Studies on the Induction and Repression of Enzymes in Rat Liver

IV. EFFECTS OF CORTISONE AND PHENOBARBITAL*

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SUMMARY

The effects of cortisone on the dietary induction and repression of serine dehydrase, ornithine transaminase, and tyrosine transaminase were studied. In rats pretreated with cortisone dietary induction was affected relatively little, but carbohydrate repression was largely eliminated.

The effects of cortisone and phenobarbital on the dietary induction of serine dehydrase and ornithine transaminase were studied. Pretreatment with phenobarbital had little effect on the induction of serine dehydrase and ornithine transaminase, but pretreatment with cortisone and phenobarbital together caused an enhanced response of these enzymes to casein hydrolysate feeding, with respect to both total amount of enzyme produced and activity per g of liver protein. Neither cortisone nor phenobarbital acted as an inducing agent when administered alone or in combination; their effects were manifested only in the presence of dietary inducer.

Studies in our laboratory have shown that certain amino acid-catabolizing enzymes can be rapidly induced in rat liver by the forced feeding of dietary casein hydrolysate (1, 2) and that this induction is probably independent of the action of glucocorticoids (2). Under the conditions of these experiments the feeding of carbohydrate repressed the induction of those enzymes (1, 3) and this repression was accompanied by the rapid accumulation of large quantities of liver glycogen (3). Carbohydrate feeding also markedly repressed the induction that was produced by the injection of glucagon, although the repression was less marked than when dietary casein hydrolysate was the inducer. The induction produced by the injection of glucagon into rats receiving dietary glucose was accompanied by a decrease in liver glycogen concentration (3). Thus, according to our findings there is an apparent reciprocal relationship between the levels of liver glycogen from dietary sources and the levels of amino acid-catabolizing enzymes. However, other workers have found that treatment with glucocorticoids causes a parallel increase in both glycogen (4-10) and amino acid-catabolizing enzymes (11-17). In view of this apparent paradox it became of interest to us to study the effects of glucocorticoids on the dietary induction and repression of amino acid-catabolizing enzymes in rat liver under our experimental conditions.

Jervell, Christofferson, and Morland (18) have shown that the drug-metabolizing enzyme, dimethylaminozobenzene reductase, has metabolic control characteristics (induction and carbohydrate repression) similar to those we observed for the amino acid-catabolizing enzymes, threonine dehydrase and ornithine transaminase (1-3). This similar response in enzymes of such dissimilar metabolic functions suggests the existence of interdependence in the metabolic controls governing the activities of these enzymes. In order to obtain further information on the possibility of such interdependence we studied dietary induction in rats that had been chronically exposed to nontoxic doses of phenobarbital, an agent which is known to stimulate drug metabolizing systems in rat liver (19-23).

The present paper describes studies on the effects of cortisone on dietary induction and repression, and the effects of phenobarbital, alone and in combination with cortisone, on dietary induction.

EXPERIMENTAL PROCEDURE

Materials

The following substances were obtained from the sources indicated: D-glucose, Allied Chemical Company; casein hydrolysate and a protein-free diet in pellet form, General Biochemicals, Inc.; cortisone acetate, Merck, Sharp and Dohme; phenobarbital sodium, Winthrop; insulin (Iletin), Eli Lilly.

Methods

Male albino rats (Holtzman) weighing 130 to 150 g were used throughout. In all experiments rats were fed a protein-free diet ad libitum for 6 days. At the end of this period the rats were fasted overnight (12 hours) and the experiments were begun at 6 a.m., at which time the rats weighed 100 to 115 g.
Effects of Cortisone on Dietary Induction and Repression—Fig. 1 (A, B, and C) shows the effects of cortisone on the induction and repression of serine dehydrase, ornithine transaminase, and tyrosine transaminase, respectively. In the case of serine dehydrase (Fig. 1A) induction produced by the feeding of casein hydrolysate was completely repressed by the simultaneous feeding of glucose at a level of 1 or 2 g per feeding (left side of figure). However, if the rats were pretreated with cortisone, a much greater induction by casein hydrolysate occurred in the face of dietary glucose (right side of figure), the most striking difference between the treated and untreated rats occurring when 1 g of glucose was fed. Cortisone alone was unable to cause enzyme induction in rats fed on a protein-free diet, and caused a slight suppression of the induction produced by casein hydrolysate.

Ornithine transaminase (Figure 1B), a mitochondrial enzyme (26), also showed glucose repression (left side of figure) although it was relatively less sensitive to the 1-g level of glucose. Results which were similar to those for serine dehydrase, glucose failed to repress ornithine transaminase induction in cortisone-treated rats (right side of figure), although cortisone alone did not cause induction and had little effect on the induction in rats fed casein hydrolysate without glucose. Indeed, previous studies (2) have shown that ornithine transaminase is completely unresponsive to prolonged cortisone administration in rats fed a chow diet ad libitum.

The induction of tyrosine transaminase (Figure 1C) was not continuous but reached a peak and decreased in the face of further

| Casein hydrolysate, with or without glucose, was fed in 3 ml of water by stomach tube. Cortisone and phenobarbital were given by intramuscular injections, always beginning after the rats had been on the protein-free diet for 1 or 2 days. Dosage quantities and schedules, along with other details for individual experiments, are given in the appropriate figure legends.

Rats were killed by decapitation and their livers were analyzed for serine dehydrase by an automated procedure (24). Tyrosine transaminase was also assayed automatically (24) by a modification of the method of Lin and Knox (25). Ornithine transaminase was assayed by the method of Peraino and Pitot (26).

In all figures each point on the curves represents an average of values from six to nine rats. In Figs. 1 and 3 the maximum standard error for each curve is given in a table in the figure legend. The error for each curve is expressed as a percentage of the average enzyme activity at the point where the maximum error occurred.

### RESULTS

<table>
<thead>
<tr>
<th></th>
<th>Without cortisone</th>
<th>With cortisone</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>C.H.</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>C.H. + 1 g glucose</td>
<td>32</td>
<td>11</td>
</tr>
<tr>
<td>C.H. + 2 g glucose</td>
<td>30</td>
<td>25</td>
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<tr>
<td>Cortisone alone</td>
<td></td>
<td>27</td>
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administration of inducer. This unusual phenomenon resembles that observed by Tomkins, Garren, Howell, and Peterkořsky in adrenalectomized rats treated with hydrocortisone (27). The feeding of glucose with casein hydrolysate produced a marked repression which was dependent on the dosage of glucose (left side of figure). Pretreatment of the rats with cortisone abolished the repressive effect of glucose and in fact enhanced the induction (right side of figure). Again, it is seen that cortisone alone did not cause induction in rats fed a protein-free diet and had little effect on the induction produced by casein hydrolysate.

If cortisone administration was begun on the day of the experiment, i.e. if the pretreatment was deleted, carbohydrate repression of all three enzymes was not affected. The necessity for pretreatment might indicate either that the hormone was slowly converted to an active metabolite, or that its effect was to modify gradually the nature of components of the induction repression system so that the functioning of the system was altered.

Effects of Other Hormones on Dietary Induction and Repression—Various steroid hormones were administered according to the procedure described for cortisone in an attempt to obtain information on the specificity of the response. It was found that triamcinolone (9a-fluoro-11,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione 16α,21-diacetate) produced an effect closely similar to that of cortisone, but deoxy cortisol, and estrogen had no measurable effect on either the induction by casein hydrolysate or the repression of this induction by dietary glucose.

In addition, a study was performed to test the possibility that the phenomenon of glucose repression might in fact result from the action of insulin, the release of which would be stimulated by the intubation of glucose (28). The possible involvement of insulin was suggested by the findings of Ishikawa, Ninagawa, and Suda (29) who reported a 5-fold increase in serine dehydrase in alloxan-diabetic rats, and a prevention of this increase by insulin administration. In our experiments insulin was administered subcutaneously (0.5 unit every 6 hours, an amount just below the lethal dose under these conditions) to rats receiving 1 g of casein hydrolysate plus 0.5 g of glucose every 6 hours. The amount of glucose fed was sufficient to produce measurable but submaximal repression. By comparison with controls receiving casein hydrolysate with or without glucose but no insulin, it could be determined whether insulin would decrease or enhance the effect of the low dosage of glucose. It was necessary to take this approach rather than to study the effect of insulin on casein hydrolysate induction in the absence of glucose because extremely low doses of insulin were highly toxic to these rats unless they also received a small amount of glucose.

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**Fig. 2.** Effect of insulin on partial repression of serine dehydrase by low levels of dietary glucose. C.H. 1 g of casein hydrolysate given as described in Fig. 1. C.H. + 0.5 g glucose, 1 g of casein hydrolysate + 0.5 g of glucose given as described in Fig. 1. C.H. + 0.5 g glucose + insulin, 1 g of casein hydrolysate + 0.5 g of glucose given as described in Fig. 1 + 0.5 unit of insulin (Iletin, Eli Lilly) given by subcutaneous injection at the same 6-hour intervals. Enzyme activity is expressed as micromoles of pyruvate per g of liver per hour.

**Fig. 3.** Effect of phenobarbital and phenobarbital plus cortisone on the dietary induction of ornithine transaminase and serine dehydrase. C.H., casein hydrolysate was fed as described in Fig. 1. C.H. + pretreat with phenobarb, each rat was given a single daily injection of 8 mg of phenobarbital for 4 days during the protein depletion period. Phenobarbital treatment was stopped 36 hours before zero time. Casein hydrolysate was given as described above. C.H. + pretreat with cort and phenobarb, each rat was given a single daily injection of 5 mg of cortisone and a single daily injection of 8 mg of phenobarbital according to the schedule for C.H. + pretreat with phenobarb (above). Casein hydrolysate was given as described above. C.H. + phenobarb on day of exp, single injection of 8 mg of phenobarbital given to each rat at zero time only. Casein hydrolysate was given as described above. Enzyme activity is expressed as micromoles of product (see Fig. 1) per liver per 100 g of body weight per hour. Maximum standard errors for each curve, expressed as percentages (as in Fig. 1), are tabulated below.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ornithine transaminase</th>
<th>Serine dehydrase</th>
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<tbody>
<tr>
<td>C.H.</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>C.H. + pretreat with phenobarb</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>C.H. + pretreat with cort and phenobarb</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>C.H. + phenobarb on day of exp</td>
<td>19</td>
<td>10</td>
</tr>
</tbody>
</table>
It was observed that for all three enzymes the results obtained with rats receiving casein hydrolysate plus glucose plus insulin were identical with results from rats receiving only casein hydrolysate plus glucose. (Fig. 2 is a representative experiment showing results obtained with serine dehydrase.) Therefore, since insulin neither enhanced nor diminished the repression produced by the low level of glucose, it is unlikely that insulin is the primary agent in the repression phenomenon.

Effect of Phenobarbital and Cortisone on Dietary Induction—Fig. 3 shows the effect of phenobarbital, alone and in combination with cortisone, on the induction of ornithine transaminase and serine dehydrase by casein hydrolysate feeding. When rats were given phenobarbital at zero time on the day of the experiment, the induction of serine dehydrase was somewhat less than in rats given casein hydrolysate alone (right side of figure) while the induction of ornithine transaminase was not affected (left side of figure). If, however, the rats were pretreated with phenobarbital for 4 days and the treatment was terminated 36 hours before zero time, a slight increase in the induction of both enzymes was produced. Thus, there is a large difference in the degree of induction between rats receiving phenobarbital on the day of the experiment and those pretreated with phenobarbital, suggesting that there are metabolic effects of acute phenobarbital administration that differ from those produced over a period of time by chronic administration.

When rats were pretreated with both phenobarbital and cortisone the induction produced by casein hydrolysate was substantially greater than in untreated rats or those treated with phenobarbital alone. The effect of pretreatment with these two agents combined was also quite different from the effect of pretreatment with cortisone alone (compare curves for casein hydrolysate plus and minus cortisone pretreatment in Fig. 1 with curves for casein hydrolysate plus and minus pretreatment with cortisone and phenobarbital in Fig. 3). Thus cortisone and phenobarbital act together to enhance the induction produced by casein hydrolysate. Neither substance produced induction in the absence of casein hydrolysate administration. Figs. 4 and 5 further indicate the synergistic interaction of phenobarbital and cortisone in enhancing the induction by casein hydrolysate. In these figures the results are calculated as enzyme activity per g of liver protein, and expressed as percentage of untreated control values. Untreated control, casein hydrolysate was fed as described in Fig. 1. C.H. + pretreat with cort, treated as described in Fig. 1. C.H. + pretreat with phenobarb, treated as described in Fig. 3. C.H. + pretreat with cort and phenobarb, treated as described in Fig. 3.
substantially higher in rats pretreated with cortisone than in the other groups (left side of figure) while the ornithine transaminase values were similar in rats treated with cortisone and those treated with both cortisone and phenobarbital. It is important to note that the comparisons made in Fig. 4 are based on very low absolute enzyme levels (see zero time values in other figures), since the pretreatments with phenobarbital or cortisone (or both) did not in themselves cause induction.

During the process of induction (Fig. 5, A and B) the enzyme activity per g of protein in the cortisone-treated rats was well below that of the control rats at all time intervals. This suggests that there was a dilution of enzyme owing to a preferential synthesis of other protein under the influence of cortisone. In rats pretreated with phenobarbital the specific activity was generally below that of the control rats at all time intervals. This suggests that there was a preferential enrichment of these enzymes with respect to other proteins during the interval for serine dehydrase). Thus there was a preferential enrichment of these enzymes with respect to other proteins during dietary induction, as a result of the combined pretreatment.

DISCUSSION

Carbohydrate repression in rat liver in vivo has now been observed with respect to the induction of certain amino acid-catabolizing enzymes (1, 3); 8-aminolevulinic acid synthetase, an enzyme involved in porphyrin biosynthesis (30); dimethylaminooazobenzene reductase, a drug-metabolizing enzyme (18), and phosphoenolpyruvate carboxykinase, an enzyme of carbohydrate metabolism (31). The uniformly repressive effect of carbohydrate on these elements of relatively unrelated metabolic pathways suggests that there is a common component in the machinery controlling the levels of these enzymes which is sensitive to the repressive action of a carbohydrate metabolite. In the present paper it was found that pretreatment with cortisone permitted dietary induction of three amino acid-catabolizing enzymes to occur in the presence of amounts of dietary glucose that would ordinarily cause complete repression, while cortisone itself could not produce induction in the absence of dietary amino acids. However, our previous work showed that dietary induction could proceed in the absence of glucocorticoids (2). Thus, in the present experiments cortisone probably did not act as the primary inducer, but instead altered the intracellular milieu in such a manner that these enzymes responded differently to the primary induction repression stimuli. Combined pretreatment with phenobarbital and cortisone also exerted an indirect influence on the induction process, i.e., these agents caused the dietary inducer (casein hydrolysate) to exert an enhanced effect. Consequently there was an increase both in the absolute levels of the enzymes and in their levels in relation to other liver proteins. The effects of phenobarbital observed in these experiments support the suggestion (see introductory section) that there is an area of interdependence in the control systems for the regulation of amino acid and drug-catabolizing enzymes.

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