Hormonal and Substrate Induction of Tryptophan Pyrrolase in Regenerating Rat Liver

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SUMMARY

The induction of tryptophan pyrrolase by hydrocortisone has been studied in rats following 70% hepatectomy. An alteration in the rate of induction was observed in both sham-operated and 70% hepatectomized rats early in the postoperative period. Normal maximal values were eventually reached, however. Later in the postoperative period, induction patterns were similar to those observed in unoperated rats. During the period in which DNA synthesis is known to occur following 70% hepatectomy, the induction of tryptophan pyrrolase by hydrocortisone was depressed in the 70% hepatectomized but not in the sham-operated rats. At this latter time period, induction of tryptophan pyrrolase by L-tryptophan was not diminished in the regenerating liver.

It has been suggested by other workers that alterations in enzyme control mechanisms, such as the absolute or relative failure of tryptophan pyrrolase induction by cortisone in minimal-deviation hepatomas, may represent a fundamental biochemical alteration in tumors. This study shows that a depressed response to hormonal enzyme induction also occurs in non-neoplastic hepatic cells preparing to divide.

INTRODUCTION

The administration of glucocorticoids to rats results in an increased synthesis of tryptophan pyrrolase (TP) by the hepatic cells (11, 19). Well-differentiated hepatocellular carcinomas, the so-called "minimal-deviation" hepatomas (13), generally show little or no increase in TP activity after cortisone administration to the host animal (3, 4). The levels of activity of other enzymes in these tumors appear relatively unresponsive to physiologic alteration of the host (1). It has been hypothesized as a result of these studies that impairment of normal enzyme regulatory mechanisms may be a critical factor in both the initiation and maintenance of the neoplastic state (16).

In a previous report, it was demonstrated that the induction of TP by hydrocortisone was reduced during the period of DNA synthesis in the regenerating rat liver (20). The purpose of these experiments was further to analyze TP induction by hydrocortisone 21 hours after 70% hepatectomy. At this time, DNA synthesis in the residual liver has been shown to occur at a maximal rate (2). Induction of TP by L-tryptophan was also studied at this time.

A delayed response to hydrocortisone at an early postoperative time was demonstrated, but maximal levels of TP activity were eventually achieved. An absolute depression of TP induction by hydrocortisone throughout the period of deoxyribonucleic acid synthesis, after 70% hepatectomy, was also demonstrated. Substrate induction, on the other hand, was not depressed.

MATERIALS AND METHODS

Treatment of Animals

Sprague-Dawley male rats (Charles River Farms) weighing approximately 250 grams were used throughout the experiments. When specified, the rats were adrenalectomized by the dealer through a single, dorsal incision approximately 5-7 days before the experimental period. Seventy percent hepatectomy (10) or a sham operation (laparotomy and cutting of liver ligaments) were performed by one operator between 8 and 11 A.M. to eliminate diurnal variation. All rats were fed Purina rat chow ad libitum and the adrenalectomized rats 0.9% saline ad libitum. The operations were accomplished in 2 minutes, and the total time under light ether anesthesia was 3 minutes. At various times after operation, hydrocortisone phosphate (5 mg/100 gm body weight) or L-tryptophan (50 mg/100 gm body weight suspended in isotonic saline) was administered intraperitoneally. When indicated, an identical dose of steroid was administered into the femoral vein via a small skin incision. At times specified, the animals were stunned and then sacrificed by decapitation. The right posterior lobe of the liver was then removed for enzyme assay. To compare the efficacy of induction among groups of animals, the assay was performed on rats killed 4 hours after hydrocortisone injection; this will be referred to as the "4-hour point." Rats administered L-tryptophan were sacrificed 5 hours after injection.

A second group of rats identically prepared by operation and steroid or L-tryptophan administration was treated with intraperitoneal colchicine (1.0 mg/kg) at 26 hours and were sacrificed at 32 hours. Three sections of liver were prepared from each rat by standard histologic techniques (hematoxylin and eosin). All nuclei were counted on each of the 3 sections for an average count of 12,000 nuclei per rat, and the mitotic index was then determined.

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2 Career Scientists of the Health Research Council of the City of New York (I-275, I-207).

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Nonadrenalectomized rats had a mortality of less than 1% regardless of the operation or drug administration. Unoperated and sham-operated adrenalectomized rats had an overall mortality of less than 5% after operation. Adrenalectomized, 70% hepatectomized rats demonstrated an overall mortality of 40%, death usually occurring within the first 12 hours after operation. The 60% of animals that survived the operation appeared well.

Enzyme Assay

 Portions of liver were prepared from the right posterior lobe, rinsed several times in iced 0.9% NaCl, and homogenized in a glass-Teflon tissue grinder in 3 volumes of cold 0.14 M KCl containing 0.02 M phosphate buffer, pH 7.0. The homogenate was centrifuged at 15,000 × g for 10 minutes in a Sorvall RC-2 refrigerated centrifuge. The supernatant was carefully withdrawn to minimize contamination with the appreciable amounts of fat which formed the surface layer. This supernatant fraction was then centrifuged for 1 hour at 100,000 × g in a Spinco model L ultracentrifuge. The resulting clear supernatant was used as the enzyme source. Tryptophan pyrrolase was assayed by the method of Knox et al. (12) which involves a preincubation in a solution containing 5.0 mM L-tryptophan, 1.0 mg of methemoglobin per ml (dissolved in 0.14 M KCl-0.02 M phosphate, pH 7.0), and 60 mM freshly neutralized ascorbic acid. Equal volumes of the liver supernatant and the preincubation solution were mixed, flushed with nitrogen, and incubated in stoppered test tubes at 37°C for 60 minutes.

 Tryptophan pyrrolase activity was determined by observing the increase in absorbance at 360 mµ at room temperature in a Zeiss PMQ II spectrophotometer. The molar extinction coefficient used for kynurenine was 4530. The standard assay mixture had a total volume of 3.0 ml and contained 0.7 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.2 ml of 0.05 M L-tryptophan, 0.1 ml of freshly neutralized 0.3 M ascorbic acid, 1.8 ml of H2O, and 0.2 ml of the preincubated premicrosomal fraction.

 The enzyme activity was calculated from the increase in absorbance over the first 15 minutes, during which time the reaction was linear under the conditions of the assay. Protein was determined by the biuret method using bovine serum albumin as a standard (6). Enzyme activities were expressed as micromoles of kynurenine formed per hour per gram protein.

 Methemoglobin (twice crystallized horse hemoglobin) and L-tryptophan were purchased from Nutritional Biochemicals Corp. Hydrocortone phosphate (Merck hydrocortisone-21-phosphate) was used throughout the experiments. The other chemicals were of reagent grade.

 RESULTS

The Effect of Hydrocortisone and Adrenalectomy on Mitotic Activity following 70% Partial Hepatectomy. The data in Table 1 show that neither prior adrenalectomy nor administration of hydrocortisone or L-tryptophan at any time after 70% hepatectomy caused an appreciable change in the resultant mitotic index. In addition, sham operation did not stimulate mitotic activity. Induction of TP by Hydrocortisone in the Regenerating Liver of Intact Rats. Because of the possibility that adrenalectomy alone was responsible for the diminished induction of TP in the regenerating liver at 18 hours, similar studies were conducted on intact rats during the period of this depression. During the course of these experiments, it was found that sham operation or 70% hepatectomy alone caused a rapid rise in TP induction.
Irving Seidman, George W. Teebor, and Frederick F. Becker

CHART 1. Induction of tryptophan pyrrolase by hydrocortisone in adrenalectomized sham-operated and 70% hepatectomized (HEP) rats. Hydrocortisone phosphate (5 mg/100 gm body weight) was injected intraperitoneally at the times indicated on the abscissa and tryptophan pyrrolase activity assayed 4 hours later. The points represent the average of 5-7 animals and the brackets indicate ±S.E.

activity, presumably due to endogenous cortisone secretion. However, by 21 hours after either operation, TP activity had returned to base line values (Chart 2).

From 18 to 30 hours after 70% hepatectomy, TP activity, determined 4 hours after hydrocortisone administration, was 50% that achieved by sham-operated animals. This is similar to the difference observed in adrenalectomized rats. This depression of response lasted through 48 hours. The partial recovery observed in the adrenalectomized group by 48 hours was not seen in the intact rats (Chart 2).

Kinetics of TP Induction by Hydrocortisone in Adrenalectomized Rats Immediately after Operation. Sham-operated and 70% hepatectomized adrenalectomized rats were given a single dose of hydrocortisone intraperitoneally within 2 minutes following operation and sacrificed at intervals thereafter to determine if the induction of TP was absolutely depressed or merely delayed. Chart 3 demonstrates that both groups of operated rats displayed a delayed response. Sham-operated rats reached peak values at 9 hours and 70% hepatectomized rats achieved similar values at the same time. Intravenous administration of hydrocortisone yielded similar results, indicating that delayed absorption of hydrocortisone from the peritoneal cavity was not a factor.

Kinetics of TP Induction in Intact Rats by Hydrocortisone 21 Hours after Operation. Because of the altered rate of TP induction immediately after operation, identical studies were performed beginning at 21 hours after operation. Intact rats were used because of the relatively high mortality of 70% hepatectomy on adrenalectomized rats (vide supra). The use of intact rats was felt to be appropriate as (a) both adrenalectomized and intact rats had displayed a similar depression of TP induction at this time period under study and (b) other workers have demonstrated that TP induction by cortisone is identical in intact and adrenalectomized animals (18).

Chart 4 demonstrates that TP activity in both sham-operated and 70% hepatectomized rats reached maximal values at approximately the same time. In contrast to the data summarized in Chart 3, TP activity in 70% hepatectomized rats never achieved the values observed in the controls. The slope of the induction curve in both sham-operated and 70% hepatectomized rats at 21 hours is similar. The maximal value for 70% hepatectomized rats was 30% less than controls.

Induction of TP by L-Tryptophan in Intact Rats 21 Hours following Operation. Intact rats were used to study the changes of TP activity produced by intraperitoneal L-tryptophan. Five hours after L-tryptophan administration, the degree of TP induction is normal in sham-operated rats and somewhat elevated in 70% hepatectomized rats (Table 2).
Chart 2. Induction of tryptophan pyrrolase by hydrocortisone in intact sham-operated and 70% hepatectomized (HEP) rats. The procedure is the same as Chart 1.

Chart 3. Kinetics of tryptophan pyrrolase induction by hydrocortisone in adrenalectomized unoperated, sham-operated, and 70% hepatectomized (HEP) rats immediately following operation. Hydrocortisone phosphate (5 mg/100 gm body weight) was injected intraperitoneally at time zero and tryptophan pyrrolase activity assayed at the times indicated on the abscissa. Each point represents the average of 4 or 5 animals and the brackets ±S.E.
TABLE 2

Induction of Tryptophan Pyrrolase by L-Tryptophan

L-Tryptophan (50 mg/100 gm body weight) was injected intraperitoneally. Five hours later, the rats were sacrificed and tryptophan pyridase activity was assayed. Values represent the average of 5 rats per group ± S.E.

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<tr>
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<th>Tryptophan pyrrolase activity (μmoles kynurenine/gm protein/hr)</th>
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<tr>
<td>Unoperated</td>
<td>392 ± 18.1</td>
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<tr>
<td>Sham-operated (21 hr)</td>
<td>365 ± 15.4</td>
</tr>
<tr>
<td>70% hepatectomized (21 hr)</td>
<td>508 ± 17.5</td>
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DISCUSSION

Knox and Auerbach (11) demonstrated that the administration of cortisone to rats resulted in a 4 to 5-fold increase in TP activity within a few hours. It was later shown that the increase in enzyme activity was the result of an increased rate of enzyme synthesis (19) and could be blocked by either actinomycin D (AMD) or puromycin (8). The induction of TP by its substrate, L-tryptophan, is blocked by puromycin but not by AMD (8). Schimke et al. (19) concluded that L-tryptophan exerted its effect by stabilizing the enzyme, thereby delaying its normal degradation.

The induction of TP by cortisone and L-tryptophan has been studied in transplantable rat hepatocellular carcinomas, particularly in the Morris “minimal-deviation” lines (15). These slow-growing carcinomas are similar in enzyme content to normal liver (13) suggesting that the enzyme deletions observed in other hepatomas may not represent a primary biochemical alteration related to the initiation of neoplasia but are rather a secondary phenomenon related to rapid tumor growth (17). Tumors with slow growth rates do demonstrate, however, differences in enzyme regulation, and this parameter is being studied in an attempt to define a fundamental alteration in tumor metabolism. TP induction by cortisone was found to be absent or markedly reduced in most hepatomas (3, 4) and substrate induction was only occasionally present (15). The latter induction, when present, was usually low and dependent on the presence of adrenal glands in the host rat or the injection of small amounts of steroids in adrenalectomized rats. From the results of these studies, Pitot and Heidelberger (16) proposed several models based upon abnormal regulatory metabolic circuits whereby neoplasia might be initiated and maintained.

Goldstein and Knox (5) and Greengard and Feigelson (7) demonstrated a depression of TP induction by cortisone in...
regenerating rat liver 48 hours post 70% hepatectomy. In a previous study, it was shown that a depression of TP induction by hydrocortisone occurred in regenerating rat liver corresponding in time to the initial period of DNA synthesis (20). This raised the possibility that the results reported in tumors may be a result of cell division rather than the neoplastic state.

The data of Chart 1 showed a depression of induction 4 hours after injection of hydrocortisone in the early hours after operation in both sham-operated and 70% hepatectomized adrenal-resected rats. A kinetic analysis of this response, however, revealed that although the rate of induction was diminished, maximal values were ultimately attained in both groups of rats (Chart 3). The reason for this alteration in kinetics is obscure but seems related to the operative procedure since it is observed in both groups of animals and since the “4-hour response” increases with time following operation (Chart 1).

The second depression of TP induction occurs only in partially hepatectomized rats, be they adrenalectomized or intact. Kinetic studies at this time reveal a pattern of induction virtually identical to that of unoperated or sham-operated rats but showing an absolute depression of 25–30% in the partially hepatectomized rats (Chart 4). Grisham has demonstrated that approximately 40% of the hepatocytes partake in DNA synthesis at this time (9), and it is possible that the curve of Chart 4 represents a normal response of only 60–70% of hepatic cells, those not synthesizing DNA, and that the DNA synthesizing cells are totally refractory to hormonal stimulation.

In these experiments, induction by substrate during the period of DNA synthesis was found to be undiminished. Nemeth (14) presented similar data for substrate induction 48 hours after partial hepatectomy. Because substrate induction of TP depends upon a continuing basal synthesis, it is apparent that translational events, at least at basal rate, are able to occur in the regenerating liver.

It seems possible, therefore, that the lessened response to hydrocortisone in hepatic cells preparing to divide is related to repression of transcriptional rather than translational mechanisms and that the duplicating genome itself may be unable to participate simultaneously in other functions.

Therefore, the failure or diminution of TP induction by steroids in hepatomas is not a specific property of neoplastic cells but may be common to dividing cells.

REFERENCES

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