Interaction of Glucocorticoid and Thyroxine in the Responses of Rats to Starvation-Refeeding

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ABSTRACT The interaction of glucocorticoid (GC) and thyroxine (T₄) in the generation of the hepatic enzyme overshoot and lipid response to starvation-refeeding was studied. Male Sprague-Dawley rats were either left intact, or treated with propylthiouracil (PTU), or adrenalectomized (ADX), or ADX and/or PTU treated and treated with GC and/or T₄. One-half of each of these treatment groups was fed a 65% glucose diet while the remaining rats were starved for 48 hours and re-fed the glucose diet for 48 hours. After decapitation, hepatic lipid and glucose-6-phosphate dehydrogenase (G6PD) activity were determined. Rats treated with only PTU had less of an enzyme overshoot than nontreated rats, and the full overshoot response was restored with T₄ treatment. ADX rats did not have the typical enzyme overshoot response to starvation-refeeding. However, ADX rats had their overshoot response restored with GC. PTU-treated ADX rats had more of an overshoot response than did ADX rats. When T₄ was administered to PTU-treated ADX rats there was less of an enzyme overshoot; however, when both T₄ and GC were administered to the PTU-treated ADX rats, the overshoot response was fully restored. The liver lipid response to starvation-refeeding followed a similar pattern except that in PTU-treated rats the liver lipid levels were significantly higher in the starved-refed rats than in the ad libitum-fed rats. These results indicate that T₄ and GC play a role in the G6PD and liver lipid response to starvation-refeeding. J. Nutr. 113: 2260–2265, 1983.

INDEXING KEY WORDS starvation-refeeding • thyroxine • glucocorticoid

Starvation-refeeding is a technique widely used to increase the hepatic lipogenic response to dietary carbohydrate (I–II). While different investigators may use different time intervals, in our hands, this procedure consists of a 48-hour starvation period followed by a 48-hour period of refeeding a 65% glucose diet. The responses to this treatment typically include an increase in the activity of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), other NADP-linked enzymes, increased incorporation of ³HOH into fatty acids and an increase in liver lipid.

The magnitude of the lipogenic response depends largely on the presence of the adrenal glucocorticoids (6–9). Adrenalectomized (ADX) rats have difficulty withstanding the starvation-refeeding treatment and fail to show the typical large increase in G6PD activity and liver lipid at the end of the refeeding period (6–10). Replacement of glucocorticoid in the ADX rat results in the full development of the enzyme overshoot and liver lipid response to starvation-refeeding. The mechanisms of the involvement of glucocorticoid in the enzyme overshoot has been thought to be that of stimulating the de novo synthesis of the enzyme protein (7).

Thyroid hormone has also been shown to be involved in the responses to starvation,
starvation-refeeding, and in the control of the activity of a variety of lipogenic enzymes (12–16). Hyperthyroid rats have been found to have fatty livers (14, 16). Hypothyroid rats likewise have been found to have fatty livers (17). These findings suggest that the thyroid hormones may be involved in the genesis of the fatty liver. Since rats given high doses of glucocorticoid and either fed ad libitum or periodically starved and refed do not become fatty nor develop fatty livers (9, 18), the question arises as to whether the thyroid hormones interact with the glucocorticoids such that they suppress the glucocorticoid-induced hyperlipogenic response to starvation-refeeding. Thus, the present work was conducted to determine the respective roles of thyroid hormone and glucocorticoid in the generation of the liver lipid and enzyme overshoot response to starvation-refeeding.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (145 to 160 g initial weight, Sprague-Dawley, Madison, WI) were fed a commercial stock diet (Purina Laboratory Animal Chow,Ralston Purina Co., St. Louis, MO) for 3 days prior to use. The rats were housed in individual wire-mesh cages in a room controlled for temperature (21 ± 1°C) and humidity (45–50%) with equal 12-hour periods of light and dark (lights on at 0600 hours). After acclimation, the rats were allocated into 18 groups of six rats each. In some groups additional rats were used because of the expected mortality due to the treatments.

Treatments. Twelve groups of rats were anesthetized with intraperitoneal injections of sodium pentobarbital (3 mg/100 g body weight) and bilaterally ADX via a dorsal incision. Four days were allowed for recovery during which time they continued to gain weight. When they reached at least 200 g in weight the experiment commenced. During the recovery period, the ADX rats continued to be fed the stock diet. Their drinking water contained 0.9% NaCl. Twelve groups of rats were given daily propylthiouracil (PTU) injections (10 mg PTU/100 g body weight in 0.1 ml of 10% gum acacia, 6-N-propyl-2-thiouracil, Sigma Chemical Company, St. Louis, MO) subcutaneously. PTU injections were given 3 days prior to the starvation-refeeding treatment and continued until the end of the experiment. PTU was used to suppress the production of thyroid hormone by the thyroid gland (19).

Intact, ADX, PTU and ADX-PTU rats were fed either ad libitum or not fed for 48 hours and refed for 48 hours a 65% glucose diet. This diet contained by weight the following ingredients: 1:1 casein:lactalbumin, 20%; glucose, 65%; corn oil, 5%, fiber (Alphacel, ICN Nutritional Biochemical Co., Cleveland, OH), 4%; Bernhardt-Tomarelli mineral mix (20), 4.9%; AOAC vitamin mix (21), 1.1%. One-third of the animals, both ad libitum-fed and starved-refed were injected intraperitoneally twice daily with a sterile isotonic (0.9% NaCl) solution of cortisol (0.075 mg/100 g body weight, injectable hydrocortisone sodium phosphate, Merck Co., Inc., West Point, PA). Cortisol was used rather than corticosterone, the most active glucocorticoid in the rat, because cortisol has a longer biological half-life than corticosterone. Also, one-third received a single daily intraperitoneal injection of thyroxine (10 μg T4/100 g body weight) (DL-thyroxine, Sigma Chemical Co., St. Louis, MO). An outline of these treatments is presented in table 1.

Experimental. Body weights and food consumption were determined daily during the refeeding period. Animals were killed by decapitation. Livers were quickly excised, chilled, weighed and used for the determination of G6PD activity (22). A 10% homogenate of the liver was prepared in ice-cold 0.14 M KCl (pH 7.4) using a Potter-Elvehjem homogenizer (Wheaton Scientific, Millville, NJ). The homogenate was centrifuged (0 to 4°C, 30 minutes, 20,000 x g) and the clear supernatant used, after the appropriate dilution with cold KCl for the assay of G6PD activity. Lipid content was determined gravimetrically by extraction with a 2:2:1 ratio of chloroform, methanol and 1 M KCl, respectively. The means were compared statistically by using the Student's t-test for groups of unequal number (23).

RESULTS

Consistent with the previous reports (1–9) starvation-refeeding resulted in the typical
enzyme overshoot and increase in liver lipid in the intact rat (table 1). Adrenalectomized rats fared less well with this treatment and did not greatly increase their enzyme activity nor were their liver lipid levels as high as their intact counterparts. The administration of glucocorticoid to ADX rats restored the overshoot and liver lipid responses to starvation-refeeding. These responses were consistent with those reported previously for ADX rats (6–9). Food intake, final body weight and liver weight were also affected by these treatments. Starvation-refeeding and ADX decreased final body weight. Except in the ADX rats, liver weights were generally greater in the starved-refed rats than in the ad libitum-fed rats. Food intake was decreased in the starved-refed ADX rat. As has been previously discussed (6–9), the ADX rats do not readily resume feeding after the starvation period and some of these rats must be given a glucose solution by mouth before they will resume feeding. If not so "primed" their hypoglycemia worsens and they die (6).

Treatment of the ad libitum-fed rat with PTU increased G6PD activity above that observed in the control rats (24.3 ± 4.0 vs. 13.8 ± 2.2), but liver lipid was significantly lower (3.92 ± 0.21 vs. 4.73 ± 0.07) than that of the control rats. The starvation-refeeding treatment of the PTU rats brought about an increase in both enzyme activity (35.2 ± 4.2 vs. 24.3 ± 4.0) and liver lipid (4.49 ± 0.14 vs. 3.92 ± 0.21). The increase in enzyme activity with starvation-refeeding was not as great in the PTU-treated rats as in the control rats. Instead of a two- to threefold increase observed in the starved-refed intact control rat (38.1 ± 3.2 vs. 13.6 ± 2.2), there was only a 50% increase (35.2 ± 4.2 vs. 24.3 ± 4.0). When T4 was administered to PTU-treated ad libitum-fed rats, the enzyme activity, though lower, was not significantly different from the PTU-treated rats and was similar to that observed in the intact rats not treated with PTU. Liver lipid in the T4- and PTU-treated rats was also similar to that of the intact ad libitum-fed rats not treated with PTU. The same

**TABLE 1**

| Diet treat. | Hormone treatments | Initial body | Final body | Avg food intake | Liver wt | G6PD units | Lipid
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<td></td>
<td></td>
<td>g</td>
<td>g</td>
<td>100 g body wt</td>
<td>g</td>
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<td>%</td>
</tr>
<tr>
<td>Ad lib</td>
<td>–</td>
<td>223 ± 4</td>
<td>60 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>13.6 ± 2.5</td>
<td>4.73 ± 0.07</td>
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</tr>
<tr>
<td>S-R</td>
<td>–</td>
<td>222 ± 3</td>
<td>6.9 ± 0.2</td>
<td>10.6 ± 0.1</td>
<td>38.1 ± 3.2</td>
<td>5.50 ± 0.29</td>
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</tr>
<tr>
<td>Ad lib</td>
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<td>190 ± 7</td>
<td>6.0 ± 0.5</td>
<td>8.7 ± 0.3</td>
<td>9.13 ± 1.78</td>
<td>4.36 ± 0.15</td>
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<tr>
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<td>214 ± 5</td>
<td>6.0 ± 1.5</td>
<td>8.36 ± 1.08</td>
<td>10.9 ± 2.06</td>
<td>3.35 ± 0.06</td>
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<tr>
<td>Ad lib</td>
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<td>111 ± 4</td>
<td>10.0 ± 0.8</td>
<td>10.3 ± 0.3</td>
<td>19.7 ± 3.4</td>
<td>4.31 ± 0.11</td>
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<tr>
<td>S-R</td>
<td>+</td>
<td>233 ± 4</td>
<td>7.0 ± 0.5</td>
<td>11.8 ± 0.37</td>
<td>35.2 ± 3.0</td>
<td>5.20 ± 0.12</td>
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<tr>
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<td>7.0 ± 0.5</td>
<td>11.8 ± 0.3</td>
<td>34.3 ± 4.0</td>
<td>3.92 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>S-R</td>
<td>+</td>
<td>219 ± 3</td>
<td>7.0 ± 0.2</td>
<td>9.6 ± 0.4</td>
<td>19.7 ± 1.7</td>
<td>4.38 ± 0.26</td>
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<tr>
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<td>9.0 ± 0.2</td>
<td>10.3 ± 0.3</td>
<td>47.9 ± 1.4</td>
<td>3.65 ± 0.17</td>
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<tr>
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<td>9.0 ± 0.2</td>
<td>10.3 ± 0.3</td>
<td>47.9 ± 1.4</td>
<td>3.65 ± 0.17</td>
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<tr>
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<td>5.0 ± 3.0</td>
<td>10.8 ± 0.3</td>
<td>4.95 ± 3.0</td>
<td>3.17 ± 0.14</td>
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<td>205 ± 5</td>
<td>6.0 ± 0.5</td>
<td>9.71 ± 1.35</td>
<td>23.5 ± 5.3</td>
<td>3.04 ± 0.10</td>
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<td>7.0 ± 0.7</td>
<td>10.5 ± 0.5</td>
<td>19.9 ± 3.6</td>
<td>3.88 ± 0.22</td>
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<td>14.7 ± 1.27</td>
<td>34.7 ± 5.9</td>
<td>4.04 ± 0.47</td>
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<td>8.8 ± 1.1</td>
<td>12.9 ± 1.9</td>
<td>5.53 ± 0.85</td>
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<td>7.0 ± 0.5</td>
<td>11.1 ± 0.5</td>
<td>22.8 ± 2.7</td>
<td>3.71 ± 0.19</td>
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<tr>
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<td>+</td>
<td>104 ± 10</td>
<td>9.0 ± 0.3</td>
<td>10.6 ± 0.5</td>
<td>31.6 ± 3.3</td>
<td>4.67 ± 0.31</td>
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<tr>
<td>S-R</td>
<td>+</td>
<td>206 ± 5</td>
<td>9.0 ± 0.2</td>
<td>13.2 ± 0.49</td>
<td>96.3 ± 5.3</td>
<td>8.49 ± 0.06</td>
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</table>

1 Abbreviations used: ADX, bilateral adrenalectomy; PTU, propylthiouracil, 10 mg(100 g body weight - day); GC, glucocorticoid, 0.15 mg(100 g body weight - day); T4, thyroxine, 10 μg(100 g body weight; G6PD, glucose-6-phosphate dehydrogenase. Initial group size, 6 rats; n is the number of rats surviving until the end of the experiment. ∗Effect of PTU on the animals at the beginning of the starvation period. **Statistical significance (P < 0.05) is indicated by letter superscript; ∗within hormone treatments, effect of starvation-refeeding is significant; ∗within dietary treatments, effect of PTU is significant; ∗within dietary treatments, effect of T4 is significant.
pattern was observed in the starved-refed rats: T₄ administration reversed the PTU effects on the enzyme overshoot such that rats treated with both PTU and T₄ responded to starvation-refeeding as did the control T₄-treated rats without PTU. Food intake, final body weight and liver weight were all somewhat affected by PTU and T₄. PTU-treated rats weighed less and had heavier livers than rats not treated with PTU. T₄ treatment of the PTU-treated rats increased the food intake of these rats when starved and refed but not when ad libitum fed.

When the PTU and ADX treatments were combined, survival was limited. Only two of the six rats fed ad libitum and three of the six rats starved and refed survived. Because of this limited survival, comparison of these groups to the intact or the ADX or PTU-treated groups may be questionable. However, those that did survive weighed less than their counterparts in their respective control groups. They also consumed less food. Their liver weights were similar to those of the control intact and PTU-treated groups and more than those of the ADX groups. These animals had the typical enzyme overshoot but not the increase in liver lipid. When glucocorticoid was administered to these ADX- and PTU-treated rats, survival rate improved. Five of the six rats in each of the dietary treatment groups survived. Food intake was increased with the restoration of this hormone as was liver weight, and the typical increase in enzyme activity and liver lipid was also observed.

When T₄ was administered to these ADX- and PTU-treated rats, survival was not improved nor were there increases in body weight or food intake. The starvation-refeeding treatment of these rats resulted in an enzyme overshoot but the liver lipid level was not increased. In fact, it was decreased compared to the ad libitum-fed rats subjected to the same treatments.

Lastly, when both hormones were restored to the ADX- and PTU-treated rats, final body weight and food intake were similar to those of the intact, untreated rats. In addition, the means for liver weight, enzyme activity and liver lipid of the starved-refed rats of these treatments far surpassed those of the intact untreated starved-refed control rats.

**DISCUSSION**

In a previous work (9, 18) we reported that rats given high doses of glucocorticoid and/or either fed ad libitum or periodically starved and refeed did not develop fatty livers. Since ADX- and GC-treated starved-refed rats do develop fatty livers, the question arises as to the mechanisms involved, which would explain why the latter treatment results in a fatty liver but the former does not. We hypothesized that the thyroid hormones may be involved. While the results of the present work demonstrate that both glucocorticoid and thyroid hormone are involved in the development of the increase in liver lipid in response to starvation-refeeding, there does not appear to be any interaction between the two. In view of the differential responses of enzyme activity and liver lipid to these two hormones, it is possible that these hormones function separately within the starve-refeed response and that there is no reason to suggest a regulatory function of the one on the other. Glucocorticoid influenced both the enzyme overshoot and the liver lipid. In the absence of thyroid hormone, or, as in this study, in the PTU-treated ADX rat, glucocorticoid replacement restored the enzyme overshoot but did not have any effect on liver lipid. In contrast, thyroid hormone administered to the PTU-treated ADX rat resulted in a small enzyme overshoot and little or no increase in liver lipid. When both hormones were replaced, both enzyme overshoot and liver lipid responses were restored.

Although there is usually a good correlation between the activity of G6PD and liver lipid (24), the results of the present work would suggest that this may be circumstantial. It appears that the induction of the enzyme overshoot via de novo enzyme protein synthesis may have been due to the action of both hormones on this process. Thyroid hormone has been shown to stimulate protein synthesis (25–27). Since treatment with PTU inhibits thyroid hormone production, the modest increase in G6PD activity in the starved-refed PTU-treated rat may have been due to a substrate-induced increase in enzyme activity and/or limited de novo enzyme protein synthesis possible through residual thyroid hormone activity assuming
that the dose of PTU used did not completely block thyroid hormone production. PTU treatment without starvation-refeeding also increased G6PD activity above that of the untreated ad libitum-fed control group, but this increase was probably not diet dependent since the PTU treatment was initiated prior to any dietary change. Perhaps, the increase in G6PD activity was due to an effect of PTU on thyroid-stimulating hormone (TSH) secretion. PTU, as an inhibitor of thyroid hormone synthesis and release, results in lower serum T₃ and T₄ levels. These decreases would signal the pituitary to produce TSH and this TSH in turn may have stimulated the pentose shunt seen as an increase in G6PD activity without an increase in liver lipid in the ad libitum-fed rats.

The decrease in liver lipid in PTU-treated rats likely was due to a decreased hepatic lipogenesis together with a decreased peripheral lipolysis and transport of lipids to the liver. A decreased lipid turnover, particularly in the ad libitum-fed rat, could account for this observation. The liver lipid levels were restored when T₄ was injected thus showing a direct effect of thyroid hormone status on liver lipid levels, an effect that was potentiated by the starvation-refeeding treatment.

LITERATURE CITED


