of the precursor pool in the 4-hour-occlusion group is largely due to the decrease in k_2* and partly due to the decrease in k_3*.

It was believed for many years that the brain is more resistant to ischemia than had been previously assumed on the basis of clinical experience. They developed a 1-hour complete ischemia model in normothermic rhesus monkeys and observed that electrocortical activity returned upon recirculation in 11 of 19 animals. No previous reports are available in regard to the preservation of the glucose transport system in the early stage of focal cerebral ischemia. Their results indirectly support our finding that k_2* and k_3* are not affected in the 1-hour-occlusion group. However, our data indicate that the glucose transport system from plasma to brain tissue (k_1) is affected in the 4-hour-occlusion group. Preservation of the glucose transport system in the early stage of cerebral ischemia could be essential for the recovery of brain function following an ischemic attack. It should be pointed out that this preservation does not contradict reports of the accumulation of lactate in ischemic tissue. Even if transport is preserved, the supply of glucose and oxygen is inadequate because of low blood flow. Lactate is formed in ischemic tissue. During prolonged ischemia, the tissue will accumulate more lactate since blood flow is not sufficient to wash away the lactate produced, thereby depleting the tissue glucose pool even further and eventually causing cell death. Insufficient data prevent us from giving a more quantitative relation between ischemia and the time required for tissue to suffer irreversible metabolic damage. However, our results suggest that between 1 and 3 hours after occlusion the tissue begins to use glucose at a lower rate (reduction in k_3*). This reduction might indicate the start of irreversible damage to the tissue.

The constants k_2* and k_3* can also be estimated from Equation 2. It should be emphasized that these values of k_2* and k_3* do not necessarily agree with those calculated from Equation 5, because the mixture of gray and white matter and ischemic tissue from which venous blood drained might differ from the mixture of these tissues seen by the coincidence detectors. Moreover, k_2*/k_3* and (k_2* + k_3*) calculated by Equation 2 were greatly influenced by variation during the first few minutes of the arterial plasma activity curve of the tracer. Theoretically, the plasma curve should be constant from t = 0; in practice this is not the case, introducing considerable error in k_2*/k_3* and (k_2* + k_3*) determined using Equation 2, particularly under pathological conditions.

**LUMPED CONSTANT.** LC was measured by Sokoloff's original method, which requires a constant tracer concentration for the initial 45 minutes. A main reason to use nonlinear least-squares fitting to estimate LC instead of the conventional asymptotic approach originally described by Sokoloff et al. is smaller error in LC. The large error resulting from the asymptotic approach is especially pronounced in the ischemic model because there is always only a fraction of the venous blood that drains from the ischemic volume of the brain. As seen from Equation 2, the error is large because there is a ratio between two small numbers. Using the entire curve should result in a more accurate estimate for LC. This was confirmed by the simulation done at this laboratory (Matsuda et al., unpublished). It was assumed that cerebral venous blood obtained from a catheter inserted into the superior sagittal sinus represented the blood circulating in the MCA region. If this assumption is valid, the measured LC should reflect changes in the arteriovenous ratio of the glucose and the glucose analogue in the brain affected by bilateral MCA occlusion. Our results show a significant increase (p < 0.05) in LC in the 4-hour-occlusion group (80%) (Tables 2 and 3). The increase in LC is not significant in the 1-hour-occlusion group. Ginsberg...
and Reivich, who measured LC in diffuse cerebral ischemia, reported an increase of 2.4 times the mean control value. (Unfortunately, they did not mention the duration of ischemia in their experiment.) Ginsberg and Reivich also suggested that LC might return to normal in the brain after a 15-minute ischemic attack followed by a 70-minute normotensive reperfusion.

There is an obvious difference between the extraction fractions of tracer \([C_{e}^{*} - C_{v}^{*}]/C_{e}^{*}\) in the sham-operated and the 4-hour-occlusion groups. The increase in extraction fraction in the 4-hour-occlusion group can be explained by the reduction of brain tissue glucose. This causes a relative increase in extraction fraction of \(^{18}\)F-2-FDG. This observed increase supports our assumption that the cerebral venous blood used in the determination of LC was perfusing ischemic brain tissue. Increased arteriovenous extraction of \(^{18}\)F-2-FDG suggests a decrease in free glucose in the ischemic brain tissue, thereby increasing the influx of tracer into the brain in the longer occlusion group.

Why does LC change in ischemic tissue? LC is composed of six separate constants: LC = \(\lambda V_{m} K_n/ (\theta V_{m} K_{n}^{*})\). Although individual constants may vary from structure to structure and condition to condition, the ratios likely remain the same under normal physiological conditions. A progressive and appreciable increase in LC has been reported in severe hypoglycemia. Indeed, theoretically LC changes whenever there is an alteration in the balance between glucose supply and glucose utilization. A major change in LC occurs only when the ratio of glucose utilization becomes limited by the supply. In pathological conditions tissue damage may disrupt the normal cellular compartmentalization, and it cannot be assumed that the ratio of the distribution volume for deoxyglucose and glucose is the same in damaged and normal tissue. There may be release of lysosomal acid hydrolases that may hydrolyze glucose-6-phosphate and alter the value of \(\theta\), causing a change in LC in ischemic tissue. Gjedde et al reported that a change in the distribution ratio influences LC more than any other factor. In analyzing the regional uptake of \(^{18}\)F-2-FDG and \(^{13}\)C-3-o-methylglucose in stroke patients, they found two types of infarcts, the phosphorylation-limited type (almost normal glucose content and LC value) and the transport/flow-limited type (low glucose content and high LC value). Since we did not measure the glucose content in ischemic tissue, these aspects cannot be addressed more specifically in our acute ischemia model. As has been speculated above, the marked increase in extraction of \(^{18}\)F-2-FDG in the 4-hour-occlusion group suggests that low glucose content in the acute stage of experimental cerebral ischemia may be due to the transport/flow limitation described by Gjedde et al. The relation between the change of LC and the state of ischemia (acute–chronic) is unclear. We have been investigating the change in LC in cats with more than 4 hours of MCA occlusion and expect to be able to say more on this after those experiments are completed.

To estimate true R, in cerebral ischemia, both transport rate constants and LC must be determined. Our method makes it possible to investigate pathophysiological changes of transfer rate constants and LC simultaneously in an experimental model of cerebral ischemia. The cause of the increase in LC in acute ischemia may be low glucose content in ischemic tissue.

Conclusion: There is a significant increase in LC in cat brain in the 4-hour-occlusion model but no significant change in the 1-hour-occlusion model. A significant change was observed in \(k_{e}^{*}\) in the 1-hour-occlusion model, and in \(k_{n}^{*}\) and \(k_{n}^{*}\) in the 4-hour-occlusion model.

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Key Words • cerebral ischemia • fluorodeoxyglucose • lumped constant • rate constants • external coincidence counting
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