Preferential Oxidation of Glycogen in Isolated Working Rat Heart

Gary W. Goodwin, Faisal Ahmad, and Heinrich Taegtmeyer
The University of Texas Houston Medical School, Department of Internal Medicine, Division of Cardiology, 6431 Fannin, Houston, Texas 77030

Abstract

We tested the hypothesis that glycogen is preferentially oxidized in isolated working rat heart. This was accomplished by measuring the proportion of glycolytic flux (oxidation plus lactate production) specifically from glycogen which is metabolized to lactate, and comparing it to the same proportion determined concurrently from exogenous glucose during stimulation with epinephrine. After prelabeling of glycogen with either ^{14}C or ^{3}H, a dual isotope technique was used to simultaneously trace the disposition of glycogen and exogenous glucose between oxidative and non-oxidative pathways. Immediately after the addition of epinephrine (1 μM), 40–50% of flux from glucose was directed towards lactate. Glycogen, however, did not contribute to lactate, being almost entirely oxidized. Further, glycogen utilization responded promptly to the abrupt increase in contractile performance with epinephrine, during the lag in stimulation of utilization of exogenous glucose, suggesting that glycogen serves as substrate reservoir to buffer rapid increases in demand. Preferential oxidation of glycogen may serve to ensure efficient generation of ATP from a limited supply of endogenous substrate, or as a mechanism to limit lactate accumulation during rapid glycogenolysis. (*J. Clin. Invest.* 1996. 97:1409–1416.) Key words: isolated working heart • glycogen • glucose • glycolysis • epinephrine

Introduction

Our interest in the relative distribution of glycogen metabolism between oxidative and non-oxidative pathways in heart arose as a consequence of reports that glycogen does not contribute to lactate in vascular smooth muscle (1, 2). This tissue is characterized by high rates of conversion of exogenous glucose to lactate. In addition, we recently developed a dual isotope technique which allows us to simultaneously trace glucose and glycogen metabolism in the isolated working rat heart (3). Using this technique, we tested the hypothesis that glycogen is preferentially oxidized in heart.

In the present study, the experimental approach was to radiolabel the glycogen from either [U-^{14}C]glucose or [6-^{3}H]glucose. Apparent glycogenolysis could then be determined continuously from the washout of either $^{14}CO_2$ plus [^{14}C]lactate, or $^{3}H_2O$ plus [^{3}H]lactate, respectively. The rates of glycogenolysis determined by this method are apparent rates, because the glycogen is incompletely enriched. Metabolism of glucose was traced similarly and concurrently by including [U-^{14}C]glucose in the presence of [^{3}H]glycogen, or [6-^{3}H]glucose in the presence of [^{14}C]glycogen. Unlike glycogen, true rates of utilization of exogenous glucose are obtained, because the specific radioactivity of the glucose is known. Production of $^{3}H_2O$ from [6-^{3}H]glucose is a valid measure of glucose oxidation in the absence of recycling of lactate by gluconeogenesis (4, 5), as in the present case, and we have previously measured glucose oxidation by this method (6). The 6-tritiated isotopomer of glucose was chosen for the present study because, by analogy to [U-^{14}C]glucose, tritium is retained upon conversion to lactate and glycogen (7).

The predominant fates of glucose or glycogen in heart, aside from glycogen synthesis, are either oxidation or release as lactate. Therefore, glycolytic flux can be estimated from the sum of oxidation plus lactate production. We sought to determine lactate flux as a percentage of glycolytic flux (oxidation plus lactate) specifically from glucose or glycogen, because the percentage is not sensitive to uncertainty regarding the specific radioactivity of glycogen. We compared this percentage between glucose and glycogen in order to ascertain if there is preferential oxidation of glycogen in comparison to exogenous glucose.

Methods

Materials. D-[6-^{3}H]glucose was obtained from Amersham Corp. (Arlington Heights, IL). Other materials were obtained as previously described (3).

Heart perfusions. These were performed using the working heart apparatus (8) modified to a closed system for collection of $^{14}CO_2$, as described (3). Rats (300–350 gram chow-fed male Sprague-Dawley) were anesthetized with pentobarbital (100 mg/Kg). Heparin (100 U) was injected into the inferior vena cava, and the hearts were immediately excised and placed in ice cold perfusate. The aorta was cannulated and retrograde perfusion begun with warm (37°C) perfusate for five min to wash blood out of the heart and to cannulate the opening to the left atrium. Perfusions were then switched to a recirculating mode, which we considered time zero of the protocol. Coronary and aortic flows were determined at 5-min intervals, and power calculated from cardiac output and mean aortic pressure (9). The perfusion pressure (afterload) was 82 cm H_2O, and the atrial filling pressure was 15 cm H_2O. The perfusate consisted of Krebs-Henseleit buffer containing 1.25 mM $CaCl_2$ and was equilibrated with 95% O_2, 5% CO_2. Glucose (5 mM) was present during the initial retrograde perfusion and the remainder of the perfusion with the exception of the first 20 min.

The perfusion protocol is shown in Fig. 1 *A*. The first portion of the protocol (to 40 min) is designed to radiolabel the glycogen, and was adapted from a previous study (3), except that β-hydroxybutyrate was used instead of lactate to stimulate glycogen synthesis.

Address correspondence to Heinrich Taegtmeyer, M.D., Ph.D., University of Texas Houston Medical School, Department of Internal Medicine, Division of Cardiology, 6431 Fannin, Houston, TX 77030. Phone: 713-794-4123; FAX: 713-792-5187.

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Hearts were perfused for the first 20 min without substrate to deplete endogenous substrates. At this time the perfusate (200 ml) was supplemented with 5 mM glucose, 10 mM β-hydroxybutyrate, and 0.05 μCi/ml of either [U-^{14}C]glucose or [6-^{3}H]glucose by injecting 1 ml of 2 M Na-D,L-β-hydroxybutyrate, 1 M glucose, and 10 μCi of either [U-^{14}C]glucose or [6-^{3}H]glucose into the stirred reservoir at the bottom of the apparatus. Glycogen resynthesis was allowed to proceed for the following 20 min, then the perfusions were switched to a nonrecirculating mode supplied by fresh perfusate between 40 and 45 min. This was performed to remove radioactive glucose and β-hydroxybutyrate from the apparatus and the heart. The [U-^{14}C]glucose in the perfusate was reduced to less than 0.5% of the concentration present before the nonrecirculating interval. At 45 min, the apparatus was recharged with 200 ml of fresh perfusate containing either [6-^{3}H]glucose or [U-^{14}C]glucose (5 mM, 0.05 μCi/ml), whichever was not included to label glycogen, and the recirculating mode of perfusion reestablished. At 51 min of the protocol, 0.2 ml of 1 mM epinephrine bitartrate was injected into the stirred reservoir, yielding a final concentration of 1 μM. Samples of perfusate (2.5 ml) from arterial and venous sides of the heart were collected simultaneously at 46 min, and at 5-min intervals between 50 and 75 min of the protocol by way of gas tight ports located at the base of the oxygenator, and from within the heart perfusion chamber, respectively, the latter to collect the coronary effluent as it drips from the apex of the heart. Beating hearts were freeze-clamped on their cannulas at 75 min with aluminum tongs cooled in liquid N_2.

Preliminary perfusions were conducted to trace either exogenous glucose with both isotopes simultaneously, or glycogen with both isotopes simultaneously, so that the two isotopic measures could be compared within the same perfusions. Perfusions were subjected to the protocol of Fig. 1 except that both isotopes were added simultaneously in order to compare metabolic rates (oxidation and lactate production) obtained by two methods: $^{14}CO_2$ plus [^{14}C]lactate from [^{14}C]glycosyl compared to $^{3}H_2O$ plus [^{3}H]lactate from [^{3}H]glycosyl, where glycosyl refers to glycogen in one perfusion, and to exogenous glucose in a separate perfusion. The two isotopes gave similar rates of utilization of either glucose or glycogen; rates of oxidation plus lactate production obtained with ^{3}H averaged 128% of values obtained with ^{14}C when compared within the same perfusions.

Analytical procedures. The content of $^{14}CO_2$ and $^{3}H_2O$ in samples of perfusate were determined as described previously (3, 10). Radioactive lactate in the perfusate was determined by paper chromatography as described previously (6, 11). In a previous study, we used ion exchange chromatography to determine radioactive lactate (3). Ion exchange and paper chromatography gave comparable results, although lower background radioactivity was obtained by paper chromatography, which was therefore adopted for routine analysis. The sample (0.3 ml) was spiked with 5 μl of 1 M carrier Na-lactate then streaked onto Whatman 1 (7.5 cm high by 15 cm wide) and air dried before subjecting to chromatography using *n*-butanol:acetic acid:water (5:1:2) as solvent system. The radioactive glucose in the sample migrates as a streak with the NaCl with $R_f < 0.3$. The R_f of lactate is 0.7. The strip of paper between $R_f = 0.5$ and 0.9 was cut into small pieces and incubated overnight in three vials, each containing 3 ml of water to elute the lactic acid before adding 10 ml of scintillation mixture (Ultima Gold; Packard, Meriden, CT). A preliminary experiment was conducted to verify the quantitative determination of a known amount (10^5 dpm) of authentic [^{14}C]lactate in a sample of perfusate.

Hearts, stored at −70°C, were weighed, powdered under liquid N_2, and a portion of the tissue powder taken for dry weight determination. Glycogen was determined on the powdered tissue as glucose using a standard enzymatic assay (12) after digestion of the tissue in hot KOH, repeated (3×) ethanol precipitation, and digestion of glycogen to glucose with amyloglucosidase (13). A portion of the amyloglucosidase digest was taken for scintillation counting to determine net ^{3}H and ^{14}C incorporation. Values were corrected for background radioactivity. We previously found that correction for contamination of the purified glycogen with radioactive glucose from the perfusate is negligible (3). Quench correction and simultaneous determination of ^{3}H and ^{14}C by spectral index analysis were performed by routines supplied with the instrument (Packard 1900 TR).

Metabolic rates were determined from the product of the venous-arterial concentration difference and the coronary flow determined at the time of sample collection. Concentration differences of $^{14}CO_2$, $^{3}H_2O$, [^{3}H]lactate, and [^{14}C]lactate were determined from the venous-arterial difference in radioactivity (dpm/ml) of the metabolite, divided by the specific activity of the glucose (dpm/μmol of [U-^{14}C]glucose or [6-^{3}H]glucose, accordingly). Thus, in the case of the [^{14}C]glycogen group, the rates for Fig. 2 were calculated as follows (V is the sample taken from the coronary effluent, and A is taken from the bottom of the oxygenator):

$$\text{rate of glucose utilization (}\mu\text{mol/min per gram dry wt)} = \frac{(V-A)\ {}^{3}H_2O \text{ plus } [{}^{3}\text{H}] \text{ lactate (dpm/ml)} \times \text{coronary flow (ml/min)}}{\text{specific activity of } [6-{}^{3}\text{H}] \text{ glucose (dpm/}\mu\text{mol)} \times \text{heart dry weight (grams)}} \quad (1)$$

$$\text{lactate flux specifically from glucose (}\mu\text{mol/min per gram dry wt)} = \frac{(V-A)\ [{}^{3}\text{H}] \text{ lactate (dpm/ml)} \times \text{coronary flow (ml/min)}}{\text{specific activity of } [6-{}^{3}\text{H}] \text{ glucose (dpm/}\mu\text{mol)} \times \text{heart dry weight (grams)}} \quad (2)$$

$$\text{apparent rate of glycogenolysis (}\mu\text{mol/min per gram dry wt)} = \frac{(V-A)\ {}^{14}CO_2 \text{ plus } [{}^{14}\text{C}] \text{ lactate (dpm/ml)} \times \text{coronary flow (ml/min)}}{\text{specific activity of } [U-{}^{14}\text{C}] \text{ glucose (dpm/}\mu\text{mol)} \times \text{heart dry weight (grams)}} \quad (3)$$

$$\text{apparent lactate flux specifically from glycogen (}\mu\text{mol/min per gram dry wt)} = \frac{(V-A)\ [{}^{14}\text{C}] \text{ lactate (dpm/ml)} \times \text{coronary flow (ml/min)}}{\text{specific activity of } [U-{}^{14}\text{C}] \text{ glucose (dpm/}\mu\text{mol)} \times \text{heart dry weight (grams)}} \quad (4)$$

The percent conversion of glucose to lactate (See Fig. 3) is (Eq. 2/Eq. 1) ×100%, and the percent conversion of glycogen to lactate is (Eq. 4/Eq. 3) ×100%. In the case of the [^{3}H]glycogen group, values were calculated similarly, although ^{14}C was used to trace glucose, and ^{3}H used to trace glycogen. Since the specific radioactivity of glucose is used to calculate flux rates, all the values are expressed in terms of glycosyl units. Further, the rates from glycogen were calculated based on the specific radioactivity of the glucose used to prelabel the glycogen. Therefore, the rates from glycogen are apparent rates, and will underestimate the true rates of glycogenolysis to the extent that isotopic dilution by unlabeled glycogen will occur. Notice that the calculation of percent conversion to lactate is independent of the coronary flow, dry weight of the heart, as well as the specific activities of the glucose and of the glycogen, so long as there is sufficient release of radioactivity by the heart for reliable determination of the ratios of [^{3}H]lactate/($^{3}H_2O$ + [^{3}H]lactate), or of [^{14}C]lactate/($^{14}CO_2$ + [^{14}C]lactate). Data are expressed as mean±standard error of the mean (SE) for $n = 5$ perfusions in each of the two groups ([^{14}C]glycogen group, and [^{3}H]glycogen group, referring to hearts in which glycogen was labeled with ^{14}C and ^{3}H, respectively, between 20 and 40 min of the protocol). A third group of perfusions ($n = 5$), in which [U-^{14}C]glucose was used to label glycogen between 20 and 40 min, was freeze-clamped at 45 min, for determination of glycogen content and ^{14}C en-

richment of glycogen at the beginning of the study period. Statistical comparison relating the [^{14}C]glycogen group to the [^{3}H]glycogen group (metabolic rates and percent conversion to lactate) was by way of two-tailed Student's *t* test for unpaired data. Statistical comparison of percent conversion of glucose to lactate vs percent glycogen to lactate within either group was by way of two-tailed Student's *t* test for paired data. Contractile performance among the three groups were compared by analysis of variance. $P < 0.05$ was considered significant.

Results

As shown in Fig. 1, metabolic activity (Fig. 1 *A*) and contractile performance (Fig. 1 *B*) were measured simultaneously. Two groups of perfusions were subjected to the full protocol. They differ with respect to the order of addition of the two glucose tracers. These two groups were otherwise subjected to the same perfusion conditions and did not differ in contractile performance. The third group depicted in Fig. 1 *B* was freeze-clamped at 45 min of the protocol; it was included to determine the enrichment of glycogen at the beginning of the study period. In this group, glycogen was labeled from [U-^{14}C]glucose. Contractile performance in the third group tended to be lower, but there was no statistical difference in the value among the three groups.

The first portion of the protocol consisted of a period of glycogen depletion by perfusion without substrate for 20 min, followed by glycogen resynthesis from radioactive precursor. Prior glycogen depletion was included to increase the degree of enrichment of the glycogen. There was a decline in power during the first 20 min of the perfusions as a consequence of the omission of exogenous substrate. However, endogenous substrates (glycogen, and probably also triglycerides) were not completely depleted (see below) so the drop in power did not result simply from a lack of substrate. Contractile performance was restored upon introduction of exogenous substrates (glucose plus β-hydroxybutyrate) at 20 min. Perfusions were continued for an additional 20 min to allow the resynthesis of radiolabeled glycogen, then the hearts were subjected to a non-recirculating interval (40 to 45 min) with fresh perfusate to wash β-hydroxybutyrate and radioactive glucose from the apparatus and the heart. The recirculating perfusion was reestablished at 45 min with fresh perfusate containing, in one group of hearts, [6-^{3}H]glucose in the case ^{14}C was used to label glycogen. In the second group of perfusions, [U-^{14}C]glucose was included at 45 min in the presence of [^{3}H]glycogen. Epinephrine (1 μM) was added at 51 min of the protocol. As expected, epinephrine produced an immediate stimulation in contractile performance.

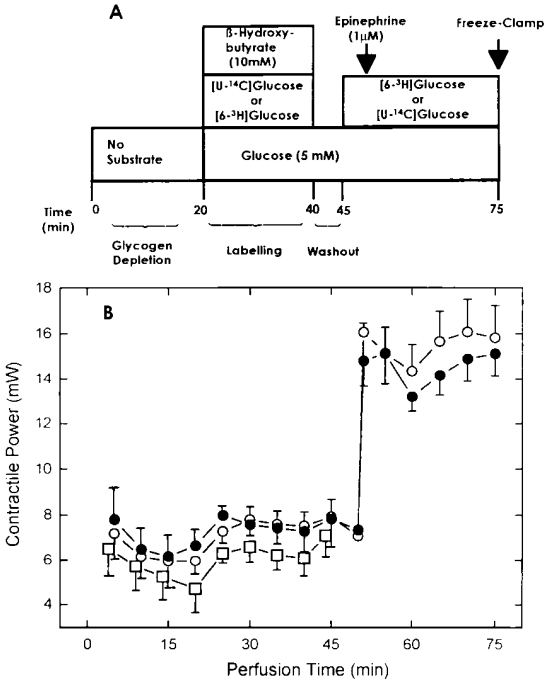

Figure 1. Perfusion protocol and contractile performance in the isolated working rat heart. The perfusion protocol (*A*) is lined up with the corresponding contractile performance (*B*). Values are the mean±SE in milliwatts ($n = 5$). The symbols are: (○), glycogen labeled with ^{14}C; (●), glycogen labeled with ^{3}H; (□), glycogen labeled with ^{14}C and freeze-clamped at 45 min of the protocol.

Hearts freeze-clamped at 45 min of the protocol contained 87.8±3.9 μmol/gram dry wt total glycogen, and 43.2±9.3 μmol/gram dry wt of [^{14}C]glycogen ($n = 5$) calculated from the ^{14}C content of the glycogen after extraction and purification, and the specific radioactivity of the [U-^{14}C]glucose. This value for the extent of radiolabeling of glycogen at 45 min of the protocol is an estimate of the amount available in the other groups for determination of subsequent glycogenolysis from label washout between 45 and 75 min. The percent enrichment is the content of radioactive glycogen as a percentage of total glycogen. This is equivalent to expressing the specific radioactivity of the purified glycogen as a percentage of the specific radioactivity of the glucose precursor. The value was 49.2±10.2% in the group freeze-clamped at 45 min. The difference between total glycogen and [^{14}C]glycogen, 44.6±9.4 μmol/gram dry wt, is an estimate of the content of nonradioactive glycogen extrapolated back to 20 min of the protocol, at the time when glycogen resynthesis was initiated. We previously found a value of 82±3 μmol/gram dry wt for rat heart in vivo (14). Therefore, in the present study, 37 μmol/gram dry wt was broken down during the first 20 min of perfusion as a consequence of omitting substrates. Hearts from fed rats otherwise synthesize glycogen during this period, if glucose is not omitted (14). These values for the balance of glycogen and the extent of glycogen enrichment achieved during the labeling period (0–45 min of the protocol) are comparable with the values we reported in a previous study using an analogous protocol (3). The ketone body, β-hydroxybutyrate, was included in the present study as an alternative energy source to divert radioactive glucose into glycogen (15). Lactate was used for this purpose in the previous study (3), and we reported 56±7% enrichment of the glycogen in that study. In the present study, β-hydroxybutyrate was used instead of lactate to insure that all of the lactate released by the hearts is of metabolic origin.

The glycogen content of the hearts at the end of the 75-min perfusions was very low as a consequence of stimulating glycogenolysis with epinephrine. The values were 8.5±0.8 μmol/gram dry weight in the [^{14}C]glycogen group, and 7.0±0.8 μmol/gram dry weight in the [^{3}H]glycogen group, averaging 7.8±0.6 μmol/gram dry weight ($n = 10$). By comparison, we previously reported that the glycogen content is 53±4 μmol/gram dry weight in hearts subjected to a comparable protocol but not stimulated with epinephrine (3). The amount of total glycogen (labeled and unlabeled) degraded between 45 and 75 min

amounted to 80 μmol/gram dry wt (87.8–7.8), or 91% degradation. In both the [^{14}C]- and [^{3}H]glycogen groups there was measurable isotopic enrichment of the glycogen both with respect to the glucose isotope included between 20 and 40 min to label the glycogen, and with respect to the isotope of glucose added at 45 min, during the period of glycogenolysis. In the [^{14}C]glycogen group, the values were 12±2% with respect to ^{14}C, and 17±2% with respect to ^{3}H. In the [^{3}H]glycogen group, the values were 24±2% for ^{14}C, and 8±1% for ^{3}H.

Fig. 2 shows the results from perfusions subjected to the protocol of Fig. 1. Metabolic rates of both the [^{3}H] and [^{14}C]glycogen groups are depicted. The two groups gave comparable rates. Flux rates obtained with the use of ^{14}C (from either glucose or glycogen) paralleled the flux rates obtained with the use of ^{3}H, although the values obtained from ^{14}C tended to be higher. The top panel of the figure shows apparent rates specifically from glycogen, and the bottom panel shows true rates specifically from exogenous glucose. The predominant fates of glucose or glycogen metabolism by heart are either oxidation or lactate production (of course glucose also goes to glycogen). Therefore, the upper curves of each panel provide an estimate of total utilization of the respective substrates. The lower curves of each panel show flux rates to lactate derived specifically from the respective substrates. The difference between the upper and lower curves in each case is an estimate of substrate oxidation, although in the case of glycogen, the values (both oxidative and nonoxidative rates) are an underestimate because only the radioactive portion is analyzed, and the glycogen is incompletely enriched.

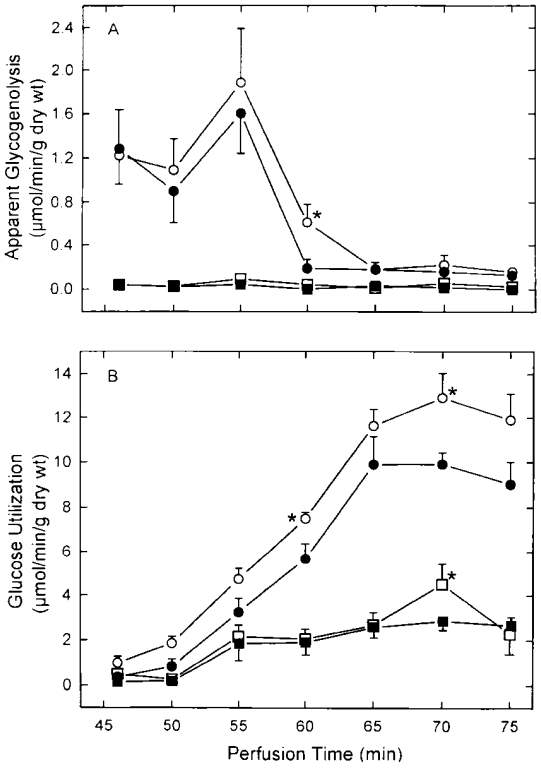

Figure 2. Rates of glucose utilization and apparent glycogenolysis in hearts subjected to the protocol of Fig. 1. *A* shows apparent flux rates from glycogen, and *B* shows flux rates from glucose. Open symbols refer to the [^{14}C]glycogen group, and closed symbols refer to the [^{3}H]glycogen group. Circles refer to substrate utilization as lactate plus oxidation, and squares refer to substrate conversion to lactate only. Thus, for the [^{14}C]glycogen group, *A* shows flux of [^{14}C]lactate (□) and of [^{14}C]lactate plus $^{14}CO_2$ (○) from [^{14}C]glycogen. *B* shows flux of [^{3}H]lactate (□) and of [^{3}H]lactate plus $^{3}H_2O$ (○) from exogenous [6-^{3}H]glucose in the same perfusions as in *A*. For the [^{3}H]glycogen group, *A* shows flux of [^{3}H]lactate (■) or [^{3}H]lactate plus $^{3}H_2O$ (●) from [^{3}H]glycogen. *B* shows flux of [^{14}C]lactate (■) and of [^{14}C]lactate plus $^{14}CO_2$ (●) from exogenous [U-^{14}C]glucose in the same perfusions as in *A*. Values are the mean±SE ($n = 5$). *$P < 0.05$ compared to the corresponding value for the [^{3}H]glycogen group.

Regardless of which isotope was used to trace glycogen (Fig. 2 *A*) epinephrine did not stimulate the release of lactate derived from glycogen. Lactate production from glycogen was not statistically different from zero either before or after addition of epinephrine. Stimulation of glycogenolysis by epinephrine was transient, probably because the glycogen became depleted.

The metabolic rates from exogenous glucose (Fig. 2 *B*), unlike glycogen, are true rates since the specific radioactivity of the glucose is known. Epinephrine induced a sustained, time dependent increase in glycolytic flux from exogenous glucose. In addition, regardless of which isotope was used to trace glucose, epinephrine stimulated lactate release from glucose. Therefore, unlike glycogen, glucose contributed to lactate efflux.

Fig. 3 presents lactate release derived specifically from glucose (top two curves) or glycogen (bottom two curves) as a percentage of the respective glycolytic rates of lactate plus oxidation, in the same perfusions as in Fig. 2. In other words, in the case of the [^{14}C]glycogen group, the top curve depicts the flux to [^{3}H]lactate as a percentage of the flux to [^{3}H]lactate plus $^{3}H_2O$ from exogenous glucose, and the bottom curve depicts the flux to [^{14}C]lactate as a percentage of the flux to [^{14}C]lactate plus $^{14}CO_2$ from glycogen. The calculations regarding the [^{3}H]glycogen group were performed similarly, but with

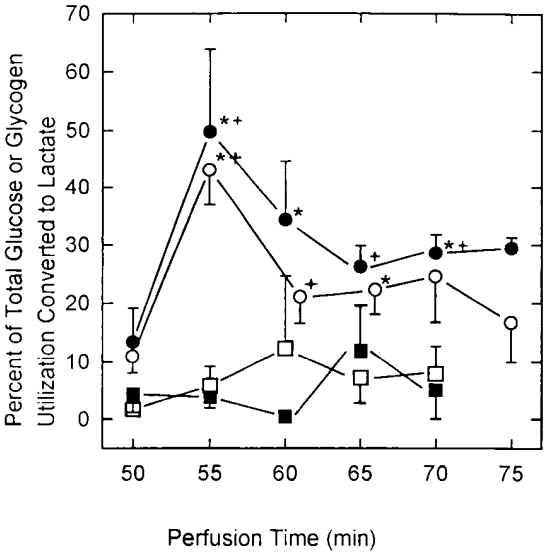

Figure 3. Percent of total glucose or glycogen utilization converted to lactate. Circles refer to lactate derived specifically from exogenous glucose. Squares refer to lactate derived specifically from glycogen. Open symbols refer to the [^{14}C]glycogen group, and closed symbols refer to the [^{3}H]glycogen group. Values are the mean±SE ($n = 5$). *$P < 0.05$ compared with the corresponding value from glycogen within the same group of perfusions. $^{+}P < 0.05$ compared to the same isotope of glycogen in the alternate group of perfusions.

isotopes in the reverse order. Both the [^{3}H] and [^{14}C]glycogen groups are depicted in the figure, and the two groups gave similar results (values not statistically different). The 46 min time point was excluded from this analysis because there was insufficient time for the system to equilibrate. The 75-min time point was excluded with respect to glycogen because of insufficient production of glycogen derived metabolites as a consequence of glycogen depletion. Immediately after stimulation with epinephrine, between 40 and 50% of glycolytic flux from glucose appeared as lactate, the remainder being oxidized. In contrast, there was minor relative conversion of glycogen to lactate, and the value remained low (< 12%) after epinephrine. The percentage of glycogenolysis (oxidation plus lactate) contributing to lactate flux was not statistically different from zero, either before or after addition of epinephrine. Therefore, glycogen was almost entirely oxidized. The difference between exogenous glucose and glycogen with respect to the relative conversion to lactate was most pronounced immediately after stimulation with epinephrine (55 min), although the difference persisted at later time points. In comparison to glucose, the values for percentage of glycogenolysis which contributed to lactate afflux were relatively constant throughout the perfusions, and averaged 6.8±3.3% for the [^{14}C]glycogen group, and 5.7±2.0% for the [^{3}H]glycogen group (n = 5 corresponding to the five time points between 50 and 70 min).

The comparison between exogenous glucose and glycogen presented in Fig. 3 was performed in two ways. In the first case, both were compared as pairs within the perfusions (see below). By the second approach, glucose was compared to glycogen between the two sets of perfusions in such a manner that the isotope of glucose used in one set was compared to the same isotope of glycogen used in the other set. This method does not require the assumption that ^{3}H release from [6-^{3}H]glucose or glycogen can be compared quantitatively, and can be interpreted the same as ^{14}C release from [U-^{14}C]glucose or glycogen. For example, immediately after stimulation with epinephrine (55 min) the relative conversion of glucose to [^{3}H]lactate in the [^{14}C]glycogen group (43±6%) is greater than the relative conversion of glycogen to [^{3}H]lactate in the [^{3}H]glycogen group (3.9±1.9%, $P < 0.05$ by unpaired t test). Similarly, the relative conversion of glucose to [^{14}C]lactate in the [^{3}H]glycogen group (50±14%) is greater than the relative conversion of glycogen to [^{14}C]lactate in the [^{14}C]glycogen group (5.9±3.3%, $P < 0.05$ by unpaired t test).

Now consider the pairwise comparison between glucose and glycogen within the perfusions. In this case, a different isotope was used to trace glucose as compared to the isotope used to trace glycogen. We became concerned that the different result from glucose compared to glycogen could result from different handling of the two isotopes in a manner not related to the relative amount of oxidative or nonoxidative pathways. Consequently, perfusions were performed both ways. In the case of the group in which glycogen is labeled with ^{14}C, the relative conversion of glucose to [^{3}H]lactate (43±6%) is greater than the relative conversion of glycogen to [^{14}C]lactate (5.9±3.3%, $P < 0.05$ by paired t test). Regarding the [^{3}H]glycogen group, the relative conversion of glucose to [^{14}C]lactate (50±14%) is greater than the relative conversion of glycogen to [^{3}H]lactate (3.9±1.9%, $P < 0.05$ by paired t test). Thus, the two isotopic methods yield comparable results regarding the relative distribution between oxidative and nonoxidative pathways with regard to both glucose and glycogen.

In other perfusions (not shown), we tested whether a substantial portion of glycolysis from glycogen could be made to contribute to lactate by inhibiting oxidative metabolism. Perfusions were subjected to the usual glycogen labeling protocol, then oxidative metabolism inhibited either by addition of 5 mM KCN at 50 min, or the heart subjected to total ischemia at 50 min and reperfusion at 60 min. It was found that both exogenous glucose and glycogen are converted almost entirely to lactate by addition of 5 mM KCN to block electron transport, confirming the previous report in vascular smooth muscle (2). Similarly, glycogen and glucose conversion to lactate were increased during 10 min of ischemia, by analysis of coronary effluent during early reperfusion (data not shown).

Discussion

The results depicted in Fig. 3 suggest that glycogen is preferentially oxidized in comparison with exogenous glucose. To demonstrate a preference, we have shown that glycogen does not contribute to lactate at the same time that exogenous glucose does by comparing the proportion of glucose converted to lactate relative to the proportion of glycogen to lactate. Epinephrine produced an expected increase in flux from exogenous glucose to lactate. The unexpected finding is that, despite enhanced glycogen oxidation, a corresponding increase in lactate flux from glycogen was not seen. Lactate production from glucose or glycogen was minor before stimulation with epinephrine. Therefore, epinephrine unmasked the difference between glucose and glycogen with respect to the disposition between oxidative and nonoxidative pathways.

We chose to use the two glucose isotopes in both orders; either glycogen was labeled with ^{14}C, then [6-^{3}H[glucose included to simultaneously trace exogenous glucose, or vice versa. By this approach, it was possible to demonstrate preferential oxidation of glycogen relative to glucose regardless of the order in which the two isotopes were employed. The preference was demonstrable in the case where glucose and glycogen were compared within the same perfusions by using a different isotope to trace glucose as compared to the isotope used to trace glycogen. Alternatively, the preference was demonstrable when the two substrates were compared between separate sets of perfusions, using the same isotope to trace glucose in one set, as used for glycogen in the other set. Nevertheless, we compared the absolute rates of ^3H_2O and [^{3}H]lactate production from [6-^{3}H]glycosyl to the values for $^{14}CO_2$ and [^{14}C]lactate production from [U-^{14}C]glycosyl, both with respect to glucose and glycogen (see Fig. 2 and Methods). The variation in absolute rates obtained between the two isotopic methods were small compared to the larger difference between glucose and glycogen with respect to the preference for oxidative vs. nonoxidative metabolism.

The residual enrichment of glycogen at the end of the protocol with respect to the isotope of glucose included during the period of glycogen labeling, although small, may reflect partial scrambling of label into the remainder of the glycogen pool. The glycogenolysis induced by epinephrine was extensive; 80 μmol/gram dry weight was broken down, as compared to the prior incorporation of 43 μmol/gram dry wt of radiolabel into glycogen. The glycogen would therefore be expected to be devoid of label if the most recently synthesized radioactive glycogen were the first to be removed during subsequent glycogenolysis, since the extent of glycogenolysis exceeded the

extent of label incorporation by nearly twofold. We recently reported partial scrambling of label into the remainder of the glycogen pool (3). Time dependent scrambling of label throughout the glycogen pool has also been observed in the case of ^{13}C-NMR of vascular smooth muscle (16).

In both groups of perfusions, glycogen was enriched both with respect to the glucose isotope included during the period of glycogenolysis (45 to 75 min) as well as the isotope used to label glycogen between 20 and 40 min, in spite of the stimulation in glycogenolysis by epinephrine. Therefore, the hearts synthesized glycogen from exogenous glucose during the period of glycogenolysis confirming the occurrence of glycogen turnover, as we recently described (3).

Glycogen in skeletal muscle is traditionally thought to provide a substrate reserve during increased demand (17), and probably plays the same role in heart. The response to catecholamine stimulation in heart includes glycogen mobilization along with enhanced contractile activity, which are coordinated, in part, by increased cytosolic Ca^{2+}. Preferential oxidation of glycogen would ensure that the limited supplies of endogenous substrate are used for efficient generation of ATP to support the increased energy demand, as opposed to the low yield of ATP during anaerobic metabolism to lactate. We will estimate the effective increase in energy storage capacity of glycogen as a consequence of preferential oxidation at the 55-min time point, when the effect was maximal. The yield of ATP per glycosyl unit of glycogen is 37 for complete oxidation, and 3 for conversion to lactate. Since an average of 6% of glycogen was converted to lactate, and 94% oxidized (Fig. 3), the ATP yield per glycosyl is 35. However, in the absence of preferential oxidation, the glycogen would be handled like exogenous glucose. At 55 min, an average of 55% of the glucose was oxidized, and 45% was converted to lactate (Fig. 3). In this case, the ATP yield per glycosyl of glycogen is decreased to 21.7. Consequently the energy yield from the glycogen mobilized at 55 min was increased by 61% (from 21.7 to 35) as a consequence of preferential oxidation. The increase in energy yield from glycogen at later time points is roughly 30%, reflecting that at the time points beyond 55 min, a lesser portion of glucose went to lactate (about one-quarter), with the remainder being oxidized.

Absolute rates of glycogen oxidation can be estimated from the apparent rates provided in Fig. 2 *A* taken together with knowledge of the degree of enrichment (specific radioactivity) of the glycogen. However, the analysis is complicated by the fact that glycogen is incompletely enriched, and the label is probably not uniformly distributed throughout the glycogen pool. Consider the two extreme situations regarding the degree of uniformity of label incorporated at a representative site of action of phosphorylase. One extreme situation corresponds to absolute molecular order in the synthesis and subsequent degradation of glycogen (the "last on, first off hypothesis") (18, 19). In this case, the rates depicted in the figure would be the true rates of glycogen oxidation up until all the radiolabel was released. After that time, glycogen oxidation would continue, but degradation of the remaining unlabeled glycogen would not be detected. At the other extreme, glycogenolysis would be completely random with respect to the temporal order of incorporation of the label. In this case, the rates depicted in Fig. 2 *A* would parallel the true rates, and would need to be adjusted upward by the factor 1/0.49 to obtain the true rates, reflecting the 49% enrichment of the glycogen that was achieved in the present study. The actual situation probably exists between these two extremes, as we recently reported (3). In that study (3), the preference of glycogen oxidation for newly synthesized glycogen was only partial. According to this interpretation, the apparent rates of glycogen oxidation depicted in Fig. 2 *A* will underestimate the true rates by an amount which increases during the course of the experiment, reflecting progressive decrease in the specific radioactivity of the glycogen being degraded at any given time during the study. By this reasoning, the ratio of the apparent rate over the true rate of glycogen oxidation will vary from a value closer to unity at 45 min to a value closer to zero at the end of the study, and will have a value of ~ 0.49 if averaged over the entire study, since the average enrichment was 49%, and $> 90\%$ of the glycogen was degraded.

The energy yield from glycogen immediately after stimulation with epinephrine is substantial in comparison to the energy from exogenous glucose. In part, this is because most of the glycogen is oxidized. The relative energy yields at 55 min of the protocol can be calculated from the average of the rates of the two groups depicted in Fig. 2. The ATP yield from oxidation of glucose contributes 86 μmol/min per gram dry wt (2.4 μmol glucose/min per gram dry wt × 36 μmol ATP/μmol glucose). The contribution by lactate glycolysis of exogenous glucose is 2.0 μmol glucose/min per gram dry wt × 2 ATP/glucose = 4 μmol ATP/min per gram dry wt, and the total yield is therefore 90 μmol ATP/min per gram dry wt. The energy yield from glycogen can be calculated by using an average of the apparent rates of oxidation depicted in the figure, and this will be a minimum estimate. The ATP yield from oxidation of glycogen contributes 1.67 μmol glycosyl/min per gram dry wt × 37 μmol ATP/μmol glycosyl = 62 μmol/min per gram dry wt, and the energy yield from conversion of glycogen to lactate is negligible. Therefore, immediately after stimulation of glycogen oxidation with epinephrine, ATP production from glycogen is $\sim 74\%$ of the value from exogenous glucose ($62/90 \times 100\%$). As discussed above, this value may be a minimum estimate because the enrichment of the glycogen being degraded at the time of stimulation with epinephrine may be less than one. The contribution of glycogen to ATP production relative to glucose becomes minor at later time points because of glycogen depletion, and because the response of glucose utilization to stimulation with epinephrine is comparatively slow.

The different time courses for glycogen oxidation compared with glucose after epinephrine (Fig. 2) suggest that glycogen provided for the immediate increase in energy demand of the heart with epinephrine (Fig. 1 *B*) when the increase in utilization of exogenous glucose was incompletely developed. We recently observed a delayed response to epinephrine of uptake of a positron emitting glucose analogue, [^{18}F] 2-deoxy-2-fluoroglucose (20). The delayed regulation of glucose utilization may result from slow, time dependent recruitment of the glucose transporter in heart (GLUT4) by translocation onto the sarcolemma (21). Regardless of the mechanism, the initial increase in substrate utilization necessitated by the acute increase in contractile activity with epinephrine was provided, in part, by rapid glycogen mobilization. Increased levels of cytosolic Ca^{2+}, through the action of Ca^{2+}-calmodulin, would lead to the simultaneous activation of myosin light chain kinase and of phosphorylase kinase, providing the observed temporal coordination of glycogen oxidation with enhanced contractile activity.

Lactate accumulation in heart is associated with impaired contractile performance (22, 23). Therefore, preferential oxidation of glycogen would provide the additional advantage that lactate accumulation from glycogen, and associated contractile dysfunction, are avoided during the bursts of glycogen mobilization that are coordinated with enhanced contractile activity. The magnitude of the lactate sparing effect can be estimated from the data provided in Fig. 2, using the average value for the two groups. At 55 min, lactate production from exogenous glucose amounts to 2.0 μmol/min per gram dry weight, which is 45% of glucose utilization. At the same time, lactate production from glycogen is negligible. However, in the absence of preferential oxidation, the glycogen would be handled like exogenous glucose, with 45% conversion to lactate. The apparent rate of glycogen oxidation at 55 min is 1.67 μmol/min per gram dry weight, which is a minimum estimate of the true rate. Therefore, in the absence of preferential oxidation, a minimum estimate of lactate flux from glycogen would be 45% of 1.67, or 0.75 μmol/min per gram dry weight, and total lactate flux from glucose plus glycogen would be at least 2.0 + 0.75 = 2.75 μmol/min per gram dry wt. The calculation predicts that lactate flux would be at least 38% higher (from 2.0 to 2.75) in the absence of preferential oxidation of glycogen, although the increase would be transient.

In an earlier study relating to recovery of contractile performance following ischemia and reperfusion, we found an inverse relation between glycogen and lactate content of the heart during reperfusion, and suggested that the glycogen prior to ischemia contributed to lactate accumulation during ischemia (14). The observation of the present study that glycogen is converted to lactate by inhibiting oxidative metabolism with cyanide or ischemia is therefore consistent with our previous study.

One possible explanation for the different handling of glycogen compared to exogenous glucose is cellular heterogeneity within the myocardium. By this reasoning, the population of myocytes responsible for the majority of glycogen oxidation is different from the population which is responsible for glycolysis of exogenous glucose to lactate. This is consistent with the known transmural heterogeneity of the myocardium with respect to workload, blood flow (24), and enzymes pertaining to glycogen and glucose metabolism (25, 26). The phenomenon of glycogen turnover, or simultaneous glycogen synthesis and degradation which we recently described in heart (3) could also be explained by cellular heterogeneity. An alternative explanation is that glycolysis is functionally compartmentalized in the cytosol, as was previously suggested in vascular smooth muscle (1, 2). There is precedence for the general concept of compartmentation in the cytosol in heart (27, 28). The occurrence of locally high reaction rates relative to diffusion could provide functional compartmentation, as could the channeling of substrates between enzymes of heteroenzyme complexes (29).

In summary, evidence is provided that the heart, as a whole, displays preferential oxidation of glycogen in comparison with exogenous glucose following stimulation with epinephrine. The effect is to increase the effective energy storage capacity of glycogen, insuring efficient generation of ATP from a limited supply of endogenous substrate. Further, preferential oxidation results in a modest, transient decrease in overall lactate generation upon stimulation with epinephrine, reducing the potential for contractile dysfunction as a consequence of lactate accumulation. The results preclude conclusions regarding the level of organization responsible for the observed difference between glycogen and exogenous glucose since we studied the metabolism of the heart as a whole. The ability of the heart to use glycogen rapidly in response to the acute increase in contractile activity with epinephrine, when compared to the more slowly developing increase in utilization of exogenous glucose, suggests that glycogen provides a buffer for rapid changes in substrate demand.

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