Glucocorticoid Control of the Liver Glycogen Synthetase-activating System*

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SUMMARY

The activity of the enzyme system which converts hepatic glycogen synthetase to the active form disappears from the livers of fasted, adrenalectomized rats. The level of active synthetase in such preparations is nil. After the administration of glucocorticoid there is a return of activating enzyme activity and gradual increases in the level of active glycogen synthetase, the rate of glycogen synthesis, and the glycogen content of the liver. We propose that the changes in activating enzyme activity are the proximate cause of the changes in the level of active synthetase and the consequent alterations in hepatic glycogen content in response to the glucocorticoid state of the animal.

The kinetics of the a and b forms1 of liver glycogen synthetase (UDP-glucose:glycogen glucosyltransferase, EC 2.4.1.11) indicates that the former is virtually fully active under physiological conditions and the latter is virtually totally inactive (2). These findings indicate that the basis of the control of this enzyme lies in the interconversion of the active and inactive forms by what we have referred to as an "on-off mechanism" (2). The b form is convertible to the a form by an enzymatic process (3) which, by analogy with the muscle synthetase system (4), presumably involves hydrolysis of a synthetase-bound phosphate group.

The studies reported in this paper are addressed to the problem of the role of the glycogen synthetase system in the glucocorticoid control of glycogen synthesis in the liver. The findings were that a combination of adrenalectomy and fasting led to a complete loss of the activating enzyme activity and to a reduction in the level of the active form of the synthetase from about 15% to 20% of the total activity to about 3% or less. Glucocorticoid treatment at this point brought about a restoration of activating enzyme activity and of synthetase a activity to the normal level. Deprivation or administration of glucocorticoid or fasting caused relatively little variation in the total synthetase activity.

These results suggest that the effect of glucocorticoid on the glycogen synthetase-activating enzyme precedes and leads to activation of glycogen synthetase and consequent stimulation of glycogen synthesis.

MATERIALS AND METHODS

Both control and adrenalectomized Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories, Brookline, Massachusetts. The latter were given 0.9% NaCl in the drinking water. Glycogen (rabbit liver, Type III) and UDP-glucose were obtained from Sigma; UDP-glucose-14C and d-glucose-6-3H, from New England Nuclear. Counting was performed in a Beckman scintillation counter at 49% efficiency with a background of 15 cpm for 14C and at 34% efficiency with a background of 26 cpm for 3H under the counting conditions.

Livers were homogenized in 3 ml per g of a solution containing 10 mM MgCl2, 10 mM GSH, and 100 mM glycylglycine, pH 7.4. The supernatant fraction from centrifugation of the homogenate at 8,500 × g for 10 min at 0° is referred to as the crude extract. For some experiments the crude extract was subdivided into a pellet and a soluble fraction by centrifugation in a Spinco model L-2 at 50,000 rpm for 40 min at 0°. The pellet was resuspended in the homogenization buffer to the original volume. This suspension is referred to as the glycogen pellet (containing the microsomal fraction and other particulate matter as well (5)).

Assay of the a (active) form of the enzyme was carried out at 37° for 2 min in a volume of 1 ml containing 4 μmoles of UDP-glucose-14C (30,000 cpm), 5 mg of rabbit liver glycogen, 1 μmole of EDTA, 45 μmoles of glycylglycine, pH 7.4, and 0.1 ml of enzyme preparation. For assay of total activity (a (active) + b (inactive)), 4 μmoles of glucose 6-phosphate were present in addition. Glycogen was isolated and its radioactivity was counted as previously described (6). Under these conditions amount of product formed is proportional to time and enzyme added (6). Since the activity of the b form in the absence of glucose 6-phosphate may be as much as 5% of its activity in the presence of glucose 6-phosphate in the above assays (2), any...

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1 These have previously been referred to as the "I" and "D" forms, respectively (1). Our reasons for preferring the more noncommittal terminology, a and b, are explained in a previous publication (2).
value for “a activity” less than about 5% of the total activity may actually reflect no a activity at all.

For conversion of synthetase b to synthetase a in vitro, the preparation was incubated at 20° and aliquots were removed periodically for assay as previously described (6).

Glycogen was assayed by the anthrone method (7).

Relative rates of glycogen synthesis in vivo were determined by measurements of incorporation of 1-glucose-6-3H into glycogen.

RESULTS

Activation of Glycogen Synthetase—Time courses for the conversion of glycogen synthetase b to a are illustrated in Fig. 1. The lag is less marked than in experiments reported previously (6)—a consequence of the presence of Mg²⁺ and GSH in the present experiments. The most dramatic features of the results expressed in Fig. 1 are the total absence of activation of glycogen synthetase in the adrenalectomized, fasted animals (Curve E) and its restoration upon administration of glucocorticoid (Curve F). Five of six livers from rats killed 1 hour after hormone administration exhibited a small increase in a activity during the incubation in vitro (average increase of the five was from 5.1 ± 2.2% to 11.3 ± 3.3% of total activity after 70 min), whereas in nontreated animals there was no semblance of an increase (Curve E). The activation curve obtained 4 2 hours after hormone administration (Curve F) was essentially normal in all animals tested. Neither fasting nor adrenalectomy alone nor glucocorticoid treatment of normal animals produced significant effects on the activation curves.

To establish whether the lack of activation was a result of a lack of activating enzyme activity or a defect in the synthetase itself, fractions from normal animals containing activating enzyme activity were added to a glycogen synthetase preparation from an adrenalectomized, fasted animal. Under these conditions there was activation of the synthetase (Table I).

Time Course of Reappearance of Active Synthetase after Administration of Glucocorticoid—It may be noted from Fig. 1, Curve E, that the initial synthetase a activity in fasted, adrenalectomized animals was reduced to a very low value (in fact, indistinguishable from zero; see “Materials and Methods”), and was restored to a normal level 42 hours after glucocorticoid treatment (Curve F), in confirmation of the findings of Hornbrook, Burch, and Lowry (8) and DeWulf, Stalmans, and Hirs (9). Fig. 2 shows the time course of this reappearance of synthetase a activity. One to two hours after the administration of hormone a activity reappeared at a significant level, reaching a peak at 44 hours. As is also illustrated in Fig. 2, adrenalectomy alone reduced the fraction of a activity by about half, whereas fasting alone had no effect. Administration of hormone to fasted, intact animals produced a more than 2-fold increase in the fraction of a activity at 3 hours. Little or no response to the glucocorticoid was observed in fed, intact animals. Changes in total activity were relatively small, declining about 25% with adrenalectomy.

Time Course of Reappearance of Glycogen Synthesis after Administration of Glucocorticoid—With the above results at hand it remained to correlate the changes in synthetase-activating activity and level of active synthetase with the rate of glycogen

<table>
<thead>
<tr>
<th>Enzyme fraction</th>
<th>Preliminary incubation</th>
<th>Glycogen synthetase activity</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Supernatant + buffer</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Pellet + buffer</td>
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<tr>
<td>Supernatant + factor</td>
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Fig. 1. Effects of adrenalectomy, fasting, and glucocorticoid treatment on activation of glycogen synthetase in vitro. The abbreviations used are: NORM, normal animals; ADRX, adrenalectomized animals; FED, fed ad libitum; STARV, fasted for 48 hours; PRED, 2 mg of prednisolone acetate per rat, administered 3 hours (C) or 4 2 hours (F) before the animal was killed. Each point is an average of data from at least four animals. The abscissa is minutes of incubation of the crude extract at 20°; the ordinate is the a activity as a percentage of the total activity. The absolute values of the total activity are available from Fig. 3.
synthesis and the glycogen content. As Fig. 3 shows, the rate of glycogen synthesis from labeled glucose (Fig. 3A) and the level of glycogen in the liver (Fig. 3B) rose in parallel in the fasted animals (both normal and adrenalectomized) after administration of glucocorticoid. It is of interest that, in addition to the expected decrease in glycogen levels with fasting, there was also a fall in the rate of glucose incorporation. The decline in incorporation rate cannot be a consequence of a lack of precursors, but rather implies a fasting-induced decrease in the activity of one or more enzymes in the pathway from glucose to glycogen.

There was a close reciprocal relationship between the glycogen content of the liver and the fraction of total glycogen synthetase not sedimentable at 50,000 rpm (Fig. 3C). There are two additional points to be made about the soluble enzyme. (a) It became resedimentable in its entirety upon addition of glycogen (15 mg per ml). (b) It was in the b form, but convertible to the a form in the presence of activating factor (see Table I).

**DISCUSSION**

As with virtually all other hormonal effects, the chain of events leading to the glucocorticoid-induced elevation in hepatic glycogen levels is largely unmapped. Persuasive evidence exists that the glycogen synthetase system is an early and important control site in this process, and the characteristics of this system have led us to propose that the manner by which modulation of this reaction is effected is by interconversion of the active and inactive forms of the synthetase (2). A corollary of this conclusion is that changes in the activity of one or both of the enzymatic systems which bring about these interconversions precede and lead to the changes in synthetase activity itself.

Friedman and Larner have shown that the interconversion of

\[ S.E. = \sqrt{\frac{\Sigma D^2}{n(n-1)}} \]

Fig. 2. Effects of adrenalectomy, fasting, and glucocorticoid treatment on rate of glycogen synthesis, glycogen content, and fraction of glycogen synthetase not sedimentable at 50,000 rpm. Relative rates of glycogen synthesis, in counts per min per g of liver, were obtained by administration of \( 3.7 \times 10^4 \) cpm of \( D\)-glucose-$\text{N}^15 \) 15 min before the animals were killed, followed by isolation of glycogen for counting, as well as chemical assay. Percentage soluble enzyme was obtained from the total activity in the 50,000 rpm fraction relative to the total activity in the crude extract (8,500 X g fraction). The symbols on the abscissa are explained in the legend to Fig. 2.
glycogen synthetase in muscle involves phosphorylation and dephosphorylation reactions (4), and Hizukuri and Larner have shown that the characteristics of the liver system are also consistent with this mechanism (3). The liver glycogen synthetase-activating enzyme, like the synthetase itself, sediments with the glycogen pellet, but can be separated from these substances, albeit still in particulate form, by sonic oscillation (3).

The evidence presented here confirms that at least one of the interconverting enzyme systems, the one producing the active form of glycogen synthetase, is affected by alterations in the glucocorticoid status of the animal. Furthermore, the disappearance and reappearance of this activity are coordinated with the level of active glycogen synthetase, the rate of glycogen synthesis, and glycogen levels in a manner consistent with the proposal of a causal relationship of the synthetase-activating system to the latter parameters.

On the basis of the above considerations, the goal of defining the sequence of events from changes in glucocorticoid availability to changes in hepatic glycogen level appears to be one step closer to realization. We have not yet established whether the loss and reappearance of synthetase-activating activity reflects a loss and reappearance of the enzyme per se or a change in another parameter on which its activity depends. The answer to this question, which is currently under investigation, will dictate the direction of the continuing search for the primary glucocorticoid effect leading to elevated glycogen levels.

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