Glucose delivery is a major determinant of glucose utilisation in the ischemic myocardium with a residual coronary flow

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Abstract

**Background:** Experimental data from isolated rat hearts suggest that glycolysis in severe myocardial ischemia is inhibited by accumulation of glycolytic metabolites. In contrast, positron emission tomography (PET) in patients with myocardial ischemia records a ‘mismatch’ between the decreased coronary flow in viable ischemic tissue and an increased fluorodeoxyglucose (¹⁸FDG) signal. To resolve this contradiction, we investigated glucose uptake at very low coronary flows in the isolated rat heart model. **Methods:** Rates of glucose uptake were measured in the isolated Langendorff-perfused Wistar rat heart, at control (12–15 ml/g wet wt/min) and low coronary flows (0.1, 0.2 and 0.5 ml/g wet wt/min) and at a range of glucose concentrations (2.75, 5.5, 11 and 22 mM). **Results:** The steady-state rate of glucose uptake versus glucose concentration could be described by a double rectangular hyperbola at each coronary flow. Glucose uptake fell to levels significantly below control at low coronary flows. However, the extraction of glucose (glucose uptake as % of glucose delivered) rose sharply, from 1% at control coronary flows, to 25±30% at low coronary flows. Crossover analysis of glycolytic intermediates in freeze-clamped tissue indicated little inhibition at any specific site, although phosphofructokinase activity was restricted when glycolytic substrate availability was high. Insulin and preconditioning both increased glucose uptake with 11 mM glucose, possibly by increasing membrane transporter density and thus increasing glucose delivery to the cytosol. **Conclusions:** Despite the reduction in absolute glucose uptake at low coronary flow-rates, the extraction of glucose was greatly increased, possibly following GLUT4 translocation. Delivery of glucose to the glycolytic pathway appears to be a major controlling site of glycolysis in low-flow ischemia. Downstream regulation is then distributed along the pathway with no one site exerting greater inhibition than reduced glucose delivery. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Glycolysis; Ischemia; Metabolic regulation; Preconditioning; Rat

1. Introduction

Over 20 years ago, Neely and Rovetto reported that absolute rates of glucose uptake and glycolysis are reduced in ischemia in isolated hearts, a phenomenon attributed to inhibition of the glycolytic pathway at glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [1,2] by a build-up of metabolites [1,3–5].

In contrast, positron emission tomography (PET), which measures the transfer of ¹⁸F-labelled deoxyglucose (¹⁸FDG) into tissue in vivo, shows an increased ¹⁸FDG uptake in relation to the fall in the coronary flow-rates in viable heart muscle of patients with coronary artery disease [6]. The difference between the increased ¹⁸FDG signal and the fall in coronary flow is called ‘metabolism/perfusion mismatch’ [7]. These observations suggest that myocardial glucose uptake could be enhanced in ischemia, and that glycolysis may not be inhibited. Other investigators have reached similar conclusions with in vivo models [8], but these concepts have not been clarified in an isolated heart, the model in which glycolytic regulation was initially described.

We examined glucose uptake in the isolated perfused rat heart over a range of low coronary flows, thought to be more comparable to in vivo ischemic coronary flow-rates (defined as those coronary flows found in the subendocardium of an area perfused by an artery which is then

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ligated, i.e., 0.07–0.15 ml/g wet wt/min in pigs and dogs [8]). The crucial issue is whether, when compared to the reduction in coronary flow, the rate of glycolysis is reduced proportionately, or is upregulated relative to coronary flow, as suggested by observations including ‘mismatch’. We also investigated the effect of insulin and preconditioning on glucose uptake and utilisation within the cell, both being factors which may increase translocation of GLUT4 glucose transporters to the membrane [9,10].

2. Methods

2.1. Isolated rat heart perfusions

2.1.1. Experimental apparatus

Male Wistar rats (263±18 g) fed ad libitum (mean fresh heart weight 1.05±0.01 g) were anaesthetised with ether, following which 200 i.u. heparin was injected into the exposed femoral vein. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985). The hearts were excised, arrested in ice-cold buffer, and mounted on a Langendorff apparatus (perfusion pressure 76 mmHg), perfused with modified Krebs Henseleit solution (in mM NaCl 113.5; KCl 4.75; KH2PO4 1.18; NaHCO3 25, CaCl2 1.36, Na-acetate 5) and gassed with 95% O2, 5% CO2 to maintain pH at 7.4. Glucose was added to all perfusates in concentrations as described below. Five millimolar acetate was present as an alternate substrate in all perfusions such that when glucose was present only at very low concentrations, adverse effects associated with limited substrate perfusion [11,12] were reduced. In addition, acetate is a fatty acid analogue, the major substrate used by the heart. Acetate may influence glucose utilisation in normoxia. However, our values were similar to those obtained in the absence of acetate, especially in low coronary flow conditions (data not shown).

2.1.2. Experimental protocol

A dose-response to glucose was obtained at a range of coronary flows. Hearts perfused with one of four different glucose concentrations (2.75, 5.5, 11 and 22 mM) were subjected to 15 min aerobic perfusion, followed by 30 min global ischemia with low coronary flows of 0.1, 0.2 or 0.5 ml/g wet wt/min (n=6/group). An infusion pump was used to deliver oxygenated buffer during low-flow ischemia. The temperature was maintained at 37°C by water jacketing, and monitored by a thermistor probe in the right ventricle. Coronary effluents were collected for 15 s after 15 min perfusion, and over each 5-min period during ischemia. Two hundred microlitre samples were taken for assessment of glucose uptake.

In a second series of experiments, hearts were perfused with 11 mM glucose with or without insulin (1 U/l), and subjected to 30 min of global ischemia with no flow, or a low flow of 0.2 ml/g wet wt/min. Two additional groups of hearts (n=6/group) (with or without insulin) were preconditioned with 5-min ischemia and 5-min reperfusion prior to low-flow ischemia. Glucose uptake and lactate washout were measured during low flow ischemia.

Hearts were clamped at the onset, after 15-min and after 30-min ischemia in the glucose and glucose+insulin groups with Wollenberger tongs kept in liquid nitrogen, for analysis of tissue metabolites. 6 hearts were used for each time point. Crossover analysis of the glycolytic intermediates was then performed.

2.1.3. Biochemistry

Glucose uptake was assessed by the rate of conversion of glucose 6-phosphate to fructose 6-phosphate, measured by the rate of 3H2O production from 3H2-glucose [1,3]. A trace amount (0.087 μM; 0.2 mCi/l) of 3H2-glucose (Amersham, Amersham, Bucks, UK) was added to the perfusate. The 3H2O was separated from 3H2-glucose by Dowex columns (Dowex 1X8-200, chloride form, Sigma, St Louis MO), and the 3H2O counted in 10-ml scintillation cocktail (Ready-Safe, Beckman Instruments, Fullerton CA).

Ventricular tissue was freeze-dried, and metabolites extracted with perchloric acid [13] for determination of high-energy phosphates and glycolytic intermediates, or with ethanol and NaOH for analysis of glycogen content [14]. The extracts were then assayed using spectrophotometric assays adapted for use on a Cobas Fara centrifugal analyser (Roche Diagnostics, Berne, Switzerland) [13]. Values were expressed as μmol/g wet wt. Glycogen was expressed as μmol 6-carbon units/g wet wt, after assaying the extract for glucose.

2.2. Expression of results and curve fitting

Results were expressed as mean±SEM. All data were expressed as per gram wet weight (wet weight=5×dry weight from previous determinations). The data were fitted using Origin (MicroCal, Northampton, MA) computer programme.

3. Results

3.1. Glucose uptake at different coronary flows and glucose concentrations

3.1.1. Changes in glucose uptake in control and ischemic hearts

Preischemic glucose uptake rates were dependent on the perfusate glucose concentration, as shown in Figs. 1 and 2. At each low coronary flow, glucose uptake fell sharply from control levels, and then increased to peak at 15–20
3.1.2. Steady-state glucose uptake—effect of concentration and coronary flow

The steady-state glucose uptake per min, taken as the uptake at 15-min ischemia, was plotted against glucose concentration for each coronary flow. Double rectangular hyperbolic relationships were obtained (Fig. 2), equivalent to Michaelis–Menten kinetics, such that glucose uptake increased linearly from 0–5 mM, and plateaued at concentrations greater than 11 mM. Similar trends were observed at coronary flows of 0.2 and 0.5 ml/g wet wt/min, as well as with control coronary flows (Fig. 2), although the curves were shifted upwards, and to the left with the higher coronary flows.

The right shift in $K_m$ for glucose uptake at low coronary flows (Fig. 2) suggests that, in ischemia, a higher min ischemia (see Fig. 1). With 2.75 mM glucose, glucose uptake declined after 15 min ischemia at each low coronary flow, such that by 30 min ischemia, the rates of glucose uptake were similar regardless of coronary flow (cf. Fig. 1a, b, c). With higher glucose concentrations, glucose uptake was maintained at a plateau level throughout ischemia at each coronary flow rate (Fig. 1). With a coronary flow of 0.5 ml/g wet wt/min, rates of glucose uptake with 11 and 22 mM glucose were similar to those in preischemic hearts (Fig. 1c).
mum glucose concentration, of at least 11 mM glucose, is required to sustain glucose uptake than is necessary in normoxia. The increased transit time of the perfusate through the coronary vasculature with a reduced coronary flow may lead to early extraction of the available glucose, in the initial portion of the perfused tissue. The concentration gradient across the cell membrane at the terminal portion of the vasculature with the lower glucose concentrations would then be insufficient to sustain facilitated diffusion. This is in agreement with standard concepts of membrane transport kinetics.

An additional explanation for the difference in glucose uptake observed at low flows and low glucose concentrations is inhomogeneity of flow in the tissue. Some portions of the hearts will be subjected to a much higher flow than others, especially as ischemia progresses. The steepness of the perfusion gradient will vary depending on the residual flow rate, the glucose concentration and glucose uptake (determinants of viability and thus of vessel compliance), but may result in the same mean glucose uptake as a heart with a lesser gradient. However, within limits, we believe that we can draw certain conclusions from the data. This issue is discussed further in Section 4.6.

Rates of glucose uptake neared saturation at glucose concentrations greater than 11 mM. Only an increase in coronary flow could increase absolute glucose uptake at high glucose concentrations. Thus the maximum rate of glucose uptake (apparent $V_{max}$) was dependent on coronary flow (Fig. 2). This finding can be explained by improved metabolism within the cell and thus increased glucose demand, as well as increased delivery.

### 3.2. Glucose uptake at a glucose concentration of 11 mM

#### 3.2.1. Absolute rates of glucose uptake

The standard concentration of glucose used in isolated rat heart perfusions in the absence of insulin is 11 mM. This concentration is twice the physiological concentration of glucose in plasma and ensures adequate glucose uptake in the absence of insulin and alternate substrates. We plotted the results for 11 mM glucose as a function of coronary flow (Fig. 3a).

The values at higher coronary flows are open to interpretation, with modulation by oxygen consumption, work rate, insulin and alternate substrate availability. Glucose uptake is not determined by coronary flow under these conditions. At low coronary flows, the dependence of glucose uptake on coronary flow increased (Fig. 3a), with a fall in glucose uptake.

Absolute glucose uptake is frequently reported in the literature. However, the uptake of glucose relative to its availability to the tissue has not been established for isolated rat hearts. In order to establish the relationship between glucose uptake and delivery of glucose, where:

$$ \text{delivery (}\mu\text{mol/min/g wet wt)} = \text{glucose concentration (}\mu\text{mol/ml}) \times \text{coronary flow (ml/g wet wt/min)} \quad \text{(1)} $$

(glucose concentration of the perfusate), the term percentage glucose extraction was derived,

$$ \% \text{Glucose extraction} = \frac{\text{glucose uptake (}\mu\text{mol/min/g wet wt)}/\text{delivery}}{(\mu\text{mol/min/g wet wt})} \times 100 \quad \text{(2)} $$

When percentage extraction of each data point in Fig. 3a was plotted against coronary flow, a negative double-exponential relationship was found (shown in Fig. 3b), described by:

$$ \% \text{extraction} = 6.81e^{-(x/31.78)} + 14.91e^{-(x/10.38)} \quad \text{(3)} $$

with $x = \text{coronary flow (ml/g wet wt/min)}$ and $\chi^2 = 14.91$. At the 'normal' range of coronary flows for an isolated perfused heart (12–16 ml/g wet wt/min), % extraction was very low, less than 3% of that delivered to the myocardium, even though glucose was the main external substrate. There was a sharp increase in glucose uptake at coronary flows less than about 1 ml/g wet wt/min, from less than 3% up to 25–28% at very low flows.

When the percentages of glucose extraction for the different glucose concentrations (from Fig. 2) were plotted against delivery of glucose (coronary flow $\times$ concentration) rather than coronary flow, to allow for comparison of the data, a similar negative exponential relationship was found (Fig. 3c), with a sharp increase in glucose extraction at reduced glucose delivery. The pattern is the same for the full range of glucose concentrations.

#### 3.3. The effects of insulin and preconditioning on glucose uptake and lactate washout

Data from hearts subjected to 30-min low flow ischemia (0.2 ml/g wet wt/min) with 11 mM glucose were compared to baseline values with zero flow ischemia, in the presence or absence of insulin (1 U/l). Insulin increased preischemic glycogen synthesis (21.40 ± 1.08 vs 16.27 ± 0.44 μmol/g wet wt; $p < 0.05$; Table 1) as well as ischemic glucose uptake (at 15 min 0.8 ± 0.1 vs 0.5 ± 0.1 μmol/min; $p < 0.05$; Fig. 4a). Lactate production was increased in insulin-treated hearts (Fig. 4b), although lesser tissue accumulation was found in the low flow hearts because of washout (Table 1). Total lactate production (tissue level + washout) was unchanged in low flow vs zero flow hearts (26.65 μmol vs 27.04 μmol).

Preconditioned hearts showed an increased glucose uptake compared to control hearts with only 11 mM glucose (Fig. 4a). However, in the presence of insulin, preconditioning had no additional effect (Fig. 4a). Preconditioned hearts had a lower lactate production (Fig. 4b) because of reduced preischemic glycogen content [15].
Fig. 3. (a) Glucose uptake versus coronary flow in isolated rat hearts perfused with buffer containing 11 mM glucose. The data is fitted separately for low flows (0.1–0.5 ml/g wet wt/min $y = 1.79x + 0.13; R = 0.99$) and high flows ($y = -0.01x + 1.28; R = 0.55$). Only at low flows is there a significant relationship between coronary flow and glucose uptake. At coronary flows greater than 12 ml/g wet wt/min, factors other than coronary flow determine glucose uptake, and these values may vary widely (see Section 3.2.1). (b) Individual data points from Fig. 3 (a) expressed as a percentage of glucose delivery i.e. extraction (delivery = flow $\times$ concentration), with a glucose concentration of 11 mM, versus coronary flow. The data were fitted with a double-negative exponential equation, where $y = 30.87 e^{-14.91x} + 6.81 e^{-14.91}$ with $\chi^2 = 14.91$. At flows below 1 ml/g wet wt/min, the percentage extraction increases sharply. (c) Steady-state glucose uptake at 15 min ischemia expressed as a function of delivery of glucose to allow for different glucose concentrations and coronary flows. The percentage extraction for each point was then calculated, and plotted against delivery. The data points from Fig. 3 (b) (11 mM glucose) were also replotted as a function of delivery. The points were then fitted with a negative exponential relationship, described by $y = 20.15 e^{-14.58x} + 4.23 e^{-14.58x}$. $\chi^2 = 14.58$. 
3.4. Tissue metabolites

Tissue levels of glycolytic intermediates, glycogen and high-energy phosphates, are shown in Table 1. Insulin-enhanced glucose uptake and glycogen content, thereby limiting the effect of reduced substrate delivery on glycolytic flux rates in ischemia and allowing regulatory sites further downstream to be indicated.

Glycogen is an important contributor to glycolytic flux. The majority of glycogen is broken down in the initial minutes of ischemia (12.4 μmol/g wet wt in the first 15 min of glucose zero flow), and does not really contribute to glycolytic flux after this time (2.5 μmol/g wet wt utilised in last 15 min). The small amount of residual glycogen may well be proglycogen, which is more resistant to intermediate accumulation. Thus, product formation greatly outweighs any intermediate accumulation, and negates significant inhibition at any point along the pathway.

Analysis of intermediate accumulation by the crossover theorem [1,18] highlighted the accumulation of G6P, F6P and end products, lactate and α-glycerophosphate (Fig. 5). Contrary to previous authors, who reported increases of 700% and 300% in DHAP and GAP respectively [1], we found no significant accumulation of these glycolytic intermediates. DHAP levels in our hearts in fact decreased significantly during the more severe ischemia used in the present study (Table 1). Levels of intermediates tended to be higher at 15 min than at 30 min in zero-flow ischemia (Table 1: Fig. 5), indicating that glycolysis was more limited after 30 min, with all available intermediates used up to provide ATP in the absence of substrate. With a maintained residual coronary flow, the fall in glycolytic intermediates was not observed. Glycolysis was maintained at a steady state throughout the 30 min ischemic period, as suggested both by glucose uptake and metabolite accumulation.

The glycolytic intermediates downstream from glucose can be broadly grouped into intermediates (G6P, F6P, FDP, GAP, DHAP, pyruvate) and end-products (L-alanine, α-glycerophosphate, lactate). The percentage of intermediates to intermediates + products declined during ischemia, as the product accumulation greatly outweighed that of the intermediates (Table 1). In addition, with a residual low coronary flow, a large amount of the products, especially lactate, will be washed out, further reducing the relative intermediate accumulation. Thus, product formation greatly outweighs any intermediate accumulation, and negates significant inhibition at any point along the pathway.

αGP levels increased significantly during ischemia, indicative of a redirection of DHAP to αGP, instead of to GAP and eventually lactate. However, the amount of αGP was significantly less than that of lactate. While this redirection may indicate some inhibition at GAPDH, the αGP shuttle is important in restoring NAD+ levels, as is the reduction of pyruvate to lactate. An accumulation of αGP and restoration of the redox potential may thus be a protective mechanism, although this is still to be confirmed.

In the presence of insulin, G6P and F6P levels increased greatly during ischemia (Table 1, Fig. 5), as a result of increased glycogen and glucose uptake. Levels of FDP, GAP and DHAP were lower after 30 min compared to the 15-min values in insulin hearts, despite high G6P and F6P values, possibly indicating some enzyme inhibition (at
PFK) with excess glycolytic substrate in the presence of insulin. A ‘bottle-neck’ effect was seen, which was less significant with a residual flow as G6P and F6P were lower with attenuated glycogenolysis in the presence of glucose, and metabolites which could inhibit PFK (i.e. lactate, H\(^+\)) would be washed out. Lactate accumulation was, however, significantly higher by the end of ischemia in insulin hearts, despite any constriction. The percentage intermediate to total metabolites was higher in all insulin vs. noninsulin hearts, although there was still little evidence of significant GAPDH inhibition. αGP tissue accumulation was greater in insulin hearts, showing more redirection when substrate was increased.

4. Discussion

We observed that the extraction of glucose from the perfusate is consistently increased as the coronary flow is reduced, even to very low values. We found an increased removal of glucose from the perfusate, even though the absolute glucose uptake was decreased in severe ischemia.
although there may be downstream regulation at sites "poured" into it. The amount of substrate entering the colysis in this model is related largely to substrate supply, is controlled largely by the amount of glucose which is very low coronary flows (Fig. 6). Thus, control of glycolysis can be seen conceptually as a "funnel", the efflux from which, where it becomes the substrate [26]. Thus glycolysis can be pictured as constrictions on the funnel spout, leading to some accumulation of "fluid" in the funnel, but these are not sufficient to prevent the final outflow. Thus there is some regulation of flux by enzyme inhibition distributed along the pathway, but these individual points do not appear to be significantly rate-limiting. While PFK may have been inhibited when substrate delivery was in excess, even when PFK was maximally inhibited (insulin and low-flow ischemia) the overall rate of glycolysis was still very high (estimated by lactate accumulation). These concepts rely on the distinction between control and regulation of a pathway, where control is defined as a change in the level of a control factor exerting a change in glucose detritiation. This is a gross oversimplification, but within the limitations of the technique, we feel that this assumption is justified (see Section 4.6).

Because accumulation of glycolytic intermediates was small compared to rates of substrate flux through glycolysis, assessed through end-product accumulation (Table 1), we made the assumption that the glucose uptake measured by [2-\textsuperscript{3}H]-glucose detritiation approximates glycolytic flux. This approximation also relies on the assumption that all glucose taken up is phosphorylated (see Section 4.6). Thus, for the purposes of the present discussion, glucose entering the cell and subsequently phosphorylated was assumed to be largely converted to pyruvate, and then to lactate under anaerobic conditions. Any possible intracellular accumulation of glucose is unlikely to alter the results significantly, given that we are measuring the rate of the initial steps of glucose utilisation. In addition, in ischemia any glucose in the cell is likely to be used up rapidly, as the requirement for ATP increases, and glycerol synthesis is unlikely. Glycogenolysis contributes substrate to the glycolytic pathway, the extent of which was estimated from gross glycogen content in the hearts prior to and at the end of ischemia. Previous calculations, using data from hearts perfused with a coronary flow of 0.2 ml/g wet wt/min, and 11 mM glucose [12], show that glucose uptake and glycogen utilisation can account for all the lactate produced, which conversely implies that all the glucose taken up in these conditions is converted to lactate [12]. Residual oxygenation can oxidise only about 3–4% of glycolytic substrate under these conditions [12].

4.2. Control vs. regulation of glycolysis

The above assumptions invoke the concept of glycolysis as a "metabolon", or single unit made up of closely associated multiple enzymes [25], which allows efficient "channelling" of the product of one reaction to the next, where it becomes the substrate [26]. Thus glycolysis can be seen conceptually as a "funnel", the efflux from which, is controlled largely by the amount of glucose which is "poured" into it. The amount of substrate entering the pathway can largely be accounted for by the amount that eventually leaves [12] (see Fig. 6). Enzyme inhibition can be pictured as constrictions on the funnel spout, leading to some accumulation of "fluid" in the funnel, but these are not sufficient to prevent the final outflow. Thus there is some regulation of flux by enzyme inhibition distributed along the pathway, but these individual points do not appear to be significantly rate-limiting. While PFK may have been inhibited when substrate delivery was in excess, even when PFK was maximally inhibited (insulin and low-flow ischemia) the overall rate of glycolysis was still very high (estimated by lactate accumulation). These concepts rely on the distinction between control and regulation of a pathway, where control is defined as a change in the level of a control factor exerting a change in
Fig. 6. Possible sites of control and regulation of glycolysis. (1) Glucose delivery is decreased in ischemia as the coronary flow falls, resulting in reduced absolute glucose uptake. A reduction in coronary flow below 1–2 ml/g wet wt/min appears to stimulate glucose transport, resulting in increased extraction of glucose from that delivered to the cells. Glucose uptake and phosphorylation are major controlling steps of glycolysis, even when these factors are significantly reduced as in ischemia. (2) Glycogen is rapidly depleted during ischemia (most is utilised within the first 15 min, Table 1). With total global ischemia, or when glucose is present only at very low concentrations, glycogen is the only significant glycolytic substrate, and the tissue content at the onset of ischemia determines subsequent glycolytic rates. (3) Phosphofructokinase (PFK) is thought to be the major site of glycolytic inhibition under normal conditions when ATP and CP levels are sufficient. Citrate and acidic pH are the major inhibitors of the enzyme [31,32]. When ATP and CP levels are depleted, inhibition of PFK is removed allowing glycolysis to continue. Glycolysis is stimulated at this step by fructose 2,6-bisphosphate [41]. However, recent isolated myocyte work and metabolic control analysis shows that in normoxia with sufficient substrate (glucose and insulin), glucose uptake and phosphorylation are the main rate-determining factors of glycolytic flux [23,24], rather than PFK. In ischemia, the site of glycolytic regulation was thought to be GAPDH rather than PFK [1]. However, if high glucose and insulin are present, regulation of glycolysis at the level of PFK, possibly due to the drop in pH [31,32] may become of greater importance. A large accumulation of G6P and F6P was found in ischemia, especially in insulin hearts (see Table 1 Fig. 5), suggesting that with sufficient or excess substrate, some feedback is present to prevent too excessive an accumulation of end product. (4) GAPDH catalyses the conversion of GAP to 2,3-bisphosphoglycerate, with the reduction of NAD\(^+\) to NADH\(^+\)\(_2\). In ischemia, as lactate accumulates because of increased production and reduced washout, balancing the lactate dehydrogenase equilibrium should theoretically lead to an accumulation of NADH and H\(^+\). Both NADH and lactate are then thought to inhibit glycolysis at GAPDH [1,2]. However, the importance of this mechanism in the overall regulation of glycolysis in ischemia is challenged by the findings that: (1) glycolysis in ischemia is limited by supply of substrate, in accordance with normal substrate–product relationships [22] (Fig. 2); (2) at low coronary flows, glucose extraction is increased (Fig. 3b); (3) hypoxia, associated with greatly increased glycolysis, is also associated with a high cytosolic NADH content; (4) regulation of glycolysis in control conditions with excess substrate is limited to less than 25% at all steps below phosphoglucoisomerase [23]; and (5) crossover analysis of glycolytic intermediates does not point to inhibition at the level of GAPDH (Table 1 Fig. 5). In addition, with high glucose at low coronary flows, lactate washout remains at a steady state for an extended period, without any evidence of attenuation of glycolysis by feedback inhibition [12]. Tissue lactate levels were also increased if insulin was present (Table 1). While the role of lactate-mediated inhibition of glycolysis cannot be excluded, its importance appears relatively small. Only if excess external lactate is added [40], may GAPDH inhibition significantly attenuate the rate of glycolysis, although this mechanism has not been shown directly. The process illustrated in the figure can be regarded as a ‘funnel’. Hypothetically, in ischemia, if glucose is taken into a cell and phosphorylated, its eventual fate must be lactate if the cell is still viable. Some constriction may be present to slow down the rate, but this does not affect the eventual outcome.

Overall flux (e.g. workload, hormones, substrate concentration, coronary flow etc.) while regulation occurs at points within the pathway which modulate the flux at that point (e.g. enzyme activity, cofactors etc) [27]. Our results call for metabolic control analysis to determine the sites of regulation of glycolysis in ischemia, as has been performed...
on glycolysis in normoxic hearts perfused with different substrates [23].

4.3. Glucose uptake and extraction versus coronary flow in the isolated rat heart

Glucose uptake and extraction relative to acute changes in coronary flow were investigated using 11 mM glucose (with no insulin), as is used in the majority of isolated rat heart perfusions. Coronary flow, oxygen consumption and contractile function are tightly coupled, to regulate heart function, and determine viability. At 'normal' coronary flows in the isolated rat heart (12–16 ml/g wet wt/min), glucose uptake is also modified, by the perfusion model used, the heart and work rates, coronary perfusion pressure, oxygen availability, alternate substrates and metabolic status of the heart (diabetic, hypertrophied, starved) [11,28]. Rates of glucose uptake are therefore not closely dependent on coronary flow at 'control' coronary flows. However, despite variations in absolute glucose uptake with different models, the percentage extraction of available glucose is very low at these flows (Fig. 3b). At the moderate reductions in coronary flows used by other authors (20–60% of normal coronary flows with crystallloid perfusions), mean glucose uptake values may also vary, but tend to be higher than those at higher flows [1,4]. This increase can be attributed to reversal of the Pasteur effect, with a relative hypoxia, and a fall in citrate and ATP levels.

Glucose uptake, and glycolytic ATP production, is an important determinant of viability, especially when the heart is compromised by a reduced flow [11,12,29]. The fate of glucose changes at a lower flow (6 ml/min vs 14 ml/min [30], as the ratio of anaerobic to aerobic glucose utilisation increases with reduced oxygen availability [30]. With more severe reductions in coronary flow, as used in our study, mechanical function is severely curtailed, or absent, with evidence of ischemic injury [12]. At these coronary flows, anaerobic glycolysis is the primary source of ATP [11]. Previously, glycolysis was thought to be inhibited in severe ischemia because glucose utilisation in an isolated rat heart at a coronary flow of 0.6 ml/g wet wt/min was less than that in the normally perfused myocardium [4]. However, as coronary flow decreases below about 1 ml/g wet wt/min in our model ('ischemia'), the percentage extraction rises sharply (Fig. 3b), suggesting that the capacity of tissue to take up glucose is enhanced. Further, glycolytic flux appears to be limited largely by the availability of glucose. A similar prediction was made with computer modelling at low coronary flows [21] i.e. that reduced glucose uptake (and glycolysis) results primarily from reduced delivery of substrate [21].

We have confirmed these findings with ultra-low coronary flows and a range of glucose concentrations in an isolated heart.

We did not find evidence of major accumulation of GAPDH during ischemia, suggesting that any inhibition at the level of GAPDH did not have a major limiting effect on flux through glycolysis [1,3–5] (see Fig. 6). Evidence of some inhibition at PFK [31] could be found, especially when the substrate supply was greatly increased by the addition of insulin (Table 1). In these conditions, the drop in pH may be greater from increased glycogen breakdown [12], such that acidic inhibition of PFK is also greater [32]. However, this inhibition did not significantly limit the overall rate of glycolytic flux. The metabolic response of the tissue to excess substrate adds to the concept of glycolysis as a 'funnel' (Fig. 6); at some point the amount of substrate entering the pathway may transiently exceed that which can exit. However, eventually output will be increased because of a greater accumulated input. Delivery of glucose (or availability of glycolytic substrate) and enzyme inhibition thus compete to determine the final rate of glucose uptake and subsequent utilisation.

4.4. Does this relationship occur in vivo?

In ischemia following coronary artery ligation, coronary flows in the subendocardium in large animals are usually in the range of 0.07–0.15 ml/g wet wt/min [8] i.e., about 3–8% of normal coronary flows (1–2 ml/g wet wt/min) [8]. Thus the coronary flows used in the present study (0.1–0.5 ml/g wet wt/min, about 2–8% of normal in vivo coronary flows in the rat—5–6 ml/g wet wt/min [33]) are more ‘physiological’ than flows of 0.6 ml/g wet wt/min and higher as used in previous rat heart studies [1,3–5]. In the pig heart in vivo, there is no change in absolute glucose uptake as the coronary flow falls to 0.07–0.1 ml/g wet wt/min but the same negative logarithmic relationship between glucose extraction and coronary flow exists [8] as found in the rat heart. A similar increase in extraction relative to coronary flow occurs in the dog heart with low-flow ischemia induced by LAD ligation [34]. Thus in the rat, pig [8], dog [10] and human heart [7], glucose extraction is increased with impaired coronary flow.

4.5. Possible mechanism of increased glucose extraction

A component of delivery of glucose is the ability of the membrane to transport glucose, which is determined by the number of glucose transporters in the membrane. Insulin significantly enhances glucose uptake by increasing the density of GLUT4 glucose transporters in the membrane, and can thus increase delivery of substrate to the cytosol. Insulin shifts the glucose uptake versus glucose concentration curve significantly upwards (increased apparent \( V_{\text{max}} \)), indicating that membrane transport becomes rate-limiting when sufficient substrate is available, supporting the concept that the initial steps of the glycolytic path are of major importance in the regulation of glycolysis. The mechanism of increased glucose extraction in acute ischemia may also be increased porosity of the sarcolemma...
mediated by translocation of glucose transporters (GLUT 1 or GLUT 4) to the membrane, shown to be triggered by ischemia [9,10]. Insulin can also further increase glucose uptake in ischemia, as we have shown, overcoming the limitation of glucose transporter density in the membrane as a determinant of glucose delivery. In the absence of insulin, only 25–30% of available glucose is taken up, leaving a large ‘reserve’ of glucose. Insulin increases this extraction to about 60–70% leaving a small ‘reserve’, at which point the gradient of glucose across the membrane limits uptake ($K_m$ of GLUT4=5 mM [35]). Preconditioning has also been shown to induce translocation of GLUT4 to the membrane, thereby increasing membrane affinity for glucose. We found increased glucose uptake in preconditioned hearts subjected to low-flow ischemia. The lack of effect of insulin in these hearts suggests a similar mechanism of action on GLUT4 translocation to the membrane. However, glucose uptake is not necessarily related to the increased recovery of function in preconditioned hearts [36].

Membrane transporter activity may also be upregulated by factors associated with ischemia including formation of adenosine [37], increased cytosolic Ca$^{2+}$ [38] or tissue cyclic AMP [38]. In addition, contraction-mediated glucose transport differs from insulin-mediated transport, indicating the involvement of different pathways in the recruitment of glucose transporters. Further work is required to confirm these hypotheses. Chronic adaptation to ischemia may also involve changes in GLUT expression and distribution.

4.6. Reservations

1. We measured the amount of $\delta$[2-$^3$H]-glucose detritiated in the phosphoglucoisomerase reaction (G6P to F6P), equated to glucose uptake. These data do not differ significantly from measurements of arteriovenous glucose differences (data not shown), although the radioactive methods are more precise. $\delta$[2-$^3$H]-glucose detritiation is regarded as the ‘gold standard’ for measuring glucose uptake, and has also been used to reflect glycolytic flux rates [11,12]. Although use of $\delta$[5-$^3$H] glucose may be a more accurate measure of glycolysis because it is detritiated further down the glycolytic pathway, at enolase, we found no difference between results from the two isotopes in low-flow ischemia (data not shown). This result also implies that the majority of glucose taken up in ischemia is converted to pyruvate.

2. $\delta$[2-$^3$H]-glucose detritiation does not distinguish between rates of glucose transport across the sarcolemma and subsequent phosphorylation. Rates of phosphorylation may be affected by ATP availability, which is decreased in ischemia. This effect needs to be investigated.

3. Alternative fates of glucose, including glycogen and intermediate accumulation, are not considered in the overall analysis of glucose uptake/glycolysis, for reasons as follows. Only about 5% of glucose taken up is incorporated into glycogen in the normally beating heart in the absence of insulin [39], but this value is presumably even lower in the ischemic heart where ATP formation is required, rather than energy storage. An accumulation of intermediates may lead to a disparity between values for glucose uptake and glycolytic flux (lactate production). However, the sum of glycolytic intermediates, other than the products of lactate, $\alpha$GP and L-alanine, does not account for more than 7.5% of the total metabolite accumulation, a value which decreases during ischemia (see Table 1, and from [1]). While $\alpha$GP accumulates to quite a large extent, the values are still small compared to both total glycolytic substrate, and overall lactate production. Thus these factors do not significantly affect the comparison of glucose uptake measured by $\delta$[2-$^3$H]-glucose with glycolytic flux.

4. An important reservation of our model is that at very low coronary flows, the distribution of coronary flow in the rat heart is likely to be heterogeneous, with consequently differing degrees of cell metabolism and viability. However, a similar restriction could apply to the studies in the previous studies of low flow ischemia in the rat heart [1,3–5,11,12,29,30,40] and investigations in the human. PET takes an average measurement over a volume of tissue, which is similar to glucose extraction by the whole isolated rat heart exposed to global decreases in coronary flow. However, despite the lack of cellular homogeneity in the response of the isolated rat heart to ischemia, the finding of an increased glucose extraction is supported by large animal data [8,10,19], and we believe this to be an important observation.

5. Summary

The rate of delivery of substrate is a major determinant of absolute glucose uptake at very low coronary flows. While absolute glucose uptake remains the same (in vivo), or falls as the coronary flow falls, the percentage extraction increases greatly, indicating an ability of the myocardium to upregulate its capacity to transport glucose and thereby provide ATP essential for maintained cell function. Similar conclusions have been reached by others using different methods of analysis [8,21,29]. A low coronary flow (<1 ml/g wet wt/min in the rat heart <10% of normal) thus appears to trigger an adaptive response in the myocardium, possibly involving translocation of glucose transporters to the membrane.

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