PRELIMINARY NOTE

Assessment of the [^{18}F]Fluorodeoxyglucose Kinetic Model in Calculations of Myocardial Glucose Metabolism during Ischemia

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The lumped constant—a term in the operational equation of the Sokoloff tracer kinetic model for deoxyglucose that accounts for the difference in transport and phosphorylation between glucose and its analog, deoxyglucose—could potentially vary from normal to ischemic conditions in the heart. To test the stability of the lumped constant during ischemia, we evaluated the ratio of the extraction fraction for (F-18)-fluorodeoxyglucose (FDG) to that for glucose (a measure of the lumped constant if there is no significant dephosphorylation of FDG-6-PO_4) and the rate constant for dephosphorylation of FDG-6-PO_4 (k_4*) in the isolated, arterially perfused interventricular septum of the rabbit during moderate and severe demand-induced and reduced-flow ischemias. The lumped constant and k_4* in each of the four ischemic experimental conditions were found not to be significantly different from the value obtained from the nonischemic controls.

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Quantification of regional exogenous glucose utilization is possible with positron computed tomography (PCT) and 2-[^{18}F]fluoro-2-deoxyglucose (FDG) when coupled with an appropriate tracer kinetic model (*1*,*2*). The model used to measure regional exogenous glucose utilization must not only be compatible with data obtained with PCT but must also describe accurately the behavior of FDG in the myocardium under normal and disease conditions. Similar to 2-[^{14}C]deoxyglucose (DG), FDG is transported into the cell and phosphorylated competitively with glucose (*3*,*6*–*8*). However, neither FDG nor DG is a substrate for other glycolytic enzymes or for glycogen synthesis, and both accumulate in the myocardium as the phosphorylated products FDG-6-PO_4 and DG-6-PO_4. If glucose metabolism is in a steady state, the kinetics of FDG-6-PO_4 (or DG-6-PO_4) accumulation over a given period of time can reflect the net phosphorylation rate of exogenous glucose, and, therefore, the rate of exogenous glucose utilization.

Phelps and co-workers (*1*,*2*) have developed an extension of the tracer kinetic model originated by Sokoloff et al. for DG (*4*) and validated its use for the measurement of cerebral glucose utilization using FDG and positron CT. This model assumes three compartments (Fig. 1): a plasma compartment, a tissue compartment for glucose and FDG, and a tissue compartment for phosphorylation of glucose and FDG to glucose-6-PO_4 and FDG-6-PO_4. The four first-order rate constants (k_i*) represent the bidirectional rate constants of transport and phosphorylation/dephosphorylation. If one knows the plasma glucose concentration, the time function of the change in plasma FDG concentration, and the tissue F-18 concentration, it is possible to calculate the rate of exogenous glucose utilization using the operational equation of the model developed by Phelps and co-workers.

A term referred to as the lumped constant in the operational equation of the model corrects for the different rates of membrane transport and phosphorylation of

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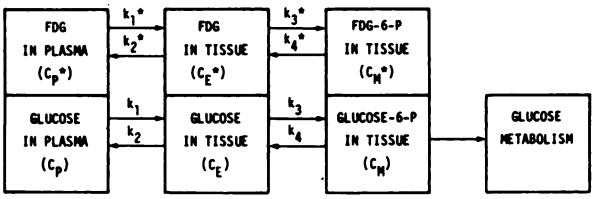

FIG. 1. Three-compartment model used to describe kinetics of myocardial glucose and competitive substrate analog FDG in terms of membrane transport and phosphorylation. Compartments 1 and 2 contain FDG and glucose in plasma and tissue, respectively. First-order rate constants, k_1*, k_2*, k_1, and k_2 are bidirectional rate constants of transport between plasma and myocardial tissue. Similarly, Compartment 3 contains FDG-6-PO_4 and glucose-6-PO_4 with k_3*, k_4*, k_3, and k_4, rate constants of phosphorylation and dephosphorylation of FDG and glucose, respectively.

glucose and its competitive analogs, DG or FDG. The lumped constant (LC) is a combination of (a) the ratio of the K_m and V_m for phosphorylation of FDG (or DG) relative to glucose, (b) the ratio of the distribution volumes of FDG (or DG) and glucose, and (c) the fraction of glucose-6-PO_4 that proceeds through glycolysis. The lumped constant has been postulated to be a source of instability in the FDG (or DG) tracer kinetic model under conditions of altered metabolic rate and substrate supply in the brain (*5*).

Recently the FDG tracer kinetic model for measuring regional exogenous glucose utilization has been extended to the heart under nonischemic conditions (*6–9*). The purpose of the present study was to evaluate the stability of the lumped constant under conditions of altered metabolic rate produced by myocardial ischemia in the highly controlled environment of the isolated, arterially perfused, interventricular septum of the rabbit.

METHODS

Experimental preparation and conditions. The lumped constant was evaluated in 27 isolated, arterially perfused interventricular septa, obtained from male New Zealand rabbits (1 kg to 2 kg). After the administration of heparin and pentobarbital by ear vein, the heart was rapidly excised through a median sternotomy. Following removal of both atria and the right ventricle, the septal artery was identified by opening the left coronary artery through its ostium in the aorta. The septal artery was cannulated with a small polyethylene cannula, secured in place with a 6.0 silk suture. An interval of no more than 5 min elapsed from the time the heart was excised to completed cannulation. Flow was maintained constant at 3.0 ml per min by a peristaltic pump. The perfusate contained (in m*M*): NaCl 130; $CaCl_2$ 2.5; KCl 6.0; MgCl 1.0; NaH_2PO_4 0.435; $NaHCO_3$ 12.0. The substrate was dextrose, 5.6 m*M*, with 25 mU/ml insulin added to ensure adequate availability of glucose to the myocardium. The perfusate was equilibrated continuously with 98% oxygen and 2% carbon dioxide, yielding a stable pH of 7.3 to 7.4.

Well-perfused tissue was identified visually, and a triangular segment (weight $\cong$ 1.0 g) was cut out with the cannula at its base. The perfused myocardium was suspended between two clamps at its base and tied to a strain-gauge type tension transducer at its apex using a 5.0 silk suture. After suspension of the septum, electrodes from a stimulator were attached to the clamps and stimuli (4–6 mV, 0.4 msec) were delivered at a rate of 72 per min.

The 27 septa were divided into five groups for study: controls (n = 7); moderate (n = 5) and severe (n = 5) demand-induced; and moderate (n = 5) and severe (n = 5) reduced-flow ischemic conditions. During control studies, flow and stimulation rate were maintained constant at 3.0 ml/min and 72 stimuli/min, respectively. Moderate and severe demand-induced ischemia were produced by introducing a paired stimulus at 50 and 150/min (100 and 300 total stimuli, respectively) at a perfusion rate of 1.5 ml/min. The marked increase in oxygen demand produced by paired stimulation without an appropriate increase in oxygen supply creates the condition of demand-induced ischemia (*10*). Moderate and severe reduced-flow ischemia were produced by reducing flow to 1.0 and 0.4 ml/min while maintaining stimulis at 72/min. Since the FDG compartmental model assumes the existence of steady-state glucose metabolism, ischemia was produced 15 min before introduction of FDG to allow attainment of a steady state for exogenous glucose utilization.

Data collection and analysis. FDG with a specific activity of 10–20 mCi/mg and a radiochemical purity of greater than 95% (*11*) was infused continuously at a concentration of 1 μCi/ml. Tracer infusion was initiated as a step function. Starting at time zero, arterial and venous samples were obtained at 5-min intervals for a total of 60 min. FDG activity was measured in a well counter and corrected for radioactive decay ($T_{1/2}$ = 109.8 min). Exogenous glucose utilization was evaluated by ^3H_2O production from 2-[^{3}H]glucose (*12*). Following separation of ^3H_2O and [^{3}H]glucose by anion-exchange chromatography (*13*), arterial and venous samples were dissolved in scintillation cocktail and counted in a liquid-scintillation counter.

The lumped constant was calculated as the ratio of the fractional extraction of FDG to the fractional conversion of 2-[^{3}H]glucose to ^3H_2O (*4*). If the rate constant of dephosphorylation of FDG-6-PO_4 (k_4*) is zero or insignificant, the value of the lumped constant can be evaluated using this approach. To evaluate the magnitude of k_4*, the extraction fraction of FDG was plotted as a function of time and fitted with a predicted curve based on the kinetic equation derived from the FDG compartmental model as described previously (*1,2*).

The lumped constant was also calculated from the

formula:

$$LC = \frac{[Glc]}{Ri} \cdot \frac{k_1^* k_3^*}{k_2^* + k_3^*}, \quad (1)$$

where LC = lumped constant; [Glc] = perfusate glucose concentration; Ri = rate of myocardial utilization of exogenous glucose, determined from flow rate times the arteriovenous difference for glucose; and $(k_1^* k_3^*)/(k_2^* + k_3^*)$ = the fractional rate at which perfusate FDG is transported into the cell and phosphorylated. The calculation of $k_1^* k_3^*/k_2^* + k_3^*$ was performed from the curve-fitting results as previously described by Ratib et al. (*9*). The calculated lumped constant using this equation does not assume k_4^* to be zero (*2*). Therefore, comparison of the values obtained from the ratio of the extraction fractions of FDG and glucose with the values obtained from Eq. (1) provides an assessment of the effect of k_4^* on the fractional extraction of FDG at steady state. Once the lumped constant is known, the myocardial metabolic rate for exogenous glucose (MMRGlc) can be calculated directly from the FDG compartmental model by rearranging Eq. (1) to give:

$$MMRGlc = \frac{[Glc]}{LC} \cdot \frac{(k_1^* k_3^*)}{(k_2^* + k_3^*)} \quad (2)$$

Statistical differences between experimental groups were determined by Student's t-test.

RESULTS

Figure 2 plots the ratio of the extraction fractions of FDG and glucose as a function of time, as obtained from an individual control experiment. Initially the extraction fraction of FDG relative to glucose is high, reflecting primarily the forward transport of FDG into the myocardium. At about 20 min, tissue FDG and FDG-6-PO_4 concentrations have approached near steady-state conditions, such that back transport of FDG out of the myocardium is nearly constant and the rate of change in the extraction fraction of FDG is slow. The fluctuations in the extraction-fraction ratio after 20 min are due primarily to measurement errors that are unavoidable due to the small arterovenous differences encountered in the septum under nonischemic conditions (*10*).

Figure 3 illustrates the average extraction-fraction ratios for five runs during moderate reduced-flow ischemia. Because of the larger number of samples per data point and the larger extraction fraction of glucose and FDG during ischemia, there is considerably less variability in the data compared with that shown in Fig. 2. The fit obtained for the predicted curve from the kinetic equation of the FDG compartmental model and the directly measured extraction fraction ratios is also shown. In all of the experiments performed under ischemic conditions, curve fitting was performed on the average data from five experiments as shown here.

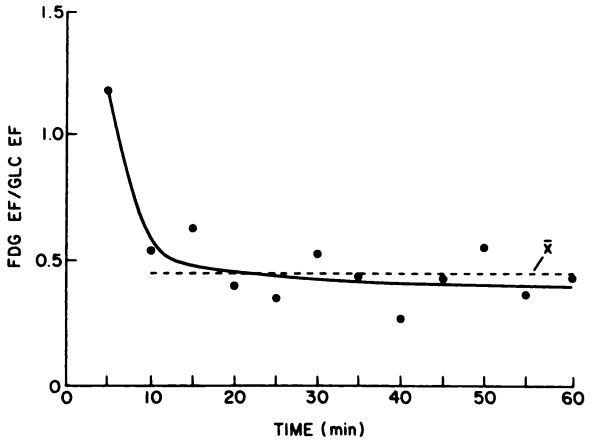

FIG. 2. Ratios of extraction fraction (EF) of FDG to glucose from individual control experiment plotted as function of time. (—) = Visual fit. (- - -) = Mean steady-state value for ratio of extraction fractions of FDG to glucose, which would be lumped constant if k_4^* were zero. Although averaging results of all seven control experiments reduced variability, estimates of combined rate constant obtained by curve-fitting had large standard errors of estimate, and no attempt was made to calculate metabolic rate and lumped constant using Eqs. (1) and (2).

The results for the five experimental groups are detailed in Table 1. Because of the dephosphorylation of FDG-6-PO_4, the value of the extraction-fraction ratio at the later times varies slowly as a function of time. Therefore, the lumped constant values for each experimental group reported here reflect ratios of the averaged extraction fraction between 20 and 40 min after initiation of FDG infusion (*1*,*4*). The value of the lumped constant calculated from the extraction-fraction ratios obtained during control experiments (0.44 ± 0.14) is lower than the previously reported values of 0.60 ± 0.10 in the isolated septum (*8*) and 0.67 ± 0.1 in the in vivo dog heart (*9*), obtained under nonischemic conditions

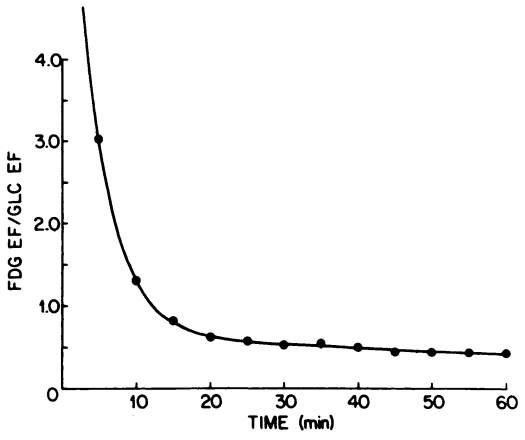

FIG. 3. Average ratios of extraction fraction (EF) of FDG to glucose as function of time from five experiments evaluated during ischemia produced by moderate flow reductions. Dots represent actual measured values, and solid line is fitted curve obtained from kinetic equation of FDG compartmental model using iterative curve-fitting routine. For discussion, see text.

TABLE 1.† VALUES FOR LUMPED CONSTANT, MYOCARDIAL METABOLIC RATE FOR GLUCOSE AND k_4*

				LC	
	k_4*	MR_{Fick} (unit: LC assumed 0.64 μmol/min/g)	MR_{FDG}	EF FDG/ Glucose	Eq. (1)
Control	0.00626	0.384		0.44	
	±0.00682	±0.100	—	±0.14	—
DII-M	0.00841	0.542	0.528	0.51	0.61
	±0.00169	±0.042	±0.026	±0.05	±0.03
DII-S	0.00834	0.917	1.055	0.51	0.72
	±0.00231	±0.067	±0.061	±0.06	±0.04
RFI-M	0.00824	0.567	0.563	0.52	0.62
	±0.00115	±0.167	±0.021	±0.04	±0.02
RFI-S	0.00727	0.465	0.466	0.51	0.63
	±0.00080	±0.109	±0.010	±0.03	±0.01

† Average results for five experimental groups (data from curve-fitting results on average data during ischemic experiments expressed as mean ± standard error of estimate [k_4*, MR_{FDG}, LC from Eq. (1)]; remainder of data are expressed as mean ± standard deviation). Abbreviations: MR = metabolic rate; EF = extraction fraction; LC = lumped constant; DII-M = Demand-induced ischemia, moderate; DII-S = Demand-induced ischemia, severe; RFI-M = Reduced-flow ischemia, moderate; RFI-S = Reduced-flow ischemia, severe.

using Eq. (1). This underestimation of the lumped constant is expected and is due to dephosphorylation of FDG-6-PO_4. The values for the lumped constant calculated from the extraction-fraction ratios during moderate and severe demand-induced and reduced-flow ischemia (0.51 ± 0.05, 0.51 ± 0.06, 0.52 ± 0.04, 0.51 ± 0.03, respectively) were not significantly different from the value obtained in control experiments. Similarly, the values for k_4* in the four ischemic experimental groups (0.00841 ± 0.00169, 0.00834 ± 0.00231, 0.00824 ± 0.00115, 0.00727 ± 0.0008) were not significantly different from control (0.00626 ± 0.00682). The lack of any significant change for both the lumped constant calculated from the extraction-fraction ratios and k_4* between ischemic and control experiments indicates that the lumped constant is stable under conditions of altered metabolic rate due to either demand-induced or reduced-flow ischemia.

From the curve-fitting results it was possible to calculate the combined rate constant of FDG transport and phosphorylation, $(k_1{*}k_3{*})/(k_2{*} + k_3{*})$, in the four ischemic experimental groups. (Due to the small extraction fraction of glucose under nonischemic conditions in the septum, large standard errors of estimation were obtained for the combined rate constant under control conditions, yielding no useful information, so these are not reported in Table 1). Using Eq. (1), it was then possible to calculate the lumped constant by an approach that takes into account the dephosphorylation of FDG-6-PO_4. The values for the lumped constant obtained during moderate and severe demand-induced and reduced-flow ischemia (0.61 ± 0.03, 0.72 ± 0.04, 0.62 ± 0.02, 0.63 ± 0.01, respectively) are in agreement with the previously reported values obtained under nonischemic conditions in the heart (*8*,*9*), providing additional evidence for the stability of the lumped constant during myocardial ischemia. The mean value of the lumped constant obtained using Eq. (1) for the four ischemic experiments (0.64 ± 0.05) was significantly higher than the mean value calculated from the extraction-fraction ratios (0.51 ± 0.03, $p < 0.01$). This observation is compatible with dephosphorylation of FDG-6-PO_4 (i.e., $k_4{*} \neq 0$).

DISCUSSION

This study provides two lines of supporting evidence for the stability of the lumped constant during altered myocardial metabolism produced by ischemia. First, the value of lumped constant calculated from the extraction-fraction ratios did not change significantly between control and ischemic experiments. Although the absolute value of the lumped constant was underestimated with this approach due to reduction in steady-state extraction of FDG from dephosphorylation of FDG-6-PO_4, constant values for both the extraction-fraction ratios and the rate constant of dephosphorylation (k_4*) provide evidence that the lumped constant does not change during myocardial ischemia. Second, values for the lumped constant calculated from Eq. (1) in ischemic experimental groups agree well with previously reported values obtained under nonischemic conditions in the in vitro septal preparation (*8*) and the in vivo dog models (*9*). Within the limits of current tracer techniques for

kinetic modeling, these observations suggest that the lumped constant is not altered under the ischemic conditions evaluated here.

The values of the lumped constant estimated by the ratio of FDG to glucose extraction fractions were obtained from the averaged values between 20 min and 40 min. This time window was used because the extraction-fraction ratios after 20 min have been used by others (*4*) for calculating the lumped constant in the brain, and because the initial rapid change in extraction-fraction ratios due to the transient net transport of FDG from perfusate to myocardium had disappeared. Due to the constancy of k_4* among the different study conditions, the stability of the lumped constant values estimated from FDG to glucose extraction-fraction ratios is not likely to be a consequence of the selected time window. To further exclude this possibility, ratios of extraction fractions between FDG to glucose were computed using the time window of 40–60 min. The variability of these estimates among the five groups was again found to be insignificant, although the values calculated from the later time window were lower, averaging 84% of the values obtained from the 20- to 40-min time window.

Because of the low temporal resolution of sampling, the values for the individual rate constants for bidirectional membrane transport and phosphorylation have large standard errors of estimate and are not reported. However, unlike the rate constants for membrane transport and phosphorylation, which are sensitive to the initial portion of the FDG extraction curve, the rate constant for dephosphorylation (k_4*) is primarily reflected in the later slowly changing portion of the curve, and therefore could be determined with acceptable accuracy at the currently used temporal resolution of sampling. Similarly, the combined rate constant reflecting the myocardial fractional extraction of FDG from the perfusate, $(k_1{*}k_3{*}/(k_2{*} + k_3{*})$, could also be obtained with acceptably low standard errors of estimates from the curve-fitting results of the present study (*9*). In the future, further validation of the FDG tracer kinetic model for use in myocardial ischemia will require the determination of absolute rate-constant values for bidirectional membrane transport, phosphorylation, and dephosphorylation by sampling with high temporal resolution using coincidence detection circuitry (*8,9*). Myocardial FDG and FDG-6-PO_4 concentrations predicted from the kinetics by the model should also be validated by comparison with values directly measured by chemical assay of tissue samples under conditions of altered myocardial metabolism produced by ischemia.

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