Activation of Glycogen Synthase in Rat Adipocytes by Insulin and Glucose Involves Increased Glucose Transport and Phosphorylation*

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JOHN C. LAWRENCE, JR.† AND JOSEPH LARNER

From the Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22903

Incubation of fat cells with insulin, concanavalin A, or H2O2 stimulated the incorporation of d-[U-14C]glucose into glycogen. Incubation with either concanavalin A or insulin alone activated glycogen synthase. H2O2 alone did not activate glycogen synthase, but in the presence of glucose it increased the percentage of glycogen synthase I activity. The activation of glycogen synthase by H2O2 and glucose was abolished by phloridzin or cytochalasin B. These inhibitors of glucose transport also abolished the action of glucose to potentiate the effect of concanavalin A on glycogen synthase.

Glucose, mannose, and 2-deoxyglucose increased glycogen synthase I activity and potentiated the effect of insulin on glycogen synthase. Incubation of cells with 3-O-methylglucose, galactose, or fructose was without effect on glycogen synthase I activity and did not result in a potentiation of the effect of insulin. Compared to other hexoses, 2-deoxyglucose was much more effective in activating glycogen synthase. Increases in 2-deoxyglucose-6-P produced during 5-min incubations with varying concentrations of 2-deoxyglucose paralleled the increases in glycogen synthase I activity, although phosphorylase a activity and concentrations of ATP and cAMP were unchanged.

Glucose-6-P and 2-deoxyglucose-6-P (but not glucose, mannose, or 2-deoxyglucose) increased the activity of glycogen synthase phosphatase which was monitored by following the increase in endogenous glycogen synthase I activity.

These results suggest that glucose-6-phosphate formation is required for the activation of glycogen synthase by glucose. Stimulation of glucose transport that results in increased cellular concentrations of glucose-6-P is a mechanism by which insulin and other agents increase glycogen synthase I. Activation of glycogen synthase by this glucose transport-coupled and phosphorylation-dependent mechanism may involve an increase in glycogen synthase phosphatase activity. A model is presented relating this mechanism and the glucose transport-independent mechanisms for activation of glycogen synthase.

We previously demonstrated that activation of glycogen synthase in rat adipocytes by insulin is much greater in the presence of, than in the absence of, glucose (1). On the basis of this and other evidence, we proposed that in the presence of glucose, activation of glycogen synthase by insulin in fat cells occurred via both glucose transport-dependent and independent mechanisms. To explore this idea further, we investigated the effects of H2O2 and concanavalin A, two agents that have been shown to mimic the effect of insulin on activation of glucose transport (2-5), on glycogen synthase of adipocytes. Because glycogen synthase activation has been shown to be a primary effect of insulin in rat skeletal muscle (6), perfused rat liver (7), and rat adipocytes (1), it was of interest to determine what effect these two agents had on glycogen synthase of fat cells. Furthermore, because we had previously observed that activation of glycogen synthase in adipocytes could occur as a result of increased glucose entry into these cells, we predicted that H2O2 and concanavalin A should increase glycogen synthase in the presence of glucose (by stimulating glucose transport) regardless of their effects in the absence of glucose. The results presented in this report provide strong evidence that stimulation of glucose transport is a mechanism by which insulin activates glycogen synthase in adipocytes and that phosphorylation of glucose is an obligatory step in this glucose transport-coupled pathway of glycogen synthase activation. Data are also presented that demonstrate that activation of synthase by this pathway may result from an effect of glucose-6-P to increase the activity of glycogen synthase phosphatase.

**MATERIALS AND METHODS**

Adipocytes were prepared from the epididymal adipose tissue of rats weighing 120 to 180 g (Wistar strain) fed ad libitum (8). All experiments with adipocytes were conducted in plastic bottles or tubes, and in a medium composed of Krebs-Ringer phosphate buffer (128 mM NaCl, 1.4 mM CaCl2, 1.4 mM MgSO4, 5.2 mM KCl, and 10 mM Na2HPO4, pH 7.4) and 30 mg/ml of bovine serum albumin (Fraction V, Sigma). Cell number was estimated by direct counting using a hemocytometer and 0.5-106 cells/ml were used.

Labeled CO2 production from d-[U-14C]glucose was measured as described previously (1). Labeled glucose incorporation into glycogen was measured after the reaction was stopped by adding 0.2 ml of 10 N KOH to 0.2 ml of cells containing 1 mM d-[U-14C]glucose (approximately 106 cpm) and additions as indicated in Fig. 1. The polypro-
Hexose Transport-coupled Activation of Glycogen Synthase

RESULTS

Lectins (2, 3) and H₂O₂ (4, 5, 15) have been shown to share with insulin the ability to stimulate glucose oxidation and glucose transport system activity in adipocytes. We therefore investigated the effects of H₂O₂ and concanavalin A on glycogen synthesis in fat cells to determine whether these agents also mimic the effect of insulin in stimulating the incorporation of glucose into glycogen. As shown in Fig. 1, incubation of cells with 0.4 mM H₂O₂ or 40 μg/ml of concanavalin A resulted in increased rates of ¹⁴CO₂ formation from 1 mM D-[U-¹⁴C]glucose (Fig. 1A) and in increased incorporation of D-[U-¹⁴C]glucose into glycogen (Fig. 1B). The stimulation of D-[U-¹⁴C]glucose incorporation into glycogen by concanavalin A and H₂O₂ prompted investigation of their effects on glycogen synthase, the enzyme most directly involved in glycogen synthesis.

In the presence of glucose, incubation of cells at 37°C for 10 min with H₂O₂ produced an increase in glycogen synthase I activity (Fig. 2). Activation of glycogen synthase by 0.4 mM H₂O₂ was observed when the medium glucose concentration was above 1 mM. Incubation of cells with H₂O₂ alone did not increase glycogen synthase I activity. We have tested concentrations of H₂O₂ between 0.05 and 8 mM for periods of incubation up to 30 min without observing activation of glycogen synthase. Concentrations of H₂O₂ above 4 mM actually inactivated glycogen synthase. For example, incubation of cells with 4 mM H₂O₂ for 10 min at 37°C decreased the percentage of glycogen synthase I activity from 14.8 ± 1.0 to 8.7 ± 1.6 (mean values ± S.E. from seven experiments).

Unlike H₂O₂, concanavalin A produced an increase in glycogen synthase I activity in the absence of glucose. The time course of glycogen synthase activation by 40 μg/ml of concanavalin A in both the presence and absence of 5 mM glucose is presented in Fig. 3. The effects of concanavalin A were maximal after 10 min of incubation and represented an increase in the percentage of glycogen synthase I activity of

1 J. C. Lawrence, and J. Larner, unpublished observations.
FIG. 2. Dependence of the activation of glycogen synthase by H$_2$O$_2$ on the concentration of glucose. Adipocytes were incubated at 37°C in 5 ml of medium (0.75 x 10$^6$ cells/ml) with 0.4 mM H$_2$O$_2$ and the indicated concentrations of glucose for 10 min before glycogen synthase activities were assayed.

FIG. 3. Time course of adipocyte glycogen synthase activation by concanavalin A in the absence and presence of glucose. Adipocytes were incubated in 5 ml of medium (0.7 x 10$^6$ cells/ml) at 37°C for 30 min before the incubations were stopped. Concanavalin A (40 µg/ml), glucose (5 mM), or concanavalin A plus glucose were added after the appropriate times to give the periods of exposure indicated before termination of the incubations.

FIG. 4. The effect of increasing concentrations of glucose on the activation of isolated fat cell glycogen synthase by concanavalin A. Adipocytes were incubated at 37°C for 10 min in 5 ml of medium (0.7 x 10$^6$ cells/ml) with or without 40 µg/ml of concanavalin A and the indicated concentrations of glucose before the incubations were terminated and glycogen synthase activity for each experimental condition was assayed.
by inhibitors of glucose transport. As shown in Fig. 6, incubation of cells with 10 μg/ml of cytochalasin B or 4 mM phlorizin abolished the effects of 5 mM glucose in both the absence and presence of 0.4 mM H₂O₂ or 40 μg/ml of concanavalin A.

Therefore, it seems likely that agents such as H₂O₂, concanavalin A, and insulin can activate glycogen synthase in the presence of glucose by stimulating glucose entry into the cell. To determine whether other hexoses could substitute for glucose, cells were incubated at 37° for 10 min with different hexoses at a concentration of 5 mM in the presence and absence of 100 microunits/ml of insulin before glycogen synthase I activity was assayed (Fig. 7). As previously reported (1), incubation of cells with insulin alone increased the percentage of glycogen synthase I activity and glucose potentiated the effect of insulin. Mannose alone increased glycogen synthase I activity to the same extent as incubation with glucose alone. In contrast to glucose and mannose, the hexoses fructose, galactose, and 3-O-methylglucose did not increase glycogen synthase I activity or potentiate the effect of insulin. In fact, incubation of cells with galactose and fructose partially blocked the effect of insulin on glycogen synthase.

As also shown in Fig. 7, incubation of cells with 2-deoxyglucose resulted in a marked increase (about five times control) in glycogen synthase activity. Experiments were performed (including fold dilutions of homogenates and Sephadex G-50 chromatography) that negated the possibility that 2-deoxyglucose was present in the extracts at sufficient concentrations to produce allosteric activation of glycogen synthase D that could account for this large increase in activity. Similar controls were previously performed showing that concentrations of glucose-6-P in homogenates following incubation with glucose or insulin plus glucose are too low to produce allosteric activation of glycogen synthase D (1).

The results presented in Fig. 8 represent the percentages of glycogen synthase I activity found in fat cells following two 10-min incubation periods. In the first period, adipocytes were incubated with (A and B) or without (C and D) 0.4 mM 2-deoxyglucose. This incubation was terminated by washing the cells two times and suspending them in medium not containing 2-deoxyglucose. In the second incubation period, 0.4 mM 2-deoxyglucose was added to cells not previously exposed to the hexose (B) and to cells that were incubated with 2-deoxyglucose in the first period (D). Insulin (2.5 milliunits/ml) was added to cells at the start of the second incubation period. In the absence of insulin, the same percentage of glycogen synthase I activity was observed in cells that were incubated with 2-deoxyglucose only in the second incubation (B) as in cells that were incubated with 2-deoxyglucose and washed free of the hexose (C). The absolute change in glycogen synthase I activity due to insulin in cells washed free of 2-deoxyglucose (C) was equal to the increase produced by the hormone in cells not incubated with 2-deoxyglucose (A). By comparing B and D to C, it can be seen that only when 2-deoxyglucose was present in the medium with insulin were the effects of insulin and 2-deoxyglucose greater than additive. This requirement of extracellular 2-deoxyglucose is consistent with the hypothesis that the potentiation of the effect of insulin by the hexose results from a stimulation by 2-deoxyglucose uptake.

The major proportion of 2-deoxyglucose that enters adipocytes is found as 2-deoxyglucose-6-P (16). This compound accumulates as a comparatively stable metabolite in cells incubated with 2-deoxyglucose, because further metabolism occurs only to a limited extent and the phosphate group renders it relatively impermeable to the plasma membrane. This property could explain why the effects of 2-deoxyglucose on glycogen synthase persist after removal of extracellular 2-deoxyglucose (Fig. 8), while the effects of glucose or insulin plus glucose are rapidly reversed by washing. The remaining experiments in this report were designed to investigate further the effects of 2-deoxyglucose and the hypothesis that phosphorylation of glucose to glucose-6-P is the link to glycogen synthase activation in the glucose transport-coupled pathway of synthase activation.

Incubation of adipocytes for 10 min at 37° with 0.5 mM or 5 mM 2-deoxyglucose alone did not decrease the concentration of cAMP under conditions in which the percentage of glycogen synthase I activity was increased (Table I). These concentrations of 2-deoxyglucose were also without effect on the concentration of cyclic nucleotide observed in the presence of 100 microunits/ml of insulin.

In the experiments presented in Fig. 9, cells were incubated with 1 mM 2-deoxyglucose for periods ranging from 0 to 60 min, before the percentages of glycogen synthase I and phosphorylase a activities and the concentrations of 2-deoxyglucose-6-P, cAMP, and ATP were determined. It was of interest to monitor phosphorylase activity under these conditions, because it has been suggested that decreases in phosphorylase a in liver due to glucose can lead to glycogen synthase activation (17, 18). With 1 mM 2-deoxyglucose, the percentage of glycogen synthase I activity was increased from about 10%...
Hexose Transport-coupled Activation of Glycogen Synthase

Fig. 6 (left). Inhibition of the effects of glucose on glycogen synthase by cytochalasin B and phloridzin. Adipocytes were incubated with or without 10 µg/ml of cytochalasin B or 4 mM phloridzin in the absence and presence of 5 mM glucose, 0.4 mM H₂O₂, 40 µg/ml of concanavalin A, 5 mM glucose plus 0.4 mM H₂O₂, and 5 mM glucose plus 40 µg/ml of concanavalin A. After 10 min the incubations were terminated and glycogen synthase activities assayed.

Fig. 7 (right). The effect of different hexoses on adipocyte glycogen synthase in the absence and presence of insulin. Fat cells were incubated at 37°C with the hexoses indicated at a concentration of 5 mM in the presence and absence of 100 microunits/ml of insulin. After 10 min the incubations were terminated and glycogen synthase I activity was determined. The results presented represent the mean values ± S.E. of three experiments performed on different days.

TABLE I

| Glycogen synthase | cAMP     |
|-------------------|----------|
| 2-Deoxyglucose | Without insulin | 100 microunits/ml of insulin | Without insulin | 100 microunits/ml of insulin |
| mM               | % synthase I activity | pmol/10⁶ cells                | % synthase I activity | pmol/10⁶ cells                |
| 0                | 10.2 ± 1.5 | 16.0 ± 1.9 | 8.8 ± 0.5 | 6.7 ± 0.6 |
| 0.5              | 27.3 ± 3.2 | 52.8 ± 3.1 | 9.0 ± 0.9 | 7.6 ± 0.6 |
| 5.0              | 43.8 ± 4.7 | 53.8 ± 2.7 | 9.3 ± 0.9 | 7.2 ± 0.5 |

at 0 min of incubation with 2-deoxyglucose to about 50% after 30 min of incubation. The percentage of phosphorylase α activity was slightly decreased over the 60-min incubation period. However, it should be noted that no detectable decreases in phosphorylase α activity were observed until after 12 min, at which time the percentage of glycogen synthase I was increased about 3-fold. Thus phosphorylase inactivation did not precede synthase activation. During the time course of incubation with 1 mM 2-deoxyglucose, the concentration of 2-deoxyglucose-6-P rose progressively, while the concentration of ATP remained essentially unchanged. The concentration of cAMP was also unchanged. The increase in glycogen synthase I activity was linear...
through 8 min of incubation with 1 mM 2-deoxyglucose (Fig. 9). Therefore, the effects of different concentrations of 2-deoxyglucose on phosphorylase activity and on concentrations of ATP and 2-deoxyglucose-6-P were investigated after 5 min, so that these parameters could be assayed under conditions in which initial rates of glycogen synthase activation were observed (Fig. 10). Glycogen synthase activation was half-maximal at 0.8 mM 2-deoxyglucose and essentially maximal at 4 mM 2-deoxyglucose. The accumulation of 2-deoxyglucose-6-P closely followed the activation of glycogen synthase. The concentration of 2-deoxyglucose-6-P found at half-maximal activation of glycogen synthase was about 8.5 nmol/10⁶ cells. In these experiments, the percentages of phosphorylase activity and concentrations of ATP remained essentially unchanged. The failure of 2-deoxyglucose to decrease ATP concentrations may seem to contradict the observations of Chandramouli and Carter (19), in which large decreases (50%) in the adenine nucleotide were seen with 2-deoxyglucose. A 60-min incubation period with higher concentrations of 2-deoxyglucose (10 to 20 mM) was used by these investigators when ATP measurements were made. Differences in experimental conditions may explain the apparently discrepant results. For example, we found that when cells were incubated at 37°C for 10 min with 5 mM 2-deoxyglucose, the concentration of ATP was decreased from a control value of 15.8 ± 0.6 to 12.8 ± 0.7 nmol/10⁶ cells (mean values ± S.E. from three experiments). Under these conditions, incubation with 100 micro-units/ml of insulin plus 5 mM 2-deoxyglucose decreased cellular ATP from 15.7 ± 0.7 (obtained with insulin alone) to 7.0 ± 0.4 nmol/10⁶ cells.
TABLE II
Effect of insulin and glucose on adipocyte glycogen synthase and concentrations of glucose-6-P and ATP

Fat cells were incubated at 37° for 10 min with or without 5 mM glucose and 100 microunits/ml of insulin. The incubations were terminated and glycogen synthase I activity and the concentrations of ATP and glucose-6-P were assayed as described under "Materials and Methods."

| Additions                  | Glycogen synthase | Glucose-6-P | ATP |
|----------------------------|-------------------|-------------|-----|
|                            | % synthase I activity | nmol/10^6 cells | nmol/10^6 cells |
| None                       | 9.2               | 0.21        | 16.4 |
| Insulin, 100 microunits/ml | 15.3              | 0.28        | 16.2 |
| Glucose, 5 mm              | 12.6              | 1.1         | 16.5 |
| Insulin, 100 microunits/ml, plus glucose, 5 mm | 30.0              | 2.4         | 16.1 |

Incubation of cells with 5 mM glucose or 100 microunits/ml of insulin plus glucose did not alter the concentration of ATP (Table II). In the presence of glucose, insulin more than doubled the concentration of glucose-6-P.

Evidence from in vitro studies has accumulated indicating that glucose-6-P can increase the activity of glycogen synthase phosphatase from various tissue sources (20–23). However, such an effect of glucose-6-P has, to our knowledge, never been demonstrated using enzymes from fat cells. Because of problems associated with purification of glycogen synthase and phosphatase from rat adipose tissue, we investigated the effects of glucose-6-P on the activity of glycogen synthase phosphatase by monitoring the increase in endogenous glycogen synthase I activity in extracts of isolated fat cells. Use of endogenous glycogen synthase could circumvent problems that arise from using purified glycogen synthase D from other tissues. The results of experiments in which extracts were incubated for 5 min at 30° with increasing concentrations of glucose-6-P (0.33 to 10 mM) are presented in Fig. 11. The incubations were terminated by adding 100 mM KF before a two-step procedure (described in detail under "Materials and Methods") involving ammonium sulfate precipitation and filtration through Sephadex G-25 was used to remove glucose-6-P. Without glucose-6-P, incubation at 30° resulted in an increase in the percentage of glycogen synthase I activity from about 10 to 38%. In the presence of glucose-6-P the percentage of glycogen synthase I activity was increased (from 38 to 65% with 10 mM glucose-6-P). Addition of 100 mM KF before the incubation with glucose-6-P greatly decreased phosphatase activity. As a control, glucose-6-P was added after 5 min of incubation at 30°. Because no further increase in glycogen synthase I activity was observed, it is unlikely that glucose-6-P was carried through the procedures designed for its removal. As shown in Fig. 12, 2-deoxyglucose-6-P increased glycogen synthase phosphatase activity, although to a lesser degree than glucose-6-P (Fig. 11). We have also found that mannose-6-P increased glycogen synthase phosphatase activity, although this compound was also less effective than glucose-6-P.

Because glucose, mannose, and 2-deoxyglucose are thought to enter adipocytes via a facilitated diffusion system, their intracellular concentrations should never be higher than their extracellular concentrations. Therefore, in the experiments presented in Fig. 7, 5 mM would represent an upper limit for the concentration of the free sugars within the cell. However, 5 mM glucose, 5 mM mannose, and 5 mM 2-deoxyglucose did not increase glycogen synthase phosphatase activity.
Hexose Transport-coupled Activation of Glycogen Synthase

DISCUSSION

The ability of H2O2 to activate glycogen synthase only in the presence of glucose is in contrast to that of insulin, since the hormone produces an increase in glycogen synthase I activity in the absence of medium glucose. Czech et al. (5, 24) proposed that the stimulation of glucose transport by insulin and H2O2 might involve a common mechanism, namely, the oxidation of key cellular sulfhydryl groups to the disulfide forms. However, the failure of H2O2 alone to increase glucose synthase I activity supports the hypothesis that the activation of glycogen synthase by insulin in the absence of glucose does not involve oxidative events that may occur during glucose transport system activation.

Unlike H2O2, concanavalin A increased the percentage of adipocyte glycogen synthase in the absence of glucose (Figs. 3 to 5). One interpretation of these results is that the site of concanavalin A action is closer to the site of action of insulin than that of H2O2. Cuatrecasas’ studies suggested that concanavalin A bound to a region of the insulin receptor thus preventing the binding of insulin (25). Perhaps our finding that concanavalin A reduced the activation of glycogen synthase observed with insulin relates to an effect of the lectin at the level of the insulin receptor. Binding of concanavalin A to a region of the receptor near the insulin binding site might be sufficient to promote a partial activation of glycogen synthase, with the “insulin-insulin receptor” interaction responsible for mediating the effects of the hormone on glycogen synthase. Evidence that concanavalin A interferes with normal insulin binding was also obtained by DeMayo et al. who reported that concanavalin A abolished negative cooperativity between insulin receptors (26).

Both concanavalin A (Figs. 3 to 6) and H2O2 (Figs. 2 and 6) increased the percentage of glycogen synthase I activity in the absence of glucose. Because both agents are known to activate glucose transport in isolated fat cells (2-5), these findings are consistent with the hypothesis that stimulation of glucose entry into the adipocyte is a mechanism for glycogen synthase activation (1). Potentiation of the effects of these agents by glucose was blocked by inhibiting glucose transport with phloridzin or cytochalasin B (Fig. 6), which adds further evidence favoring this hypothesis.

The results presented in this report provide evidence that phosphorylation of glucose to glucose-6-P is involved in this hexose transport-coupled pathway for the activation of glycogen synthase. For example, incubation of cells with 3-O-methylglucose and galactose did not result in activation of glycogen synthase. The uptake of 3-O-methylglucose occurs in fat cells via the β-glucose transport system and its transport is stimulated in adipocytes by insulin, although the hexose is not phosphorylated (5, 27-29). Galactose transport is not well characterized in fat cells; however, in red blood cells galactose has a high affinity for the β-glucose transport system (30). Galactose uptake is stimulated by insulin in skeletal muscle where the hexose is metabolized very slowly (31). Galactose is also slowly metabolized in fat cells (32). The results in the present report obtained with 3-O-methylglucose and galactose are consistent with the idea that a certain level of cellular metabolism is necessary for glycogen synthase activation by hexoses. Mannose, a hexose that is metabolized in adipose tissue much like glucose (33), produced essentially the same effects as glucose. No activation was observed with fructose. The reason glucose and mannose, but not fructose, activate glycogen synthase and potentiate the effect of insulin on increasing glycogen synthase I activity may relate to differences in the transport and metabolism of fructose (34). More investigation is needed to clarify this point.

The strongest indication that hexose phosphorylation leads to glycogen synthase activation came from results with 2-deoxyglucose. Transport of 2-deoxyglucose occurs by the β-glucose transport system (35). The hexose is phosphorylated and 2-deoxyglucose-6-P accumulates in the cell because further metabolism is very limited. Czech (16) has shown that insulin can triple the concentrations of 2-deoxyglucose-6-P. It is possible that these properties explain why 2-deoxyglucose was so effective in activating glycogen synthase and potentiating the effect of insulin to increase glycogen synthase I activity (Figs. 7 to 10).

Gilbow and Nuttall (36) reported that physiological concentrations of ATP inhibited rat liver glycogen synthase phosphatase. ATP is also required for the conversion of glycogen synthase I to D in the protein kinase-catalyzed reaction (6). Therefore, a decrease in cellular ATP could result in glycogen synthase activation. This was considered as a possible mechanism for the increase in glycogen synthase I activity resulting from incubation of cells with 2-deoxyglucose, since, under certain conditions, the concentrations of ATP were decreased by incubating cells with this hexose. However, this mechanism seems unlikely because glycogen synthase I activity was increased over four times by 2-deoxyglucose under conditions in which the concentration of ATP was not detectably changed (Figs. 8 and 9). For the same reason, it is unlikely that a decrease in ATP mediates the effect of glucose to activate glycogen synthase or to potentiate the effect of insulin to increase glycogen synthase I activity (Table II).

A decrease in cAMP could also bring about an increase in the percentage of glycogen synthase I activity by lowering the activity of cAMP-dependent protein kinase. However, it seems unlikely that this mechanism is involved in the activation of glycogen synthase by 2-deoxyglucose, since cAMP concentrations were not detectably decreased by the hexose under conditions in which 2-deoxyglucose increased glycogen synthase I activity (Table I).

Stalans et al. proposed that inactivation of phosphorylase is involved in the activation of hepatic glycogen synthase by glucose (17, 18). They further suggested that only when 2-phosphorylase a are decreased to a certain threshold does glycogen synthase activation occur. By their model, phosphorylase inactivation should always precede glycogen synthase activation. There is no evidence that such a scheme fits the activation of glycogen synthase by 2-deoxyglucose in adipocytes, because phosphorylase inactivation clearly did not precede glycogen synthase activation (Fig. 9).

Hizukuri and Takeda (20) first demonstrated an increase in glycogen synthase phosphatase activity with glucose-6-P using a partially purified enzyme preparation from bovine spleen. Kato and Bishop (21) later demonstrated a similar effect using purified enzymes from rabbit skeletal muscle. Increases in glycogen synthase phosphatase activity in the presence of glucose-6-P have also been demonstrated by Nakai and Thomson (32) using a purified heart enzyme and by Killilea et al. (23) using a highly purified rabbit liver phosphatase. These studies have not completely eliminated the possibility that glucose-6-P directly stimulates glycogen synthase phosphatase. However, as Kato and Bishop (21) suggested, the stimulation may arise from an effect of glucose-6-P to alter the conformation of glycogen synthase D, rendering it a better substrate for the phosphatase.
Regardless of the mechanism by which glucose-6-P increases glycogen synthase phosphatase activity, it seems possible that such a mechanism might be operative in adipocytes, since glucose-6-P increased the activity of fat cell glycogen synthase phosphatase (Fig. 11). Calculations suggest that intracellular concentrations of glucose-6-P following incubation of cells with glucose are in the range of concentrations that increase phosphatase activity. If the intracellular water space is assumed to be 1.5 pl/cell (37), then the intracellular concentration of glucose-6-P in cells incubated with 5 mM glucose for 10 min can be estimated at about 0.7 mM and 1.6 mM in cells incubated with insulin plus glucose (calculated from data in Table II). If the concentrations of intracellular 2-deoxyglucose-6-P resulting from incubating cells with 2-deoxyglucose are estimated using the same assumptions (from Figs. 9 and 10), these also are in the range of 2-deoxyglucose-6-P concentrations that increased phosphatase activity (Fig. 12). Estimates of the intracellular concentrations of metabolites are only approximations since the metabolites probably do not randomly distribute within the cytosol and may not be free in solution, but bound to some extent to cellular constituents. It should also be stressed that conditions of the phosphatase assay are vastly different from those present in the cellular milieu. However, glucose, mannose, and 2-deoxyglucose did not increase phosphatase activity. These results are consistent with the proposal that hexose phosphorylation is involved in mediating the effects of glucose to activate glycogen synthase.

In summary, the results presented in this report indicate that activation of glycogen synthase by glucose occurs subsequent to the transport and phosphorylation of the hexose. Fig. 13 depicts a model for glycogen synthase activation by insulin and glucose. The interaction of insulin with its receptor triggers cellular events leading to activation of glycogen synthase by a direct, hexose transport-independent mechanism. Activation by this pathway is observed in the absence of glucose, or with glucose under conditions in which glucose transport is inhibited, as in the presence of phloridzin or cytochalasin B. This direct pathway is also activated by concanavalin A in the absence of glucose (Fig. 3). Evidence obtained from experiments with skeletal muscle (38, 41), liver (42), and adipose tissue (43, 44) indicates that the mechanism of this direct pathway of glycogen synthase activation by insulin may involve a decrease in cAMP-dependent protein kinase activity. The insulin-insulin receptor interaction also results in the activation of the hexose transport system. In the presence of glucose, mannose, or 2-deoxyglucose, this leads to increased sugar entry and phosphorylation that results in activation of glycogen synthase by a hexose transport-coupled and phosphorylation-dependent pathway. In the presence of glucose, activation by this pathway can be effected by concanavalin A and H2O2, by virtue of their effects to stimulate glucose transport. This mechanism may involve accumulation of hexose phosphate and an increase in glycogen synthase phosphatase activity.

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REFERENCES
1. Lawrence, J. C., Jr., Guinovart, J. J., and Larner, J. (1977) J. Biol. Chem. 252, 444–450.
2. Czech, M. P. and Lynn, W. S. (1973) Biochim. Biophys. Acta 297, 368–377.
3. Czech, M. P., Lawrence, J. C., Jr., and Lynn, W. S. (1974) J. Biol. Chem. 249, 7498–7505.
4. Czech, M. P., Lawrence, J. C., Jr., and Lynn, W. S. (1974) J. Biol. Chem. 249, 5421–5427.
5. Czech, M. P. (1976) J. Biol. Chem. 251, 1164–1170.
6. Larner, J., and Villar-Palasi, C. (1971) Curr. Top. Cell. Regul. 3, 195–226.
7. Miller, T. B., Jr., and Larner, J. (1973) J. Biol. Chem. 248, 3483–3488.
8. Rodbell, M. (1964) J. Biol. Chem. 239, 375–380.
9. Thomas, J. A., Schleider, K. K., and Larner, J. (1968) Anal. Biochem. 25, 486–499.
10. Gilboe, D. F., Larson, K. L., and Nuttal, F. Q. (1972) Anal. Biochem. 47, 20–27.
11. Williamson, J. R., and Corkey, B. E. (1969) Methods Enzymol. 13, 434.
12. Harper, J. F., and Brooker, G. (1975) J. Cyclic Nucleotide Res. 1, 207–218.
13. Renner, E. D., Plagemann, P. G. W., and Bernlohr, R. W. (1972) J. Biol. Chem. 247, 5765–5776.
14. Larner, J., Villar-Palasi, C., and Brown, N. E. (1969) Biochim. Biophys. Acta 178, 470–479.
15. Czech, M. P., Lawrence, J. C., Jr., and Lynn, W. S. (1974) J. Biol. Chem. 249, 1001–1006.
16. Czech, M. P. (1976) J. Clin. Invest. 57, 1523–1532.
17. Stalmans, W., De Wulf, H., and Hers, H. G. (1971) Eur. J. Biochem. 18, 582–587.
18. Stalmans, W., De Wulf, H., Hue, L., and Hers, H. G. (1974) Eur. J. Biochem. 41, 197–194.
19. Chandramouli, V., and Carter, J. R., Jr., (1977) Biochim. Biophys. Acta 496, 278–291.
20. Himukuri, S. and Takeda, Y. (1970) Biochim. Biophys. Acta 211, 378–381.
21. Kato, K., and Bishop, J. S. (1972) J. Biol. Chem. 247, 7420–7429.
22. Nakai, C., and Thomas, J. A. (1974) J. Biol. Chem. 249, 6459–6467.
23. Killilea, S. D., Brandt, H., Lee, E. Y. C., and Whelan, W. J. (1976) J. Biol. Chem. 251, 2369–2368.
24. Czech, M. P., Lawrence, J. C., Jr., and Lynn, W. S. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4173–4177.
25. Coutrecoosa, P. (1970) J. Biol. Chem. 245, 3528–3534.
26. Demeyt, P., Gavin, J. R., III, Roth, J., and Neveille, D. M., Jr. (1974) Diabetes 23, (suppl. 1) 865.
27. Crofford, O. B., and Renold, A. E. (1965) J. Biol. Chem. 240, 2112.
Hexose Transport-coupled Activation of Glycogen Synthase

2113

28. Crofford, O. B. (1967) Am. J. Physiol. 212, 217–220
29. Chang, K.-J., and Cuatrecasas, P. (1974) J. Biol. Chem. 249, 3119–3120
30. Le Fevre, P. G., and Marshall, J. K. (1958) Am. J. Physiol. 194, 333–337
31. Resnick, O., and Hechter, O. (1958) J. Biol. Chem. 224, 941–964
32. Kuo, J. F., and Dill, I. K. (1969) Biochim. Biophys. Acta 177, 17–26
33. Wood, F. C., Jr., Leboeuf, B., Renold, A. E., and Cahill, G. F., Jr. (1961) J. Biol. Chem. 236, 13–21
34. Froesch, E. R. (1965) in Adipose Tissue, Handbook of Physiology (Renold, A. E. and Cahill, G. F., Jr., eds) section 5, pp. 281–293, The American Physiological Society, Washington, D.C.
35. Czech, M. P., Lynn, D. G., and Lynn, W. S. (1973) J. Biol. Chem. 248, 3636–3641
36. Gilboe, D. P., and Nuttal, F. Q. (1974) Biochim. Biophys. Acta 337, 57–67
37. Czech, M. P. (1977) Mol. Cell. Biochem. 11, 51–63
38. Villar-Palasi, C., and Wenger, J. J. (1967) Fed. Proc. 26, 563
39. Shen, L. C., Villar-Palasi, C., and Larner, J. (1967) Physiol. Chem. Phys. 2, 536–544
40. Miller, T. B., and Larner, J. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 2774–2777
41. Walkenbach, R., and Larner, J. (1978) Mol. Cell. Biochem. in press
42. Miller, T. B., Jr., and Larner, J. (1973) J. Biol. Chem. 248, 3483–3488
43. Soderling, T. R., Corbin, J. D., and Park, C. K. (1973) J. Biol. Chem. 248, 1822–1829
44. Guinovart, J. J., Lawrence, J. C., Jr., and Larner, J. (1978) Biochim. Biophys. Acta, in press
Activation of glycogen synthase in rat adipocytes by insulin and glucose involves increased glucose transport and phosphorylation.

J C Lawrence, Jr and J Larner

J. Biol. Chem. 1978, 253:2104-2113.