The Sequential Increase in the Rates of Synthesis of Enzymes in Rat Liver after Glucocorticoid Administration

YEE S. KIM

Department of Pharmacology, Saint Louis University School of Medicine, Saint Louis, Missouri 63104

(Received August 20, 1967, and in revised form November 29, 1967)

SUMMARY

The relative rate of synthesis of glutamic-alanine transaminase (GAT) in rat liver was measured at various times after the administration of massive doses of prednisolone sodium phosphate or hydrocortisone succinate by the L-leucine-^{14}C pulse-labeling method. The rate of synthesis was unchanged 3.5 hr after the administration of prednisolone and 6.5 hr after the administration of hydrocortisone. However, the rate was increased approximately 2-fold after 6.5 hr of prednisolone administration, and approximately 3-fold after 2 days of prednisolone or 5 days of hydrocortisone. Thus, the first noticeable increase in the GAT synthesis, occurring some 6 hr after the hormone administration, lags behind those of glutamic-tyrosine transaminase (GTT) and tryptophan pyrrolase (TP) which have been shown to occur within 3 hr after an administration of hormone. This observation indicates that the stimulatory effect of glucocorticoid hormone on the synthesis of liver proteins are sequential rather than simultaneous.

INTRODUCTION

One of the important questions concerning the mechanism of glucocorticoid hormone action which has not been satisfactorily answered is whether the stimulatory effect of the hormone on the synthesis of liver enzymes is simultaneous for all the responsive enzymes, irrespective of the time courses of enzyme accumulation in the tissue. The GAT activity level in a rat liver does not reach its maximum for 5 days after glucocorticoid treatment, but the rate of synthesis of the enzyme has been shown to reach a maximum as early as 12 hr after an administration of the hormone (1). This finding suggests the possibility that an increase in the rate of GAT synthesis may begin much earlier, possibly within 2–3 hr of hormone treatment, at which time the synthesis of other enzymes such as GTT and TP is known to have already been stimulated maximally. However, results of experiments designed to determine if the rate of GAT synthesis was increased within a short time after a hormone administration were inconclusive (1). The experiments described in the present report show that under the influence of even a massive dose of hormone, the rate of GAT synthesis does not increase in 3.5 hr, but an increase in the rate is noticeable in 6.5 hr.

MATERIALS AND METHODS

Male Sprague-Dawley rats of 200 g body weight were used. Each rat was given intraperitoneally 4 mg/rat of prednisolone sodium phosphate every hour for 3 hr in one group of animals and for 6 hr in another group. In order to assess the effect of a long period of hormone treatment, 3 rats were given the same amount of the hormone every 12 hr for 2 days. Similar experiments were also carried out with 20 mg/rat of hydrocortisone succinate. For the assessment of long-term effect, the same amount of hydrocortisone succinate was

given to animals every 12 hr for 5 days. The control groups received 0.9% saline solution instead of a hormone. Thirty minutes after the last hormone (or saline) administration, 20 μC/rat of uniformly labeled L-leucine-^{14}C (305 mC/mmole) were administered intraperitoneally, and 30 min thereafter the animal was sacrificed by decapitation. The liver was isolated, rinsed in the potassium phosphate buffer, 0.01 M, pH 7.4, containing 0.25 M sucrose, and quickly frozen in dry ice. The frozen liver was stored at —30°C until used.

Each liver was homogenized in the phosphate buffer with sucrose, and the samples of soluble protein and the enzyme-antiserum precipitate were prepared as described previously (2, 3). An aliquot of soluble protein was precipitated with 0.5 N perchlorate. Usually 100 units of GAT was precipitated with the equivalent amount of antiserum, and the enzyme-antiserum precipitate was washed three times with 0.9% saline solution containing 0.1% L-leucine. The purity of the enzyme used to elicit the antiserum and the specificity of antiserum have been discussed previously (4, 5). Both the perchlorate-precipitated soluble protein and the enzyme-antiserum precipitate were further washed with 0.5 N perchlorate and the ether-ethanol-chloroform mixture as described by Korner (6), and suspended in known amounts of water. The samples were then solubilized with 0.05 N NaOH to make the final concentration of 0.025 N in NaOH and less than 2 mg/ml in protein. Glass counting vials containing 18 ml of scintillation fluid and 0.5 ml of the samples were counted twice to the counting error of within 1% in the Beckman CPM 100 scintillation counting system with the carbon-14 channel wide open. The scintillation fluid consisted of 2,5-diphenyloxazole (PPO), 5 g; ethanol, 310 ml; ethyleneglycol, 20 ml; and toluene to make up the final volume of 1 liter. The background was 40 cpm, and the counting efficiency of carbon-14 under the condition was 82.5%. Details of the counting procedure will be described elsewhere (manuscript in preparation). Protein concentrations of all samples were determined by the method of Lowry *et al.* (7), and GAT activities were assayed according to the Method II described by Segal *et al.* (8).

RESULTS AND DISCUSSION

Although the previous experiments (1) failed to yield definite evidence to show increase in the rate of GAT synthesis after 3 hr and 6 hr of hormone treatment, possibilities that the insoluble form of prednisolone acetate may have taken a longer period of time for absorption than the soluble forms and/or that the dose may have been insufficient to elicit the stimulatory effect could not be completely excluded. In order to eliminate these possibilities, massive doses of prednisolone sodium phosphate or hydrocortisone succinate (soluble forms) were administered to the animals. In the present experiments a higher dose of the radioactive amino acid was administered to animals, and the counting efficiency of radioactivity was higher than the previous experiments (1, 2). These modifications of the procedure were made in an attempt to minimize the counting error in the measurement of radioactivity.

The results observed are summarized in Tables 1 and 2. The enzyme activity per gram of liver was not increased for 6.5 hr of either prednisolone or hydrocortisone treatment, but was increased approximately 2-fold after 2 days of prednisolone, and approximately 4-fold after 5 days of hydrocortisone. The relative rate of enzyme synthesis measured by the extent of radioactivity incorporation, on the other hand, shows no changes in 3.5 hr of prednisolone treatment or 6.5 hr of hydrocortisone treatment. Approximately 2-fold increase in the rate was observed after 6.5 hr of prednisolone, and approximately 3-fold increase after 2 days of prednisolone or 5 days of hydrocortisone. The long-term experiments suggest that the extent of the maximal stimulation in the rate of GAT synthesis by prednisolone sodium phosphate or hydrocortisone succinate is approximately three times that of control. The short-term experiments indicate that an increase in the rate of GAT synthesis is noticeable 6 hr (or more) after the initial

TABLE 1
Effect of prednisolone sodium phosphate on the radioactive leucine incorporation into rat liver glutamic-alanine transaminase

Parameter	Control animals (8)[a]	Hormone-treated animals 3.5 hr (8)	6.5 hr (7)	2 days (3)
GAT activity				
Units/g liver	28	26	29	50
H/C[b]	—	0.86	1.04	1.77
Radioactivity in soluble protein				
Cpm/g liver[c] ($\times 10^{-5}$)	3.01 ± 0.29	2.65 ± 0.31	2.69 ± 0.23	2.83 ± 0.38
H/C	—	0.87	0.87	0.94
Radioactivity in GAT				
Cpm/g liver[d]	238 ± 32	239 ± 28	506 ± 75	619 ± 109
H/C	—	1.00	2.13	2.60

[a] Numbers in parentheses indicate the number of animals in each group.
[b] The ratio of the measured values in the hormone-treated (H) to the control animals (C).
[c] Cpm in the protein per gram of liver with standard deviations.
[d] Cpm in the GAT per gram of liver was normalized to 3.00×10^5 cpm of soluble protein per gram of liver, since the radioactivity in the soluble protein is the measure of the size of radioactive precursor pool (2, and unpublished observation). The cpm in soluble protein and GAT of normal animals are higher than those in previous experiments (1, 2). The higher radioactivity in both the precursor pool and the enzyme are due to the higher dose of radioactive leucine administered and a higher counting efficiency in the present experiments.

administration of hormones, and the rate increases to a maximal level several hours thereafter.

A significant observation from this study is that the rate of GAT synthesis is unchanged up to 6.5 hr of hydrocortisone administration. In contrast to this result, Schimke observed that the rate of TP synthesis was maximally (*ca.* 4-fold) stimulated 3 hr and 20 min after a single administration of *ca.* 5 mg/100 g body weight of hydrocortisone in an experiment using the pulse-labeling technique very similar to that employed in the experiments described in this report (9).

Time courses of changes in the activities of various liver enzymes following glucocorticoid administration vary markedly.

TABLE 2
Effect of hydrocortisone succinate on the radioactive leucine incorporation into rat liver glutamic-alanine transaminase

Parameter	Control animals (3)[a]	Hormone-treated animals 3.5 hr (3)	6.5 hr (4)	5 days (3)
GAT activity				
Units/g liver	28	32	30	100
H/C[b]	—	1.14	1.07	3.58
Radioactivity in soluble protein				
Cpm/g liver[c] ($\times 10^{-5}$)	2.96 ± 0.49	2.99 ± 0.36	3.24 ± 0.29	2.56 ± 0.40
H/C	—	1.01	1.09	0.87
Radioactivity in GAT				
Cpm/g liver[d]	248 ± 30	247 ± 35	266 ± 67	768 ± 100
H/C	—	0.98	1.07	3.10

[a–d] Footnotes to Table 1 apply also to this table.

For example GTT and TP activities reach a maximum within a few hours after a glucocorticoid administration (10, 11), whereas the peak of GAT activity is not reached for 5 days (2, 12, 13) and that for arginase still later (14). However, the increase in the tissue enzyme activity level depends on the accumulation of enzyme protein, which is a function of turnover rate rather than the rate of synthesis alone (1, 3, 15, etc.). On the other hand, it has been shown that glucocorticoid hormone has either very little effect (10) or a considerable stimulatory effect (2) on the rates of degradation of the enzymes responsive to the hormone. The substantial increase in the GTT activity in rat liver within 2–3 hr after a single administration of hormone is therefore attributed to the result of a large increase in the rate of synthesis (10). Furthermore, Kenney has presented evidence indicating that stimulation of nuclear RNA synthesis upon glucocorticoid treatment begins after a lag of about 30 min duration, and accumulation of the induced GTT (or increase in the rate of GTT synthesis) begins about 30 min after the effect on RNA is apparent (16).

Summarizing the evidence discussed above, there is no stimulation of GAT up to 6.5 hr of hormone treatment, during which time the rates of synthesis of TP and GTT are already stimulated maximally. Thus, although the increase in the rate of GAT synthesis starts much earlier than is manifested by the increase in the enzyme activity, the time at which the increase in GAT synthesis begins lags behind that of GTT or TP. Since the GAT synthesis was measured in the livers of intact well fed rats, while the synthesis of GTT and TP was studied with livers of either starved or fed adrenalectomized animals, there is a possibility that the lag in the stimulation of GAT synthesis discussed in the present report may be due to the differences in the strains and types of animals and/or their diet. However, the possibility is an unlikely event in view of the following observations: Most significantly, the half-life of GAT (an index of rates of synthesis and degradation of the enzyme) determined by kinetic studies of the enzyme activity following cortisone administration to adrenalectomized male Osborne-Mendel rats (14) was exactly the same as the value determined by the similar experiment with intact male Holtzman rats following prednisolone administration (2). This observation indicates that the time course of GAT stimulation and its return to the basal state in the adrenalectomized rats of one strain is identical to that in the intact rats of another. Furthermore, it has been demonstrated that the time course of radioactive amino acid incorporation into the total liver protein in either starved or fed adrenalectomized rats before and after glucocorticoid administration were the same, and that glucocorticoid administration had very little effect on the accumulation of radioactivity in the total liver protein of well fed intact rats (2, 10, unpublished observation). These observations are interpreted as indicating that the dietary factor (up to 24 hr of starvation), adrenalectomy and differences in the strain of animals render very little effect on the time course of glucocorticoid stimulation of liver protein synthesis. It is therefore concluded that the apparent effects of glucocorticoid hormone on the synthesis of liver proteins are sequential rather than simultaneous.

ACKNOWLEDGMENT

This work was supported by Research Grant AM 11280-01 National Institute of Arthritis and Metabolic Diseases U.S. Public Health Service.

REFERENCES

1. H. L. Segal and Y. S. Kim, *J. Cellular Comp. Physiol.* Suppl. 1 to Vol. 66, 11 (1965).
2. H. L. Segal and Y. S. Kim, *Proc. Natl. Acad. Sci. U.S.* **50**, 912 (1963).
3. H. L. Segal, R. G. Rosso, S. Hopper and M. M. Weber, *J. Biol. Chem.* **237**, PC 3303 (1962).
4. P. W. Gatehouse, S. Hopper, L. Schatz and H. L. Segal, *J. Biol. Chem.* **242**, 2319 (1967).
5. H. L. Segal, Y. S. Kim and S. Hopper, "Advances in Enzyme Regulation" (G. Weber, ed.), Vol. 3, p. 29. Pergamon, London, 1965.
6. A. Korner, *Biochem. J.* **73**, 61 (1959).

7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. H. L. Segal, D. S. Beattie and S. Hopper, *J. Biol. Chem.* **237**, 1914 (1962).
9. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *J. Biol. Chem.* **240**, 322 (1965).
10. F. T. Kenney, *J. Biol. Chem.* **237**, 3495 (1962).
11. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *J. Biol. Chem.* **240**, 322 (1965).
12. F. Rosen, N. R. Roberts, L. E. Budnick and C. A. Nichol, *Science* **127**, 287 (1958).
13. F. Rosen, N. R. Roberts and C. A. Nichol, *J. Biol. Chem.* **234**, 476 (1959).
14. C. M. Berlin and R. T. Schimke, *Mol. Pharmacol.* **1**, 149 (1965).
15. R. T. Schimke, *J. Biol. Chem.* **239**, 3808 (1964).
16. F. T. Kenney and F. J. Kull, *Proc. Natl. Acad. Sci. U.S.* **50**, 493 (1963).