Regulation of Glycogen Utilization, but Not Glucose Utilization, by Precontraction Glycogen Levels in Vascular Smooth Muscle†

Christopher D. Hardin* and Tina M. Roberts

Department of Physiology, University of Missouri, Columbia, Missouri 65212

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ABSTRACT: These experiments were designed to determine whether glycogenolysis was influenced by the glycogen concentration of vascular smooth muscle. Segments of hog carotid artery smooth muscle were allowed to synthesize variable amounts of 1-[^{13}C]glucosyl units of glycogen. Artery segments were then isometrically contracted in the presence of 2-[^{13}C]glucose. Prior to and after isometric contraction, measurements were made of tissue glycogen content and superfusate glucose and lactate concentrations. 2-[^{13}C]Lactate and 3-[^{13}C]lactate peak intensities in the superfusate were measured using ^{13}C-NMR spectroscopy. The tissue glycogen content decreased exponentially during the 4.5 h of isometric contraction ($R^2 = 0.990$), despite more than a 3-fold range of glycogen concentration prior to contraction. The extent of glycogen utilization during a 3 h isometric contraction varied linearly with the precontraction glycogen concentration ($R^2 = 0.727$). Lactate production specifically from glycogen breakdown increased with an increase in precontraction glycogen concentration ($R^2 = 0.620$). During a 3 h isometric contraction neither the glucose utilization ($R^2 = 0.007$) nor lactate production specifically produced from glucose ($R^2 = 0.000\,02$) varied with the precontraction glycogen concentration. It is concluded that the rate of glycogenolysis is determined by the content of glycogen during prolonged contractions. In addition, precontraction glycogen levels influence the pathway for glycogen utilization but not the pathway for glucose utilization. Therefore, glycolysis and glycogenolysis behave independently in vascular smooth muscle.

Glycogen storage in muscle can be an important mechanism to clear glucose from the blood, and glycogen stores are an important metabolic fuel for a variety of cell types. The regulation of glycogen utilization during muscle work has received considerable investigation over the last few decades. It is well-known that the rate of glycogenolysis is determined by the activation of glycogen phosphorylase and hence by the activation of phosphorylase b kinase. In muscle, cytoplasmic free [Ca^{2+}] is traditionally thought to regulate phosphorylase b kinase activity and AMP levels to regulate the phosphatase activity. However, in skeletal muscle, the calcium activation of phosphorylase b kinase and the subsequent activation of glycogen phosphorylase occurs only during the first few minutes of a contraction and then decreases activation of phosphorylase despite continued contraction (for example, see ref 1). Therefore, regulation of glycogenolysis may not be explained by changes in cytoplasmic free [Ca^{2+}] and AMP levels during prolonged contractions.

The decline in phosphorylase activity despite high levels of calcium and AMP, phosphorylase reversal, has been proposed to result from the release of phosphorylase activity from a glycogen−enzyme complex during glycogen degradation (*2*, *3*). The release of glycogen phosphorylase from the glycogen particle has been proposed to uncouple phosphorylase activation from the phosphorylase kinase activity (*3*). There has been considerable controversy about whether the level of glycogen stores in a muscle can act as a regulatory mechanism for glycogenolysis (*5*−*7*). Part of the difficulty in the interpretation of experiments designed to test this hypothesis has been that in working skeletal muscle calcium levels can alter on a twitch by twitch basis and levels of ATP, ADP, and AMP change considerably during exercise.

Tonic vascular smooth muscle, such as that derived from hog carotid artery, is ideally suited to test the hypothesis that the rate of glycogenolysis can be regulated by the size of the glycogen store during prolonged contraction. During activation and sustained contraction, the levels of ATP and hence ADP and AMP do not change in tonic vascular smooth muscle (*8*, *9*). In addition, after the activation phase, cytoplasmic free calcium does not change during sustained contraction. Therefore, if the glycogen utilization rate is regulated by the glycogen content of the cell, then it would be predicted that the rate of glycogenolysis would decrease exponentially with a decrease in glycogen content. In the present study, we measure glycogen utilization in a vascular smooth muscle preparation with variable glycogen content and find that the glycogen utilization rate varies exponentially with the glycogen content of the tissue.

In addition, vascular smooth muscle has been proposed to have separate compartments for glucose utilization and for glycogen utilization. By use of ^{13}C-NMR and specifically labeled glucose and glycogen, we were able to measure the flux of each of these two compartmented pathways. We were able to determine that the flux of glycogenolysis was altered by the glycogen content of the tissue but glucose

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* Address all correspondence to Christopher Hardin, Ph.D., Department of Physiology, MA-415 Medical Sciences Building, University of Missouri, Columbia, MO 65212. Phone: 573-884-6209. Fax: 573-884-4276. E-mail: physcdh@muccmail.missouri.edu.

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utilization and lactate production from glucose were unaltered. These results are consisted with a compartmentation of the pathways of glucose utilization and glycogen utilization in vascular smooth muscle.

METHODS

Tissue Handling. Hog carotid artery segments are obtained at a local abattoir within ~30 min of slaughter and transported to the laboratory in cold (~5−10 °C) physiological saline solution (PSS)[1] consisting of (mM) NaCl (116), KCl (4.6), KH_2PO_4 (1.16), $NaHCO_3$ (25.3), $CaCl_2$ (2.5), and $MgSO_4$ (1.16). At the laboratory, the arteries were placed into fresh PSS equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at 37 °C. Segments were dissected free of loose fat, connective tissue, and adventitia. Throughout all manipulations, PSS was equilibrated with the gas mixture. Segments of the carotid artery were selected to be within 5 cm of the bifurcation into the internal and external carotid artery. Carotid segments (5−7 cm in length) were chosen that had a diameter of the lumen of ~2−2.2 mm.

Time Course of Glycogen Depletion. A series of experiments was conducted to determine the time course of glycogen utilization during contraction in hog carotid artery segments with variable levels of glycogen prior to contraction. Each carotid segment was cut into 12 rings (~40−80 mg each) and allowed to synthesize glycogen stores for up to 16 h at 37 °C with 5 mM unlabeled glucose as the substrate. At the end of the glycogen repletion incubation, three carotid rings from each segment were then blotted dry on filter paper and rapidly weighed and frozen in liquid N_2 for measurement of glycogen content. The remaining nine carotid rings were mounted on glass tubes such that the lumen of the ring was stretched to approximately 1.5 times the resting length and incubated at 37 °C in PSS with added 80 mM KCl and 5 mM glucose. At 1.5, 3.0, and 4.5 h of contraction, three additional rings from the same artery were blotted dry on filter paper and rapidly weighed and frozen in liquid N_2 for measurement of glycogen content.

Repletion of Glycogen Stores with ^{13}C-Labeled Glycogen. Each carotid segment was cut into six rings (~40−80 mg each) and placed in PSS with 80 mM added KCl (no glucose present) at 37 °C for 20 min to decrease endogenous glycogen stores (*10*, *11*) to approximately 1 μmol/g wet weight. All rings cut from the same artery segment were kept together by stringing suture through the lumen. Some individual carotid rings from different arteries were blotted dry on filter paper, weighed, and rapidly frozen in liquid N_2 and stored for assay of glycogen content prior to glycogen synthesis. The remaining unmounted carotid rings were incubated in PSS with 5 mM 1-[^{13}C]glucose for periods of 1.5, 6, or 12−16 h at 37 °C to incorporate ^{13}C-label into varying levels of newly synthesized glycogen stores (1-[^{13}C]-glycogen).

Incubations of Carotid Segments during Isometric Contraction. Since approximately 50% of glycogen stores were depleted after 3 h of isometric contraction (see Results), experiments that utilized ^{13}C-labeled substrates were performed on tissues that were contracted for 3 h. After synthesis of variable 1-[^{13}C]glycogen stores (as described above), the carotid rings were rinsed in PSS for 30 min for a total of 3 consecutive rinses to remove residual 1-[^{13}C]-glucose. Three of the carotid rings from each artery were then blotted dry on filter paper and rapidly weighed and frozen in liquid N_2. The remaining three carotid rings were mounted on glass tubes such that the lumen of the ring was stretched to approximately 1.5 times the resting length. The three mounted rings from the same artery were then placed into an incubation chamber filled with 3 mL PSS with added 80 mM KCl and 2-[^{13}C]glucose and incubated at 37 °C for 3 h. The carotid rings were then removed from the glass tubes, briefly rinsed with fresh PSS, blotted dry on filter paper and rapidly weighed and frozen in liquid N_2. A 2.5 mL sample of the superfusate was rapidly frozen in liquid N_2 and stored for NMR analysis.

Measurements of Glycogen and Glucose Utilization. The glycogen content of carotid rings was assayed using enzyme-linked assays as previously described (*11*) using a Hitachi U2000 dual beam spectrophotometer. Prior to the initiation of the experiments, the criteria for exclusion of tissues from analysis was set as being when an artery did not synthesize at least 1 μmol/g wet weight of glucosyl units per hour for the first 6 h of the incubations. We have previously shown that hog carotid artery can synthesize glucosyl units of glycogen from glucose at a rate of approximately 1.3 μmol/g wet weight per hour (*10*). Data were also excluded when an individual measurement of glycogen content in glycogen repleted tissues deviated more than 50% from the mean (based on triplicates) for that artery. Total glycogen flux during the contraction (micromoles per gram of wet weight per 3 h) is defined as the difference between the mean glycogen content measured before contraction and that measured at the end of the contraction for each tissue.

The concentration of glucose at the start of the contraction was 5 mM. Glucose concentration of the superfusate at the end of the contraction was measured using standard enzyme-linked assays using a Hitachi U2000 dual beam spectrophotometer. The extent of glucose utilization during the contraction was calculated by subtracting the content of glucose in the superfusate at the end of the contraction from the starting glucose content (3 mL superfusate of 5 mM) and normalizing to the mass of tissue in the incubation chamber during the contraction.

Measurement of Lactate Production Specifically from Glucose or Glycogen by ^{13}C-NMR. Since glucose stores were labeled with ^{13}C at the 2nd carbon, and glycogen stores were labeled with ^{13}C at the 1st carbon of the glucosyl units, measurement of the 2-[^{13}C]lactate and 3-[^{13}C]lactate levels by ^{13}C-NMR provided an index of the lactate production specifically derived from glucose or glycogen. Frozen superfusates (2.5 mL aliquots) were lyophilized in a Speed Vac (Savant Instruments, Inc.). The powder was resuspended in 1 mL of 99% D_2O with 25 mM 3-(trimethylsilyl)-1-propanesulfonic acid (DSS) as a chemical shift reference. A 650 μl aliquot was transferred to a 5 mm NMR tube. ^{13}C-NMR spectroscopy was performed on a Bruker AMX 500 spectrometer with the following acquisition parameters: 300 scans with 16 dummy scans, 30° pulse angle at 125.77 MHz, 33 333 Hz sweep width, and a 1 s predelay. The number of points acquired and processed with 1 Hz line broadening prior to Fourier transform was 32 768. All spectra were broad-band proton decoupled and referenced with DSS at 0 ppm. NMR data were processed using Bruker software for peak magnitude determination. All peaks are expressed relative to the DSS peak at 0 ppm, and no corrections were

[1] Abbreviations: PSS, physiological saline solution; DSS, 3-(trimethylsilyl)-1-propanesulfonic acid.

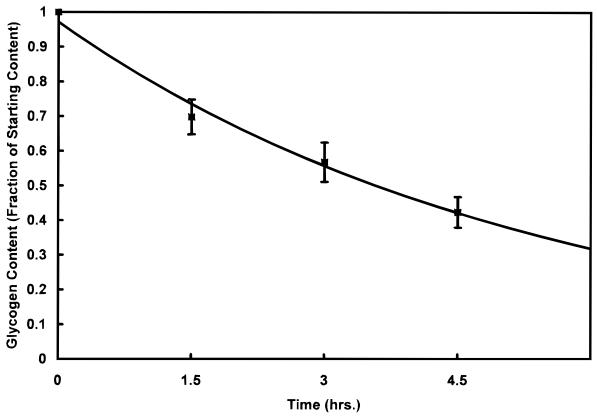

FIGURE 1: Glycogen content of hog carotid artery segments normalized to the precontraction glycogen concentration during isometric contraction for 1.5, 3.0, and 4.5 h. Precontraction glycogen concentrations ranged from 3.03 to 11.17 μmol/g wet weight glucosyl units of glycogen. Since rings from each artery segment were frozen at each time point, data from each time point are self-paired. Data points represent the mean ± SEM for 24 carotid arteries.

made for nuclear Overhauser effects which were assumed to be unchanged for all experiments. ^{13}C-NMR signal intensities were scaled for the tissue mass in each organ chamber during the contraction. 3-[^{13}C]Lactate peak intensity normalized to the DSS peak and tissue mass provides a relative measure of the 1-[^{13}C]glycogen that was converted to lactate. 2-[^{13}C]Lactate peak intensity normalized to the DSS peak and tissue mass provides a relative measure of the 2-[^{13}C]glucose that was converted to lactate.

Statistics. Statistics and curve fits were calculated using Microsoft Excel, version 7.0, for the PC for least-squares regression analysis.

RESULTS

Carotid segments that were used for the determination of the time course of glycogen repletion synthesized between 3.03 and 11.17 μmol/g wet weight glucosyl units of glycogen. Of the 24 tissues used for these experiments, the glycogen content of the individual tissues prior to contraction (in micromoles per gram of wet weight glucosyl units of glycogen) was less than 4 for seven arteries, between 4 and 8 for 11 arteries, and greater than 8 for six arteries. When these arteries were isometrically contracted, glycogen content was measured after 1.5, 3.0, and 4.5 h of contraction (Figure 1). Regardless of the precontraction glycogen levels, by the end of 1.5, 3.0, and 4.5 h of contraction, artery segments had 70% (± 5%), 57% (± 6%), and 42% (± 4%) respectively (mean ± SEM). Therefore, the absolute rate of glycogen utilization during contraction depended on the level of glycogen stores in the artery. The decline in the rate of glycogen utilization over time was fit with an exponential curve ($y = 0.9719\ e^{-0.1857}$, $r^2 = 0.990$). At 3 h of isometric contraction, approximately half of the glycogen stores remained regardless of the precontraction glycogen content. This flux readily allowed measurement of glycogen stores and lactate production from either glucose or glycogen.

When hog carotid artery segments were incubated at 37 °C in PSS with 5 mM 1-[^{13}C]glucose, they were capable of synthesizing glycogen up to levels of approximately 13 μmol/g wet weight (see Figure 2). To achieve variable precontraction tissue glycogen contents, we incubated carotid segments for variable times (1.5, 6, and 14−16 h) prior to contraction. When carotid segments were then isometrically contracted in the presence of 5 mM 2-[^{13}C]glucose, approximately half of the tissue glycogen was utilized during the 3 h of contraction (Figure 2). The extent of glycogen utilization during the 3 h contraction was linearly dependent on the precontraction glycogen content of the tissue [$R^2 = 0.7265$ ($N = 58$)]. The slope of the regression line (0.5307) provides an index of the fraction of the precontraction glycogen content that remained at the end of contraction. Thus, 53% of the glycogen stores remained after 3 h of contraction regardless of the precontraction glycogen level. This number is remarkably similar to the 57% that was determined by the experiments depicted in Figure 1. Therefore, consistent with the data in Figure 1, the rate of glycogen utilization during an isometric contraction was determined by the size of the glycogen stores prior to contraction.

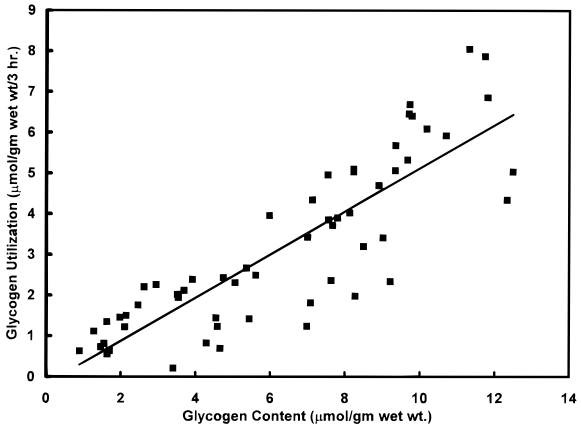

FIGURE 2: Scatter plot of the glycogen utilization during a 3 h isometric contraction vs the precontraction glycogen concentration of the carotid artery segment. The slope of the least-squares regression line is an index of the percentage of precontraction glycogen that is utilized during the prolonged contraction.

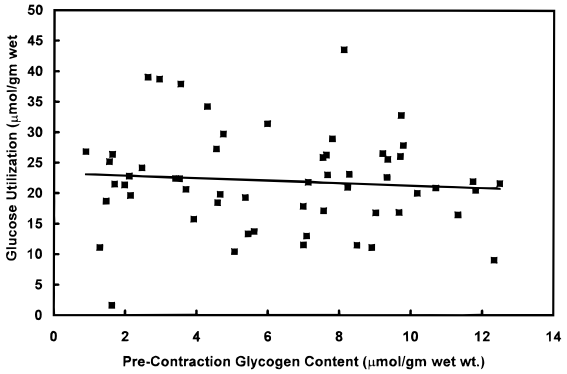

FIGURE 3: Scatter plot of glucose utilization during a 3 h isometric contraction vs the precontraction glycogen concentration of the carotid artery segment. The least squares regression line has an R^2 of 0.007 indicating no relationship between the two variables.

The regulation of the rate of glycogen utilization during contraction depended on the size of the glycogen stores prior to contraction. A high rate of glycogenolysis will produce substantial glucose-6-phosphate leading to an inhibition of hexokinase. Therefore, it might be expected that the enhanced glycogenolysis at high glycogen content would inhibit glucose uptake and utilization. Since we labeled glucose as 2-[^{13}C]glucose and glycogen as 1-[^{13}C]glycogen, we were able to determine whether high rates of glycogen utilization resulted in an inhibition of glucose uptake and utilization. Shown in Figure 3 is the relationship between glucose utilization and the precontraction glycogen content

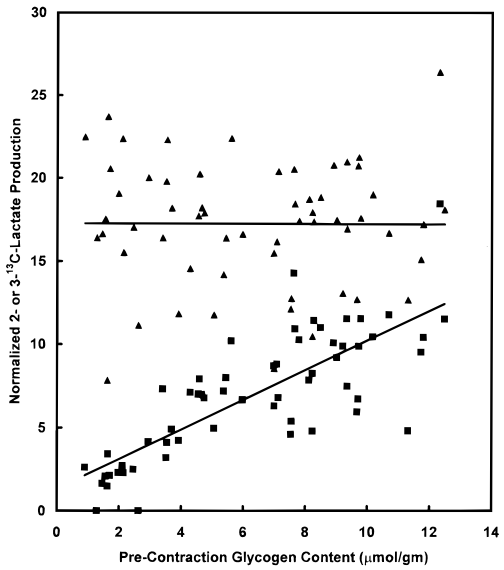

FIGURE 4: Scatter plot of lactate production from specifically either from glucose or from glycogen vs the precontraction glycogen concentration of the carotid artery segment. 3-[^{13}C]lactate (squares) was specifically derived from 1-[^{13}C]glycogen while 2-[^{13}C]lactate (triangles) was derived specifically from 2-[^{13}C]glucose. The lactate peak intensities were normalized to the tissue mass in each organ chamber during the contraction and normalized again to the DSS peak at 0 ppm. Least-squares regression lines are shown for 3-[^{13}C]-lactate (squares, bottom line) and for 2-[^{13}C]lactate (triangles, top line).

of the artery segments. On the basis of the least-squares linear regression line, there was no dependence of glucose utilization on precontraction glycogen content ($r^2 = 0.007$). Therefore, an increase in the glycogen content of an artery segment prior to contraction resulted in increased glycogen utilization but no change in glucose utilization during contraction.

Although the pathways for glycogen utilization and for glucose utilization share nine common enzymatic steps in the conversion from glucose-6-phosphate to pyruvate, the substantial changes in glycogenolysis observed with changes in precontraction glycogen levels did not influence the overall rate of glucose utilization. In the experiments depicted in Figures 2 and 3, glucose was labeled with ^{13}C at the 2nd carbon while the glucosyl units of glycogen were labeled at the 1st carbon. The source of lactate was readily determined since 2-[^{13}C]lactate will exclusively be made from 2-[^{13}C]-glucose and 3-[^{13}C]lactate will be exclusively made from 1-[^{13}C]glycogen. Shown in Figure 4, the lactate production from glucose (triangles) did not change with increasing precontraction glycogen levels. However, the lactate production from glycogen (squares) increased approximately linearly with increases in precontraction glycogen levels. Therefore, the overall rate of glycogen conversion to lactate was varied approximately with the precontraction glycogen concentration, but no alteration in either the extent of glucose uptake or the conversion of glucose to lactate was observed.

Since the production of lactate is likely to be derived from only the breakdown of glucose and glycogen, total lactate production would be expected to increase with increased precontraction glycogen content. Shown in Figure 5 is the measured lactate content of superfusates plotted as a function of precontraction glycogen content. As expected, there is a small increase in total lactate production with an increase in precontraction glycogen content.

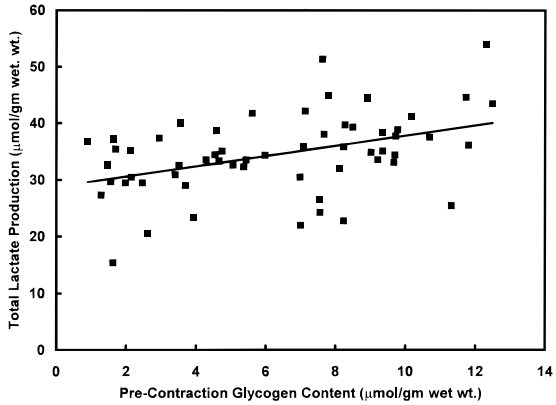

FIGURE 5: Scatter plot of total lactate production by carotid segments during 3 h of isometric contraction vs the precontraction glycogen concentration of the carotid artery segment. Total lactate production was taken from measurements of superfusate lactate concentrations (see Methods for details). Shown is the least-squares regression line.

DISCUSSION

Regulation of Glycogenolysis by Glycogen Content. Considerable controversy surrounds the proposition that the rate of glycogen utilization may be controlled by the glycogen content of the tissue. Glycogen utilization is controlled by the activity of glycogen phosphorylase, which is in turn controlled by phosphorylase b kinase and phosphatase activities. It has been proposed that the enzymes responsible for the regulation of glycogen utilization are part of a substrate−enzyme complex termed the glycogen particle (*3*). During glycogen utilization, it has been proposed that the degradation of glycogen results in the release of glycogen phosphorylase from the glycogen particle thereby uncoupling the action of phosphorylase b kinase from glycogen phosphorylase. The clear prediction of such a model is that, in the absence of changes in other regulatory elements of the system, as the glycogen content of a tissue decreases, the rate of glycogen utilization should decrease. Experiments aimed at measuring a relationship between glycogen content and the rate of glycogenolysis in skeletal muscle have been difficult to interpret because of issues such as motor unit recruitment, alterations in cellular phosphorylation state, and hence AMP levels, and varying levels of cytoplasmic free calcium concentrations (*4*−*7*). Therefore, skeletal muscle has proved to be a difficult tissue to use for a direct test of the hypothesis that glycogenolytic rate can be determined by the glycogen content of the tissue.

Vascular smooth muscle is well suited to the study of the regulation of metabolism for several reasons. Like skeletal muscle, the rate of energy utilization can be readily controlled by changing the activation state of the muscle. Unlike skeletal muscle, tonic vascular smooth muscle can sustain prolonged contractions with minimal fatigue and, after the activation phase of the contraction, levels of ATP and free calcium do not change during prolonged contraction (*8*, *9*). In addition, vascular smooth muscle is a highly glycolytic tissue (*12*) and capable of storing substantial amounts of glycogen (*10*). Therefore, tonic vascular smooth muscle is well suited to test the hypothesis that the rate of glycogenolysis may be determined by the glycogen content of the cell.

If phosphorylase is released due to glycogen degradation and hence uncoupled from the actions of phosphorylase b kinase, then it would be expected that as the glycogen stores

are utilized that the rate of glycogen utilization would decrease proportionately. Therefore, the content of glycogen stores during a prolonged contraction in vascular smooth muscle should decline exponentially. Shown in Figure 1, over 4.5 h of contraction the level of glycogen declined. The fit of the data to an exponential had a better correlation coefficient (0.990) than if the data were fit to a straight line ($r^2 = 0.956$). Thus, the data are consistent with the hypothesis that cellular glycogen levels are a regulator of the rate of glycogenolysis over a 3-fold range of glycogen levels.

The data in Figure 1 are plotted as the fraction of precontraction glycogen content over time during a prolonged contraction. If the data were plotted as absolute glycogen content over time during a prolonged contraction, the data would be represented by a family of curves described by the same exponential with differing intercepts. The absolute amount of tissue glycogen that had been utilized by a given time point during contraction plotted against the absolute precontraction glycogen content should yield a linear relationship. When the extent of glycogen utilization during 3 h of contraction was plotted against the precontraction glycogen level (Figure 2), the data are best fit by a straight line ($r^2 = 0.727$) rather than an exponential ($r^2 = 0.669$). Therefore, the data are consistent with the glycogen content of contracting vascular smooth muscle playing a role in the regulation of the rate of glycogenolysis.

The interpretation of the data presented in Figures 1 and 2 depend on glycogen phosphorylase being the only controller of glycogen levels during contraction. If glycogen synthase was active during contraction, then net glycogen utilization would be determined by the balance of the synthase and phosphorylase activities. In resting hog carotid artery, we have demonstrated a simultaneous synthesis and degradation of glycogen (*10*), yet in contracting hog carotid artery we found no evidence of such a glycogen substrate cycling (*13*). Therefore, during contraction, there is no measurable glycogen synthesis and glycogen levels are determined entirely by the activity of phosphorylase.

The relationship between glycogen content and the rate of glycogen utilization may be readily measured in tonic vascular smooth muscle. However, in skeletal muscle, the existence of such a relationship has proved controversial (*4*−*7*, *14*). Generally, in studies using very short stimulation protocols, the precontraction glycogen levels did not effect the rate of glycogenolysis while a longer period of stimulation revealed that precontraction glycogen content correlated with the rate of glycogenolysis. It has been proposed that in skeletal muscle, the rate of glycogenolysis depends on the precontraction glycogen levels during the "aerobic" phase of repetitive stimulation since no correlation of precontraction glycogen levels and the rate of glycogenolysis was observed during the shorter "anaerobic" phase (*7*). Therefore, in skeletal muscle, during glycogenolysis under well-oxygenated conditions, precontraction glycogen levels may be an important determinant of the rate of glycogenolysis (*7*). This may be a reflection that under well-oxygenated conditions levels of AMP and the cytoplasmic phosphorylation potential change less than under anaerobic conditions.

Glycogen phosphorylase kinase activity is dependent on calcium and it may be assumed that intracellular free calcium concentration is the primary determinant of phosphorylase kinase activity in vascular smooth muscle since ATP levels do not change during maintenance of a prolonged contraction (see refs *8*, and *9* for examples). In skeletal muscle, the calcium activation of phosphorylase kinase and the subsequent activation of glycogen phosphorylase occurs only during the first few minutes of a contraction, and then the activation of phosphorylase decreases despite continued contraction (*1*). Indeed, in vascular smooth muscle, it has been demonstrated that glycogen phosphorylase a activity increased 3.5-fold from rest to contraction (elicited by KCl) within 45 s of the onset of contraction (*15*). However, by 30 min of contraction, phosphorylase a levels had declined by ∼35% from the levels observed 45 s after the onset of contraction. Therefore, it would be expected that the rate of glycogen breakdown would be higher during the first few minutes of a prolonged contraction in vascular smooth muscle and decline during maintenance of contraction. Lynch and Paul (*16*) observed that the rate of glycogenolysis was greatest during the first 10−15 min of contraction and had substantially slowed by 30 min of contraction. When other investigators have measured the rate of glycogenolysis over short periods of time (∼30 min), the rates of glycogenolysis varied from approximately 0.04 μmol/g/min (*17*) to 0.057 μmol/g/min (*15*). Those rates compare only with the highest long-term, steady state rates observed in this study in tissues with greater than 10 μmol/g glucosyl units of glycogen. It is likely that a short-term burst of glycogenolysis occurred during the first few minutes of contractions in the present study. However, since sequential measurements were made over a prolonged contraction, the contribution of glycogenolysis during the brief activation phase of contraction is likely negligible to the long-term measurements made in the current study.

Therefore, we have established that the rate of glycogenolysis varies with the glycogen content of vascular smooth muscle consistent with the hypothesis that glycogen utilization results in a decreased phosphorylase activity due to the release of phosphorylase from glycogen. In smooth muscle, the importance of localized multienzyme complexes has been well-reported (*12*, *13*, *16*, *18*−*21*). Therefore, it is likely that glycogen phosphorylase is bound to glycogen in vascular smooth muscle as has been proposed for striated muscle (*3*, *2*).

Independent Regulation of Glycogenolysis and Glycolysis. Although the rate of glycogenolysis depended on the precontraction glycogen content in hog carotid artery, the rate of glucose utilization did not vary despite a 7-fold change in glycogen utilization. In working skeletal muscle, increased glycogen content resulted in decreased glucose uptake (*22*, *5*) and decreased glycogen availability resulted in increased glucose uptake (*22*). Recently, it has been reported that in intact humans, glycogenolysis is increased in muscles with increased precontraction glycogen levels, yet whole-body glucose disappearance does not change during muscular activity (*4*). However, the glucose uptake of the specific active muscles was not measured. The general finding of an inverse relationship between glycogenolysis and glucose utilization suggests that both substrates, glucose and glycogen, supply a single regulated pathway.

In vascular smooth muscle, it has been proposed that glycolysis and glycogenolysis are functionally separate at low (*16*) and high (*13*) glycogen levels. This functional separation of glycolysis and glycogenolysis has been proposed to result from differently localized sets of enzymes (*13*), one set to carry out the conversion of glucose-6-

phosphate derived from glucose breakdown to lactate (glycolysis) and one to carry out the conversion of glucose-6-phosphate derived from glycogen breakdown to pyruvate or lactate (glycogenolysis). This functional separation of the enzymes for glycolysis and for glycogenolysis may allow for the flux of glycogenolysis to be independent from the flux of glycolysis. Such an independence of flux of glycolysis and glycogenolysis would require that not only would the rate of input (glucose or glycogen) of the pathways be independent but that the rate of production of one of the pathway products (lactate) derived from either glucose or glycogen would be independent. In the experiments presented here, the glucosyl units of glycogen were labeled with ^{13}C at the 1st carbon (1-[^{13}C]glycogen) while the glucose was labeled with ^{13}C at the 2nd carbon (2-[^{13}C]glucose). Therefore, we were able to detect the lactate production specifically derived from glucose (2-[^{13}C]lactate) or from glycogen (3-[^{13}C]lactate).

Our studies demonstrate that during a 3 h isometric contraction, both glycogen utilization and lactate production specifically derived from glycogen increased with an increase in precontraction glycogen content (Figures 2 and 4). However, neither glucose utilization nor lactate production from glucose significantly changed as the precontraction glycogen content changed (Figures 3 and 4). Since lactate derived from glycogen and lactate derived from glucose will both contribute to the total lactate produced, the total lactate was slightly increased with an increase in the precontraction glycogen level of the tissue (Figure 5). The lactate production from glycogen was always lower than the lactate production from glucose (Figure 4), and thus the increase in lactate production from glycogen should have had only a small effect on the total lactate produced by the tissue (Figure 5). However, these data clearly demonstrate that the two pathways, one for glucose conversion to lactate and the other for glycogen conversion to lactate, are independent and additive. A similar analysis has recently been employed for the study of glyconeogenesis in skeletal muscle (*23*). Ryan and Radziuk (*23*) found that the conversion of lactate and other products of glucose metabolism into glycogen was independent and additive to the conversion of plasma lactate to glycogen. Therefore, some structuring of carbohydrate metabolism may also exist in skeletal muscle although it may only be detectable with measurements of gluconeogenesis or glyconeogenesis. The independent regulation of two components of carbohydrate metabolism that share nine common enzymatic steps is contrary to the commonly held notion that the enzymes for glycolysis are freely diffusing and randomly distributed within the cytoplasm. The organization of cytoplasmic enzymes that must underlie the observation of compartmented carbohydrate metabolism in the current study may reflect a general feature of cellular organization.

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