Activation of glycogen synthase in myocardium induced by intermittent hypoxia is much lower in fasted than in fed rats

Yangsong Wu, Hong Wang, David L. Brautigan, and Zhenqi Liu

Division of Endocrinology and Metabolism, Department of Internal Medicine, and Center for Cell Signaling, University of Virginia Health System, Charlottesville, Virginia

Submitted 8 September 2006; accepted in final form 19 September 2006

Activation of glycogen synthase in myocardium induced by intermittent hypoxia is much lower in fasted than in fed rats. Am J Physiol Endocrinol Metab 292:E469–E475, 2007. First published September 26, 2006; doi:10.1152/ajpendo.00486.2006.—Obstructive sleep apnea is characterized by intermittent obstruction of the upper airway, which leads to intermittent hypoxia. Myocardial glycogen is a major energy resource for heart during hypoxia. Previous studies have demonstrated that intermittent hypoxia rapidly degrades myocardial glycogen and activates glycogen synthase (GS). However, the underlying mechanisms remain undefined. Because sleep apnea/intermittent hypoxia usually happens at night, whether intermittent hypoxia leads to GS activation in the postabsorptive state is not known. In the present study, male adult rats were studied after either an overnight fast or ad libitum feeding with or without intermittent ventilatory arrest (3 90-s periods at 10-min intervals). Hearts were quickly excised and freeze-clamped. Intermittent hypoxia induced a significant decrease in myocardial glycogen content in fed rats and stimulated GS in both fasted and fed rats. However, the portion of GS in the active form increased by ~38% in fasted rats compared with a larger, ~130% increase in fed rats. The basal G-6-P content was comparable in fasted and fed animals and increased approximately threefold after hypoxia. The basal phosphorylation states of Akt and GSK-3B and the activity of protein phosphatase 1 (PP1) were comparable between fasted and fed control rats. Hypoxia significantly increased Akt phosphorylation and PP1 activity only in fed rats. In contrast, hypoxia did not induce significant change in GSK-3B phosphorylation in either fasted or fed rats. We conclude that hypoxia activates GS in fed rat myocardium through a combination of rapid glycogenolysis, elevated local G-6-P content, and increased PP1 activity, and fasting attenuates this action independent of local G-6-P content.

Glucose 6-phosphate; glycogen synthase kinase 3; heart; protein kinase B; protein phosphatase 1

Obstructive sleep apnea is a common health problem affecting many obese humans and is characterized by repeated obstruction of the upper airway and intermittent hypoxia. It has been well known that people with obstructive sleep apnea have higher cardiovascular morbidities and mortalities (39, 44, 48, 49). However, the underlying mechanisms are unclear. Because myocardial glycogen is a major energy source for heart during hypoxia when glycogenolysis provides substrate for glycolysis to maintain cardiomyocyte survival, it is possible that myocardial glycogen synthesis and degradation may play important roles in this process. Previous studies (26, 37) have indeed demonstrated that intermittent hypoxia rapidly degrades myocardial glycogen and activates glycogen synthase (GS), a rate-limiting enzyme in the glycogen synthesis pathway, whereas reoxygenation is associated with glycogen repletion. GS activity is regulated by complex multisite phosphorylation as well as allosteric factors (29, 52). Phosphorylation of GS at specific sites decreases, whereas dephosphorylation increases GS activity. The phosphorylation of GS by glycogen synthase kinase-3 (GSK-3) and dephosphorylation by protein phosphatase 1 (PP1) appear particularly important. GSK-3 is a key component of the Wnt and phosphatidylinositol 3-kinase (PI 3-kinase)/protein kinase B (Akt) signaling pathways and has many pivotal biological roles, in addition to the regulation of glycogen synthesis (14). GSK-3 phosphates and inactivates GS (14, 29, 52). On the contrary, inhibition of GSK-3 decreases GS phosphorylation and increases GS activity. In turn, the phosphorylation/activity of GSK-3 is tightly regulated by Akt, a key threonine-serine protein kinase in the PI 3-kinase signaling pathway. Phosphorylation of specific residues on Akt activates the kinase, and activated Akt inhibits GSK-3 by phosphorylating Ser343 (GSK-3α) and Ser473 (GSK-3β) (14, 29, 46), thereby increasing GS activity. Among protein phosphatases, the glycogen-bound PP1 is the major enzyme responsible for the dephosphorylation and activation of GS (18, 40). Glucose 6-phosphate (G-6-P) is another major regulator of GS activity, acting both by allosterically activating GS and enhancing GS dephosphorylation by making it a better substrate for PP1 (16, 24, 28, 36, 47).

Previous studies examining intermittent hypoxia-stimulated GS activation were all done in ad libitum-fed animals. Because obstructive sleep apnea usually happens at night in both the postprandial and postabsorptive states, it is unclear whether those findings can be extrapolated to the fasted (postabsorptive) state. The major purpose of the present study was to examine whether intermittent hypoxia stimulates GS equally in the fasted and fed states in rat myocardium and the mechanisms underlying the intermittent hypoxia-induced GS activation. Our data indicate that intermittent hypoxia dramatically stimulated GS activity, which was associated with rapid glycogenolysis, enhanced Akt phosphorylation, and PP1 activity in rat myocardium in fed rats, and fasting attenuated these actions.

Materials and Methods

Animal preparation and experimental protocols. The study protocol was approved by the Animal Care and Use Committee at the University of Virginia. Four groups of male Sprague-Dawley rats (n = 6–12 for each group), weighing 250–300 g, were studied after...
either an overnight fast (2 groups) or an overnight ad libitum feeding (2 groups). Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg; Abbott Laboratories, North Chicago, IL). The carotid artery and trachea were cannulated via a midline neck incision. A thoracotomy was performed, and mechanical ventilation with room air was started via a trachea cannula using a Harvard rodent ventilator (Harvard Instruments, South Natick, MA). The arterial catheter was connected through a three-way stopcock to a pressure probe. Mean arterial pressure was monitored throughout the study (Transonic Systems, Ithaca, NY). Pentobarbital sodium was infused intra-arterially at variable rates to maintain a steady level of anesthesia throughout the study.

After a 30-min baseline period to assure hemodynamic and anesthetic stability, rats were assigned to one of the following two protocols. J) Hypoxia: rats were subjected to three 90-s periods of ventilatory arrest (by turning off the ventilator) at 10-min intervals, and the hearts were excised immediately after last hypoxia episode; 2) control: rats were maintained on a ventilator throughout the study, and the hearts were excised at the end of the experiment. All hearts were freeze-clamped in liquid nitrogen immediately after excision and then stored at −70°C until analysis.

**Measurement of GS activity.** Heart muscle GS activity was measured in cell-free homogenates, using previously established methods (45, 50), and was expressed as the rate of incorporation of [U-14C]uridine diphosphoglucose (Amersham Biosciences, Piscataway, NJ) into glycogen (μmol.g−1.min−1). The activities of the G-6-P independent form (GS-I) was determined in the presence of 0.17 mM (physiological concentration) G-6-P. The total GS activity (GS-D) was measured in the presence of 7.2 mM G-6-P. The ratios of GS-I to GS-D represent the fraction of GS in the active form.

**Measurement of heart muscle glycogen content.** Approximately 30–40 mg of heart muscle was powdered in liquid nitrogen and then dissolved in 30% (wt/vol) potassium hydroxide. Glycogen was precipitated and washed three times with ice-cold ethanol. After being digested with amyloglucosidase, the glucose concentration was measured using glucose oxidase method (7), and the glycogen content was expressed as milligrams of glycogen per gram wet tissue.

**Measurement of myocardial G-6-P content.** The G-6-P content was measured using a previously described method (38). Briefly, heart muscle samples (~100 mg) were powdered in liquid nitrogen and extracted for 24 h in 5 volumes of 6% perchloric acid. After neutralization, the extracts were then mixed with an assay solution containing 0.2 M triethanolamine (pH 7.6), 0.2 mM nicotinamide adenine dinucleotide phosphate, and 5 mM MgCl2. The absorbance was measured before and after addition of 340 μl G-6-P dehydrogenase, and the amount of G-6-P in the tissue was calculated against G-6-P standard curve and expressed as micromoles per gram of tissue.

**Quantitation of Akt and GSK-3 phosphorylation.** Pieces (~40 mg) of frozen heart muscle were powdered in frozen 25 mM Tris-HCl buffer (26 mM KF and 5 mM EDTA, pH 7.5) and then disrupted by sonication using a Fisher XL2020 sonicator (Fisher Scientific, Pittsburgh, PA). The homogenate was centrifuged at 2,000 rpm for 2 min. Aliquots of the supernatant containing ~60 μg of protein were diluted with an equal volume of SDS sample buffer and electrophoresed on a 10% polyacrylamide gel. After being blocked with 5% low-fat milk in Tris-buffered saline plus Tween-20, membranes were incubated with antibodies against Akt, phospho-Akt (Ser473), GSK-3β, or phospho-GSK-3β (Ser9) (New England BioLabs, Beverly, MA) overnight at 4°C. This was followed by a donkey anti-rabbit IgG coupled to horseradish peroxidase, and the blots were developed using an enhanced chemiluminescence Western blotting kit (Amersham Life Sciences, Piscataway, NJ). Autoradiographic films were scanned by densitometry (Molecular Dynamics, Piscataway, NJ) and quantitated using Imagequant 3.3. Both the total and phospho-specific densities were quantitated, and the ratios of phospho-specific density to total density were calculated.

**Assay of PPI activity.** PPI activity was assayed using 32P-labeled rabbit glycogen phosphorylase a as substrate (6). Briefly, 32P-labeled phosphorylase a was prepared by adding ~30 mg of phosphorylase b and 15–20 μCi [γ-32P]ATP to a reaction buffer containing 20 mM Tris-HCl, pH 8.2, 70 μM CaCl2, 28 mM Na β-glycerol phosphate, 28 mM β-ME, 1 mM ATP, 2 mM MgCl2, 10 nM microcystin, 1 μl of PKA, and 50 units of phosphorylase kinase and incubating at 30°C for 1–1.5 h. An equal amount of saturated ammonium sulfate was then added, mixed well, and incubated on ice for 30 min. After centrifugation at 12,000 g for 10 min at 4°C, the pellet was washed with 45% ammonium sulfate 4–5 times to remove all free [32P]ATP. The final pellet was resuspended in 1 ml of solubilization buffer (50 mM MOPS, pH 7.0, 0.1 mM caffeine, 0.1% β-ME) and dialyzed at 4°C overnight against 1 liter of solubilization buffer. Glyceral was added to 50% final concentration, and the solution was stored on ice/water slurry for immediate use or at −20°C for later use. Specific radioactivity of the substrate was ~6 × 105 cpn/mg phosphorylase a and gave a yield of ~90–95% 32P-labeled phosphorylase a.

Approximately 20 mg of heart muscle were powdered in liquid nitrogen and then homogenized in 0.17 mM (physiological concentration) glucose 6-phosphate (Amersham Biosciences, Piscataway, NJ) into glycogen (μmol.g−1.min−1). The activities of the G-6-P independent form (GS-I) was determined in the presence of 0.17 mM (physiological concentration) G-6-P. The total GS activity (GS-D) was measured in the presence of 7.2 mM G-6-P. The ratios of GS-I to GS-D represent the fraction of GS in the active form.

**Statistical analysis.** All data are presented as means ± SE. Statistical comparisons between two different groups were made using a two-tailed, unpaired t-test. All statistical analyses were performed using SigmaStat 3.0 software. A statistical significance is defined as a P value of ≤ 0.05.

**RESULTS**

**Effect of intermittent hypoxia on blood glucose concentrations, myocardial glycogen and G-6-P content, and insulin concentrations.** Blood glucose concentrations increased significantly after intermittent hypoxia in both fasted and fed animals, and the increase was much more dramatic in fed rats (Fig. 1A). Myocardial glycogen content was higher in fed than in fasted control rats after >1 h of mechanical ventilation (7.22 ± 0.44 vs. 5.50 ± 0.51 mg/g heart tissue, P < 0.03; Fig. 1B). Intermittent hypoxia significantly decreased myocardial glycogen content in fed animals (P < 0.0005), consistent with hypoxia-induced glycogenolysis. Glycogen content also decreased in fasted rats; however, this decrease was not statistically significant (P = 0.1). The myocardial content of G-6-P (an intermediate metabolite in the glycogen synthesis and degradation pathway) was comparable between fasted and fed control animals (0.17 ± 0.02 vs. 0.14 ± 0.01 μmol/g tissue, P = 0.21) and increased about threefold in fasted (0.51 ± 0.10 μmol/g tissue, P < 0.02) and fed (0.38 ± 0.06 μmol/g tissue, P < 0.002) rats after intermittent hypoxia (Fig. 1C). The G-6-P contents were not significantly different between the fasted and fed hypoxia groups (P = 0.348).

The plasma insulin concentrations were higher in fed than in fasted animals (239 ± 30 vs. 119 ± 12 μM, P < 0.015). Hypoxia induced a further decrease in insulin concentrations in fasted rats (86 ± 21 μM, P < 0.04). On the contrary, the plasma insulin concentrations did not change significantly in fed rats after hypoxia (230 ± 37 μM, P = 0.24). This is consistent with previous reports that hypoxia decreases insulin...
secretion by isolated islets (11) and inhibits pancreatic insulin response to hyperglycemia via sympathetic inhibition in vivo (22).

Basal mean arterial blood pressure was higher in fed than in fasted control animals (129 \pm 3 \text{ vs. } 101 \pm 4 \text{ mmHg}, P < 0.0005) and stayed stable throughout the experiments. Hypoxia induced a significant increase in mean arterial blood pressure in fasted rats from 102 \pm 4 \text{ to } 120 \pm 4 \text{ mmHg} (P < 0.03). Although mean arterial blood pressure also increased in fed animals after hypoxic episodes, it was statistically nonsignificant (126 \pm 6 \text{ vs. } 141 \pm 8 \text{ mmHg}, P = 0.25). The increases in the mean arterial blood pressure were comparable between the fasted (by an average of 18 mmHg) and fed animals (average increase of 15 mmHg).

**Fig. 1.** Effect of intermittent hypoxia on blood glucose, cardiac glycogen, and glucose 6-phosphate (G-6-P) content. A: blood glucose concentrations. Compared with respective control: \( \ast P < 0.05, \ast\ast P < 0.00001 \); compared with respective fasting group: \#P < 0.0002. B: cardiac glycogen content. Compared with control: \( \ast P < 0.0005 \); compared with respective fasting group: \#P < 0.03. C: cardiac G-6-P content. Compared with respective control: \( \ast P < 0.02, \ast\ast P < 0.002 \); compared with fasting hypoxia group: \#P = 0.348.

Effect of intermittent hypoxia on myocardial GS activity. As shown in Fig. 2, intermittent hypoxia significantly stimulated GS in both fasted and fed animals. However, this stimulatory effect was much more dramatic in fed than in fasted rats. The portion of GS in the active form increased by \( \sim 38\% \) in fasted rats (from 0.13 \pm 0.01 to 0.18 \pm 0.02, \( P < 0.05 \)) compared with an \( \sim 130\% \) increase in fed rats (from 0.18 \pm 0.03 to 0.41 \pm 0.08, \( P < 0.03 \)).

**Fig. 2.** Effect of intermittent hypoxia on myocardial glycogen synthase activity. Compared with respective control; \( \ast P < 0.05, \ast\ast P < 0.03 \).

Effect of intermittent hypoxia on Akt phosphorylation in myocardium. Basal Akt phosphorylation was comparable (0.28 \pm 0.08 vs. 0.29 \pm 0.04, \( P = 0.85 \); Fig. 3) between fasted and fed control rats (expressed as the ratio of phospho-Akt/total Akt). In fasted rats, hypoxia did not alter Akt phosphorylation. However, in fed animals hypoxia significantly increased the fraction of phosphorylated Akt (0.44 \pm 0.04, \( P = 0.03 \)).

**Fig. 3.** Effect of intermittent hypoxia on rat myocardial Akt phosphorylation. Intermittent hypoxia significantly enhanced myocardial Akt phosphorylation at Ser\(^{473} \) only in fed animals. Compared with control: \( \ast P = 0.03 \); compared with respective hypoxia group: \#P < 0.02.

Effect of intermittent hypoxia on GSK-3\( \beta \) phosphorylation in myocardium. Because GSK-3\( \beta \) is downstream of Akt and regulates GS activity, we further analyzed the phosphorylation state of GSK-3\( \beta \). Phosphorylation of GSK-3\( \beta \) at Ser\(^{9} \) inhibits its kinase activity, which in turn leads to increased GS activity (14). As shown in Fig. 4, the phosphorylation status of GSK-3\( \beta \) was comparable between fasted and fed control rats (0.83 \pm 0.07 vs. 0.71 \pm 0.06, \( P = 0.25 \)), and intermittent
Hypoxia did not induce significant change in GSK-3β phosphorylation in either fasted or fed rats. Therefore, the changes in GS activity could not be attributed to inactivation of GSK-3β.

Effect of intermittent hypoxia on myocardial PP1 activity. In fasted rats, hypoxia did not induce a significant change in PP1 activity. However, in fed rats the PP1 activity was significantly higher in the hypoxia group compared with control rats (3.1 ± 0.2 vs. 4.0 ± 0.3 nmol·μg protein⁻¹·min⁻¹, P < 0.04; Fig. 5). The fed hypoxic group also had significantly higher PP1 activity than the fasted hypoxic rats (P < 0.02).

DISCUSSION

Using a rat model of intermittent ventilatory arrest, the present study examined the modulatory role of feeding status on GS activation in the myocardium in response to intermittent hypoxia. The results showed that in fed animals intermittent hypoxia induced significant glycogenolysis, activated GS, stimulated Akt phosphorylation without increasing GSK-3β phosphorylation, and enhanced PP1 activity. It was remarkable that these changes all occurred at constant circulating insulin concentration. In contrast, in fasted animals the extent of GS activation was much less dramatic, and it was not associated with increased Akt phosphorylation, GSK-3β phosphorylation, or PP1 activity. There was comparable myocardial G-6-P content between the fasted and fed rats. These findings indicate that fasting attenuates intermittent hypoxia-stimulated GS activation in rat myocardium independent of G-6-P content.

Consistent with previous data from fed rats (26, 37), we observed a significant myocardial glycogen breakdown during hypoxia in fed animals in the present study, which was associated with a significant increase in the GS activity. However, in fasted rats the glycogen content decreased only slightly (statistically insignificant), and the activation of GS was much less dramatic (~38% increase in fasted rats vs. ~130% increase in fed rats) after hypoxia. Prior evidence has suggested that myocardial GS activation is a rapid, proportionate, wholly intrinsic response to glycogen breakdown in the myocardium during periods of hypoxia (37). Therefore, the rapid glycogenolysis may have contributed significantly to the myocardial GS activation during hypoxia in the fed group and the difference in GS activation between the fed and the fasted rats in the present study. It is also possible that fasted animals exhibited less glycogenolysis and GS activation due to less severe hypoxic insult and/or less mechanical workload than fed rats. Though we did not measure blood PO2 and pH during hypoxia, it is unlikely that the extent of hypoxia differed between fasted and fed rats in the present study, since all animals were subjected to intratracheal intubation and equal length of ventilatory arrests. A previous study (37) has demonstrated that cardiac mechanic work is a major determinant of hypoxic GS activation, since pretreatment of rats with β-blocker propranolol blunts the magnitude of glycogenolysis and GS activation during subsequent hypoxia. That hypoxia induced comparable increases in the mean arterial blood pressure in fasted (by an average of 18 mmHg) and fed animals (average increase of 15 mmHg) suggests that the workload in the fasted rats was at least not dramatically lower than that in the fed rats.

Previous evidence (4, 12, 41, 42) suggests that fasting increases myocardial glycogen content. We have carried out additional experiments to examine the impact of feeding status on myocardial glycogen content and GS activity in rats, and our results confirmed that cardiac glycogen content was significantly higher in the overnight-fasted rats than in the ad libitum fed rats (9.53 ± 0.74 vs. 5.83 ± 0.18 mg/g heart tissue, n = 6 for each group, P < 0.003). On the contrary, myocardial GS activity was much lower in the overnight-fasted than ad libitum fed rats (0.14 ± 0.04 vs. 0.26 ± 0.03, n = 6 for each group, P < 0.05). Although the myocardial GS activity is much higher in fed rats after three episodes of intermittent hypoxia (Fig. 2), the glycogen content in the myocardium was not higher than that in the fasted rats (Fig. 1B). This is secondary to the lack of time for glycogen reaccumulation to occur. Laughlin et al. (26) have previously demonstrated that it takes ~40 min to replenish myocardial glycogen following intermittent hypoxia.

In rats subjected to intubations and mechanical ventilation, we observed a lower myocardial glycogen content in the fasted than in the fed control rats. This is not entirely surprising because fasted rats have lower hepatic glycogen stores than fed rats, and it is therefore very likely that fasted rats may have relatively more enhanced myocardial glycogenolysis to fulfill

![Phospho-GSK-3β and Total GSK-3β](image)

**Fig. 4.** Effect of intermittent hypoxia on GSK-3β phosphorylation in rat myocardium. Intermittent hypoxia had no significant impact on myocardial GSK-3β phosphorylation in either fasted or fed rats.

![Effect of intermittent hypoxia on myocardial PP1 activity](image)

**Fig. 5.** Effect of intermittent hypoxia on myocardial protein phosphatase 1 (PP1) activity. Intermittent hypoxia significantly increased myocardial PP1 activity only in fed group. Compared with control: *P < 0.04; compared with fasting hypoxia group: #P < 0.02.
myocardial energy needs during mechanical ventilation. This lower myocardial glycogen content in the fasted rats may also explain the discrepancy between the present study and previous reports using isolated hearts and low-flow ischemia, where glycogen degradation in the fasted rats was either similar (12) or higher (4) than in the fed rats. However, important distinctions exist between these two models, such as in vivo vs. in vitro, intact animals vs. isolated hearts, intact vs. low coronary flows, and short duration (90 s) vs. longer duration (15 to 30 min), making direct comparisons unrealistic.

Both hypoxia and myocardial ischemia stimulate cardiomyocytic glucose transport (13, 20) and glycogenolysis (26, 35–37), leading to increased intracellular G-6-P concentrations. As stated before, G-6-P not only allosterically activates GS, it also changes the configuration of GS, making it a better substrate for PP1. When assayed in vitro, McNulty and Luba (36) demonstrated that GS phosphatase activity increased by ~60% after the G-6-P concentration in the assay mixture was increased from 0.17 to 0.5 mM. This strongly suggests that increased local G-6-P plays an important role in activating GS. In the present study, the G-6-P content increased comparably between fed and fasted rats during hypoxia. Inasmuch as GS was also significantly activated in the fasted rats, the extent of GS activation was much less dramatic in the fasted rats than the fed ones. Therefore, an increase in the local G-6-P content during hypoxia was only partially responsible for hypoxia-induced GS activation; at least it is the case in the fed animals that other mediators of GS activity must have also contributed to hypoxia-stimulated GS activation.

Akt is a key intermediate in the PI 3-kinase signaling pathway, and its activation leads to many biological effects, including the regulation of cell survival and apoptosis (9, 10, 15, 34). Activation of Akt stimulates the prosurvival signaling and inhibits apoptotic signaling. Several groups (2, 8, 51) have previously demonstrated that myocardial ischemia or exposure of cultured cells to hypoxia induces a transient increase in Akt phosphorylation. Both agents activations PI 3-kinase/Akt signaling and transfecting myocardium with adenovirus containing constitutively active Akt have been shown to reduce myocardial infarct size in rodent ischemia-reperfusion model (1, 15, 23, 34). In the present study, we observed a significant increase in Akt phosphorylation after intermittent hypoxia in the fed animals, but not in the fasted rats. This suggests that fasting attenuates the animals’ capability to increase Akt phosphorylation via as-yet-undefined mechanism(s), and whether this translates into less protection against intermittent hypoxia during fasting warrants further investigation.

Akt-GSK-3 signaling pathway plays a very important role in insulin-stimulated GS activation in muscle (21, 29, 33, 46). It is of interest that intermittent hypoxia enhanced the phosphorylation of Akt, but not that of GSK-3 in the myocardium of fed animals in the present study. This raises a possibility that intermittent hypoxia-induced Akt signaling may differ from that of insulin. This is not surprising, because previous studies (20) have shown that insulin and ischemia stimulate glycogenolysis in heart muscle by acting on the same targets, but through different and opposing signaling pathways. To further clarify this issue, we quantitated the phosphorylation status of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and 70-kDa ribosomal protein S6 kinase (p70S6K), two downstream effectors of Akt in the PI 3-kinase/Akt signaling pathway. Insulin stimulates the phosphorylation of 4E-BP1 and p70S6K by activating Akt and mammalian target of rapamycin (25, 27, 32, 43). Consistent with no increase in GSK-3β phosphorylation, intermittent hypoxia did not change the phosphorylation status of either 4E-BP1 or p70S6K (data not shown). Thus Akt was activated, but it was uncoupled from its best-characterized effectors.

PP1 has many important biological functions in vivo, including the regulation of glycogen synthesis (29, 40). PP1 dephosphorylates GS and, hence, enhances its activity and increases glycogen synthesis. PP1 activity is regulated by many factors, such as the amount and/or phosphorylation status of various regulatory proteins, and the PI 3-kinase signaling pathway (3, 5, 17–19, 30, 31, 40). In the present study, we observed a significant increase in PP1 activity in hearts of the fed hypoxic animals. As discussed above, Akt phosphorylation was also significantly increased in this group of rats after hypoxia. Whether the increase in PP1 activity was coupled to increased Akt phosphorylation in the present study remains unclear and deserves further investigation. The fact that, in fasted rats, Akt was not activated and neither was PP1 activity increased suggests such a possible link. There was no change in the amount of PP1c protein and PP1 regulatory subunit G protein after hypoxia (data not shown). Therefore, the activity change was probably due to regulatory phosphorylation. Because G-6-P content increased to an equal extent in both fed and fasted groups and G-6-P can allosterically activate GS and make GS a better substrate for PP1, we speculate that the combination of high G-6-P and increased PP1 activity contributed to the much higher GS activation seen in the fed hypoxic group.

In summary, our data indicate that, in fed animals, intermittent hypoxia induces significant glycogenolysis, activates myocardial GS, stimulates Akt phosphorylation, and enhances PP1 activity. Although GS activity was also significantly increased in fasted animals, it was much less dramatic and was not associated with an increase in Akt phosphorylation or PP1 activity. This difference is not attributable to local G-6-P content, which was comparable between fasted and fed rats. We conclude that hypoxia activates GS in fed animal myocardium through a combination of rapid glycogenolysis, elevated local G-6-P content, and increased PP1 activity, whereas fasting attenuates this action independent of G-6-P.

ACKNOWLEDGMENTS

We thank Dr. Eugene J. Barrett for helpful discussion and critical reading of this manuscript.

GRANTS

This work was supported by a research grant from the American Diabetes Association (Z. Liu) and National Institutes of Health Grants RR-15540 (Z. Liu), AT-01304 (D. L. Brautigan), and P30-DK-063609 to the University of Virginia Diabetes Endocrinology Research Center.

REFERENCES


