Inability of Hyperglycemia to Counter the Ability of Glucagon to Increase Net Glucose Output and Activate Glycogen Phosphorylase in the Perfused Rat Liver

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We examined the ability of hyperglycemia to alter the ability of glucagon to activate phosphorylase and stimulate glucose output in perfused rat livers. The livers were perfused with a Krebs-Henseleit buffer containing washed bovine erythrocytes and albumin at 37°C for 90 or 120 minutes. In the first 60 minutes, the livers were perfused with insulin (10 μU/mL), glucagon (11 pg/mL), and glucose (105, 230, or 440 mg/dL). In the second 30 or 60 minutes, the glucagon concentration in the perfusate was elevated to 44, 88, 176, or 352 pg/mL or the infusion of glucagon was terminated. In the presence of glucose at 105 mg/dL, the termination of glucagon infusion decreased phosphorylase activity and glucose output. In contrast, the elevation of glucagon from 11 to 352 pg/mL activated phosphorylase and increased net glucose output in a dose-dependent manner. A linear correlation was observed between net glucose output and glycogen phosphorylase activity. An elevation of the glucose concentration from 105 to 230 or 440 mg/dL decreased net glucose output from 0.81 ± 0.03 to 0.66 ± 0.09 or −0.004 ± 0.21 mg/min/100 g body weight, respectively, but did not cause significant change in phosphorylase-a activity (105 mg/dL, 50 ± 11; 230 mg/dL, 40 ± 2; 440 mg/dL, 69 ± 3 mU/mg protein). The elevation of the glucagon concentration from 11 to 88 μU/mL in the presence of glucose at 105, 230, or 440 mg/dL increased net glucose output by 0.65 ± 0.06, 0.61 ± 0.08 or 0.64 ± 0.26 mg/min/100 g body weight and raised phosphorylase-a activity by 65 ± 5, 82 ± 11, or 55 ± 4 mU/mg protein, respectively. These results suggest that hyperglycemia decreases net hepatic glucose output without changing the activity of phosphorylase-a. Further hyperglycemia does not alter the ability of glucagon to activate phosphorylase or to stimulate net hepatic glucose output.

GLUCAGON, INSULIN, and the plasma glucose level play major roles in regulating glucose production by the liver.[1] Glucagon is the primary stimulator of glucose production and its effect is countered by insulin. Induction of hyperglycemia has been shown to cause a rapid suppression of net hepatic glucose output in vivo[1-4] and in vitro.[5,6] In vivo studies have suggested that, unlike insulin, hyperglycemia might not affect the ability of a physiological increment in glucagon to increase glucose output by the liver.[7,8] It would thus appear that while hyperglycemia can suppress basal glucose production, it can not interfere with the ability of glucagon to induce additional glucose output.

Glucagon increases glucose production primarily by stimulating glycogenolysis via an activation of phosphorylase.[1,5,9,10] Glucose is also believed to inhibit glycogenolysis by binding to phosphorylase-a and then facilitating the conversion of phosphorylase-a to phosphorylase-b by protein phosphatase-1.[5,9-12] The dephosphorylation of phosphorylase-a releases the inhibitory effect of active phosphorylase on glycogen synthase phosphatase, which then activates glycogen synthase.[5,9,10] In in vivo studies, however, an ability of hyperglycemia to suppress the activity of phosphorylase has been controversial. Stalman et al,[13] Newman and Armstrong[14] have shown marked inactivation of phosphorylase and activation of glycogen synthase following intravenous injection of glucose in anesthetized fed or fasted rats. By contrast, Niewoehner et al[15,16] and Nuttall et al[17] reported a slight decrease or no change in phosphorylase activity following intravenous or oral administrations of glucose in anesthetized fasted rats, despite simultaneous activation of glycogen synthase by these treatments. Since glucose administration in vivo is followed by changes in plasma insulin and glucagon levels,[16] it is difficult to know if there was a direct effect of hyperglycemia on the liver. On the other hand, liver perfusion allows for examination of the direct effect of glucose on the liver without the complication of other hormonal changes. In most in vitro experiments, however, the effects of large changes in glucose have been examined in the absence of glucagon and insulin. One in vitro study reported that the phosphorylase-a activity to be lowered by glucose.[18] Another showed that the extent of the decrease in phosphorylase-a activity caused by the addition of 40 mmol/L glucose was reduced by simultaneous addition of glucagon.[19] The effect of glucose on the ability of glucagon to activate phosphorylase cannot, however, be evaluated from these studies because they did not show phosphorylase activity in the presence of 10 mmol/L glucose alone,[18] or the extent of the change in phosphorylase activity by the addition of glucagon alone.[19] Thus it is not possible to say whether the ability of glucagon to activate phosphorylase and increase glucose output by the liver is compromised in a hyperglycemic state.

We previously described a perfusion system which sustained the liver in vivo sensitivity to insulin and glucagon over the physiological range.[20] Using this system, we perfused the liver in fed rats with physiological concentrations of glucose, glucagon, and insulin and examined the ability

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of physiological changes in glucose to alter the effect of glucagon on glucose output and phosphorylase activation.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250 to 310 g were maintained on a 12-hour day-night rhythm with free access to tap water and standard laboratory chow. Experiments were performed between 11:00 AM and 1:00 PM. The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care Guidelines, and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee, Nashville, TN.

Liver Perfusion

Surgery and perfusion were performed essentially as described previously.[20,21] After anesthesia with sodium pentobarbital (50 mg/kg body weight, intraperitoneally), the portal vein and inferior vena cava were canulated with polyethlene tubing. Livers were perfused by flow-through with 37°C oxygenated Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 3% bovine serum albumin and 20% washed bovine erythrocytes. The flow rate was 15 to 17 mL/min.

Experimental Protocol

Livers were perfused with insulin (10 μU/mL), glucagon (11 pg/mL), and glucose (105, 230, or 440 mg/dL) for 60 minutes. A portion of the left lobe of the liver was removed and frozen with liquid nitrogen and the concentration of glucagon was increased to 44 to 352 pg/mL. Liver perfusion was then continued for 30 or 60 minutes. At the cessation of the experiment, the livers were clamped and frozen with tongs cooled in nitrogen. Blood samples were taken from the portal vein and inferior vena cava at appropriate times. Hepatic glucose balance was determined as the product of the perfusate flow-rate and the hepatic-portal venous difference for glucose across the liver.

Analysis

Glucose was assayed using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Phosphorylase-a activity was determined by a modified technique of Hutson et al.[19,20] Protein concentrations were determined by the method of Lowry et al.[22]

Materials

Glucagon and insulin were purchased from Eli Lilly (Indianapolis, IN) and Squidd-Novo (Princeton, NJ), respectively.

RESULTS

Effect of Glucagon on Net Hepatic Glucose Balance and Phosphorylase-a Activity

Figure 1 shows the time course of the change in hepatic glucose production, which resulted from the termination or an eightfold increase in glucagon infusion. With basal insulin (10 μU/mL) and glucagon (11 pg/mL), hepatic glucose production decreased slowly through the experimental period. The termination of glucagon infusion resulted in a further decrease in hepatic glucose production. By contrast, when glucagon increased from 11 to 88 pg/mL, net

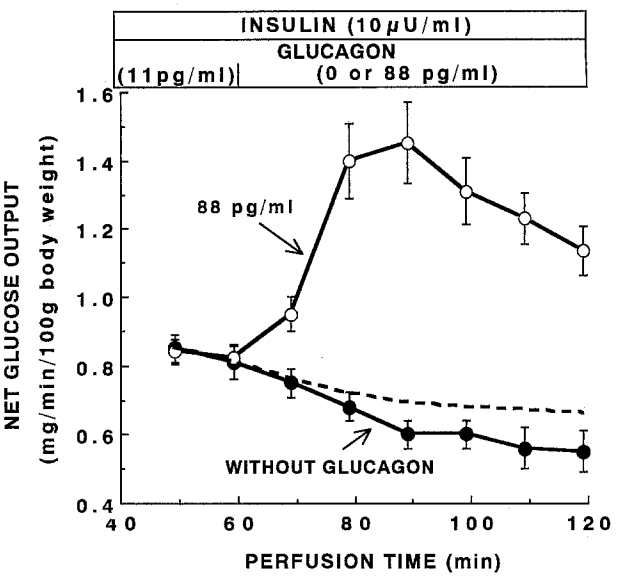

Fig 1. Time course of a change in net glucose output from rat livers perfused with various concentrations of glucagon. Livers were perfused with glucose (105 mg/dL), insulin (10 μU/mL), and glucagon (11 pg/mL) during the first 60 minutes and then the concentration of glucagon was elevated to 88 pg/mL or the infusion of glucagon was terminated. (——) Level of net glucose uptake in the liver perfused with glucose (105 mg/dL), insulin (10 μU/mL), and glucagon (11 pg/mL) during the second 60 minutes. Values are means ± SEM of 5 experiments.

glucose production increased progressively during initial 30 minutes and then decreased slowly. Figure 2 shows the net glucose production rate and the activity of phosphorylase-a in liver perfused with various concentrations of glucagon. Both parameters increased as glucagon was elevated from 11 to 352 pg/mL. As shown in Fig 3, a significant correlation ($r = .785, P < .01$) existed between the rate of net production of glucose and the activity of glycogen phosphorylase.

Effect of Increasing the Concentration of Glucose on Net Glucose Production and Phosphorylase-a Activity

In the perfused rat liver exposed to basal levels of insulin (10 μU/mL) and glucagon (11 pg/mL), elevation of the perfusate concentration of glucose from 105 to 230 or 440 mg/dL suppressed hepatic glucose production by 20% and 100%, respectively (Fig 4). However, the elevated glucose concentrations did not affect phosphorylase-a activity. An eightfold increase in glucagon produced similar increments in glucose output and phosphorylase activation, regardless of the coexistent glucose concentration (Fig 4). The effect of hyperglycemia per se was on the baseline glucose output rate, rather than on the glucagon stimulated output.

DISCUSSION

The results of the present study confirmed our in vivo observation that the ability of a physiological increment in

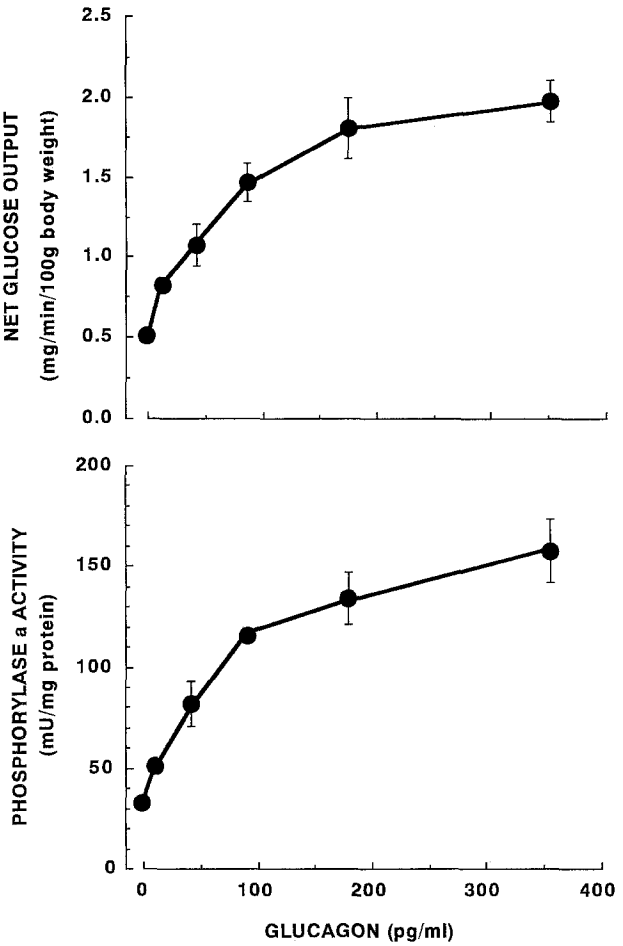

Fig 2. Effect of glucagon on the activity of glycogen phosphorylase and rate of net glucose production in livers perfused with glucose at 105 mg/dL. Livers were perfused as described in Fig 1. Results are the rates or the activities 30 minutes after the change in the concentration of glucagon. Values are means ± SEM of 5 to 12 experiments.

glucagon to increase glucose output by the liver was unaffected by the prevailing glucose level.[7,8] The results suggest that this finding can be explained by the failure of hyperglycemia to alter the ability of glucagon to activate phosphorylase. The present results also suggest that hyperglycemia can induce a decrease in net glucose production with unchanged phosphorylase activity.

The perfused rat liver preparation used in this study has been shown to be sensitive to physiological concentrations of insulin in terms of inhibition of glucagon-stimulated glucose output and activation of phosphorylase.[20] Glucagon also regulated phosphorylase activity and glucose output even at basal concentrations (11 pg/mL), as indicated by the decrease in glucose production and phosphorylase activity that occurred on termination of the infusion of basal glucagon (Figs 1 and 2). The concentrations of insulin and glucagon used in the present study are somewhat below the levels of these hormones reported to occur normally in portal venous blood.[23] The sinusoidal insulin level is usually about 20 μU/mL, while the sinusoidal glucagon level is approximately 35 pg/mL.[24]

The activity of phosphorylase-a is determined by the balance of the opposing actions of phosphorylase kinase, which phosphorylates and activates phosphorylase, and phosphorylase phosphatase, which dephosphorylates and inactivates phosphorylase. Glucagon has been well established to activate phosphorylase via activation of protein kinase-A and subsequent activation of phosphorylase kinase following elevated concentration of cyclic adenosine monophosphate (AMP). Glucose has been reported to have no effect on the glucagon-induced increase in cyclic AMP and subsequent activation of protein kinase-A and phosphorylase-b kinase.[21,25-27] Furthermore, the administration of glucagon has been reported to induce phosphorylation and activation of inhibitor-1,[28] which inhibits the activity of protein phosphatase-1.[29] Protein phosphatase-1 plays a major role in dephosphorylating phosphorylase-a in the parenchymal cell.[11] Phosphorylase-a by itself has also been shown to have an inhibitory effect on protein phosphatase-1.[11] By contrast, glucose inhibits phosphorylase-a by forming a glucose-phosphorylase complex, which is a better substrate for protein phosphatase-1. Indeed, the effect of glucose to inhibit phosphorylase has been reported to be dependent on the activity of protein phosphatase-1.[30] Since glucagon inhibits protein phosphatase-1, glucose might be less effective in inhibiting phosphorylase in the presence of glucagon. Pretreatment with glucagon has been showed to cause a marked decrease in the ability of intravenously injected glucose to reduce phosphorylase activity in an in vivo study using fed rats.[19]

Glucose administration induces an increase in glycogen synthase activity[5,9,10,31,32] and glycolytic flux[33,34] and those

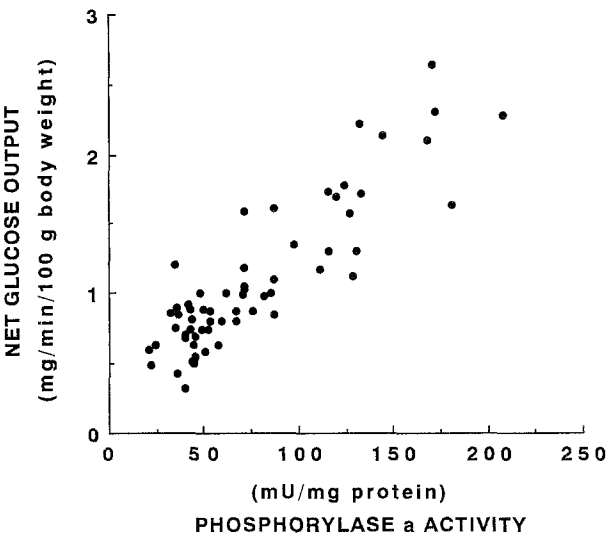

Fig 3. Correlation between the activity of glycogen phosphorylase-a and rate of net glucose production in perfused liver. Livers were perfused as described in Fig 1, except in some experiments glucagon was elevated from 11 to 44, 176, or 352 pg/mL. Results are the rates and the activities after 30 minutes of the change in the concentration of glucagon.

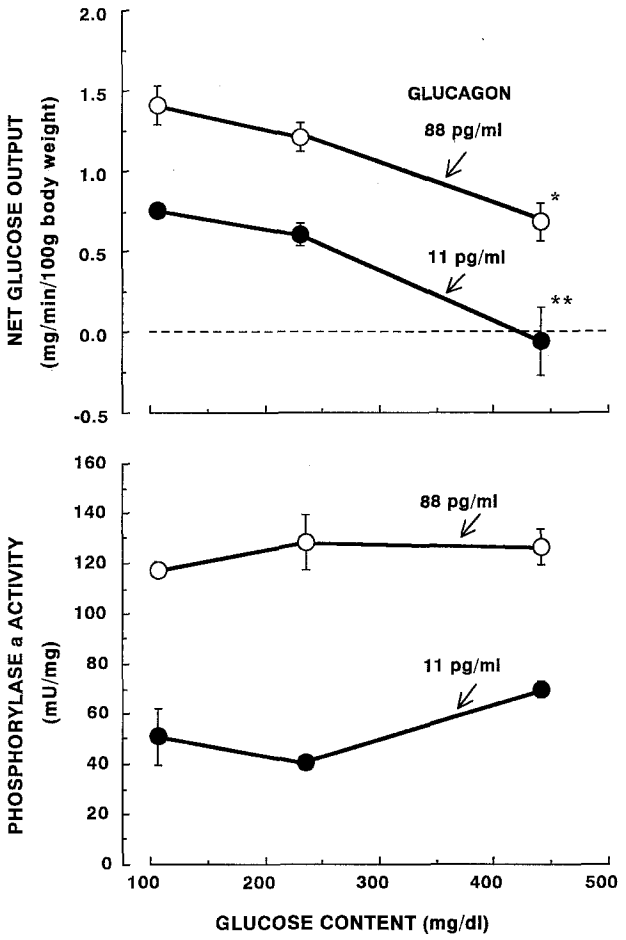

Fig 4. Effect of hyperglycemia on the rate of net glucose output and glycogen phosphorylase-a activity in the rat liver perfused with 11 or 88 pg/mL. Livers were perfused as described in legend to Fig 1, except that in some experiments glucose added at 230 or 440 mg/dL instead of 105 mg/dL. Results are the rates or the activities 30 minutes after the change in the concentration of glucagon. Values are mean ± SEM of 5 to 8 experiments. *$P < .05$, **$P < .01$ *v* corresponding value for livers perfused with glucose concentration of 105 mg/dL.

effects of glucose are secondary to an increase in hexose-6-phosphate.[31-33] Increased hepatic content of glucose-6-phosphate activates glycogen synthase phosphatase catalyzing the activation of glycogen synthase, and inhibits kinase(s) catalyzing the inactivation of glycogen synthase.[35,36] Since fructose-6-phosphate is the substrate of fructose-6-phosphate,2-kinase and an inhibitor of fructose-2,6-bisphosphatase,[37] the increase in the hexose phosphate may result in increased fructose-2,6-phosphate, which is the most potent activator of phosphofructokinase. Recently, glucose has been also reported to increase fructose-2,6-phosphate via dephosphorylation of fructose-6-phosphate,2-kinase:fructose-2,6-phosphatase.[38] On the other hand, an increase in a content of glucose-6-phosphate has no effect on the activity of phosphorylase-a.[31] Glucose administration to isolated rat hepatocytes, perfused rat livers, or anesthetized mice causes an increase in hexose-6-phosphates[18,33,34,39-41] and the rate of phosphorylation of glucose by glucokinase depends on the concentration of glucose.[39,40,42] Glucokinase is characterized by a Michaelis constant (Km) that exceeds the usual concentration of glucose. Glucagon appears to be ineffective in modifying glucose phosphorylation by glucokinase.[40] An increase in glucose-6-phosphate content,[41] activation of glycogen synthase,[41] increased flux of glycolysis,[34,42] and intracellular glucose recycling[40] induced by the administration of glucose in the perfused rat liver could have become pronounced when glucose was added above 10 mmol/L. Furthermore, it has been reported that preincubation of hepatocytes with 10 to 30 mmol/L glucose induced a translocation of glucokinase to an alternative site that might favor the partitioning of glucose-6-phosphate toward glycogen.[43] Since in the present study the glucose-induced suppression of net glucose output was observed clearly when glucose was added to obtain a concentration greater than 10 mmol/L (Fig 4), glucose-induced suppression of net glucose output with unchanged activity of phosphorylase-a might be due to increased recycling in glycogen/glucose-6-phosphate and/or in glycolytic pathway.

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