Influence of Dietary Tryptophan on the Induction of $\gamma$-Glutamyltranspeptidase-positive Foci in the Livers of Rats Treated with Hepatocarcinogen

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ABSTRACT

The ingestion of an elevated level (2%) of L-tryptophan (TRP) in a purified diet was investigated to determine whether it would influence the induction of $\gamma$-glutamyltranspeptidase (GGT)-positive foci in the livers of rats exposed to a hepatocarcinogen. Subtotal hepatectomies were performed, and 18 h later, the rats were given injections i.p. of diethylnitrosamine (30 mg/kg). Ten days later, groups of male rats were placed on choline-supplemented (CS), CS + TRP, choline-deficient (CD), or CD + TRP diets for 10 wk. In two separate experiments, the rats fed the CS + TRP diet or the CD diet developed more and larger GGT+ foci than did rats fed the CS diet. Rats fed the CD + TRP diet revealed similar changes to those found in rats fed the CD diet. The liver weights of the rats fed the CD or the CD + TRP diet were greater than those of rats fed the CS or the CS + TRP diet. Hepatic GGT activity was somewhat elevated in rats fed the CS + TRP diet and markedly elevated in rats fed the CD or the CD + TRP diet. Hepatic ornithine decarboxylase activity was increased in rats fed the CD + TRP diet. The results suggest that increased dietary tryptophan has a promoting effect on liver carcinogenesis as measured by the induction of GGT+ foci in the livers of rats exposed to diethylnitrosamine. A potentiating effect by tryptophan was not observed in the livers of rats fed a CD diet.

INTRODUCTION

Awareness that diet in general or specific dietary components may play a role in tumorigenesis has stimulated research directed toward determining which nutritional components are involved and how they may act. Currently, based upon epidemiological and experimental evidence, a number of nutritional components have been described as being influential in the process of tumorigenesis (1-9). Among the implicated nutrients are protein, fat, vitamins, and minerals. A number of these dietary components have been considered to act at the promotional stage of tumorigenesis.

In our laboratory we have been concerned for a number of years with the effects of dietary tryptophan on the livers of experimental animals (mice and rats). Indeed, tryptophan appears to have a unique effect on hepatic protein synthesis (10-12). Therefore, in conjunction with our interests in liver tumorigenesis due to chemical carcinogens, we became concerned in the present study with the possible effect of tryptophan as a promoting agent on liver tumorigenesis due to chemicals.

Review of the literature reveals that some investigators have reported that tryptophan enhances tumorigenesis due to selected chemical carcinogens. Tryptophan has been implicated in carcinogenesis of the bladder in earlier (13-17), as well as in more recent (18-20), experimental studies. On the other hand, the effect of tryptophan on liver tumorigenesis is conflicting; an enhancing effect has been reported by some workers (13, 14, 21), while a decreasing effect has been reported by other workers (22, 23).

In this study we have investigated whether the ingestion of an elevated level of L-tryptophan in a purified diet would influence the induction of GGT+ positive foci of livers of rats exposed to a hepatocarcinogen. The enzyme-altered foci which develop in the livers of rats treated with a hepatocarcinogen are considered to be the precursor lesions to neoplastic nodules and hepatomas (24, 25). Also, we determined whether an elevated tryptophan level in a choline-deficient diet would further influence the induction of GGT-positive foci which has been reported by others (26-28). Our findings indicate that elevated dietary tryptophan increases the incidence of GGT-positive foci in the livers of rats fed a choline-supplemented diet but not those fed a choline-deficient diet for 10 wk.

MATERIALS AND METHODS

Animals and Treatments. Male rats of the Sprague-Dawley strain (Hartman Sprague Dawley, Bethesda, MD) weighing on the average 202 g (in Experiment 1, 239 g, and Experiment 2, 172 g) were used in all experiments. Animals were kept in a temperature-controlled room with alternating 12-h cycles of light and dark. The rats were fed ad libitum a commercial ration (Wayne Lab-Blox; Allied Mills, Inc., Chicago, IL) for at least 1 wk before the start of the experiments and also for 10 days after subtotal hepatectomy. Purified CS and CD diets were prepared as described by Shinozuka et al. (26). The tryptophan-supplemented diets were the CS and CD diets to which was added 2% TRP at the expense of sucrose. Standard two-thirds partial hepatectomies were performed on all rats according to Higgins and Anderson (29). Eighteen h later, the rats were given injections i.p. of DEN (Eastman Organic Chemicals, Rochester, NY) in saline at a dose of 30 mg/kg. Ten days later, groups of rats (6-12 rats/group) were placed on the CS, CS + TRP, CD, or CD + TRP diets for 10 wk. All animals were weighed at weekly intervals, and food consumptions were monitored on 2 consecutive days at weekly intervals. After an overnight fast, the rats were killed by decapitation. The livers were rapidly removed and weighed, and pieces of each liver were frozen on dry ice for sectioning or were added to cold buffer solutions for chemical determinations. Blood samples were taken for serum enzyme assays.

Assay for GGT+ Foci. For analysis of GGT+ foci, frozen pieces of livers were cut at 8-μm sections with a cryostat and then stained according to the procedure of Rutenburg et al. (30). GGT+ foci were counted using three slides from three different areas of each liver. The major and minor diameters of each focus were measured using an ocular...
micrometer. Assuming that each focus was elliptical in shape, the area of each focus was calculated. The area of each tissue section was measured using an Apple II+ computer with graphics tablet accessory. The size of each focus, the number of foci per cm², and the percentage of liver occupied by the foci were calculated for each liver. Statistical comparisons were made by the Fisher F test and the Student t test (31).

Other Analyses. The activity of ODC in the liver was assayed by the modification of the method of Russell and Snyder (32) and described earlier by us (33); the activities of cytochrome P-450 and cytochrome b₅ were assayed according to the methods described earlier (34, 35); and the activity of GGT was assayed as described previously (36). Analyses of the size distributions of total hepatic polyribosomes were evaluated as described earlier (37), and in vitro [¹⁴C]leucine incorporation into proteins using total hepatic microsomes was performed as described earlier (37). Free amino acid concentrations in liver and serum were determined using a Spinco Model 118C amino acid analyzer. Levels of SGPT and serum GGT were assayed on a SMA 12/60 (Technicon Instruments).

RESULTS

The effects of feeding the four dietary regimens (CS, CS + TRP, CD, and CD + TRP) for 10 wk on rats that had received subtotal hepatectomy (18 h) were studied in two experiments. The results relating to the numbers and sizes of GGT+ foci in the livers of the four groups of rats are summarized in Table 1. In both experiments, rats fed the CS or CD diet developed more and larger GGT+ foci than did rats fed the CS diet. In general, the effect of the CD diet was somewhat greater than that of the CS diet. In Experiment 1, rats fed the CD + TRP diet developed fewer GGT+ foci which occupied less areas within the livers than was the case of rats fed the CS + TRP or the CD diet. However, in Experiment 2, rats fed the CD + TRP diet developed essentially the same numbers and similar sizes of GGT+ foci as in the livers of rats fed the CS or the CS + TRP diet. The combined results of Experiments 1 and 2 are summarized in Table 1 and reveal that the addition of TRP to the CD diet appears to potentiate the number and size of GGT+ foci, while the addition of TRP to the CD diet has little influence on the stimulatory effect of the CD diet itself.

Chart 1 reveals the body weight changes in rats fed the four diets for 10 wk in Experiment 2. Rats fed the CS diet gained the most weight, while rats fed the other three diets gained somewhat less weight; the rats fed the CD or the CD + TRP diets gained the least amount of weight. In Experiment 2, the average daily diet intake (g) per rat for the four groups was as follows: CS, 16.7; CS + TRP, 15.8; CD, 15.2; and CD + TRP, 15.8. The changes in body weights in Experiment 1 were similar to those in Experiment 2, but overall, the rats gained much less, particularly during the last 3 wk. At autopsies, the rats of Experiment 1 revealed evidence grossly of pneumonia and lung abscesses in many of the animals. This pathology probably contributed to lower mean body weight gains along with less diet consumption for the rats in Experiment 1 compared to those in Experiment 2.

In Experiments 1 and 2, the liver weights of the rats fed the CD or the CD + TRP diet were greater (19–27%) than those of rats fed the CS or the CS + TRP diet, the latter two groups having similar liver weights (Table 1). Biochemical assays (ornithine decarboxylase activity, GGT activity, cytochrome P-450 and b₅ activities, status of polyribosomes and in vitro protein synthesis of the livers, SGPT and serum GGT activities, and free amino acid concentrations of livers and sera) were conducted on control and experimental animals. In general, there were few significant differences between the groups, and therefore only selected findings of differences are cited. Hepatic ODC activity was significantly increased in rats fed the CD + TRP diet. Hepatic GGT activity was somewhat elevated in rats fed the CS + TRP diet but significantly elevated in rats fed the CD or the CD + TRP diet. Since the results of liver assays were determined using total livers in control and experimental groups, it is difficult to evaluate them, since the livers of the experimental animals contained altered populations of liver cells. Whether certain subpopulations of the altered cells demonstrate significant biochemical differences can only be determined in future experimental studies using isolated subpopulations of the livers of rats fed the CD or the CD + TRP diet.
TRYPtopHAN AND LIVER CARCINOGENESIS

In earlier studies, the effects of tryptophan upon hepatic protein metabolism in short-term (hours or days) experiments were investigated (10–12). It might be appropriate to review a few of these findings in relation to the present findings in long-term (weeks) experiments concerned with hepatic carcinogenesis. In searching as to how tryptophan may act as a promoter in our present experimental model (Table 1), we may assume that tryptophan conceivably acts to affect or influence gene expression. Some evidence exists that a potential basis for the phenotypic expression of chemically induced neoplasms occurs by the release of nuclear restricted RNA, and of possibly other RNA species, into the cytoplasm (38, 39). Indeed, a number of investigators have described the enhanced outflow of mRNA from hepatic nuclei after treatment with chemical carcinogens (40–43) or after treatment with a promoter, such as phenobarbital (44). Tryptophan administration rapidly induces enhanced nucleocytoplasmic translocation of mRNA in the liver (10, 12, 45), and the possible importance of this similarity merits further investigation. Along with this early and rapid stimulation of cytoplasmic mRNA in the liver due to tryptophan, enhanced hepatic protein synthesis occurs (10, 12). This response may involve a number of enzymes. Stimulation of the activities of many important hepatic enzymes due to tryptophan (10, 46, 47) and to certain chemical carcinogens (48, 49) may influence the process of hepatocarcinogenesis. For example, much attention has been directed toward establishing a correlation between elevation of ODC activity and promotion in experimental carcinogenesis of skin (50, 51) and bladder (52). Tryptophan does stimulate hepatic ODC activity (47) as does feeding a choline-deficient diet (53). However, in the present study, hepatic ODC activity was significantly increased in rats fed the CD + TRP diet over levels in rats fed the other diets, yet this was not associated with a potentiation by tryptophan on the CD diet effect of inducing GGT+ foci in the liver (Table 1). Further studies are needed to determine which of the many effects that tryptophan has on the liver are involved in the process of promotion.

A few other possible mechanisms in relation to increased dietary tryptophan and promotion of liver cancer need to be considered. (a) It is conceivable that tryptophan may be acting via a metabolite or related compound as is thought to be the case for bladder carcinogenesis (15). To date, normal metabolites of tryptophan have not been reported as being carcinogenic or as being promoters in the liver. However, pyrolysis products of tryptophan have been reported to be hepatocarcinogenic (54). (b) Tryptophan may act secondarily to influence the process of promotion. Apropos to this, it has been reported that the addition of tryptophan to a purified diet containing adequate cysteine and methionine caused a decrease in total glutathione in the liver (55, 56). Hepatic glutathione is involved in the rapid metabolism of lipid peroxides, toxic substances, to innocuous products and thereby protects membranes against lipid peroxidation (57). Thus, if elevated dietary tryptophan induces a decrease in hepatic glutathione levels, this could conceivably render the liver more susceptible in the process of progression to cancer.

Review of earlier experimental studies dealing with elevated dietary tryptophan along with chemical carcinogens on the induction of liver cancer reveals conflicting findings. An increased incidence of liver cancers was reported in rats fed increased dietary tryptophan (1.0–4.3%) with 2-acetylaminofluorene (13, 14), β-naphthylamine (14), or diethylnitrosamine (21), while a decreased incidence of liver cancers was observed in rats fed elevated dietary tryptophan (1–1.4%) with dibutyl nitrosamine (22), 3′-dimethylaminobenzidine (23), or diethylnitrosamine (23). The discrepancies between these experimental results may be attributed to differences in experimental design or conditions. On the other hand, a number of investigators have reported that the promotional effect of feeding a CD diet on the induction of GGT+ foci and on hepatocarcinogenesis can be potentiated by using ethionine (58), 2-acetylaminofluorene (28, 59), or phenobarbital (28). In the present study, we observed a potentiating effect of increased dietary tryptophan on the induction of GGT+ foci with the CS diet, but none was found with the CD diet (Table 1). These results suggest that the effects on promotion due to elevated dietary TRP in the CS diet or due to the CD diet alone may be different, yet they are not potentiating, or that the effects may be along a similar pathway, whereby the effect is maximal with either one, and therefore, one does not potentiate the other.

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*Cancer Res* 1985;45:4844-4847.

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