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Further Enhancement by Tryptophan of Hepatic Protein Synthesis Stimulated by Phenobarbital or Cortisone Acetate[1,2] (40181)

ETHEL VERNEY AND HERSCHEL SIDRANSKY

Department of Pathology, University of South Florida, College of Medicine, Tampa, Florida 33620 and Department of Pathology, The George Washington University Medical Center, Washington, D.C. 20037

In earlier studies from our laboratory and from other laboratories, it has been demonstrated that the administration of tryptophan has a stimulatory effect on hepatic polyribosomes and protein synthesis in fasted and in fed animals (1–6). In addition, it has been reported that tryptophan administration produced an improvement in the state of polyribosomal aggregation and in protein synthesis in the livers of animals treated with hepatotoxic agents such as ethionine (7), actinomycin D (8), puromycin (9), hypertonic NaCl (10), or CCl_4 (11).

The present study was concerned with whether tryptophan administration would have a stimulatory effect on hepatic polyribosomes and protein synthesis in animals which already had an enhanced effect induced by agents such as phenobarbital or cortisone acetate. Phenobarbital (12–14) and cortisone or hydrocortisone (3, 15–19) have previously been demonstrated to enhance hepatic protein synthesis. Our present results reveal that tryptophan administration further enhanced hepatic polyribosomal aggregation and protein synthesis in animals which already had been previously stimulated.

Methods and materials. Female Sprague–Dawley rats weighing 75–130 g were used in these experiments. They were housed in suspended wire cages in an air-conditioned room and were fed a commercial diet (Wayne Lab Blox, Allied Mills Inc., Chicago, IL). Water was available *ad libitum,* but was removed after animals were force-fed tryptophan or distilled water.

Phenobarbital (Phenobarbital–Sodium USP crystalline, Mallinckrodt Chemical Works, St. Louis, MO), 10 mg/ml, was injected twice daily intraperitoneally at a level of 4 mg/100 g body wt for 1–3 days. Cortisone acetate (Cortisone acetate, Merck, Sharpe and Dohme, West Point, PA) saline suspension 25 mg/ml was injected at a level of 10 mg/100 g body wt intraperitoneally 14 hr before sacrifice. All rats were fasted overnight and on the following morning were divided into equal groups (two to four rats/group) that were force-fed distilled water (3 ml/100 g body wt) or *L*-tryptophan (30 mg in 3 ml distilled water/100 g body wt) 1 hr before sacrifice.

Animals were killed by decapitation. Livers were removed, weighed, and homogenized with a Polytron PT 20 using 3 vol of 0.25 *M* sucrose in TKM buffer (0.05 *M* Tris, pH 7.6, 0.025 *M* KCl and 0.005 *M* $MgCl_2$). A postmitrochondrial supernatant was prepared using a JA-20 rotor in a Beckman J-21 preparative centrifuge. All work was performed at 2°.

A deoxycholate-treated postmitochondrial supernatant was loaded onto a 12 ml 0.3 *M*–1.1 *M* sucrose gradient in TKM and spun in a SW 41 rotor at 38,000 rpm for 1 hr in a Beckman L3-40 ultracentrifuge. The gradient, after being punctured at the bottom of the tube, was scanned in a Gilford 2000 spectrophotometer using a flow cell of 4 mm and absorbancy at 260 nm was monitored on a strip chart recorder.

The degree of hepatic polyribosomal aggregation was evaluated from the patterns obtained by sucrose density gradients. This was conducted by calculating the relative distribution of monomer-dimers in relation to total ribosomes by measuring the area under the monomer and dimer peaks and the area under the entire pattern (monomer-dimers plus the other polyribosome fractions) of each gradient pattern (8).

In vitro incorporation studies were mea-

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sured using [^{14}C]leucine, uniformly labeled (10 mCi/mmol), in a system using postmitochondrial supernatant or ribosomes (1). The results were expressed as cpm incorporated into acid precipitable proteins per mg RNA.

Results. Figure 1 presents the hepatic polyribosomal profiles of rats that received intraperitoneal injections of phenobarbital twice daily for 1, 2, or 3 days and then received a tube-feeding of distilled water or tryptophan 1 hr before killing. It is apparent that in the control rats the administration of phenobarbital increased the degree of the hepatic polyribosomal aggregation (more heavier aggregates and fewer monomer-dimers), the degree of improvement appeared to increase with the duration of administration. Rats that received tryptophan 1 hr before killing showed further improvement in hepatic polyribosomal aggregation than comparable controls, rats receiving nothing or those receiving phenobarbital for 1, 2, or 3 days. In an attempt to quantitate the changes in hepatic polyribosomal aggregation in the control and experimental groups, the profiles of three to four experiments were analyzed. The ratios of monomer-dimers to total ribosomes were determined and compared. Rats that received tryptophan following phenobarbital for 1, 2, or 3 days revealed, respectively, a decrease in the monomer-dimer/total ribosomes of 8, 23, and 25% in comparison with those that received phenobarbital alone. Statistically, the change after 3 days had a P value of less than 0.02.

Table I summarizes the effect of tryptophan administration after phenobarbital treatment for 1, 2, or 3 days upon *in vitro* hepatic protein synthesis, in which [^{14}C]leucine incorporation into protein using hepatic postmitochondrial supernatants was measured. Rats that received phenobarbital alone for 3 days revealed a 26% increase in *in vitro* protein synthesis in comparison to controls that did not receive phenobarbital. The increases due to tryptophan administration ranged from 29 to 44%. In four experiments in which rats received phenobarbital for 3

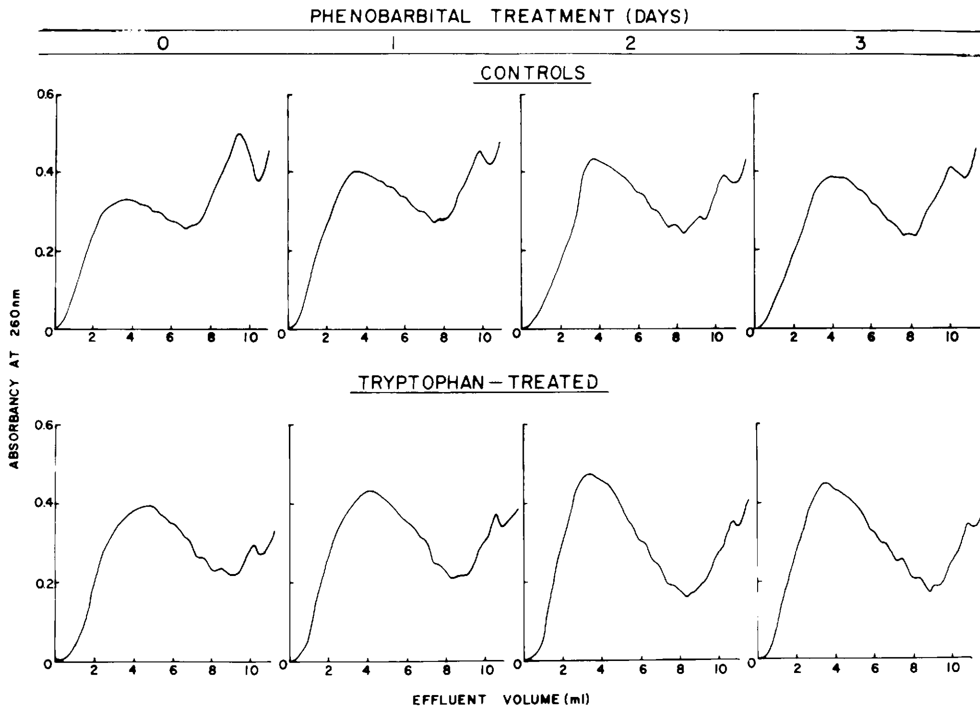

FIG. 1. Sucrose density gradient patterns of hepatic polyribosomes of deoxycholate-treated postmitochondrial supernatants of rats treated with phenobarbital for 1, 2, or 3 days and then receiving tryptophan or water 1 hr before killing. Nonribosomal materials from each group, as measured on supernatants obtained by centrifuging deoxycholate-treated postmitochondrial supernatants in a 65 rotor at 65,000 rpm for 22 hr in a Beckman L5-75 ultracentrifuge to pellet ribosomes, revealed no contributions to the 260 nm profiles.

days and then tryptophan before killing, ribosomes were also used to assay *in vitro* hepatic protein synthesis using a constant cell sap. The tryptophan-treated group revealed a 21% ($P < 0.01$) improvement over the control group.

Figure 2 summarizes the hepatic polyribosomal profiles of rats that received cortisone acetate 14 hr before killing and tryptophan or distilled water by stomach tube 1 hr before killing. Rats that received cortisone acetate revealed a shift toward greater polyribosomal aggregation than that in the control (distilled water) rats. Rats that received tryptophan revealed greater polyribosomal aggregation than did those not receiving tryptophan whether the rats were pretreated with cortisone acetate or not. Quantitation of the changes in the degree of hepatic polyribosomal aggregation in the control and experimental groups of nine experiments revealed that the ratios of monomer-dimers/total ribosomes were decreased 21% for the tryptophan-treated rats compared with the controls and 22% for the tryptophan-treated, cortisone acetate-treated group compared with the cortisone acetate-treated group. These differences were statistically significant ($P < 0.01$).

TABLE I. EFFECT OF TRYPTOPHAN ADMINISTRATION AFTER PHENOBARBITAL TREATMENT FOR 1–3 DAYS UPON *in Vitro* HEPATIC PROTEIN SYNTHESIS.

Phenobarbital treatment days	No. of experiments	[^{14}C]Leucine incorporation into hepatic protein using postmitochondrial supernatants/% increase in tryptophan-treated vs. controls[a]
1	3	28.8 ± 3.34[b]
2	3	34.6 ± 9.09
3	4	43.6 ± 11.07[b]

[a] All groups received phenobarbital for 1–3 days.
[b] $0.05 > P > 0.01$.

Table II summarizes the effect of tryptophan administration on control or cortisone acetate-treated rats upon *in vitro* hepatic protein synthesis, in which [^{14}C]leucine incorporation into protein using hepatic postmitochondrial supernatants was measured. The increase with cortisone acetate alone was sim-

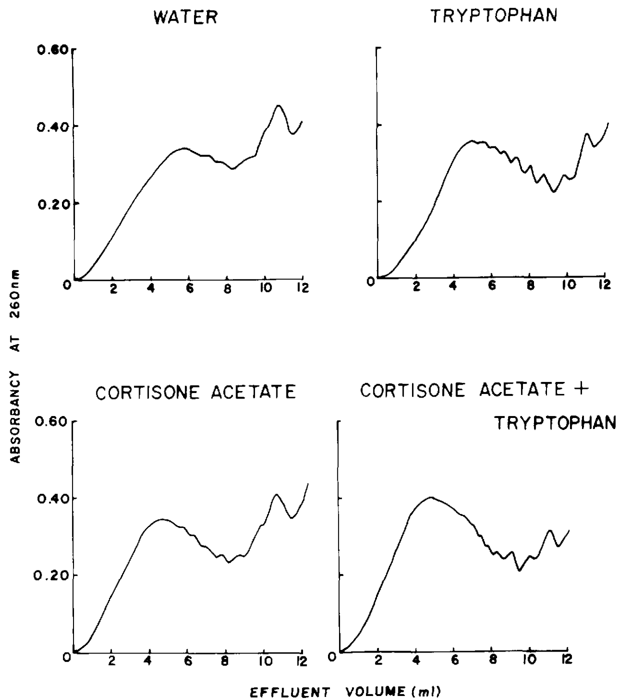

FIG. 2. Sucrose density gradient patterns of hepatic polyribosomes of deoxycholate-treated postmitochondrial supernatants of rats treated with cortisone acetate 14 hr and tryptophan 1 hr before killing. Nonribosomal material from each group, as measured on supernatants obtained by centrifuging deoxycholate-treated postmitochondrial supernatants in a 65 rotor at 65,000 rpm for 22 hr in a Beckman L5-75 ultracentrifuge to pellet ribosomes, revealed no contributions to the 260 nm profiles.

TABLE II. *In Vitro* Hepatic Protein Synthesis in Rats Treated with Tryptophan, Cortisone Acetate or Both.

Group[a]	[^{14}C]Leucine incorporation into hepatic protein using postmitochondrial supernatants % increase[b]
Cortisone	29 ± 10.91
Tryptophan	74 ± 10.97[c]
Cortisone + tryptophan	115 ± 23.84[c]

[a] Results of six experiments.
[b] Each group compared with controls.
[c] $P < 0.01$.

ilar to that reported earlier (19) and also the increase with tryptophan alone was similar to that reported earlier (1, 5, 6). Rats that received tryptophan showed increases over comparable controls (rats that received cortisone acetate pretreatment or not).

Discussion. This study reveals that tryptophan administration to rats that have had hepatic protein synthesis enhanced by other means (administration of phenobarbital or cortisone acetate) further stimulated hepatic polyribosomes toward heavier aggregation and hepatic protein synthesis (*in vitro*). Earlier studies have reported a stimulatory effect of tryptophan on hepatic polyribosomes and protein synthesis under experimental conditions where these parameters were at low (fasted state) or suppressed (hepatotoxic agents) levels (1–5, 7–11). Also it has previously been demonstrated that when hepatic polyribosomes and protein synthesis are influenced by nutritional means such as in fed mice (6) or by force-feeding a threonine-devoid diet to rats (20), where the parameters are normal or enhanced, tryptophan administration can have a further stimulatory effect. Now it is evident that tryptophan can also have a stimulating effect on hepatic protein synthesis even when this metabolic process has been enhanced by selected drugs.

The potentiating effect of tryptophan upon hepatic protein synthesis after using stimulatory drugs such as phenobarbital or cortisone acetate raises many interesting problems. Conceivably, each of these agents may be acting to enhance hepatic protein synthesis in different ways. The different mechanisms considered to be involved have been reviewed in earlier reports (17, 21–26). Thus by investigating a combination of two systems, such as a nutritional component like tryptophan and commonly used therapeutic agents like phenobarbital or cortisone acetate it is possible to obtain information which may be utilized in approaches to improve liver protein metabolism in normal and in diseased states. A similar approach using combinations of chemotherapeutic agents or drugs has proved to be of great value in the treatment of malignancies.

Our laboratory has been concerned with the mechanism(s) by which tryptophan acts to stimulate hepatic protein synthesis. Currently there is evidence that tryptophan may act at the transcriptional (27–29) as well as at the posttranscriptional (1, 22–24, 30) level of control of hepatic protein synthesis. This may allow tryptophan to play an important role in the regulation of protein synthesis in the liver in depressed (after hepatotoxic agents or fasting (1–5, 7–11)), normal (fed (6)) or even in already stimulated (20, this study) states.

Summary. This study was concerned with the effect of the administration of tryptophan on hepatic polyribosomes and protein synthesis in rats in which hepatic protein synthesis was already enhanced by the administration of phenobarbital or cortisone acetate. Previously it has been demonstrated that tryptophan has a stimulatory effect on hepatic protein synthesis of fasted or hepatotoxin-treated animals. The present study revealed that tryptophan administration to rats that have had hepatic protein synthesis enhanced by treatment with phenobarbital (1–3 days) or cortisone acetate (14 hr) further stimulated hepatic polyribosomes toward heavier aggregation and hepatic protein synthesis (*in vitro*).

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