INDUCTION OF ALANINE TRANSAMINASE BY ADRENAL STEROIDS IN CULTURED HEPATOMA CELLS[1]

Kai-Lin Lee and Francis T. Kenney

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received June 17, 1970

SUMMARY—The capacity of adrenal steroid hormones to accelerate synthesis of alanine transaminase (L-alanine-2-oxoglutarate aminotransferase, E.C. 2.6.1.2) in cultured cells of the H-35 hepatoma was investigated. The transaminase of these cells was immunologically identical to the rat liver enzyme. By use of an isotopic-immunochemical method, it was found that transaminase synthesis was increased 150%, 24 hr after hydrocortisone was added to the medium. We conclude that the induction of this enzyme by glucocorticoids in vivo is due to direct action of the hormones of hepatic cells. Lactic dehydrogenase levels were unchanged by the steroids tested. The responses of alanine transaminase to various concentrations of hydrocortisone and to modifications of the steroid structure were like those of tyrosine transaminase in H-35 cells, except that synthesis of the latter enzyme is stimulated to a much larger extent.

It is well-known that the rat liver enzyme alanine transaminase (L-alanine-2-oxoglutarate aminotransferase, E.C. 2.6.1.2) is inducible by glucocorticoids in vivo (1, 2). Maximum increase in the enzyme level is attained only after prolonged steroid treatment, reflecting the slow turnover rate of this enzyme relative to other glucocorticoid-inducible enzymes in liver (2–4). This slow response has hitherto made it impossible to distinguish (e.g., by perfusion analyses) whether glucocorticoids act directly on hepatic cells to effect induction of this enzyme. In this report we will show that hydrocortisone and other adrenal steroids act directly on cultured cells of the Reuber (H-35) hepatoma to accelerate synthesis of this enzyme.

MATERIALS AND METHODS

Reuber (H-35) cells were grown in monolayer at 37° C in plastic tissue culture flasks (Falcon, 76 cm^2). The growth medium was Eagle's basal medium

*Research supported jointly by the National Cancer Institute and by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation.

enriched fourfold with amino acids and vitamins and supplemented with 20% fetal calf serum and 5% calf serum. All experiments were performed with early stationary phase (8- to 9-day) cultures in serum-free medium. Tissue culture materials were purchased from Grand Island Biological Company.

Cells were lysed in 0.15 M KCl-0.001 M EDTA–0.005 M α-ketoglutarate (pH 7.8) by alternately freeze-thawing three times in liquid nitrogen and a 37° C water bath. The cell lysates were centrifuged at 40,000 rpm for 35 min. Alanine transaminase of supernatant solutions was assayed as described by Segal et al. (5). A unit of enzyme is defined as the amount which produces 0.55 μmoles pyruvate per min (5). Lactic dehydrogenase was measured by the method of Kornberg (6). Protein was determined by the procedure of Lowry et al. (7) with bovine serum albumin used as standard.

RESULTS AND DISCUSSION

The soluble alanine transaminase of H-35 cells was elevated by the addition of hydrocortisone (5×10^{-7} M) to the medium, with a clearly significant change being detected 24 hr after hormone addition (Fig. 1). Enzyme continued to accumulate for 48 hr and then was maintained at a level 70 to 100% greater than the basal or uninduced level. The rate of increase in enzyme level during

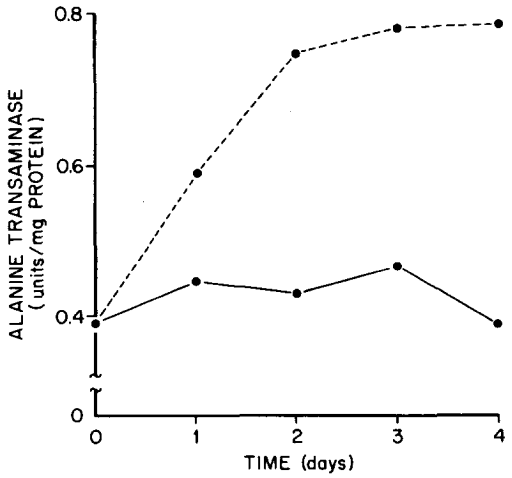

FIG. 1. Induction of alanine transaminase by hydrocortisone in H-35 cells. At zero time, growth medium was replaced with serum-free medium without (●——●) or with 5×10^{-7} M hydrocortisone (●---●). The medium was changed every 36 hr. Each point is the average of three similarly treated monolayer cultures.

the first 48 hr is comparable to that observed in vivo, but in the latter case enzyme continues to increase and reaches a new steady state after 4 to 5 days of hormone treatment (2). That the induction process appears to cease after 48 hr in these cultures probably reflects a cytotoxic effect of long-term glucocorticoid treatment. Such an effect is apparent in loss of total protein adhering to the surface of culture flasks after several days of glucocorticoid treatment.

TABLE 1

Effects of Various Steroids on Alanine Transaminase Activity

Cells were exposed to the indicated steroid for 48 hr in serum-free medium. Data are the average ± standard error of three similarly treated monolayer cultures.

Addition	Concentration	Alanine transaminase	Lactic dehydrogenase
	M	units/mg protein	
None		0.40 ± 0.001	2.33 ± 0.09
Hydrocortisone	5×10^{-7}	0.63 ± 0.014	2.56 ± 0.17
Corticosterone	5×10^{-7}	0.64 ± 0.001	2.42 ± 0.17
D-Aldosterone	1×10^{-6}	0.69 ± 0.002	2.50 ± 0.17
Progesterone	5×10^{-6}	0.40 ± 0.010	2.58 ± 0.14

Induction of alanine transaminase showed the same dependency on hydrocortisone concentration as that previously found for induction of tyrosine transaminase in these cells (8); a significant stimulation was detected at 10^{-8} M hydrocortisone, and 5×10^{-7} M was optimal. Corticosterone and D-aldosterone were also effective as inducers of alanine transaminase, while progesterone was inactive (Table 1). In this regard, too, results with induction of alanine transaminase parallel previous results wherein the structural requirements of the steroid inducer were analyzed for induction of tyrosine transaminase (9). None of these steroids altered lactic dehydrogenase of the hepatoma cells (Table 1).

By double diffusion analysis in agar gel, we found the alanine transaminase of H-35 cells to be immunologically identical to the rat liver enzyme (Fig. 2).

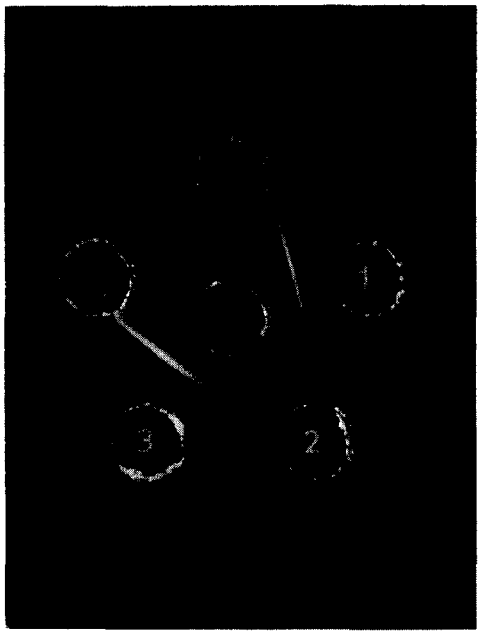

FIG. 2. Double diffusion analysis of the reaction of antitransaminase with various transaminase preparations. The central well contained 5 µl of rabbit antitransaminase serum (titer, 42.5 units/ml). To well 1 was added 0.46 unit of alanine transaminase from H-35 cells treated with hydrocortisone for 65 hr. To well 2 was added 0.3 unit of enzyme from untreated H-35 cells. Well 3 contained 0.7 unit of transaminase from adult rat liver. Preparations from H-35 cells were concentrated by 50% $(NH_4)_2SO_4$ precipitation and had specific activities of 0.891 and 2.055 units/mg protein for untreated and hydrocortisone-treated enzymes, respectively. Specific activity of the partially purified liver enzyme was 2.04 units/mg protein.

A sharp precipitin line showing identity was formed when antiserum prepared against purified enzyme from glucocorticoid-treated rats was tested against partially purified enzyme from rat liver or from H-35 cells before and after hydrocortisone treatment; there was also an ill-defined reaction with the rat liver extract that was absent from H-35 cells. Titration of crude preparations from these three sources against the antiserum yielded the same equivalence points, confirming immunological identity of the enzymes and showing that increased transaminase activity following induction in H-35 cells reflects increased transaminase protein (Fig. 3).

Segal and Kim (2) have demonstrated that glucocorticoids increase the rate of synthesis of alanine transaminase in rat liver. A comparable experiment with H-35 cells is presented in Table 2. In this experiment the transaminase level

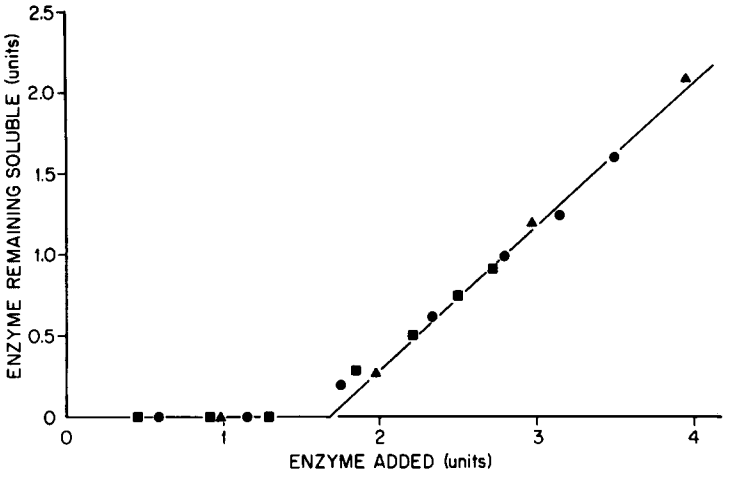

FIG. 3. Immunochemical titration of alanine transaminase preparations from rat liver and H-35 cells. Each tube contained 1.7 antitransaminase units and varying amounts of crude soluble fractions from adult liver (▲), untreated H-35 cells (■), or hydrocortisone-treated H-35 cells (●) as indicated. After 18 hr at 4°C the tubes were centrifuged and the soluble transaminase activity was determined. Specific activities (units/mg protein) of the enzyme preparations were 0.18, 0.65, and 0.32 for liver, hydrocortisone-treated cells, and control cells, respectively.

TABLE 2

Effect of Hydrocortisone on Synthesis of Alanine Transaminase in H-35 Cells

Cells were pretreated for 22 hr with serum-free medium with or without added hydrocortisone (5×10^{-7} M). At 22 hr, 75 μCi of ^{3}H-leucine (56 Ci/mmole) was added to each flask and the cells were collected 2 hr later. For each experiment 14 similarly treated monolayer cultures were pooled. Alanine transaminase was partially purified from soluble fractions by heat denaturation and ammonium sulfate fractionation (5) before immunochemical precipitation (10). Data are the averages from two separate experiments; variation was 5% or less in all values.

Treatment	Transaminase activity	Radioactivity in	
		Transaminase	Soluble proteins
	units/mg protein	cpm	cpm/mg $\times 10^{-3}$
None	0.407	1,117	94.5
Hydrocortisone	0.499	2,742	105.5

was increased about 20%, 24 hr after steroid addition to the medium; pulse-labeling measurements of the rate of enzyme synthesis showed this parameter to be increased about 150% at this time. In comparable experiments measurements of the rate of synthesis of tyrosine transaminase have shown that synthesis of this enzyme is elevated 10- to 12-fold 24 hr after hydrocortisone treatment of H-35 cells (8). Steroid treatment caused a small increase in the rate of synthesis of total soluble proteins (Table 2); this effect is variable and in many experiments no change can be detected in this parameter (8).

From these results it can be concluded, with reasonable assurance, that the reported induction of alanine transaminase in rat liver after glucocorticoid treatment is due to direct steroid action on hepatic cells, as is the case for inductions of tyrosine transaminase (11, 12) and tryptophan pyrrolase (12). Both transaminases are inducible in cultured hepatoma cells as well, but tryptophan pyrrolase is not detectable in H-35 cells and cannot be induced by glucocorticoid treatment (13).

The similarity of the responses of tyrosine and alanine transaminases to varied steroid concentrations and modifications of the steroid structure suggests that the same mechanism is involved in induction of these two enzymes. If this is so, the difference in extent of response is of special interest: at 24 hr after addition of the steroid inducer, synthesis of tyrosine transaminase is elevated 10- to 12-fold, whereas that of alanine transaminase is approximately doubled. Kim (14) has presented evidence that synthesis of these two enzymes in rat liver is stimulated in sequential, rather than coordinate, fashion after glucocorticoid treatment. These observations are reminiscent of the polarity phenomenon associated with transcription of polycistronic messenger RNA's in bacteria (15).

Acknowledgments.—We thank Dr. Harold Segal of the Department of Biology, State University of New York at Buffalo, for generously providing us with antiserum to purified rat liver alanine transaminase. We also thank Miss Jan Laney and Mr. Lawrence Roberson for excellent technical assistance.

REFERENCES

1. Rosen, F., Roberts, N. R., Rudnick, L. E., and Nichol, C. A., Science 127, 287 (1958).
2. Segal, H. L. and Kim, Y. S., Proc. Nat. Acad. Sci. U.S.A. 50, 912 (1963).

3. Segal, H. L. and Kim, Y. S., J. Cellular Comp. Physiol. 66, Suppl. 1, 11 (1965).
4. Berlin, C. M. and Schimke, R. T., Mol. Pharmacol. 1, 149 (1965).
5. Segal, H. L., Beattle, D. S., and Hopper, S., J. Biol. Chem. 237, 1914 (1962).
6. Kornberg, A., in Methods in Enzymology, S. P. Colowick and N. O. Kaplan, eds. Vol. I., p. 441. Academic Press, New York (1955).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
8. Reel, J. R., Lee, K. L., and Kenney, F. T., submitted for publication.
9. Lee, K. L. and Kenney, F. T., in preparation.
10. Reel, J. R. and Kenney, F. T., Proc. Nat. Acad. Sci. U.S.A. 61, 200 (1968).
11. Barnabei, O. and Sereni, F., Boll. Soc. Ital. Biol. Sper. 32, 1656 (1961).
12. Goldstein, L., Stella, E. J., and Knox, W. E., J. Biol. Chem. 237, 1723 (1962).
13. Lee, K. L., unpublished observations.
14. Kim, Y. S., Mol. Pharmacol. 4, 168 (1968).
15. Ames, B. N. and Martin, R. G., Ann. Rev. Biochem. 33, 235 (1964).