The Effect of Hydrocortisone on Tyrosine-α-ketoglutarate Transaminase and Tryptophan Pyrrolase Activities in the Isolated, Perfused Rat Liver*

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Administration of hydrocortisone or cortisone to rats has been shown to produce a marked increase in hepatic tryptophan pyrrolase (1, 2) and tyrosine-α-ketoglutarate transaminase (4, 5) activities. Although present evidence suggests that the rise in tryptophan pyrrolase activity involves the synthesis of new protein (6) and the rise in tyrosine transaminase activity may not (7), the mechanism of action of hydrocortisone is unknown. The question of whether or not the increased enzyme activities are the result of a direct action of hydrocortisone on hepatic cells was investigated in the present study. Since attempts to demonstrate a direct action of hydrocortisone on tryptophan pyrrolase activity in homogenates (1) or slices (8) of rat liver have been unsuccessful, as were similar attempts with tyrosine transaminase, we turned to the artificially perfused, isolated rat liver (9, 10). Hydrocortisone produced a distinct increase in activity of both enzymes in this preparation. Barnabei and Sereni (11) also have recently reported that hydrocortisone induced an increase in tryrosine transaminase activity in the isolated, perfused rat liver.

EXPERIMENTAL PROCEDURE

Animals—Male Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories, Inc. and maintained on Purina chow checkers and water for at least 4 days before use. Livers were obtained from animals weighing 300 to 350 g, whereas blood was drawn from four to six animals weighing 400 to 500 g.

Liver Slice Studies—Slices approximately 0.5 mm thick were cut with a Stadie-Riggs microtome. Approximately 0.5 g of tissue was incubated in 10 to 15 ml of a tissue culture medium (Difco, TC199) containing 0.1 mM sodium-α-ketoglutarate for 3 hours at 38° in an atmosphere of 95% O₂-5% CO₂. Sodium-α-ketoglutarate was added to prevent thermal inactivation of tyrosine transaminase which otherwise occurs on maintaining the enzyme at 38° in vitro (7). Hydrocortisone was added as the water-soluble sodium succinate ester (Solu-Cortef, Upjohn). Since there was as much tyrosine transaminase activity in the medium as in the slice at the end of the incubation period, slices were homogenized directly in the medium. The homogenate was centrifuged for 30 minutes at 11,500 r.p.m. in a Servall SS-1 centrifuge and assayed for tyrosine transaminase activity according to the procedure described by Lin et al. (12).

Liver Perfusion Studies—The technique used to remove and perfuse the isolated rat liver was that described by Miller et al. (9, 10). The operation to remove the intact liver consisted of cannulating the portal vein, thoracic vena cava, and bile duct and then freezing the liver of all other attachments except the diaphragm. The liver was then placed in the perfusion apparatus, maintained at 37-39° (9), and perfused with fresh oxygenated, heparinized blood (50 units of heparin per ml of blood) via the portal vein. The operation usually took about 30 minutes, the liver being without circulation for approximately 8 minutes.

Glucose, 500 mg, was added to the perfusion medium (approximately 80 to 90 ml) at the beginning of the experiment. A mixture containing 327 mg of essential and nonessential amino acids as described by Miller, Burke, and Haft (10), was dissolved in 6.0 ml of Ringer’s solution and infused into the blood entering the portal vein, at the rate of 1.0 ml per hour for 6 hours by means of a constant infusion pump. Hydrocortisone (Mann Research Laboratories, Inc.) was dissolved in 12 ml of Ringer’s solution and either added to the blood reservoir, perfused into the blood entering the portal vein, or both perfused into the blood entering the portal vein and added to the blood reservoir. Puromycin (6-dimethylaminono-9-[3-deoxy-3-(p-methoxy-β-phenylalanyl-amino)-β,d-ribofuranosyl]-β-purine) dihydrochloride was neutralized with NaOH and added to the blood reservoir.

Minor hepatic lobes were tied off and removed at the beginning of the experiment and after 2, 4, and 6 hours. The lobes were then blotted, weighed, frozen, and stored at -16° for 18 to 24 hours. The frozen lobes were then homogenized in 9 volumes of cold 0.14 M KCl-0.005 M NaOH by means of a motor-driven Teflon pestle; the homogenate was centrifuged at 11,500 r.p.m. in a Servall SS-1 centrifuge for 30 minutes; and the resulting supernatant fraction was assayed for tyrosine transaminase (as described above), lactate dehydrogenase (assayed at 25° according to the procedure described by Weber and Cantero (13)), and tryptophan pyrrolase activities. Control experiments showed that no loss of tryptophan pyrrolase or lactate dehydrogenase was detected.

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1 This enzyme has been given the systematic name L-tyrosine: 2-oxoglutarate aminotransferase (3) and is referred to here as "tyrosine transaminase."

2 The authors wish to thank Dr. Earl H. Dearborn, Research Division, American Cyanamid Company, for generous supplies of puromycin.
RESULTS

Attempts to demonstrate an effect of hydrocortisone on tyrosine transaminase activity in vivo were unsuccessful. In two experiments the average enzyme activity before incubation was 1.3 μmoles of HPP 8 per g of liver per minute and 1.4 μmoles of HPP 6 per g of liver per minute after incubation of the slices for 3 hours in tissue culture medium to which sodium-α-ketoglutarate had been added. Addition of 35 and 1000 μg of hydrocortisone per ml gave activities after incubation of 1.0 and 1.7 μmoles of HPP per g per minute, respectively. Modification of the incubation medium by addition of 10% horse serum, or stepwise addition of hydrocortisone did not significantly alter the results.

In contrast to the results obtained with slices, hydrocortisone produced distinct increases in both tyrosine transaminase and tryptophan pyrrolase activities in the isolated, perfused liver. The enzyme activities are shown separately in Figs. 1 and 2 from four perfusions with, and one without, hydrocortisone, in all of which both enzymes were measured. When the perfusion medium without hydrocortisone was circulated, the tyrosine transaminase activity fell during the experimental period, and the tryptophan pyrrolase activity was essentially unchanged (Curve A, Figs. 1 and 2). When perfused with hydrocortisone, the activities of both enzymes increased, although in different manners. The tyrosine transaminase activity rose promptly (Fig. 1), whereas the tryptophan pyrrolase activity rose later but steadily (Fig. 2). The mode of hydrocortisone administration appeared to have some effect on these enzyme changes. The averages from two experiments show that the largest and most prolonged increases (Curve D, Figs. 1 and 2) were obtained by adding half the dose of hydrocortisone to the blood reservoir at the beginning of the experiment and infusing the other half into the portal vein throughout the experiment. Single experiments showed lower (tryptophan pyrrolase) or more transitory (tyrosine transaminase) increases when the dose of hydrocortisone was either added entirely to the blood reservoir at the beginning of the experiment (Curve B, Figs. 1 and 2) or was entirely perfused into the portal vein during the first 15 minutes of the experiment (Curve C, Figs. 1 and 2). During the latter experiment, hepatic blood flow decreased for the 1st hour after the rapid infusion of

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\Delta \text{Activity} = \text{Activity at time } t - \text{Activity at time } 0
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and little loss (15%) of tyrosine transaminase occurred during frozen storage of the liver lobes. The procedure described by Civen and Knox (8) plus added hematin (14) was used to assay tryptophan pyrrolase activity. The assay mixture contained 18 μmoles of L-tryptophan, 400 μmoles of glucose, 30 units of glucose oxidase, 16 μg of crystalline catalase (Worthington Biochemical Corporation), 4 μmoles of hematin (prepared by dissolving hemin (Mann) in dilute NaOH immediately before use), 1.0 ml of supernatant fraction, 400 μmoles of Na2HPO4-NaH2PO4, pH 7.0, and water to make 8.0 ml. The reaction was carried out in a Dubnoff shaker at 38° in an atmosphere of O2.

FIG. 1. Change in tyrosine transaminase activity in the isolated, perfused liver with hydrocortisone (initial activity set at 0). ○—○, no hydrocortisone added to perfusion medium; □—□, 1.5 mg of hydrocortisone infused via portal vein during first 15 minutes of experiment; △—△, 1.5 mg of hydrocortisone added at beginning of experiment and 0.25 mg infused per hour for 6 hours, average of two experiments; ⬤—⬤, 3 mg of hydrocortisone added at beginning of experiment.

FIG. 2. Change in tryptophan pyrrolase activity in the isolated, perfused liver with hydrocortisone (initial activity set at 0). Same legend as in Fig. 1.

Aliquots of the reaction mixture were withdrawn at 20 and 60 minutes after initiation of reaction, by the addition of supernatant fraction, to cover the period in these experiments during which the reaction rate remained constant (14). The aliquots were mixed with 0.5 volume of 15% metaphosphoric acid and filtered. The filtrate was then neutralized and assayed for kynurenine content (1). Protein concentrations of the supernatant fractions were measured by the biuret reaction with crystalline bovine serum albumin (Mann) as the standard. Enzyme activities were expressed as micromoles of p-hydroxyphenylpyruvate formed per g of protein per minute for tyrosine transaminase; micromoles of kynurenine formed per g of protein per hour for tryptophan pyrrolase; and micromoles of nicotinamide-adenine dinucleotide, reduced form (NADH),6 oxidized per g of protein per minute for lactate dehydrogenase.

6 In accordance with the newly recommended usage (3) nicotinamide-adenine dinucleotide, reduced form (NADH), is substituted here for diphosphopyridine nucleotide, reduced form (DPNH).

6 The abbreviation used is: HPP, p-hydroxyphenylpyruvate.
the total dose of hydrocortisone, which may have contributed
to the delayed rises in enzyme activities observed (Curve C,
Figs. 1 and 2).

Despite variations possibly associated with the mode of hydro-
cortisone administration, the absolute values from all four
experiments with hydrocortisone were averaged to provide the
base line for further comparisons (Figs. 3 and 4). These averages
confirmed the facts mentioned that perfusion with hydrocortisone
more than doubled both enzyme activities over the initial values,
and that tyrosine transaminase activity rose earlier (80% of
maximal activity within 2 hours) than did tryptophan pyrrolase
activity (progressive elevation only after 2 hours).

The nature of the hydrocortisone effect on the two enzyme
activities was studied by removing the hydrocortisone after 2
hours or by adding puromycin to the perfusion medium (Figs.
3 and 4). Replacing the initial blood, which had received 3 mg
of hydrocortisone at the beginning of the experiment and had
been circulated through the liver for 2 hours, with new blood
containing no added hydrocortisone led to a fall in tyrosine
transaminase activity (Fig. 3) similar to that observed 2 hours
after hydrocortisone administration without blood replacement
(Curve B, Fig. 1) and had no effect on the rise in tryptophan
pyrrolase activity (Fig. 4). Five hourly additions of 15 mg of
puromycin to the approximately 100 ml of blood perfusing the
liver, a concentration shown to inhibit protein synthesis in vitro
(15) and in vivo (16), almost completely depressed both the hydro-
cortisone-induced rises (Figs. 3 and 4) and in other experiments,
not shown, the basal levels of tyrosine transaminase and trypto-
phan pyrrolase activities. Addition of $1.6 \times 10^{-3}$ m puromycin
to the assay mixtures had no effect on the enzyme activities.
Perfusion with medium containing added puromycin had no
significant effect on hepatic lactate dehydrogenase activity.
(Values of 1375, 1360, 1340, and 1240 amoles of NADH oxidized
per g of protein per minute were observed after 0, 2, 4, and 6
hours of perfusion.) The bile flow and protein concentration
of the liver extracts were also unaffected by perfusion with
puromycin.

**DISCUSSION**

The 2- to 3-fold increases in these enzyme activities obtained
in the isolated, perfused liver can be compared with the 2- to
5-fold increases in tryptophan pyrrolase (8) and tyrosine trans-
aminase (4) activities produced by administration in vivo of an
approximately similar dose, 3 mg, of adrenal steroid.

Civen and Knox (8) showed that hydrocortisone induced an
increase in tryptophan pyrrolase activity of adrenalectomized
rats in a manner unrelated to the induction of the same enzyme
by tryptophan and suggested that hormones may produce their
physiological effects by acting as primary inducers of cellular
enzymes. Evidence that this action of hydrocortisone is directly
on the liver is provided here by the hydrocortisone-induced rises
in both tyrosine transaminase and tryptophan pyrrolase in the
isolated, perfused liver.

The perfusion studies also revealed a different time course of
the effect of hydrocortisone on the activities of the two enzymes.
Hechter, Solomon, and Caspi (17) have shown that the isolated
rat liver perfused with blood containing 2 to 6 mg of corticosteroid
per 100 ml of blood can metabolize 15 to 20 $\mu$g of steroid per g
of liver per minute at blood flows of approximately 2.5 ml per g
of liver per minute. At this rate of metabolism, with liver

weights of about 10 g and the blood flow approximately 1 ml per
hour per g of liver per minute, the 3 mg of hydrocortisone added to nearly
100 ml of perfusion medium in the present study would have been
metabolized within 35 to 50 minutes. This suggests that the
rise of tryptophan pyrrolase activity observed after 2 hours
occurred when little or none of the added hydrocortisone re-
mained. This suggestion is supported by the fact that after 2
hours of perfusion, substituting blood containing no added
hydrocortisone for the blood which had received 3 mg of hydro-
cortisone at the beginning of the experiment did not inhibit the
rise in tryptophan pyrrolase activity. Thus, hydrocortisone
initiated a series of reactions leading to an increase in tryptophan pyrrolase which continued after most of the steroid had been metabolized or removed. In contrast, the tyrosine transaminase activity was significantly elevated within 2 hours of hydrocortisone administration and declined after 4 hours. The decline of enzyme activity could be prevented by continuous infusion of half of the dose of hydrocortisone during the experiment. This suggested that a minimal level of hydrocortisone was necessary to maintain elevated levels of tyrosine transaminase activity.

Puromycin prevented the hydrocortisone-induced increases and decreased the basal levels of both tyrosine transaminase and tryptophan pyrrolase activities without changing hepatic lactate dehydrogenase activity, the bile flow, or the protein content, and without inhibiting the activities of the two former enzyme reactions in vitro. Thus, puromycin did no general damage to the liver in the test period but exerted the relatively specific effect of preventing both the appearance of additional enzyme activities and maintenance of the basal activities of these two enzymes. These results indicate that puromycin interfered with the continuing formation of active enzymes, which on other grounds have been thought to turn over with half times of approximately 2½ hours (18, 19). However, no necessary connection was established here between puromycin's interference with the formation of the active forms of these enzymes and this compound's known action of inhibiting protein synthesis.

SUMMARY

1. Hydrocortisone produced an increase in both tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase activities in the isolated, perfused liver, although the steroid had no significant effect on the tyrosine transaminase activity of rat liver slices incubated in tissue culture medium. The former enzyme rose to maximal levels within 2 to 4 hours of hydrocortisone administration and then declined, whereas the latter increased only after 2 hours and then rose progressively for the next 4 hours.

2. Maintenance of elevated levels of tyrosine transaminase, but not of tryptophan pyrrolase, appeared to depend on a continuous supply of hydrocortisone to the perfused liver.

REFERENCES

The Effect of Hydrocortisone on Tyrosine-α-ketoglutarate Transaminase and Tryptophan Pyrrolase Activities in the Isolated, Perfused Rat Liver
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