The Effect of Insulin on Enzyme Adaptations to Diets and Hormones*

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SUMMARY
1. Insulin prevented increases in liver glucose 6-phosphatase and fructose 1,6-diphosphatase activities in response to cortisol administration, but it did not prevent increases in response to diets high in protein or in fructose.
2. Insulin increased pyruvate kinase activity in animals treated with cortisol or fed a high protein diet; both treatments decrease pyruvate kinase activity. Insulin did not increase the pyruvate kinase activity of animals receiving a high glucose diet.
3. Insulin administration had a more marked effect on glucose 6-phosphate dehydrogenase activity than on malic dehydrogenase (decarboxylating) activity.
4. The increases in serine dehydrase, glutamic-oxalacetic transaminase, and malic dehydrogenase in response to cortisol treatment were partially or completely prevented by insulin. The increases in glutamic-pyruvic transaminase and lactic dehydrogenase in response to cortisol administration were not affected by insulin. Insulin either had no effect on increases observed in these enzymes after high protein or high fructose feeding or caused even greater increases after dietary treatment. Two enzymes (glutamic-pyruvic transaminase and lactic dehydrogenase) not affected by insulin in the glucose-fed group were increased by insulin in groups receiving high protein or high fructose diets.

It has been shown that the increase in activities of liver glucose 6-phosphatase (\textit{p}-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) and fructose 1,6-diphosphatase (\textit{p}-fructose 1,6-diphosphate 1-phosphohydrolase EC 3.1.3.11) usually observed in response to injections of glucocorticoids can be prevented by insulin (1). Insulin administration has also been reported to decrease the liver glutamic-pyruvic transaminase (\textit{t}-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) activity (2), although another report indicated that this treatment neither affected glutamic-pyruvic transaminase activity nor prevented the increase in glutamic-pyruvic transaminase activity in response to glucocorticoids (3). It has been proposed that insulin is a "suppressor" of the synthesis of enzymes concerned with gluconeogenesis (1). However, since both the phosphatases (4) and glutamic-pyruvic transaminase (5) have been closely linked to gluconeogenesis, it appears that either the linkage of glutamic-pyruvic transaminase to gluconeogenesis or the suppressor theory will require modification.

Pyruvate kinase (adenosine triphosphate:pyruvate phosphotransferase, EC 2.7.1.40) activity has been related to glycolysis and observed to decrease in activity during conditions that cause increased gluconeogenesis (6). It has also been reported that insulin is a specific inducer of liver pyruvate kinase activity (7). However, this induction was studied only in diabetic rats, which have lower than normal liver pyruvate kinase activity, and insulin restored the activity to normal levels.

Since this suppressor theory has been applied to four enzymes, only two of which have been examined, it seemed of importance to examine the role of insulin as a suppressor of several enzyme systems under various conditions. The role of insulin in inducing pyruvate kinase activity in nondiabetic animals under various conditions may also indicate the generality of the induction of this enzyme by insulin.

METHODS

Male Sprague-Dawley rats were housed in screen-bottom cages and allowed food and water \textit{ad libitum}. The diets have been described (8). Insulin was injected by the following schedule per 150 g of body weight: 4 i.u. of protamine-zinc-insulin per day over the entire experiment period; in addition, 2 i.u. of zinc-insulin the 1st day and 1 i.u. of zinc-insulin the 2nd day. All injections were intramuscular. From the 3rd day, there should have been a steady state flow of 4 i.u. of insulin per day per 150 g based on a release time of about 3 days for the protamine-zinc-insulin. The cortisol group received intraperitoneally 5 mg of hydrocortisone per rat per day for the entire experimental period and were fed the high glucose diet. All animals were treated for 5 days before death. The animals were stunned by a sharp blow on the head, decapitated, and exsanguinated, and the livers were removed, weighed, and chilled. A portion of the
liver was homogenized in 19 volumes of 0.1 M potassium citrate (pH 6.5). A second portion was homogenized in 4 volumes of 0.14 M KCl, centrifuged for 30 min at 0–4° at 30,000 × g, and the resulting supernatant solution was used as the enzyme source.

The citrate preparations were used for glucose 6-phosphatase (9) and phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) (10), both at 37°. The remaining enzyme in the KCl preparation were determined with a Gilford transferase, EC 2.4.1.1) (11), malic dehydrogenase (decarboxylating) oxidoreductase (decarboxylating, EC 1.1.1.40) (12), lactate dehydrogenase (L-1actate:NAD oxidoreductase, EC 1.1.1.27) (13), malic dehydrogenase (L-malic:NAD oxidoreductase, EC 1.1.1.37) (14), pyruvate kinase (15), fructose 1,6-diphosphatase (16), serine dehydrase (L-serine hydro-lyase (dehydrating), EC 4.2.1.13) (17), glutamic-pyruvic transaminase (2-oxo-glutarate aminotransferase, EC 2.6.1.1) (19). Protein was determined (20) before and after centrifugation of the KCl preparation in order to obtain total and soluble liver protein concentrations.

The results are reported per 100 g of body weight, which is indicative of total available activity. This can be readily recalculated per g of liver or per g of liver protein, since values are also reported for grams of liver per 100 g of body weight and milligrams of liver protein per 100 g of liver weight. Enzyme activities are reported in units, a unit being defined as the amount of enzyme causing the conversion of 1 μmole of substrate in 1 min under the previously described conditions.

RESULTS AND DISCUSSION

As previously reported (1), insulin partly abolished the significant increases in glucose 6-phosphatase and fructose 1,6-diphosphatase in response to cortisol injection (Table I). Insulin also decreased the response of glucose 6-phosphatase to a high protein diet but did not prevent it. The response of fructose 1,6-diphosphatase to a high protein diet was unaffected by insulin. In the case of the high fructose regimen, insulin did not prevent or affect the increase of either of the phosphatases. In rats receiving the control glucose diet, insulin caused a decrease in glucose 6-phosphatase activity but not in that of fructose 1,6-diphosphatase. Thus, the suggestion (1) that insulin has a specific suppressor action appears unwarranted. The difference between the insulin effects on increases after high protein feeding and those after high fructose feeding are inconsistent with both phosphatases being in a single "functional genome unit" as proposed (21) but is consistent with a previous report on the selective induction of these two enzymes (4). The data are also indicative that the fructose and cortisol inductions of glucose 6-phosphatase are different, for, with the high protein feeding, the increases following some have similarity to those of cortisol. These findings are consistent with previous reports of differences between high protein and high fructose inductions of this enzyme (22) and the induction by dietary treatments with adrenalectomized rats (23).

A high protein diet and cortisol administration decreased pyruvate kinase activity, and feeding the high fructose diet increased liver pyruvate kinase activity. The insulin treatment

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Glucose</th>
<th>Cortisol</th>
<th>High protein</th>
<th>High fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With insuline</td>
<td>Without insuline</td>
<td>With insuline</td>
<td>Without insuline</td>
</tr>
<tr>
<td>Body weight</td>
<td>196 ± 8 g</td>
<td>179 ± 5</td>
<td>177 ± 13</td>
<td>134 ± 10</td>
</tr>
<tr>
<td>Liver weight/body weight</td>
<td>4.71 ± 0.24</td>
<td>4.77 ± 0.12</td>
<td>5.32 ± 0.36</td>
<td>7.15 ± 0.25</td>
</tr>
<tr>
<td>Soluble liver protein (mg/100</td>
<td>550 ± 20</td>
<td>600 ± 40</td>
<td>640 ± 60</td>
<td>1010 ± 60</td>
</tr>
<tr>
<td>Total liver protein (mg/100</td>
<td>1640 ± 60</td>
<td>1370 ± 60</td>
<td>1500 ± 150</td>
<td>1870 ± 60</td>
</tr>
<tr>
<td>Glucose 6-phosphatase</td>
<td>50.7 ± 2.6</td>
<td>74.4 ± 4.1</td>
<td>86.2 ± 9.8</td>
<td>108 ± 13</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphatase</td>
<td>22.4 ± 1.9</td>
<td>20.6 ± 0.3</td>
<td>27.7 ± 2.0</td>
<td>43.7 ± 2.2</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>67.3 ± 17.2</td>
<td>55.2 ± 7.2</td>
<td>68.5 ± 15.2</td>
<td>32.3 ± 3.5</td>
</tr>
</tbody>
</table>

* Standard error of the mean. All values are the averages of at least six rats.

Activity is reported in micromoles per min per 100 g of body weight.
Effect of Insulin on Enzyme Adaptations

Table II

Effect of insulin on the activities of several liver enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Glucose (With insulin)</th>
<th>Glucose (Without insulin)</th>
<th>Cortisol (With insulin)</th>
<th>Cortisol (Without insulin)</th>
<th>High protein (With insulin)</th>
<th>High protein (Without insulin)</th>
<th>High fructose (With insulin)</th>
<th>High fructose (Without insulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase</td>
<td>59.8 ± 7.9¹</td>
<td>74.5 ± 5.5</td>
<td>64.2 ± 3.4</td>
<td>100 ± 7</td>
<td>82.7 ± 6.6</td>
<td>39.8 ± 7.7</td>
<td>92.3 ± 3.7</td>
<td>60.7 ± 3.7</td>
</tr>
<tr>
<td>Malic dehydrogenase</td>
<td>2180 ± 260</td>
<td>1540 ± 100</td>
<td>2240 ± 150</td>
<td>3760 ± 240</td>
<td>2120 ± 130</td>
<td>1670 ± 70</td>
<td>2200 ± 150</td>
<td>1750 ± 240</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>1350 ± 250</td>
<td>1620 ± 130</td>
<td>2420 ± 30</td>
<td>2350 ± 120</td>
<td>2210 ± 440</td>
<td>1320 ± 120</td>
<td>3070 ± 700</td>
<td>1490 ± 120</td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>38.0 ± 10.4</td>
<td>22.0 ± 1.2</td>
<td>51.2 ± 10.7</td>
<td>55.4 ± 6.0</td>
<td>65.3 ± 3.8</td>
<td>27.9 ± 3.4</td>
<td>106 ± 14</td>
<td>51.8 ± 7.0</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>12.0 ± 0.8</td>
<td>15.4 ± 0.6</td>
<td>17.3 ± 2.6</td>
<td>41.1 ± 3.1</td>
<td>18.6 ± 3.2</td>
<td>14.4 ± 1.3</td>
<td>69.0 ± 11.7</td>
<td>52.6 ± 3.7</td>
</tr>
<tr>
<td>Glutamic-pyruvic transaminase</td>
<td>37.4 ± 3.7</td>
<td>36.3 ± 2.6</td>
<td>91.2 ± 12.4</td>
<td>104 ± 4</td>
<td>173 ± 12</td>
<td>95 ± 5</td>
<td>78.6 ± 6.6</td>
<td>44.6 ± 3.8</td>
</tr>
<tr>
<td>Glutamic-oxaloacetic transaminase</td>
<td>266 ± 24</td>
<td>278 ± 12</td>
<td>385 ± 29</td>
<td>780 ± 71</td>
<td>899 ± 73</td>
<td>68 ± 48</td>
<td>318 ± 24</td>
<td>246 ± 25</td>
</tr>
<tr>
<td>Serine dehydrodrase</td>
<td>5.33 ± 0.59</td>
<td>6.09 ± 0.55</td>
<td>11.5 ± 1.4</td>
<td>25.9 ± 2.9</td>
<td>30.1 ± 1.5</td>
<td>34.2 ± 3.3</td>
<td>4.10 ± 0.65</td>
<td>5.50 ± 0.85</td>
</tr>
</tbody>
</table>

¹ Activity is reported in micromoles per min per 100 g of body weight.
² Standard error of the mean. All values are the average of at least six rats.

The observation that insulin increases the activity of glucose 6-phosphate dehydrogenase in all groups with the exception of the cortisol-treated group and returned the malic dehydrogenase (decarboxylating) activity to normal in the cortisol-treated rats, whereas in the untreated rats it was substantially increased, may again indicate a cortisol-insulin antagonism since similar insulin effects are not observed in any of the other three groups. It therefore appears from these data that the two TPN-linked en-

increases in phosphorylase and lactic dehydrogenase activity after insulin treatment only in the high protein and high fructose groups may again be due to the lower circulating levels of insulin in these animals. Thus it might be expected that the effect of an excess dose of insulin would be more noticeable in animals with lower endogenous levels. Insulin treatment prevented the increases in phosphorylase and malic dehydrogenase in response to cortisol injections but not the increase in lactic dehydrogenase. The high protein regimen caused a decrease in phosphorylase activity which was reversed after insulin treatment. High protein feeding plus insulin injections increased lactic dehydrogenase activity, although high protein feeding or insulin injections alone did not affect this activity. The fructose regimen did not affect the activity of these three enzymes except after insulin treatment when it caused significant increases in phosphorylase and lactic dehydrogenase.

Although the two TPN-linked dehydrogenases behave similarly in many cases in response to dietary and hormonal treatments (24–26), they responded very differently to insulin treatment. Glucose 6-phosphate dehydrogenase activity was increased by insulin in the control, high protein, and high fructose groups, but not in the cortisol group. The malic dehydrogenase (decarboxylating) activity, in contrast, was slightly decreased by insulin in the control group and markedly in the cortisol group, but not in the high protein and high fructose groups.

The observation that insulin increases the activity of glucose 6-phosphate dehydrogenase in all groups with the exception of the cortisol-treated group and returned the malic dehydrogenase (decarboxylating) activity to normal in the cortisol-treated rats, whereas in the untreated rats it was substantially increased, may again indicate a cortisol-insulin antagonism since similar insulin effects are not observed in any of the other three groups. It therefore appears from these data that the two TPN-linked en-

returned the pyruvate kinase activity after cortisol administration to normal, but failed to raise the activity of the high protein group to that of the control value. The role of insulin in maintenance and induction of pyruvate kinase in the liver does not seem to be as clear-cut as previously suggested (7). It appears in the case of the two diets, the high protein and high fructose diets, which would be expected to elicit the lowest degree of insulin secretion, that insulin has a definite effect on increasing the pyruvate kinase activity. However, in the case of the high glucose diet in which circulating levels of insulin would be expected to be higher, this hormone had no effect on the activity. The fact that the pyruvate kinase activity of the high protein group was increased by insulin, but not returned to normal, indicates that the activity of this enzyme is regulated by other controlling mechanisms in addition to insulin and glucoconoid levels. The very high activity of pyruvate kinase after fructose feeding which has been noticed previously after sucrose feeding (6) may be related to the fact that the ratio of metabolism in the liver compared with peripheral tissue is higher for fructose than glucose. The results with the fructose group indicate that the availability of substrates particularly at the triose phosphate level may also be a factor in controlling the activity of liver pyruvate kinase. The effects of cortisol and insulin on the activity of the two phosphatases and pyruvate kinase represent a further case of the well known insulin-cortisol antagonism, without specifying at what level of involvement the antagonism occurs.

Insulin treatment of the group fed the high glucose diet caused a decrease of phosphorylase, an increase in malic dehydrogenase, and no change in lactic dehydrogenase activity (Table II). Cortisol injections caused increases in all three of these enzymes. Thus, once again, there appears to be a possible insulin-cortico-steroid antagonism in the case of phosphorylase and malic dehydrogenase but not in the case of lactic dehydrogenase. The
enzymes respond quite differently to insulin administration in all groups except the cortisol-treated group.

The three enzymes examined which are associated with amino acid metabolism were unaffected by insulin treatment in the glucose group. The activities of all three of these were increased markedly by cortisol treatment. Insulin plus cortisol treatment affected glutamic-pyruvic transaminase similarly to cortisol injection alone. This agrees with a previous report (3). Changes in the levels of glutamic-pyruvic transaminase were similar to those observed with lactic dehydrogenase; that is, exogenous insulin administration caused an increase in the activity in the fructose and high protein groups and had no effect on the activity of the other two groups. This is of particular interest in the high protein group which already had a markedly elevated activity. The further increase in activity may be related to lower blood glucose level after insulin, thus making the liver even more dependent on amino acid metabolism, or was possibly due to an increased food intake. The responses of serine dehydrase and glutamic-oxalacetic transaminase to cortisol were significantly decreased by insulin treatment. The results with serine dehydrase and glutamic-oxalacetic transaminase are suggestive again of some antagonism between cortisol and insulin. The activity of serine dehydrase after high protein feeding was not altered by insulin treatment, but the activities of the transaminases were further increased on the high protein regimen after insulin. Thus, the results with glutamic-oxalacetic transaminase appear to be a combination of the previous two enzymes, serine dehydrase and glutamic pyruvic transaminase, with an apparent cortisol-insulin antagonism and an increased dependence on amino acid metabolism. None of these three enzymes was altered in activity after feeding the high fructose diet; however, insulin treatment on this dietary regimen caused increases in both transaminases. Once again the increases in transaminases after insulin treatment with the high fructose diet may be related to increased dependence on amino acid metabolism or increased food intake; although it would be expected that serine dehydrase should show a similar pattern, this was not observed.

Insulin also prevented the marked increases in liver to body weight ratios and soluble liver protein in response to cortisol. The treatment with insulin had no significant effect on the liver to body weight ratio in the other three groups. Insulin treatment caused a significant increase in liver protein in all except the cortisol-treated rats.

Thus, although insulin prevents many inductions of enzymes by cortisol, this block does not occur in all cases. Many increases in enzyme activities produced in response to cortisol can be prevented by insulin administration but are not prevented by insulin administration in the case in which the inducing conditions are dietary, such as the high fructose and high protein diets. Thus a general suppressor activity of insulin is not consistent with the observed facts.

REFERENCES
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