Glycogen Synthase, Synthase Phosphatase, and Phosphorylase Response to Glucose in Somatostatin-Pretreated Intact Rats

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SUMMARY

The response of the glycogen synthase and phosphorylase systems of the liver to intravenous glucose in the presence and absence of short-term somatostatin blockade of insulin secretion was determined in fed and 20-h fasted rats. These enzyme systems regulate glycogen synthesis and degradation, respectively. In the presence of somatostatin, intravenous glucose (1.0 g/kg), promptly (5 min) increased the proportion of synthase in the I (active) form, and the increase was similar to that in animals that had not received somatostatin. In the same animals, phosphorylase *a* also was decreased, and the decrease was similar in all groups. When a smaller dose of glucose (250 mg/kg) was used that only modestly increased plasma glucose (139 mg/dl) and produced a less than maximal synthase response, insulin (1 U/kg) did not potentiate glucose activation of synthase either in the presence or absence of somatostatin. Phosphorylase *a* did not change significantly in any group.

Glucose, in both the presence and absence of somatostatin, also rapidly (2 min) converted synthase phosphatase from a form inhibited by EDTA to a form not inhibited by EDTA.

These data indicate that the synthase and phosphorylase systems in vivo respond primarily to a rise in plasma glucose and not to a simultaneous elevation in plasma insulin. Thus, glucose is regulating its own storage as glycogen in the liver. The effect of glucose on the synthase may be mediated through a conversion of the synthase phosphatase to a form that is not dependent on divalent cation for activity. This form presumably is more active in vivo. DIABETES *28*: 231–236, March 1979.

In an intact animal, a high level of circulating glucose results in an increased rate of glucose stored as glycogen in the liver. The rate-limiting step in this process is mediated by glycogen synthase. Glycogen synthase is present in an active form, synthase I, and an inactive form, synthase D. Synthase kinase mediates the conversion of synthase I to synthase D. Conversion of the D form back to the I form is mediated by synthase phosphatase.[1] We recently obtained data by using a ± EDTA assay that suggests that this latter enzyme, or its substrate, also is present in two forms and that interconversion is under metabolite and hormonal control.[2]

The rate-limiting step in the degradation of glycogen to glucose is mediated by glycogen phosphorylase. Phosphorylase is likewise interconvertible between an inactive form, phosphorylase *b*, and an active form, phosphorylase *a*. A phosphatase catalyzes the conversion of phosphorylase *a* to phosphorylase *b*, and a kinase catalyzes the reverse reaction.[1]

In vivo, glucose administration stimulates a rapid conversion of liver synthase D to synthase I.[3,4] However, the relative importance of a rise in glucose concentration and of a rise in insulin concentration in this process has been uncertain.[5,6,7,8]

Somatostatin rapidly inhibits the secretion of both insulin and glucagon without any apparent direct effect on the liver.[9,10] Therefore, it should be useful for studying the acute effects of glucose and insulin independently on the liver synthase, synthase phosphatase, and phosphorylase systems in vivo. Neither insulin antiserum, which neutralizes insulin, nor mannoheptulose, which prevents insulin release, is satisfactory for this purpose. Both agents stimulate glucagon release,[11] which inhibits the response to either insulin or glucose.[2,5,12,13,14,15] In the present study, the response of glycogen synthase, synthase phosphatase, and phosphorylase systems of the liver to glucose administration in normal fed and fasted rats after short-term somatostatin blockade of insulin and glucagon secretion has been deter-

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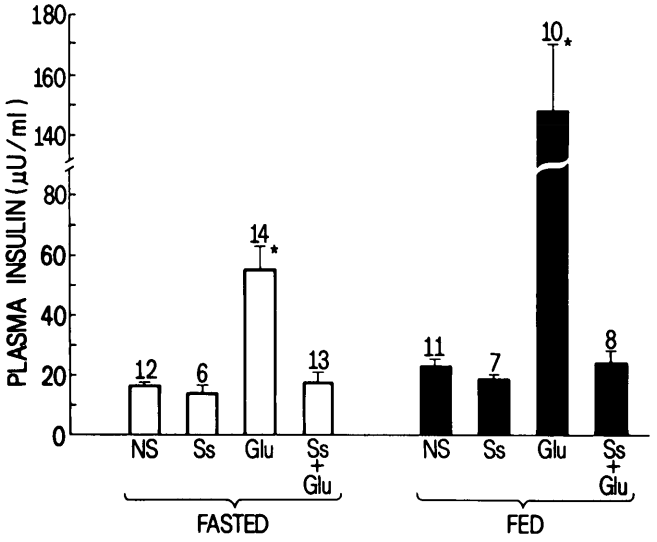


FIGURE 1. Plasma insulin concentrations in fed and fasted rats. Numbers above the bars indicate number of animals. Asterisk (*) indicates the mean difference is statistically significant compared with that of normal saline-treated animals. NS, normal saline; Ss, somatostatin; Gluc, glucose (1.0 g/kg); Ss + Glu, somatostatin given 1 min before glucose.

mined. Under the conditions used, it is clear that glucose has a potent and rapid effect on these enzyme systems that is independent of a rise in insulin. Part of this data has been published previously in abstract form.[16]

MATERIALS AND METHODS

Glucose-6-P, glucose-1-P, ATP, EDTA, UDP-glucose, rabbit liver glycogen, bovine serum albumin, and Tris buffer were purchased from Sigma Chemical. Glycogen was passed over a mixed bed, ion exchange resin (Amberlite MB-3, Mallinckrodt) before use. Glucose-labeled UDP [^{14}C]glucose, [^{14}C]glucose-1-P, and [^{125}I]insulin were obtained from New England Nuclear, sodium secobarbital from Eli Lilly, 2-(N-morpholino)ethanesulfonic acid·H_2O (MES) from Calbiochem, and rabbit antiguinea pig serum from Miles Research Division. Insulin antibodies were raised in guinea pigs in our laboratory. Somatostatin, glucagon-free insulin (lot 615-D63-1D), and rat insulin standard were gifts from Dr. Romano Deghenghi, Ayerst Laboratories (Canada), Eli Lilly, and Novo Research Institute (Bagovaerd, Denmark), respectively.

Male, Sprague-Dawley rats, 120–180 g, were used. Animals were kept in a temperature- and light-controlled animal room and fed Purina Rat Chow and water ad libitum. When fasted rats were used, food was removed at 19–21 h before the animals were killed. Animals were always killed between 0900 and 1130 h.

Rats were anesthetized with 50 mg/kg of Seconal intraperitoneally. Approximately 15 min later the rats received 1 mg/kg somatostatin (0.33 mg/ml in 0.9% NaCl), 1 U/kg insulin (1 U/ml in 0.003 N HCl/saline), 1.0 or 0.25 g/kg glucose (1.0 or 0.25 g/ml), or a combination of the above intravenously. When glucose and insulin were given together, a solution of 1 g/ml or 250 mg/ml glucose + 1 U/ml insulin was used. Control animals were given an equivalent volume of saline and were killed 5 min later. The animals in which synthase and phosphorylase activities were determined were given somatostatin 1 min before either glucose or glucose plus insulin and the rats were killed 5 min after the second injection. Animals used for the determination of synthase phosphatase activity were killed 2 min after glucose and 3 min after somatostatin administration.

The animals were killed by opening the chest rapidly and removing the heart. A piece of liver was frozen directly for synthase and phosphorylase studies by using liquid nitrogen-cooled aluminum clamps (−196°C). Tissue was stored in liquid nitrogen until it was homogenized later the same day. Blood for glucose and insulin determinations was obtained from the chest cavity after the heart was removed. Blood was collected in beakers containing heparin and immediately placed on ice. It was then centrifuged at 0–4°C, and the plasma was separated and stored at −20°C until assay.

A crude extract of frozen liver tissue was prepared for synthase and phosphorylase assay in a manner described previously.[4] Total synthase and synthase I activity was determined by the method of Thomas et al.[17] Total phosphorylase and phosphorylase *a* activity was determined on an extract that had been frozen at −80°C until the assay, using the Tan and Nuttall[18] modification of the method of Gilboe et al.[19] Synthase phosphatase activity was determined in fresh tissue by using a glycogen pellet preparation and endogenous synthase D substrate, as described by Gilboe and Nuttall.[20] Activity was assayed in the presence of 0.5 mM Mg^{++} or 5 mM EDTA.[2] Activity in the presence of 0.5 mM Mg^{++} represents total phosphatase activity, i.e., EDTA inhibitable and noninhibitable activity. Mg^{++} is added because the divalent cation concentration in some pellet preparations is slightly less than necessary for full phosphatase activity.

Plasma glucose was determined by the Nelson method,[21] glycogen by a phenolsulfuric acid method,[22] and insulin by a double antibody method.[23]

Statistical analysis of the data was done using Student's *t* test for unpaired variates. The criterion of significance is a P value of less than 0.05.

RESULTS

The mean plasma insulin concentration increased greatly 5 min after glucose was administered alone, as expected. However, the rise was considerably greater in the fed (Δ124 μU/ml) than in the fasted (Δ39 μU/ml) animals (Figure 1). In somatostatin-pretreated rats, the mean initial insulin concentration was similar to controls, and glucose did not induce a rise. In 13 animals, the insulin rise 1 min after glucose administration also was prevented by somatostatin (data not shown). Thus, it is clear that the dose of somatostatin used completely inhibited an insulin response to glucose.

The initial glucose concentration was slightly higher in both fed and fasted animals that received somatostatin (Figure 2). This reached statistical significance only in the fasted group. After administration of 1 g of glucose per kilogram body wt, the mean plasma glucose concentration was essentially identical in both groups and was not affected by somatostatin administration.

When somatostatin was given to either fed or fasted animals the percent of synthase in the I or active form was unchanged from that in the controls (Figure 3). The mean was lower in somatostatin-treated animals, but this was

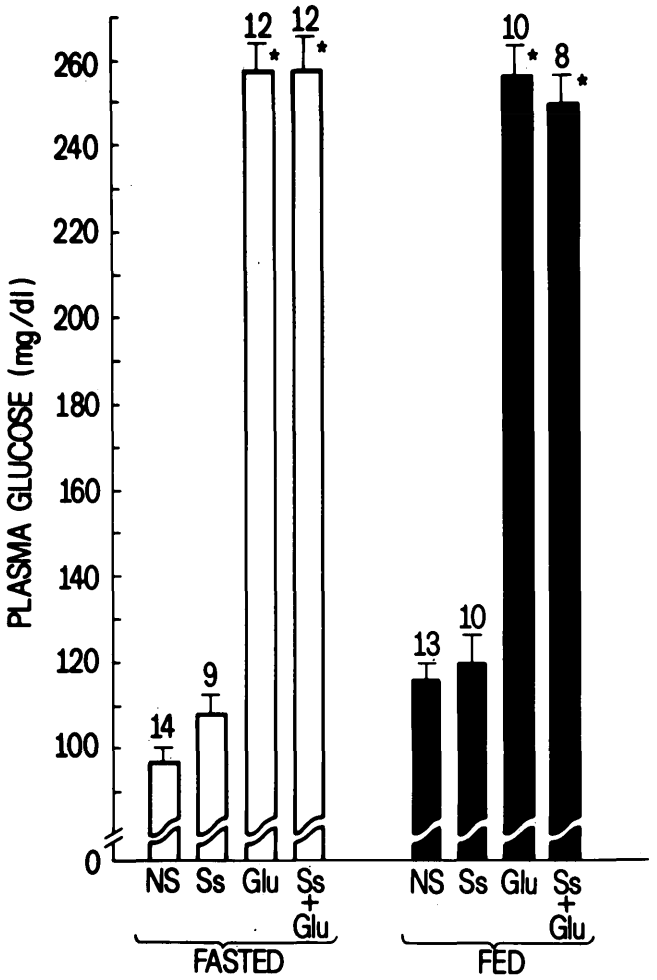


FIGURE 2. Plasma glucose concentrations in fed and fasted rats. Groups are as indicated in the legend of Figure 1. Asterisk (*) indicates the mean is statistically different from that of the normal saline-treated animals.

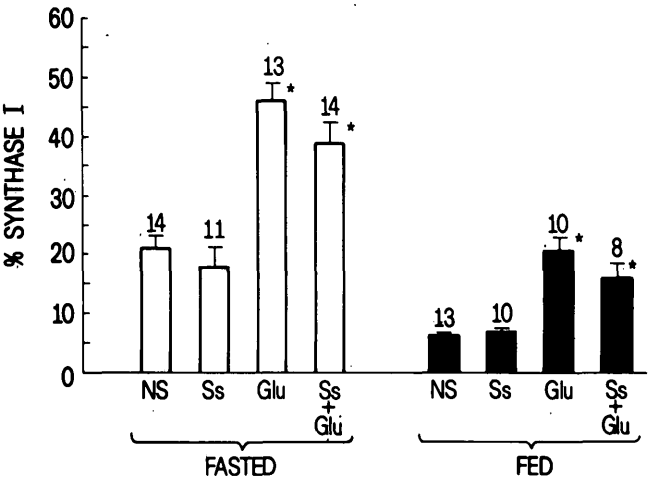


FIGURE 3. Percent synthase I in fed and fasted rats. Groups are as indicated in the legend of Figure 1. Total synthase activity was 0.8 μmol/min/g wet wt for fasted and fed rats. The difference in the mean between the glucose and the somatostatin plus glucose groups was not statistically significant in either the fed or the fasted animals. Asterisk (*) indicates the mean is statistically different from that of the normal saline-treated animals.

not statistically significant. After glucose administration, the percent synthase I increased in both the fed and the fasted animals, but the response was considerably greater in the fasted animals. Somatostatin pretreatment resulted in a slightly smaller increase in both groups, but this was not statistically significant. Total synthase activity did not change significantly in any treatment category.

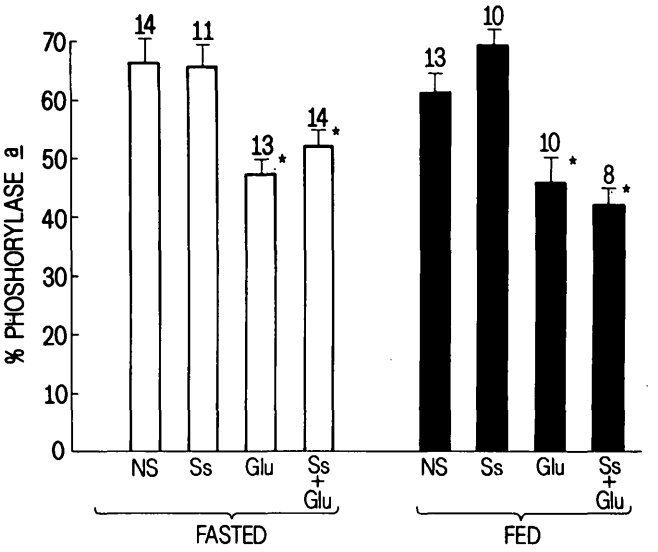
Somatostatin had no effect on the proportion of phosphorylase in the *a* form in fed or in fasted animals (Figure 4). After administration of 1 g/kg of glucose, the percent phosphorylase *a* decreased, and the decrease was similar in both groups. Pretreatment with somatostatin did not significantly influence the response. Total phosphorylase activity was not affected.

As noted previously,[6] insulin had no effect on the percent of synthase in the I form in fed rats (Figure 5). When insulin was given with 1 g of glucose per kilogram, the increase in percent synthase I was slightly, but not significantly, greater than the increase with glucose alone. Insulin also did not significantly affect the decrease in percent phosphorylase *a* induced by glucose. The decrease in percent phosphorylase *a* after glucose did not reach statistical significance in either group, but when the glucose and insulin plus glucose data are combined, the decrease is significant ($P < 0.05$).

Since the dose of glucose used in our studies resulted in a very high glucose concentration, we were interested in determining if insulin might potentiate the effect of a smaller dose of glucose. Therefore, the experiments were repeated using one-fourth the dose (250 mg/kg). Fasted rats were used because the synthase system was most responsive to glucose in this group, and, thereby, small differences would be detected more easily.

This dose of glucose resulted in an increase in plasma glucose from 98 mg/dl to 139 mg/dl. The proportion of synthase in the I form increased from a control value of 28% to 44% after glucose (Figure 6). Thus, both the increase in plasma glucose and the percent synthase I were considerably less than when 1 g of glucose per kilogram was

FIGURE 4. Percent phosphorylase *a* in fed and fasted rats. Groups are as indicated in the legend of Figure 1. Total phosphorylase activity was 19 and 15 μmol/min/g wet wt for fasted and fed rats, respectively. The difference in the mean between the glucose and somatostatin plus glucose groups was not statistically significant in either the fed or the fasted animals. Asterisk (*) indicates the mean is statistically different from that of the normal, saline-treated animals.



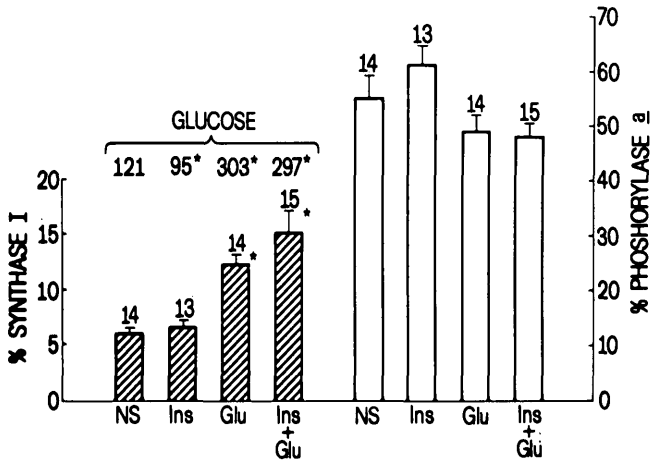


FIGURE 5. Effect of insulin on percent synthase I and percent phosphorylase *a* in fed rats. The plasma glucose values (mg/dl) are indicated above the crosshatched bars for each type of injection. Asterisk (*) indicates the mean difference is statistically significant compared with normal saline-treated animals. The difference in the mean between the glucose and insulin plus glucose groups was not statistically significant. NS, normal saline; Ins, insulin; Glu, glucose (1.0 g/kg), Ins + Glu, insulin plus glucose given as a single injection.

given. With the smaller dose of glucose, a change in percent phosphorylase *a* could not be detected. When insulin (1 U/kg) was given with glucose, the mean plasma glucose concentration was slightly, but not significantly lower (134 mg/dl) as was the percent of synthase in the I form (41%). Again, a change in percent phosphorylase *a* was not present.

In the group pretreated with somatostatin (Figure 6), the percent synthase I was lower than in those that did not receive somatostatin, but this did not reach statistical significance. The increase in percent synthase I after administration of glucose or insulin plus glucose was similar to that observed in animals not pretreated with somatostatin. The percent of phosphorylase in the *a* form did not change significantly in any of the somatostatin-pretreated groups. Thus, in animals receiving less than a maximally stimulating amount of glucose, an additive, or potentiating, effect of insulin on the synthase system could not be demonstrated. Also with this smaller dose of insulin, a change in the proportion of phosphorylase in the *a* form could not be detected.

The activity of synthase phosphatase, the enzyme responsible for catalyzing the conversion of synthase D to synthase I, was assayed in both the presence and absence of EDTA in glycogen pellet preparations from fed rats (Figure 7). In the presence of EDTA, the activity was essentially identical in extracts from saline- or somatostatin-treated animals. In glucose-treated animals, the activity was increased as noted previously,[2] and somatostatin pretreatment did not modify the response (Figure 7). When assayed in the absence of EDTA, the activity was similar in all groups.

Glycogen, particularly in the presence of ATP,[24] inhibits synthase phosphatase activity[25] and can result in a lower percent synthase I. Therefore, tissue glycogen concentrations were measured (Table 1). As expected, the concentrations were greatly reduced in fasted animals. However, over the short time period studied, there was no change in either the fasted or the fed animals after either glucose or somatostatin plus glucose administration. In fed animals receiving only somatostatin, the mean was significantly greater than it was in the control animals. However, the smallest number of determinations was done in this group, and it is unlikely that the difference is meaningful.

DISCUSSION

Glucose given intravenously[3,6] or orally[4] results in a very rapid increase in percent of synthase I (within 2 min)[6] and in glycogen synthesis in liver of normal animals.[4] In other tissues, such as skeletal muscle, the accompanying rise in insulin concentration is primarily responsible for the increased proportion of synthase in the I form,[4,26] but the role of insulin in liver is controversial. In studies using perfused liver[14,27] and isolated hepatocytes from normal fed rats,[28,29,30] it is clear that a raised glucose concentration

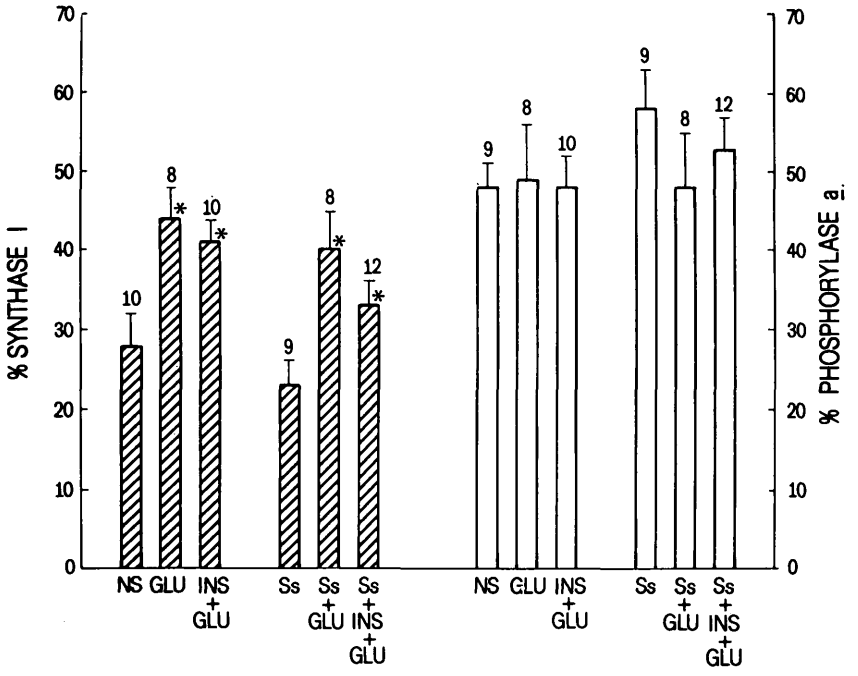


FIGURE 6. Effect of glucose and insulin on percent synthase I and percent phosphorylase *a* in fasted rats. Groups are as indicated previously. The glucose dose used in these experiments was 250 mg/kg. The respective mean plasma glucose concentrations (mg/dl) were: NS, 98; Glu, 139; Ss, 111; Ins + Glu, 134; Ss + Glu, 143; Ss + Glu + Ins, 139. Total synthase activity was 1.1 μmol/min/g wet wt. Total phosphorylase activity was 18 μmol/min/g wet wt.

can increase synthase I activity and stimulate an increased rate of radioactive glucose incorporation into glycogen independent of added insulin. Similar synthase results can be demonstrated in normal fasted rats pretreated with large amounts of insulin antiserum before glucose was administered, but only transiently.[6] This treatment leads to an increase in glucagon[11] that opposes the effect of glucose.[15]

There are several reports that insulin administration in vivo[5,12,13,31,32,33] results in a rapid increase in liver synthase I activity. In most, but not all of these studies,[5] diabetic animals have been used or glucose and insulin were infused simultaneously.[12,13] Others using normal intact animals have either not been able to demonstrate an acute increase in percent synthase I,[6,7,31] or only a small and inconsistent effect.[8]

In a perfused rat liver preparation, insulin had either no effect[14] or modestly accelerated a glucose-induced increase in percent synthase I.[32] In isolated hepatocytes, insulin alone has been reported to increase the percent synthase I and stimulate glycogen deposition.[28] Others could not demonstrate an independent insulin effect, but did show potentiation of the glucose effect.[30]

A major objective of the present study was to determine if an acute rise in insulin concentration plays a significant role in glucose activation of the synthase system or inactivation of the phosphorylase system in normal fed and fasted animals. From the data obtained, it is apparent that the acute rise in insulin concentration that results when a dose of glucose is given intravenously does not contribute significantly to an increase in synthase I activity or to a reduction in phosphorylase *a* activity. Also, the glucose induced change was not enhanced by insulin. In fact, the mean response to glucose was reduced.

It is of interest that the synthase response to glucose was greater in fasted than in fed animals, whereas the insulin response was less. This also has been reported by others.[36] However, the smaller rise in synthase I in fed animals may have been due to glycogen inhibition of synthase phosphatase,[25] since the glycogen concentration was much greater.

TABLE 1
Liver glycogen in fed and fasted animals

	Fasted	Fed
NS	1.89 ± 0.32 (13)	31.6 ± 3.3 (10)
Ss	1.77 ± 0.27 (11)	*43.8 ± 4.3 (5)
Glu	1.68 ± 0.26 (12)	34.4 ± 3.3 (10)
Ss + Glu	1.61 ± 0.22 (14)	38.3 ± 3.7 (8)

Concentration in milligrams per gram wet weight.
* Significantly different statistically from NS-fed mean.

Even though insulin does not appear to be of major importance in the acute response of the liver synthase system to an increased glucose level, it is essential in maintaining the integrity of the synthase system. In diabetic[31,34] and fasted-adrenalectomized animals[34] (which are low insulin states), liver synthase phosphatase activity was reduced and synthase D was partially converted to a form not suitable as substrate for the phosphatase.[34] Insulin administration to diabetic rats[31] or glucose administration to fasted-adrenalectomized rats[34] resulted in restoration of the phosphatase activity, but this required several hours.[34]

In the presence of chronic insulin deficiency, the circulating glucagon concentration also would be elevated[11] and liver cAMP concentrations increased,[35] which could activate the cAMP-dependent synthase kinase and oppose the effect of glucose. Thus, previously reported insulin effects in diabetic animals may be explained by a reduction in cAMP concentration and/or reduced synthase kinase activity.

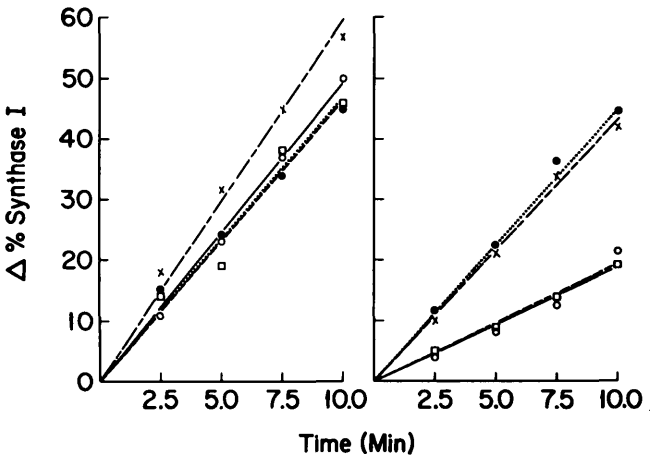
Stalmans and associates[37] have suggested that glucose activation of the liver synthase system is mediated through an effect on phosphorylase *a*, which is an inhibitor of synthase phosphatase. Glucose stimulates a decrease in the percent of phosphorylase in the *a* form both in vivo[4] and in vitro.[14,27] In their experience, a decrease in phosphorylase *a* always precedes a rise in synthase I.

We also have found purified liver phosphorylase *a* to be a potent, noncompetitive, but incomplete inhibitor of liver synthase phosphatase activity.[38] In fact, the K_i (<0.7 U/ml) was considerably lower than the usual tissue concentration (6–15 U/ml). This made physiologic interpretation of the data difficult. In addition, phosphorylase phosphatase activity was several-fold higher than synthase phosphatase activity in liver.[38] Thus, if glucose affected both phosphatases simultaneously and equally, then it would be anticipated that a decrease in phosphorylase *a* would precede an increase in synthase I and the two events need not be interrelated.

Some time ago, we reported an increase in synthase I before a decrease in phosphorylase *a* in rats given glucose by gavage.[4] This suggested that independent mechanisms exist for the control of synthase phosphatase and phosphorylase phosphatase activities by glucose. Subsequently others[36,39] also reported changes in percent synthase I independent of phosphorylase *a*.

More recently, we demonstrated a possible mechanism for the control of synthase phosphatase activity independent of a change in phosphorylase *a*. In extracts of liver from

FIGURE 7. Effect of glucose after somatostatin treatment of fed rats. Phosphatase activity was measured in the presence of either 0.5 mM Mg^{2+} (left) or 5 mM EDTA (right). Activity in the presence of 0.5 mM Mg^{2+} represents total phosphatase activity, i.e., EDTA inhibitable plus EDTA noninhibitable activity. Animals were given glucose 2 min before they were killed (●). Some animals received somatostatin 1 min before glucose treatment (X) and were killed 2 min later. Control animals received somatostatin (□) or saline (○) 2 min before they were killed. Each plot represents the mean of four animals in each group. Total synthase was similar in all groups (0.7 U/ml) and did not change during the incubation period. The initial percent synthase I was similar for all groups (approximately 20%) except the glucose group (approximately 30%).



normal fed rats, synthase phosphatase activity using endogenous substrate was inhibited approximately 50% by addition of supramaximal amounts of EDTA; this suggested the presence of two forms of synthase phosphatase, an EDTA inhibitable form and an EDTA noninhibitable form. Different forms of synthase D, the substrate, was an alternate and equally plausible explanation, but the net effect would be the same.

When glucose was given to normal fed animals, the synthase phosphatase activity was converted almost completely to an EDTA noninhibitable form within 2 min, whereas glucagon or cAMP produced the opposite effect within 15 s.[2] Insulin alone had no effect, but whether the rise in insulin as a result of glucose administration contributed to activation of the phosphatase could not be determined. In the present study, an increase in the EDTA noninhibitable phosphatase rate was observed after glucose administration both in the absence and presence of somatostatin blockade of insulin secretion. Thus, it is apparent that glucose can stimulate conversion to the noninhibitable form in the absence of a rise in insulin.

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