Opposite Effects of Hyperglycemia and Insulin Deficiency on Liver Glycogen Synthase Phosphatase Activity in the Diabetic Rat

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The specific effect of hyperglycemia on the reported decrease in liver glycogen synthase phosphatase activity was studied in STZ-induced diabetic rats with normal fasting insulinemia. Four groups of animals were investigated: control (nondiabetic), diabetic hyperglycemic (STZ), diabetic normoglycemic (STZ followed by 3-day phloridzin treatment), and a diabetic normoglycemic group injected with glucose to reinstate hyperglycemia. None of the treatments significantly altered fasting plasma insulin and glucagon concentrations. We found that hepatic synthase phosphatase activity decreased in STZ-induced diabetic rats and was further markedly reduced when glycemia was normalized in the diabetic animals. This additional decrease in phosphatase activity was almost fully reversed when hyperglycemia was restored by acute glucose infusion of the normoglycemic diabetic rats. In parallel, the levels of liver G6P and F6P were markedly reduced in the diabetic normoglycemic rats and restored with reinstatement of hyperglycemia. In contrast, liver microsomal glucose-6-phosphatase activity was enhanced and glucokinase activity was lowered in all diabetic groups, regardless of glycemia. Our results indicate that hyperglycemia per se counteracts part of the loss of hepatic synthase phosphatase in diabetic animals and provokes the stable conversion of synthase phosphatase from a less active to a more active form. *Diabetes* 42:363–66, 1993

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STZ, streptozocin; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; RIA, radioimmunoassay.

Liver synthase phosphatase activity is lowered in insulin-deficient rodents (1–5). The nature and origin of this defect are not well defined. The decrease in synthase phosphatase is dependent on the severity of diabetes (3–5), therefore on the degree of insulin deficiency (3,5), and involves the glycogen-bound form of the enzyme (4,6). This defect has been attributed to a loss of synthase phosphatase protein (7–9), but the possibility that a stable covalent modification (inactivation) of the enzyme is involved as well cannot be discarded. This possibility is particularly relevant with respect to recent findings on the control of rat skeletal muscle synthase activation by insulin that has been shown to occur as a result of phosphorylation of glycogen synthase phosphatase by an insulin-stimulated protein kinase (10). Some evidence suggests that in the liver, too, insulin can promote glycogen synthase activation and glycogen synthesis by a direct activation of the phosphatase independently of the action of the hormone to decrease cyclic AMP–dependent protein kinase activity (11–14).

Because glucose and phosphorylated hexoses (15–17) increase liver glycogen synthase phosphatase activity in vitro, it could be that hyperglycemia limits the decreased activity of the enzyme in the STZ-induced diabetic rat. To outline the selective role of hyperglycemia on the regulation of liver synthase phosphatase, we normalized blood glucose in the diabetic hyperglycemic and normoinsulinemic rat model by administering phloridzin and restored hyperglycemia in the phloridzin-treated diabetic rats by intravenous infusion of glucose without changes in fasting plasma insulin concentration. The diabetic hyperglycemic and normoinsulinemic rat is a unique model to investigate the selective role of acute hyperglycemia because plasma insulin is near normal in the postabsorptive state, and acute increase of glycemia does not significantly alter the plasma insulin and gluca-

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TABLE 1
Characteristics of diabetic animals and effects of phloridzin and glucose administration

	Control	Diabetic	Diabetic-phloridzin	Diabetic-phloridzin-glucose
Glycemia (mM)	7.9 ± 1.7	18 ± 2.9*	5.2 ± 0.4†	29 ± 2.2‡
Plasma insulin (pM)	138 ± 23	107 ± 15	74 ± 19	47 ± 8
Plasma glucagon (ng/L)	424 ± 139	290 ± 63	360 ± 52	600 ± 140

Rats were injected with 65 mg of STZ/kg, diluted in saline (diabetic rats). Some diabetic rats were treated with phloridzin as indicated in ref. 18. In addition, 50% of phloridzin-treated diabetic rats received several bolus injections of glucose, and parameters were determined 40 min later. No diabetic animal was ketotic at any time of study. Values are means ± SE for 4 animals in each group except for plasma glucagon in control group (n = 3).
*P < 0.05 vs. control group.
†P < 0.05 vs. diabetic group.
‡P < 0.05 vs. diabetic-phloridzin group.

gon concentrations. However, the animals are insulin deficient in the postprandial state and, therefore, hyperglycemic. Thus, they retain some effects of chronic insulin deficiency. With this model, we show that normalization of glycemia in diabetic animals resulted in a further decrease in hepatic glycogen synthase phosphatase activity, and that this decrease was reversible with reinstatement of hyperglycemia.

RESEARCH DESIGN AND METHODS

Animals and preparation of liver filtrates. Male specific-pathogen–free Sprague-Dawley rats from Charles River (Montreal, Quebec, Canada) were rendered diabetic by the administration of a low dose of STZ (65 mg/kg) dissolved in saline. In this peculiar diabetic model, the postprandial insulin response is impaired, but the basal (fasting state) insulin secretion remains normal for at least 10 days (18). Some diabetic rats were rendered normoglycemic by 3-day phloridzin treatment as detailed in a previous study (18). The group administered STZ and phloridzin was divided into two groups; 50% of these rats were fitted with a catheter in the jugular vein under pentobarbital anesthesia (1.0 ml/kg) 3 days before death. The day of the experiment, the conscious rats received intermittent bolus injection(s) of glucose over a 40-min period through the jugular catheter to re-establish hyperglycemia (Table 1). Plasma glucose levels were monitored every 5 min to ensure that glycemia was >20 mM during the infusion. All groups were killed by decapitation after an overnight fast. The abdomen was incised to expose the liver, which was sampled two different ways: one part was rapidly frozen between aluminium blocks cooled in liquid nitrogen, and a second part was homogenized in a Potter-Elvehjem tube (Thomas, Philadelphia, PA) with 2 vol of ice-cold buffer containing 0.25 M sucrose, 0.5 mM dithiothreitol, 50 mM imidazole, 0.5 mM benzamidine, and 0.5 mM phenylmethyl-sulfonyl fluoride (pH 7.4). Freeze-clamped livers were stored at −80°C until enzyme and metabolite determinations were made. Homogenized livers were centrifuged for 10 min at 8000 g, and 500 μl of the supernatant was applied to a column of Sephadex G-25 medium (15 ml of gel bed) equilibrated in the homogenization buffer. The fraction (700 μl) eluted with Hb (containing 15–20 mg of protein/ml) (filtrate) was used for the synthase phosphatase assay.

Assays of enzymes and metabolites and RIAs. The activity of glycogen synthase phosphatase was determined by measurement of the activation of endogenous (natural) synthase in filtrates incubated at 20°C with 30 mM glucose (19). Alternatively, the rate of activation of exogenous (added) purified dog liver glycogen synthase was measured in the same liver filtrates as described previously (20). One unit of synthase phosphatase converts one unit of endogenous or purified synthase b into synthase a per min in the specified conditions of each assay. Glycogen synthase a was measured as described previously (21). Glucokinase activity was measured after subtraction of the activity of liver hexokinase (22). Glucose-6-phosphatase was assayed at 30°C in intact and detergent-treated liver microsomes in the presence of 0.5 mM [^{14}C]G6P (23). Fluorimetric assay of G6P and F6P was performed in neutralized perchloroacetic liver extracts (24). Plasma glucose was determined with a Beckman glucose analyzer (Palo Alto, CA). Plasma insulin (18) and glucagon (25) were determined by RIA.

Statistical analysis. Results are means ± SE for the indicated numbers (n) of observations. Statistical analysis was performed with Student's t test for unpaired samples. P < 0.05 was considered statistically significant.

RESULTS

STZ-induced diabetic rats were hyperglycemic, and 3-day phloridzin treatment restored normoglycemia (Table 1). Intravenous injections of glucose maintained hyperglycemia in phloridzin-treated diabetic rats for the last 40 min before death. No statistically significant differences were found in fasting plasma insulin concentrations between control and diabetic rats, between diabetic and phloridzin-treated diabetic rats, or between the latter and phloridzin-treated diabetic animals injected with glucose. In addition, the glucagon levels are not significantly different between these experimental groups.

Figure 1C shows that liver glycogen synthase phosphatase activity, determined with natural (endogenous) synthase b substrate, was reduced by 60% in animals

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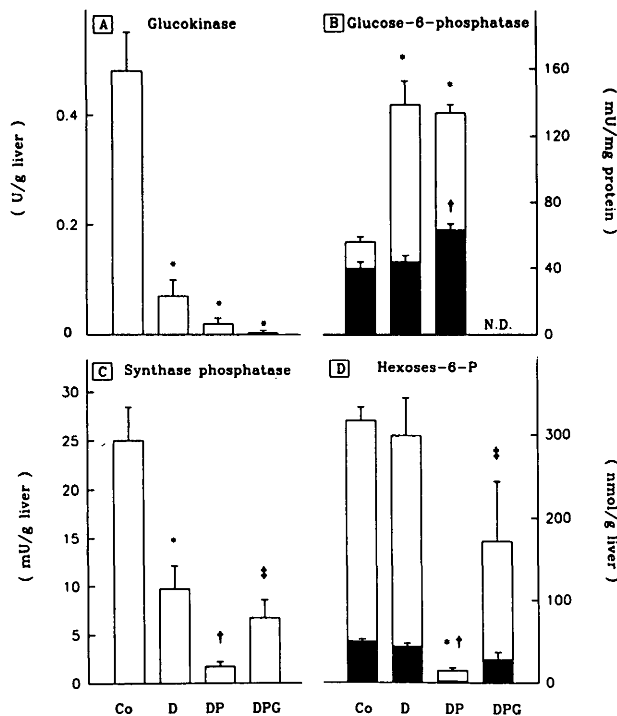

FIG. 1. Effect of diabetes and plasma glucose on liver enzyme activities and metabolite concentrations. Animals were treated as indicated in Table 1. Experimental groups were control group (Co), diabetic group (D), diabetic-phloridzin group (DP), and diabetic-phloridzin-glucose group (DPG). *A*: glucokinase; *B*: glucose-6-phosphatase (■, intact microsomes; □, disrupted microsomes); *C*: synthase phosphatase; and *D*: G6P (□) and F6P (■). ND, not determined. Values are means ± SE for 4–5 animals in each group. **P* < 0.05 vs. control group. †*P* < 0.05 vs. diabetic group. ‡*P* < 0.05 vs. diabetic-phloridzin group.

with diabetes. Treatment with phloridzin to normalize glycemia resulted in a marked further decrease (~90%) in synthase phosphatase activity in diabetic rats. Restoration of hyperglycemia in phloridzin-treated diabetic rats by intravenous glucose administration brought back the synthase phosphatase activity to near the level found in hyperglycemic diabetic animals. Similar results were obtained when synthase phosphatase activity was measured with added (exogenous) purified dog liver synthase *b* as substrate but the magnitude of the changes was ~50% less (data not shown). Liver G6P and F6P (Fig. 1*D*) were unchanged by diabetes despite fasting hyperglycemia, but were severely decreased with normalization of glycemia by phloridzin and were partially restored by acute infusion of glucose to the normoglycemic diabetic animals.

As expected, diabetes decreased and increased the activities of glucokinase and detergent-solubilized microsomal glucose-6-phosphatase, respectively (Fig. 1*A* and *B*). However, in contrast with the drastic effect of normalization of glycemia on synthase phosphatase activity in diabetic rats, the activities of glucokinase and glucose-6-phosphatase were unchanged, and that of glucokinase was not restored by subsequent glucose infusion. A small but significant increase was observed in glucose-6-phosphatase measured in intact liver microsomes from diabetic rats after treatment with phloridzin, consistent with the drop in hepatic G6P measured under the same conditions.

DISCUSSION

A specific effect of glycemia on liver synthase phosphatase. Insulin deficiency in diabetic animals leads to depressed liver glycogen synthase phosphatase activity, which impairs glycogen synthesis. Because diabetic animals are also characterized by fasting hyperglycemia, it has not been possible with most animal models to dissect the respective roles of hypoinsulinemia and hyperglycemia in the decrease in phosphatase activity. The use of the fasting hyperglycemic and normoinsulinemic diabetic rat model and phloridzin treatment to restore normoglycemia allows one to study the role of the glycemic component on the decrease of liver glycogen synthase phosphatase activity in the diabetic rat. With this strategy, we show in this paper that hyperglycemia partially compensates for the depressed liver glycogen synthase phosphatase activity. This reduction in synthase phosphatase in the untreated diabetic animal is primarily caused by postprandial insulin deficiency.

The lack of restoration by glucose of liver synthase activation in vivo (26–28) or in isolated hepatocytes (29) from diabetic rats does not necessarily contradict our findings. Indeed, in hyperglycemic diabetic animals, it might be expected from our results that further glucose administration would not enhance hepatic synthase phosphatase activity beyond that already compensated by endogenous hyperglycemia. In isolated hepatocytes from fasted normal rats, even at very high glucose concentrations (50 mM), there is no noticeable increase in G6P (19), and one may safely predict that such would not be the case in liver cells from diabetic rats exposed to glucose either (29) because of the depressed glucokinase activity. Therefore, if, as we propose below, G6P is responsible for glucose activation of synthase phosphatase in diabetic rats, this compensatory mechanism would remain unnoticed in hepatocytes.

A new mechanism for synthase phosphatase activation by glucose. Our results show that acute variations in plasma glucose, independent of insulinemia, affect liver synthase phosphatase activity in diabetic rats and are positively correlated with hepatic G6P and F6P level. Several interpretations for these changes may be disregarded. Alterations in natural (endogenous) synthase *b* that might be brought about by diabetes would not be sufficient to explain our results because qualitatively similar changes were also observed when synthase phosphatase activity was measured with added purified synthase *b* substrate. A carry over of endogenous G6P in the test, resulting in stimulation of synthase phosphatase activity, can be excluded, because G6P would be removed by Sephadex G-25 gel filtration in the assay of synthase phosphatase. It is also important to recall that, whereas allosteric stimulation of synthase phosphatase activity by G6P has been demonstrated before (15,16), the effects reported herein are stable changes in activity, such as those resulting from covalent modification of enzymes as proposed before (17). The rapid (40-min)

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activation of synthase phosphatase by administering glucose to diabetic normoglycemic rats is indeed not compatible with an increased synthesis of synthase phosphatase protein. We propose therefore as a working hypothesis that G6P provokes a conversion of synthase phosphatase from a less active to a more active form.

It can be seen from Fig. 1 that, in spite of a marked reduction in glucokinase and augmentation in glucose-6-phosphatase activities, G6P and F6P levels in livers from hyperglycemic diabetic rats are similar to those in control rats. We suggest that hyperglycemia can maintain normal hexose phosphates in the liver because glucose is a substrate of glucokinase and therefore, with hyperglycemia, flux through glucokinase is maintained at a high rate, despite the reduced amount of glucokinase; this is not the case when glycemia is normalized in the phloridzin experiment. Furthermore, the observed changes in glucokinase and glucose-6-phosphatase activities brought about by diabetes are the same in all diabetic groups regardless of glycemia. Taken together, these observations indicate that hyperglycemia in diabetic rats maintains a normal level of G6P and F6P, which can partially compensate for the decrease in liver glycogen synthase phosphatase activity, independently of glucokinase and glucose-6-phosphatase activities.

In summary, our results show that hyperglycemia counteracts part of the loss in liver synthase phosphatase activity in diabetic animals. They also explain the previously reported inability of glucose to activate liver synthase in vivo and in hepatocytes of diabetic rats and suggest a new regulatory mechanism for synthase phosphatase activation, implicating different forms of the enzyme.

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