Effect of Mechanical Work Load on the Transmural Distribution of Glucose Uptake in the Isolated Perfused Rat Heart, Studied by Regional Deoxyglucose Trapping

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SUMMARY The applicability of the 2-deoxyglucose tracer method to the study of the transmural distribution of glucose uptake in the left ventricle of the isolated, Langendorff-perfused rat heart was validated for the myocardium. The total 2-deoxy[1H]glucose trapped in the myocardium was proportional to the glucose uptake which was varied by the mechanical work load and availability of other oxidizable substrates. When the mechanical work of the heart was eliminated by K⁺-induced arrest, the glucose uptake of 2.0 µmol/min per g protein was evenly distributed across the left ventricular wall. In the beating heart, glucose uptake averaged 4.7 µmol/min per g protein, and was about 40% higher in the subendocardial layer than in the subepicardium (P < 0.001). Measurement of the transmural distribution of coronary flow by the microsphere method indicated that there were no areas of underperfusion in the left ventricle. The transmural gradient in glucose uptake probably is caused by uneven distribution of the mechanical work load. Circ Res 49: 62-69, 1981

THE transmural distribution of energy metabolism in cardiac muscle is only partially known. A transmural gradient has been observed for selected metabolites, and also in the case of the glycolytic enzymes (Lundsgaard-Hansen et al., 1967) and glycogen phosphorylase (Jedeikin, 1964) The data gathered hitherto suggest that oxygen consumption is higher in the subendocardial layer than in the more superficial layers (Howe et al., 1975, Weiss et al., 1978). The computed stress distribution within the ventricular wall indicates a gradient with higher radial, longitudinal, and latitudinal systolic stress in the endocardial layers of the myocardium (Wong and Rautaharju, 1967). The data suggest that the rate of energy consumption is highest in the innermost layers of the myocardium and that the systolic intramyocardial pressure exceeds the coronary pressure in these layers, resulting in an impediment to myocardial blood flow during systole (Archie, 1975). The physical differences between the inner and outer layers of the myocardium may be the cause of myocardial metabolic gradients.

The transmural distribution of actual glycolytic flux and glucose uptake has not been studied previously by methods yielding quantitative data. In the present case, the proportionality of deoxyglucose trapping to glucose uptake in the whole heart was confirmed and the kinetics of deoxyglucose release determined. The data allowed calculation of the transmural distribution of glucose uptake in the left ventricular wall on the basis of deoxyglucose trapping in the various layers of the myocardium. A transmural gradient of glucose uptake could be demonstrated in the beating heart.

Methods

Reagents

2-Deoxy-d-[3H]glucose and d-[3-3H]glucose were obtained from the Radiochemical Centre and non-radioactive 2-deoxy-d-glucose from Sigma Chemical Co. ¹⁴C-labelled microspheres with a diameter of 14.7 ± 1.1 µm (mean ± SD) were obtained from New England Nuclear, and standard reagents from E. Merck, A.G.

Animals

Male Sprague-Dawley rats weighing 270-330 g from the Department's own stocks were used. They were fed ad libitum on pelleted standard rodent chow (Hankkija Ltd.).

Apparatus

The perfusion apparatus was constructed essentially as described by Chain et al. (1969). The perfusion fluid reservoirs connecting glass tubes and heart compartment were thermostated at 37°C. Rapid changes in the perfusion medium were achieved by means of suitable three-way stopcocks. A multibulb oxygenator was used for recirculation perfusion.

Perfusion

The rats were anesthetized with ether and injected with 500 IU heparin intravenously before...
excision of the heart. The hearts were perfused using the Langendorff procedure (Langendorff, 1885), the perfusion medium being Krebs-Ringer bicarbonate buffer (Krebs and Henseleit, 1932) containing 2.5 mM Ca\(^{2+}\), 5 mM glucose, 5 IU insulin per liter in equilibrium with O\(_2\)/CO\(_2\) (19:1). Octanoate (1 mM) was also added to the perfusate when indicated. When we studied arrested hearts, a medium with a potassium chloride concentration of 18 mM was used (high-K\(^+\) medium), the sodium chloride concentration being lowered accordingly, and the intraventricular pressure kept at zero by perforating the myocardium with an injection needle. The perfusion pressure was 7.85 kPa (80 cm of water).

**Protocol**

After a preperfusion for 10 minutes without recirculation, pacing of the heart at a rate of 260 beats/min or arrest with a high-K\(^+\) medium was begun. One group of arrested hearts was perfused in the presence of 1 mM octanoate with an addition of 5 mM glucose. After a further 10 minutes, the perfusion was switched to recirculation with 70 ml of the same medium containing a 620 nM concentration of deoxy-[\(^3\)H]glucose (specific radioactivity 18,000 dpm/nmol). After a 20-minute recirculating perfusion with this medium, the perfusion was switched to a non-circulating system with normal medium without deoxyglucose. This period, during which the heart was beating, lasted for 10 minutes in order to wash free deoxyglucose out from the tissue. For experiments in which the half-life of tissue deoxyglucose was to be measured, this last phase of the perfusion was continued for 70 minutes. The hearts were then immersed in ice-cold Krebs-Henseleit medium and the left and right ventricles and atria were dissected free.

The left ventricle was cut longitudinally, blotted, opened, and frozen between aluminium plates precooled in liquid nitrogen so that a tissue sheet with a thickness of 3.8 ± 0.1 mm (mean ± SEM) was formed, the epicardium constituting one surface and the endocardium the other. The right ventricle and atria were also quick-frozen separately. The left ventricle was cut into 40-μm slices in a cryostat at −20°C. These slices were combined to represent six equal pads of the total thickness of the myocardium and were pulverized at −196°C. Tritiated water was separated from the perfusate by distillation using the apparatus described by Moss (1964). The perfusion conditions were similar to those used in the deoxyglucose experiments, and perfusate samples were taken at the beginning and end of phase 3 of the perfusion protocol described above.

**Glycogen Synthesis**

The myocardial glycogen synthesis from external glucose was determined by measuring the [\(^3\)H]glucose incorporation into glycogen. The radioactivity of glucose labeled as described above cannot be incorporated into non-carbohydrate compounds because of the loss of tritium during glycolysis. The freeze-clamped heart was pulverized at −196°C, 100 mg of the product was digested, and glycogen was precipitated, as described by van Handel (1965). The precipitate was then suspended in 1 ml of 66% ethanol containing 5 mM non-radioactive glucose. A sample was collected on a Millipore filter by suction and washed three times with the ethanol-glucose solution. The filter was dried in a counting vial and dissolved in 6 ml of Cellosolve, followed by 10 ml of toluene-based scintillation solution.

**Determination of the Kinetics of Tissue Deoxyglucose-6-phosphate Release**

The release of radioactivity into the perfusate and the final radioactivity of the heart were measured. The medium was the same as used in the last phase of the perfusion (see Protocol). The release was also determined in arrested hearts and in the presence of 620 nM deoxyglucose.

The proportions of labeled deoxyglucose and deoxyglucose-6-phosphate in the myocardium were determined in perchloric acid extracts (Williamson and Corkey, 1969) of quick-frozen hearts (Wollenberger et al., 1960) at 10 or 70 minutes after the beginning of the last perfusion step by separating the compounds by ion exchange chromatography [according to the method of Katz et al. (1977)] and measuring the radioactivities of the deoxyglucose and deoxyglucose-6-phosphate fractions.

**Calculation of Regional Uptake of External Glucose**

Glucose is transported into the cell by facilitated diffusion using a saturable carrier (Morgan et al., 1964). In insulin-responsive tissues such as muscle, glucose transport into the cell is limiting (Morgan et al., 1961) and the glucose is rapidly phosphorylated to glucose-6-phosphate. This represents an irreversible trapping of glucose, since the activity of glucose-6-phosphatase is extremely low in cardiac muscle (Opie and Newsholme, 1967). Glucose-6-phosphate is used mainly for glycogen synthesis or
glycolysis, the activity of glucose-6-dehydrogenase being very low in this tissue. The unidirectional rate of glycolysis can be determined by measuring \(^3\)HOH formation from \([3-\text{H}]\)glucose, as described above, and an estimate for the glycogen synthesis rate can be determined on the basis of label incorporation from \([3-\text{H}]\)glucose, also as described above.

2-Deoxyglucose shares the transport system across plasma membrane with glucose and is rapidly phosphorylated to 2-deoxyglucose-6-phosphate, which is metabolically inert (Kipnis and Cori, 1959), since it is not a substrate for phosphohexose isomerase or glucose-6-phosphatase. This, in effect, makes 2-deoxyglucose into a compound which is almost irreversibly trapped as 2-deoxyglucose-6-phosphate (but see below). If glucose (G) and 2-deoxyglucose (2-DG) compete for the same translocator, obeying Michaelis-Menten kinetics (Dixon and Webb 1971), and the concentration of free G and 2-DG is kept close to zero intracellularly by further irreversible phosphorylation to the corresponding 6-phosphate esters, the uptake rate of 2-DG is

\[
v^* = \frac{V_m^* \cdot C}{K_m^* + \frac{C}{C^*}}
\]  

(1)

and if the concentration of 2-DG is so low that it has no effect on glucose transport, the rate of the latter is

\[
v = \frac{V_m \cdot C}{K_m + C}
\]  

(2)

where \(v^*\) = uptake rate of 2-DG, \(v\) = uptake rate of G, \(V_m^*\) = maximal rate of 2-DG uptake, \(V_m\) = maximal rate of G uptake, \(C^*\) = 2-DG concentration in the perfusion medium, \(C = G\) concentration in the perfusion medium, \(K_m^*\) = plasma 2-DG concentration at \(v^* = \frac{V_m^*}{2}\), \(K_m\) = plasma G concentration at \(v = \frac{V_m}{2}\).

Then:

\[
\frac{v^*}{v} = \frac{V_m^* \cdot C^*(K_m + C)}{V_m \cdot C \cdot [K_m^* + \frac{K_m^* \cdot C}{K_m} + C^*]}
\]  

(3)

When 2-DG is present at a tracer concentration, Equation 3 becomes

\[
\frac{v^*}{v} = \frac{V_m^* \cdot C \cdot K_m}{V_m \cdot C \cdot K_m^*} = \frac{C^* \cdot V_m^* \cdot K_m}{C \cdot V_m \cdot K_m^*}
\]  

(4)

demonstrating that the uptake ratio of 2-DG to G is linearly proportional to the product of the concentration ratio \((Cv^*/C) = R\) and the factor

\[
\frac{V_m \cdot K_m}{V_m^* \cdot K_m^*} = A
\]

and

\[
v^* = v \cdot A \cdot R.
\]  

(5)

Accumulation of the tracer, deoxyglucose, is a function of transport into the cell, transport out of the cell, phosphorylation by hexokinase, and hydrolysis of deoxyglucose-6-phosphate. In the following, the sequences of inward transport and phosphorylation on the one hand, and hydrolysis and outward transport on the other, are treated as units. This is justifiable if the relative affinities of glucose and deoxyglucose for the sequence of transport systems are closely similar. The experimental results below show that this assumption is realistic and, moreover, the ratio of the affinities of glucose and deoxyglucose is close to unity throughout the whole system.

Since the intracellular metabolism of 2-DG represents a dead end, the leakage of 2-DG out of the cell must be taken into consideration (but since glucose metabolites do not accumulate, the release of glucose is not of practical significance). The leakage, as described below, becomes significant also when a wash-out phase is incorporated in the perfusion protocol to avoid error due to extracellular retention of 2-DG label in the tissue.

As shown in the Results section, the outward leakage can be approximated by a first-order reaction

\[
v_e^* = k_e \cdot C_t^*
\]  

(6)

where \(k_e\) is the rate constant, \(C_t^*\) the intracellular concentration of 2-DG metabolites and \(v_e^*\) the leakage rate of 2-DG. Although the plasma membrane translocator may be symmetrical, so that the rate constants are equal in both directions, \(k_e\) here represents the combined action of 2-DG-6-P hydrolysis (which is at least one order of magnitude slower than the rate of membrane transport) and translocation across the plasma membrane. In a closed perfusion system with a small leakage of 2-DG out of the cell and a concomitant usage of glucose, the ratio \(R = C^*/C\) is not necessarily constant, but may slowly increase during the perfusion so that

\[
R = R_0 + r \cdot t
\]  

(7)

where \(R_0\) is the 2-DG/C concentration ratio in the perfusate at time zero, \(t\) is time, and \(r\) is a proportionality coefficient.

The rate of net uptake of 2-DG can be described as

\[
\frac{dC_{t1}^*}{dt} = A \cdot v(R_0 + r \cdot t) - k_e C_{t1}^*
\]  

(8)
Integration of Equation 8, taking \( C_i = 0 \) at time zero, yields

\[
C_{00}^* = \frac{(1-e^{-kt})}{v} \left( \frac{A \cdot r - k_s \cdot A \cdot R_0}{k_s^2} \right) + \frac{v \cdot A \cdot r}{k_s}, \tag{9}
\]

and solving for \( v \),

\[
v = \frac{k_s^2 C_{00}^*}{(A \cdot r - k_s \cdot A \cdot R_0)(1-e^{-kt})} \tag{10}
\]

When \( \frac{C^*}{C} = R \) is constant, i.e., \( r = 0 \), Equation 10 yields

\[
v = \frac{k_s C_{00}^*}{A \cdot R_0 (1-e^{-kt})}. \tag{11}
\]

The constant \( A \) was experimentally determined in whole isolated perfused hearts by determining \( v \) by the [3-\( ^3 \)H]glucose method (see above) and 2-DG uptake in parallel experiments. The value of \( k_s \) was determined by an extended perfusion of a 2-DG-loaded heart in the absence of 2-DG.

Since a washout phase was always included in the 2-DG experiments, \( C_{00}^* \), the tissue 2-DG label concentration at the end of the accumulation phase (3), was extrapolated by the equation

\[
C_{00}^*(1 + t') = e^{-k_s t}, \tag{12}
\]

where \( t' \) is the duration of the wash-out period and \( C_{00}^* \rightarrow C \) the tissue 2-DG label concentration at the end of the wash-out period.

**Determination of the Myocardial Flow Distribution**

\(^{141}\)Ce-Labeled microspheres were sonicated in a solution containing 0.9% NaCl and 0.1% Tween 80. One hundred microliters of this suspension, containing 1500-2500 microspheres, were mixed with 0.9 ml of oxygenated perfusate (37°C) and immediately injected into the aortic cannula within 30 seconds at the end of step 3 of the perfusion. The hearts were prepared and sliced as described for the deoxyglucose phosphate measurements. Radioactivity was determined from dried samples with a Packard Sekelronik model 48 well-type scintillation detector. The coronary flow (ml/min per g dry wt) was calculated by multiplying the relative flow rate by the average coronary flow measured in terms of the total flow through the heart. There was obviously no leakage through the mitral valve, since the proportion of non-trapped microspheres (<1%) was the same in the beating hearts as in arrested hearts in which the intraventricular pressure had been adjusted to zero.

**Determination of Intraventricular Pressure**

A needle 0.9 mm in diameter and 5 mm long was inserted through the myocardium into the left ventricle and connected to a Statham P23 1D pressure transducer and a Statham SP1400 pressure monitor, and to observe the complete waveform of the ventricular pressure changes in a beating heart, a digital transient store (model 512A; Physical Data, Inc.) with a frequency response of 0 to 80 kHz was used.

**Protein**

Protein was determined by the biuret reaction according to Szarkowska and Klingenberg (1963).

**Statistical Analysis of the Data**

Statistical analysis of the data was performed using the Student's \( t \)-test or one-way analysis of variance.

**Results**

**Trapping and Retention of Deoxyglucose in Heart Muscle**

Figure 1 depicts the kinetics of deoxyglucose release from the perfused heart after a 20-minute exposure to a tracer amount of [\( ^3 \)H]deoxyglucose in the perfusate. After discontinuation of the labeling phase, wash-out of deoxyglucose proceeded with a biphasic kinetic pattern with two half-lives, one of 1.5 ± 0.1 min and the other of 69 ± 5 min (mean ± SEM from six hearts). This value gives \( k_s = 0.0100 \) in Equations 8-12 of the Methods section. The same \( t_1/2 \) values were observed in beating and arrested hearts. The shorter \( t_1/2 \) evidently represents the clearance from the extracellular space and the longer \( t_1/2 \) the elimination of the intracellular label, probably by hydrolysis of deoxyglucose-6-phosphate and subsequent leakage from the cell. The addition of 620 nM non-radioactive deoxyglucose into the perfusate did not affect the release of tissue deoxyglucose radioactivity in the presence of 5 mM glucose, but in the absence of glucose, non-radioactive deoxyglucose in the perfusate increased the rate of label release.

The \( t_1/2 \) of the cellular radioactivity release was lengthened by a factor of 2.3 when glucose and deoxyglucose were replaced by 1 mM octanoate. This phenomenon could be due to elimination of the countertransport of deoxyglucose and glucose by the withdrawal of the latter. Also, the resorption of deoxyglucose intracellularly is more efficient when the competing substrate, glucose, is not present. All these data suggest that glucose and deoxyglucose compete for the same transport and phosphorylation system.

The proportion of free deoxyglucose in the tissue was 8% of the total at the end of the wash-out phase of the perfusion (at 10 minutes) and 9% of the total...
Glucose Uptake Rate in the Whole Heart and Validation of the Deoxyglucose Trapping Method for Its Determination

To obtain conditions involving different glucose uptake rates, beating, arrested, or glucose + octanoate-perfused arrested hearts were studied. The results are shown in Table 1. In beating hearts, most of the external glucose was used in glycolysis, but the proportion of the label incorporated into glycogen was noticeable in the arrested and glucose + octanoate-perfused arrested hearts. The rate of glucose uptake behaved as expected from the relative rates of cellular energy consumption and inhibition of glycolysis in heart muscle by fatty acids (for a review, see Neely and Morgan 1974).

The correlation between deoxyglucose accumulation and glucose uptake was positive and close to linear. From Table 1 and the initial amounts of glucose and deoxyglucose in the perfusate, it can be calculated that the deoxyglucose:glucose ratio in the perfusate remains practically constant during phase 3 of the perfusion experiment, and the coefficient $r$ in Equation 10 is zero. Thus, by inserting the flux values from Table 1 and $k_e$ as calculated from Figure 1 into Equation 11, the coefficient $A$ of Equations 8–11 can be calculated. Its numerical value was 1.08, 0.93, and 1.00 in beating, arrested, and glucose + octanoate perfused hearts, respectively. The average value was 1.00. This indicates that, throughout the complete system of hexose uptake and phosphorylation, deoxyglucose behaves like an ideal tracer and can be used to obtain quantitative data on glucose uptake in the myocardium.

Transmural Distribution of Glucose Uptake

The regional uptake of glucose was calculated using the Equations 11 and 12 and the numerical values for $k_e$, $A$, and $R$ determined as described above. The results are depicted in Figure 2. The glycolytic rate was significantly higher in the subendocardial layer of the beating heart than in the...
subepicardial layers ($P < 0.001$ by analysis of variance), whereas it was quite even in the outer half-thickness of the myocardium. The glycolytic rate was lower in the arrested heart, and was evenly distributed in the various layers of the myocardium. In the beating heart, the glucose uptake of the right ventricle or the atria was slightly lower than that of the left ventricle, but in the arrested heart, no difference existed in the uptake of glucose into the right and left ventricles or the atria.

Transmural Distribution of Myocardial Perfusion

The microsphere technique revealed that the regional perfusion of the subendocardial layers in the isolated perfused heart is somewhat higher than in the layers on the epicardial side (Fig. 3). Perfusion of the arrested heart was lower, but the transmural distribution was in principle the same as in the beating heart. The regional perfusion of the myocardium was the same in the right and left ventricles. In hearts perfused at an aortic pressure of 7.85 kPa, the systolic intraventricular pressure was 8.8 kPa and the diastolic intraventricular pressure was 0 kPa.

In the arrested hearts, the intraventricular pressure was zero. In vivo or in a working heart, the time-averaged diastolic pressure is higher, and it remains to be established whether gradients of glucose uptake also exist in vivo.

Discussion

Accumulation of deoxyglucose labeled with $^{14}$C, or 2-deoxy-2-fluoroglucose labeled with $^{18}$F, has been employed previously for studies on the distribution of glucose in the brain, and the kinetic parameters of the enzyme for this tissue have been sufficiently well characterized to validate the method used (Sokoloff et al., 1977; Reivich et al., 1979). Only preliminary data exist on the uptake of
strate that the deoxyglucose trapping method can capillary circulation is higher in the beating heart, stress in the subendocardial layers.

The regional differences demonstrated in Table 1, also demonstrates the regulation of glucose utilization by free fatty acid availability (Hiltunen and Hassinen, 1976; Neely and Morgan, 1974). Here a half-life of 1.15 hours was observed by Neely et al. (1970) according to the pattern of glucose metabolism in the perfused working and Langendorff heart of normal and insulin-deficient rats. Biochem J 115: 537-646

The results also confirm the isolated potassium- arrested perfused heart as a reliable model for a resting muscle, and establish that this does not entail any regional underperfusion of the muscle. In the light of the higher local perfusion rates in the subendocardial layers, it is unlikely that the higher rates of glucose uptake are due to anaerobic glycolysis. It is known that the glycogen stores, and also the maximum glycogen phosphorylase activity, are higher in the subendocardial myocardium (Jedekin, 1964). A high glycogen concentration must indicate conditions in which the cellular energy state is also high, since lowering of the energy state leads to a decrease in glycogenolysis and an increase in glycogenolysis (Morgan and Parmeggiani, 1964). It is remarkable that the regional fuel stores in the myocardium are scaled according to the potential energy expenditure. The present results confirm the observation by Neely et al. (1970) according to which a reciprocal correlation exists between glycogenolysis and mechanical work load in isolated perfused hearts.

When extrapolating the present findings to in vivo conditions, it should be borne in mind that free fatty acids are always present in the blood, and that fatty acids replace carbohydrates as an oxidizable fuel in the myocardium. The transmural distribution of the fatty acid oxidizing capacity is not known, and free fatty acids may influence both the total rate of glucose uptake and its transmural distribution. In vivo studies on the problem of regional glucose uptake therefore are needed.

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