BUTTERWORTH HEINEMANN

Research Communications

Tryptophan binding to nuclei of rat liver and hepatoma

Herschel Sidransky, Rabinder N. Kurl,* Stephanie C. Holmes, and Ethel Verney

*Department of Pathology, The George Washington University Medical Center, Washington, DC USA; and *Program in Clinical Pharmacology, Brown University, Providence, RI USA*

This study was conducted to determine whether L-tryptophan binding to nuclei of rat transplantable hepatomas (5123 and 19) differed from that in host liver or normal liver of rats. Binding of L-tryptophan to rat hepatic nuclear proteins has been demonstrated to be saturable, stereospecific, and of high affinity and the nuclear envelope binding protein has been purified and characterized. Using an in vitro ^{3}H-tryptophan binding assay, the total and specific L-tryptophan binding was appreciably less in nuclei of hepatoma than in nuclei of host liver or normal liver. On Scatchard analyses, the K_D values were similar for nuclei of hepatoma and of host liver but the B_{max} values were less in hepatoma than in host liver. Free L-tryptophan levels and nuclear poly(A)polymerase activity levels in hepatoma were higher than those of host liver. Using compounds (L-tryptophan implicated in the eosinophilia-myalgia syndrome, D,L-β-(1-naphthyl)alanine, chlordiazepoxide, and 3-methylindole) that had earlier been found to diminish in vitro ^{3}H-tryptophan binding to rat hepatic nuclei, similar inhibitory effects were observed with nuclei of hepatoma or with nuclei of host liver. Using polyclonal antibodies raised against the L-tryptophan binding protein of rat hepatic nuclear envelopes, immunoblot assay was performed on nuclei of hepatoma and of normal liver. The receptor protein (67 kD) was present in both preparations. (J. Nutr. Biochem. 6:73–79, 1995.)

Keywords: tryptophan; binding; nuclei; liver; hepatoma; rat

Introduction

L-tryptophan has been demonstrated to affect hepatic RNA and protein metabolism.[1] Recently, a tryptophan-binding protein, which was identified by in vitro ^{3}H-tryptophan binding studies, has been purified to apparent homogeneity from rat hepatic nuclear envelopes.[2,3] Polyclonal antibodies raised against the tryptophan binding protein of rat liver nuclear envelopes were obtained and used to identify the receptor in nuclear preparations of rat liver by an immunoblot assay.[3] A major polypeptide with a M_r of 34,000 or 64,000 was evident in these preparations, depending upon whether or not protease inhibitors were included. Schroder et al.[4] have reported on a tryptophan binding protein of nuclear envelopes of mouse lymphoma (L5178y) cells that has similar characteristics as those of rat hepatic nuclear envelopes.

In earlier studies,[5,6] we investigated a number of effects due to the acute administration of L-tryptophan upon hepatomas and host livers of rats. Unlike the stimulatory effects of tryptophan on the livers of normal rats, the effects on hepatomas were little or none. Specifically, unlike the stimulatory effects of tryptophan upon normal rat livers, there were no changes in polyribosomal pattern, protein synthesis (measured in vitro using microsomes), poly(A)mRNA synthesis, nuclear RNA efflux, nuclear NTPase activity, and RNA polymerase I and II activity. Host livers of rats bearing transplanted hepatomas responded to tryptophan similar to livers of normal rats except that the changes were somewhat less than those observed in control livers. In view of these earlier findings with hepatomas, it was of special interest to determine whether nuclear tryptophan binding activity in hepatoma differed from that in normal liver. Although the significance of binding of tryp-

This work was supported by U.S. Public Health Service Research Grant DK-45353 from the National Institute of Diabetes and Digestive and Kidney Diseases and a research grant from Showa Denko KK.
Address reprint requests to Dr. H. Sidransky at the Department of Pathology, The George Washington University Medical Center, 2300 Eye Street, N.W., Washington, DC 20037-2337 USA.
Received June 10, 1994; accepted October 20, 1994.

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(94)00014-D

tophan to hepatic nuclear envelopes is not yet clear, it has been postulated that it may be related to the enhanced nuclear RNA efflux.[3] This study was designed to determine whether binding differences existed in hepatoma compared with host liver or normal liver.

In the present study, we investigated the in vitro ^{3}H-tryptophan binding capacity of nuclei of hepatomas as well as of host livers of rats bearing transplantable hepatomas. In view of the observed differences between the two, which consisted of diminished in vitro ^{3}H-tryptophan binding to hepatoma nuclei, we have explored other possibly related differences.

Methods and materials

Animals and tumor tissue

Female inbred Buffalo rats (Fredrick Cancer Research and Development Center, Fredrick, MD USA) were used to maintain Morris hepatoma 5123, initially induced by the ingestion of N-2-fluorenylphthalamic acid[7] or hepatoma 19 induced by the ingestion of ethionine[8] by serial subcutaneous transplantation. For these studies the hepatoma was transplanted subcutaneously in 5- to 7-week-old Buffalo rats and were removed 20 to 28 days after transplantation. Rats were kept on a commercial diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL, USA) throughout except that diet was removed overnight before killing the following morning.

Isolation of nuclei

Rat hepatic and hepatoma nuclei were prepared as described by Blobel and Potter[9] and as used in our laboratory in earlier studies.[2,3] The nuclei were washed twice with buffer A (0.05 M Tris hydrochloride, pH 7.5, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0001 M phenylmethylsulfonyl fluoride (PMSF), 0.0002 M dithiothreitol (DTT), and 0.25 M sucrose) prior to further use.

Binding of ^{3}H-tryptophan to nuclei

Rat hepatic nuclei or hepatoma nuclei were incubated with L-(5-^{3}H)tryptophan (7.5 μCi was added to the incubation mixture last at time 0) in the absence or presence of an excess of unlabeled L-tryptophan (10^{-4} M) or unlabeled test compound (10^{-4} M) at room temperature for 2 hr. Nuclei were then washed (by resuspension and centrifugation) three times with buffer A to remove free and loosely bound radioactivity. After the final wash, nuclei were suspended in buffer A and then radioactivity was measured after adding Aqueous Counting Scintillate (ACS II; Amersham/Searle, Arlington Heights, IL). Specific binding of ^{3}H-tryptophan to hepatic or hepatoma nuclei was expressed as cpm per unit protein (binding in the absence of test compound minus binding in the presence of excess of test compound). Values were then compared with values obtained using unlabeled L-tryptophan (control group).

Enzyme assay

Poly(A)polymerase activity was measured as described by Kurl et al.[10,11] with slight modifications. Rat hepatic nuclei and nuclear envelopes (preparations as described earlier[2,3]) (50 to 100 μg of protein) were incubated for 60 min at 37°C in the presence of ATP (0.5 nmol), Mn^{2+} (1.7 mM), KCl (40 mM), water or poly(A) (200 μg/ml), ^{3}H-ATP (1.5 μCi), and 0.05 M Tris (pH 8.0) contained in a total volume of 120 μL. The incubation was terminated by placing the tubes in ice, and aliquots (80 μL) were spotted on DE-81 filter discs (25 mm) which were washed four times with sodium phosphate dibasic, rinsed with distilled water, and counted in the present of ACS II (Amersham). Since Jacob et al.[12] reported that isolated hepatic nuclei contain two distinct physiologically active forms of poly(A)polymerase (chromatin-bound form and free form), we measured both forms (free determined with added poly(A) and bound without the addition).

Table 1 In vitro total and specific ^{3}H-tryptophan binding to nuclei control livers, host livers, and transplantable hepatomas (H5123 and 19) of buffalo rats

Nuclei	^{3}H-tryptophan binding to nuclear protein (%)	
	Total	Specific*
Control liver (Normal rat)	(6) 100†	(3) 64.4 ± 15.3‡
Host liver	(4) 99 ± 15	(7) 66.3 ± 4.9
Hepatoma H5123	(17) 25 ± 5§	(7) 57.3 ± 3.9
Host liver	(4) 118 ± 51	
Hepatoma 19	(4) 39 ± 16‖	

*Specific binding (%) $= \frac{\text{total binding} - \text{nonspecific binding}}{\text{total binding}} \times 100$

where nonspecific binding was binding obtained in the presence of an excess (10^{-4} M) of unlabeled L-tryptophan.
†Number of experiments in parentheses. Control value (100%) = 84,921 ± 24,991 cpm/mg of protein.
‡Means ± SEM.
§$P < 0.001$.
‖$0.05 > P > 0.01$.

Western blot analysis

Nuclear proteins were separated on polyacrylamide gels (12%) under denaturing conditions and electrophoretically transferred to nitrocellulose sheets (45 μm). The sheets were blocked with 10% BSA in phosphate buffered saline (PBS) overnight at 4°C and then probed with 1:1000 dilution of the rabbit polyclonal serum raised against the purified rat hepatic nuclear envelope protein with tryptophan receptor activity.[3] The nitrocellulose sheet was washed again and incubated with peroxidase-labeled secondary antibody. The antigen–antibody complex was visualized with 0.1 M Tris pH 7.4 containing 0.5 mg/ml of the substrate, 3,3′-diaminobenzidine and 0.005% (v/v) hydrogen peroxide.

Other determinations

Total protein free (acid-soluble) tryptophan concentrations in serum, host liver, and hepatoma were estimated spectrofluorometrically.[13] The protein content was determined as described by Lowry et al.[14] Data were analyzed by Student's *t*-test.[15]

Chemicals

L-tryptophan from U.S. Biochemical (Cleveland, OH USA) was used. In one set of experiments, L-tryptophan (SDA and SDB) was obtained from Showa Denko (Tokyo, Japan). SDA L-tryptophan contained 6 ppm 1,1′-ethylidenebis(tryptophan) (EBT) and SDB L-tryptophan contained 146 ppm EBT. Other chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO USA).

Table 2 Total protein-free tryptophan levels in tissue and in nuclei of buffalo rat liver and hepatoma 5123

Group	Protein-free tryptophan levels	
	Tissue (μg/g)	Nuclei (μg/mg of RNA)
Host liver	(12) 3.80 ± 0.42*	(4) 11.9 ± 4.0
Hepatoma 5123	(12) 8.17 ± 1.44†	(4) 21.2 ± 12.5

*Number of experiments in parentheses. Means ± SEM.
†$P < 0.01$.

Table 3 Poly(A) polymerase activity on nuclei of host livers and transplantable hepatomas (H5123 and 19) of buffalo rats

Nuclei	PAP activity*	
	Engaged (%)	Free (%)
	Nuclei	
Host Liver	(4) 100†	(5) 100‡
Hepatoma H5123	(4) 155.2 ± 17.6	(6) 145.1 ± 12.1§
	Nuclear Envelopes	
Host Liver	(4) 100	(5) 100
Hepatoma H5123	(4) 139.2 ± 12.1§	(5) 206.5 ± 30.4§

*Number of experiments in parentheses. Means ± SEM.
†100% corresponds to 13,034 cpm of ^{3}H-AMP incorporated/mg of protein for nuclei and 27,515 cpm ^{3}H-AMP incorporated/mg of protein for nuclear envelopes.
‡100% corresponds to 11,719 cpm of ^{3}H-AMP incorporated/mg of protein for nuclei and 13,024 cpm of ^{3}H-AMP incorporated/mg of protein for nuclear envelopes.
§$0.05 > P > 0.01$.

Results

In the first series of experiments, we investigated whether there were differences in in vitro total (nonspecific and specific) ^{3}H-tryptophan binding to nuclei of hepatomas and of host livers of rats bearing transplantable hepatomas. *Table 1* summarizes the results of in vitro ^{3}H-tryptophan binding experiments in which nuclei of control livers, host livers, and hepatomas (5123 and 19) were assayed. It is readily apparent that there is a statistically significant reduction in total ^{3}H-tryptophan binding to nuclei of the hepatomas in comparison with control liver or host liver.

Next, we investigated whether the diminished in vitro total ^{3}H-tryptophan binding to hepatoma nuclei compared with control liver or host liver involved specific tryptophan binding. This was conducted by assaying in vitro ^{3}H-tryptophan binding for both total and nonspecific (incubated in the presence of an excess of unlabeled tryptophan) binding and then calculating specific binding as the difference between the two determinations. *Table 1* summarizes the percent of specific tryptophan binding to nuclei of control liver, host liver, and hepatoma 5123. In addition to a significant decrease in percent of total binding (*Table 1*), there is a small decrease (11 to 14%) in percent of specific binding in hepatoma 5123 compared with normal liver or host liver (*Table 1*). Thus, if one calculates the specific binding of tryptophan per unit of nuclear protein, there is a significant decrease in the specific binding of tryptophan per unit of nuclear protein of hepatoma 5123 (−78.4%) compared with that of host liver.

To further characterize the in vitro nuclear ^{3}H-tryptophan binding of host liver and of hepatoma 5123, we determined the kinetics of binding of ^{3}H-tryptophan after different times (1/2, 1, 2, 3, and 4 hr) of incubation. Saturation occurs by 3 hr for both hepatoma and host liver, and the total binding and the specific binding at each time were appreciably greater (similar differences with that reported for 2 hr in *Table 1*) for host liver than for hepatoma. Values (cpm/mg of nuclear protein) for host liver and hepatoma 5123, respectively, at the different time intervals up to 3 hr were as follows: total binding – 1/2 hr 6,997, 5,477; 1 hr 7,852, 6,083; 2 hr 13,609, 8,603; and 3 hr 15,287, 11,893; specific binding – 1/2 hr 3,651, 2,830; 1 hr 4,347, 2,828; 2 hr 8,997, 4,649; and 3 hr 9,778, 7,351.

Next, we determined the total protein-free tryptophan levels in host liver and hepatoma 5123 in 12 experiments. The protein-free tryptophan levels (μg/g of tissue) are summarized in *Table 2* and reveal a 115% increase in hepatoma compared with liver. In four experiments, we determined the total protein-free tryptophan levels in isolated nuclei of host liver and of hepatoma (*Table 2*). The results expressed per unit of nuclear RNA revealed a 78% increase in hepatoma nuclei versus host liver nuclei.

Earlier our laboratory reported on an association of poly-(A)polymerase (PAP) with the tryptophan receptor in rat hepatic nuclei.[16] Our findings suggested that PAP and the tryptophan receptor share structural homology. In view of this association, we determined the activities of poly-(A)polymerase of nuclei and nuclear envelopes of host liver and transplantable hepatoma 5123. *Table 3* summarizes these findings. It is apparent that the PAP activities were increased in hepatoma 5123 over those in host liver. In one experiment, we determined the serum PAP activity levels of rats bearing hepatomas (5123 and 19). The rats bearing hepatomas had increased serum free PAP activity levels (+109% for 5123 and +107% for 19) compared with control rats. Similar elevations of serum PAP activity levels in hepatoma-bearing rats have been reported.[10]

In earlier studies[17–20] we reported that tryptophan binding to hepatic nuclei or nuclear envelopes of normal rats was affected by a number of agents. Therefore we investigated whether such alterations would also occur with hepatoma nuclei as well as with nuclei of host liver (controls). First, we investigated whether a tryptophan analog, D,L-β-(1-naphthyl)alanine, would affect tryptophan binding to nuclei of hepatoma 5123. Earlier, we had reported that D,L-β-(1-naphthyl)alanine competed effectively with L-tryptophan for in vitro ^{3}H-tryptophan binding to rat hepatic nuclear envelopes.[17] *Table 4* summarizes the results of the present experiments and reveals that D,L-β-(1-naphthyl)alanine and L-tryptophan compete to similar degrees for in vitro ^{3}H-tryptophan binding to nuclei of control liver, host liver, and hepatoma 5123. The inhibition of ^{3}H-tryptophan binding to hepatoma nuclei compared with control liver nuclei was diminished 34 and 38%, respectively, for L-tryptophan and for D,L-β-(1-naphthyl)alanine.

Next, we investigated the effects of L-tryptophan from

Table 4 Inhibition of total in vitro ^{3}H-tryptophan binding to nuclei of liver or hepatoma 5123 using an excess of unlabeled tryptophan or test compound

Compound*	Host Liver (% inhibition)	Hepatoma 5123 (% inhibition)
L-tryptophan	(3) 74.3 ± 19.7†	(3) 36.6 ± 18.6
D, L-β-(1-naphthyl)-alanine	(3) 59.1 ± 16.0	(3) 32.9 ± 20.3
Control L-tryptophan‡	(4) 60.3 ± 1.13	(5) 63.6 ± 1.46
SDA L-tryptophan	(4) 56.7 ± 1.99	(5) 56.9 ± 2.29‖
SDB L-tryptophan	(4) 52.9 ± 3.22§	(5) 49.9 ± 5.50‖
L-tryptophan (TRP)	(4) 66.6 ± 1.1	(4) 63.3 ± 7.9
3-methylindole (3MI)	(4) +15.8 ± 9.2**	(4) 1.7 ± 1.1**
TRP + 3MI	(4) 55.1 ± 3.2‖	(4) 50.5 ± 3.2
Chlordiazepoxide (Chl)	(4) +13.7 ± 11.1**	(4) 6.2 ± 4.2**
TRP + Chl	(4) 16.1 ± 11.3**	(4) 18.4 ± 11.3‖

*Unlabeled compounds added at 10^{-4} M. All compounds were compared with control (water-treated group). The total ^{3}H-tryptophan binding for the control groups averaged 18,707 cpm/mg of protein for host liver and 10,812 cpm/mg of protein for hepatoma 5123.
†Number of experiments in parentheses. Means ± SEM.
‡Control L-tryptophan was obtained from US Biochemical (Cleveland, OH, USA). SDA L-tryptophan contained low EBT (6 ppm) and SDB L-tryptophan contained high EBT (146 ppm) content, and both were obtained from Showa Denko (Japan).
§$0.01 > P > 0.05$, all comparisons are with L-tryptophan group.
‖$0.05 > P > 0.01$.
**$P < 0.01$.

two different sources upon in vitro ^{3}H-tryptophan binding to nuclei of host liver and of hepatoma 5123. The two sources of L-tryptophan were: (1) L-tryptophan (SDA and SDB) implicated in cases of the eosinophilia-myalgia syndrome (L-tryptophan from Showa Denko, Tokyo, Japan)[18,19] and (2) nonimplicated L-tryptophan from U.S. Biochemical (Cleveland, OH USA). The results are summarized in *Table 4*. It is apparent that the diminished inhibitory binding response with implicated L-tryptophan especially for SDB, compared with nonimplicated L-tryptophan for hepatoma 5123 is similar to that of host liver. In a previous study using nuclear envelopes of normal livers of Sprague–Dawley rats,[18] similar results were obtained as for nuclei of host livers of Buffalo rats.

In another group of experiments, we investigated the effects of two other compounds, chlordiazepoxide and 3-methylindole, when administered alone or together with L-tryptophan on in vitro ^{3}H-tryptophan binding to nuclei of host liver or hepatoma 5123. In earlier studies,[19,20] we reported that, using normal rat hepatic nuclei, each of the two compounds added to unlabeled L-tryptophan caused an appreciable decrease in in vitro ^{3}H-tryptophan binding when compared with that of unlabeled L-tryptophan alone. The results of the present experiments are summarized on *Table 4*. While 3-methylindole and chlordiazepoxide alone had no inhibiting effects, the additions of each to L-tryptophan caused appreciable decreases in inhibition compared with that of L-tryptophan alone. Overall, the effects are similar for nuclei of host liver and of hepatoma. The findings for host liver are similar to those reported earlier using chlordiazepoxide[20] or using 3-methylindole[21] for hepatic nuclei or nuclear envelopes of normal Sprague–Dawley rats.

A group of experiments were conducted to analyze the dissociation constant (K_D) and concentration (B_{max}) of ^{3}H-tryptophan binding protein of nuclei of hepatoma 5123 and of host liver according to Scatchard. Earlier we had reported on the K_D and B_{max} values when using hepatic nuclear envelopes of normal Sprague-Dawley rat[2,21] and of normal Lewis[22] rats. Our present findings were as follows: hepatoma 5123 (n = 11), K_D = 13.3 ± 2.36 nM, B_{max} = 281 ± 103 fmol/mg of protein; and host liver (n = 4), K_D = 18.1 ± 8.81 nM and B_{max} = 1,803 ± 661 fmol/mg of protein. While the K_D values for nuclei of hepatoma 5123 and host liver were essentially similar, the B_{max} for hepatoma 5123 was significantly less ($P < 0.05$) (−84%) than that of the host liver. Also, both values for host livers were similar to those obtained from normal Buffalo rat livers or normal Sprague–Dawley rat livers. With Sprague–Dawley rats, the previously reported data using normal hepatic nuclear envelopes were: K_D = 18.1 nM,[2] 12.1 nM,[21] and 17.1 nM,[22] and B_{max} = 3,273 fmol/mg of protein,[2] 3,163 fmol/mg of protein,[21] and 2,285 fmol/mg of protein[22] for the low affinity binder. The somewhat lower B_{max} value for Buffalo host livers is probably related to the use of nuclei rather than nuclear envelopes for the assays.

In earlier studies, the rat hepatic nuclear envelope tryptophan binding protein was purified to apparent homogeneity using either concanavalin A–agarose or tryptophan-agarose.[3] Now we desired to compare the nuclear tryptophan binding protein of hepatoma 5123 with that of normal liver. Nuclear proteins of each were separated on a polyacrylamide gel under denaturing conditions and then the proteins were electrophoretically transferred to a nitrocellulose sheet. Polyclonal antibodies raised against the tryptophan binding protein[3] were then used to identify the receptor protein in the two different nuclear preparations by an immunoblot assay (*Figure 1*). It is apparent that the receptor protein (67 kD) was present in the nuclei of hepatoma 5123 as it was in the nuclei of normal liver.

Discussion

The results of the present study reveal that upon in vitro assay there appears to be a diminished number of tryptophan receptors in nuclei of hepatoma in comparison with those in host liver. These findings are consistent with earlier data regarding other selected biochemical responses of the two tissues to tryptophan.[5,6] In general the tryptophan-stimulated responses, involving polyribosomal pattern, protein synthesis, poly(A)mRNA synthesis, nuclear RNA efflux, and nuclear NTPase activity, in normal or host liver, were diminished in hepatoma relative to normal or host liver. Why there is a diminished number of tryptophan receptors in hepatoma nuclei compared with that in hepatic nuclei is not clear. One possible explanation is that it is related to the elevated free tryptophan levels in hepatoma cells over that in hepatic cells.

In this study (*Table 2*) as well as in another study,[6] it has been found that the basal level of free tryptophan is higher (3.1 to 3.9 fold) in the hepatoma than in normal liver or host liver. Within 30 min following the administration of tryptophan to tumor-bearing rats, the levels rise appreciably in

both hepatoma and in host liver with the hepatoma maintaining a 107% higher level than the of host liver.[6]

The elevated hepatoma levels of free tryptophan may be due to a variety of factors. Plasma-free amino acid pool is considered to be the main direct source of amino acids for the tumor. While the free plasma amino acid concentrations under normal conditions show relatively little variations,[23] this balance can be perturbed in the presence of a tumor through a variety of different means: (1) by variations in ingested protein,[24] (2) by changes in intestinal absorption,[24] (3) by alterations in nonessential amino acid biosynthesis in liver,[25] (4) by changes in tissue oxidative catabolism of amino acids,[26] (5) by differences between protein synthesis and tissue proteolytic activities,[27] and (6) by tumor demand for essential and nonessential amino acids needed for tumor proliferation.[28] In regard to aromatic essential amino acids, there is a sharp decrease in their plasma and liver concentrations 48 hr after tumor inoculations.[29,30] A net flux from host tissues to tumor occurs.[30] It appears that L-tryptophan may be metabolized differently in the hepatoma than in the host liver. Tryptophan oxygenase, an enzyme that metabolizes L-tryptophan, is reported to be absent in hepatoma tissue.[31,32] Thus, the increased flux of L-tryptophan to the hepatoma and the decreased capacity to metabolize the L-tryptophan may both contribute to the elevated free L-tryptophan levels in hepatomas. Whether these high levels of L-tryptophan influence hepatoma protein synthesis is unknown, although it is known that hepatoma protein synthesis, especially in rapidly growing hepatomas, is at a higher rate than that of normal liver.[33] As for the effect of L-tryptophan on hepatic protein synthesis in rapidly dividing or growing livers of rats, it has been demonstrated that L-tryptophan administration to rats 1 or 2 days following partial hepatectomy induces increased protein synthesis in the regenerating liver.[6] In normal (fasted or fed) animals, L-tryptophan rapidly stimulates hepatic protein synthesis.[1]

Evidence exists that supports the concept that elevated, endogenous, free tryptophan levels in tissues lead to increased in vivo receptor binding as reflected by measurement of subsequent in vitro ^{3}H-tryptophan binding which reveals decreased binding. Previous studies with normal rats revealed that after appreciably raising serum and liver-free tryptophan levels, by tube-feeding L-tryptophan to overnight fasted rats 20 min before killing, there was a marked decrease (-57%) in in vitro ^{3}H-tryptophan binding to hepatic nuclei.[18] This indicates that, at least in normal rats, elevation of circulating tryptophan levels can rapidly increase hepatic receptor binding in vivo (as determined indirectly by subsequent in vitro ^{3}H-tryptophan binding assays). Whether and to what degree elevated circulating and hepatoma free L-tryptophan in tumor-bearing rats as reported in *Table 2* may tie up in vivo receptor binding for tryptophan in hepatoma nuclei more than for host liver nuclei remain to be determined.

In this study we evaluated whether the ^{3}H-tryptophan binding to hepatoma nuclei was affected by selected compounds, reported earlier to inhibit binding by added excess unlabeled L-tryptophan[17,18,20,21] using normal hepatic nuclei. Our data using Showa Denko L-tryptophan, D,L-β-(1-naphthyl)-alanine, 3-methylindole, and chlordiazepoxide (*Table 4*) reveal similar effects with nuclei of hepatoma 5123 as with nuclei of host liver. This indicates that at least some of the receptor responses in hepatoma are functionally similar to those of normal rat liver nuclei. Earlier, we reported that nuclei of different cell types (brain and macrophages)[34,35] reveal some differences in receptor binding responses to selected chemicals.

In an earlier study[10] we investigated PAP activity in the sera of rats with transplantable hepatomas 5123 and 19. The serum levels of the enzyme increased with time (2 to 5 weeks) after subcutaneous transplantation. Our present results indicate that the tumor nuclei have elevated activity of PAP (*Table 3*) which could probably contribute enzyme to the blood. An association of PAP with tryptophan receptor in nuclei of rat liver has been reported.[16] This association may also be present in hepatoma tissue.

In an earlier report,[22] we observed a decrease in the affinity of in vitro L-tryptophan binding to hepatic nuclei and nuclear envelopes of Lewis rats compared with Sprague–Dawley rats. While the K_D values were similar, the B_{max} values were significantly less in Lewis rats compared with the Sprague–Dawley rats. These results are similar to those observed in this study when comparing hepatoma with host liver. However, the tissue-free L-tryptophan levels and also tryptophan oxygenase activity levels were similar in the livers of both strains of rats while the hepatoma had increased free L-tryptophan levels and decreased (absent) tryptophan oxygenase levels[31,32] in comparison with host liver. Thus, the earlier observed strain differences in tryptophan binding affinity appear to be quite different than the difference between hepatoma and host liver.

How elevated free L-tryptophan levels in hepatoma tissue may affect tumor protein metabolism is not clear. Earlier reports revealed that hepatoma protein synthesis is increased over that of normal liver,[33] turnover of mRNA of hepatomas occurs at a slower rate than that of host liver,[36] and RNase activity is less in hepatoma than in host liver.[36] Administration of L-tryptophan to fasted or fed rats enhances hepatic protein,[1] but it fails to do likewise in hepatoma when administered to tumor-bearing rats, yet is does affect host liver.[5,6]

This difference in response could be due to enhanced hepatoma protein synthesis already stimulated by the increased levels of free L-tryptophan in hepatoma. However, it cannot be explained only by enhanced protein synthesis in hepatoma over that of liver since animals subjected to other conditions whereby hepatic protein synthesis is enhanced, such as by cortisone or phenobarbital administration[37] or in regenerating liver after partial hepatectomy,[6] are still able to show a stimulatory response to the administration of L-tryptophan. The enhanced hepatoma PAP activity over that in host liver may likewise be explained by the increased free L-tryptophan levels in hepatomas. In normal animals the administration of L-tryptophan rapidly increased hepatic PAP activity.[38] Thus, the diminished hepatoma nuclear tryptophan receptor activity when measured in vitro as in our present experiments compared with that in host liver may be a reflection of enhanced in vivo hepatoma nuclear receptor binding due to the increased free L-tryptophan levels. To clarify whether the above speculation is valid, one needs to investigate hepatoma tissue devoid of increased

free L-tryptophan. We plan to investigate this in tissue culture using hepatoma whereby the tumor cell levels of L-tryptophan may be diminished and then the response to added L-tryptophan levels can be followed.

By Western blotting analysis, the presence of the binding protein was comparable in hepatoma 5123 and normal liver (*Figure 1*) though the number of binding sites were markedly reduced in the tumor tissue (*Table 1*). Thus, the difference in the number of binding sites between the two tissues may not be due to differential expression of the gene encoding the protein. These differences in binding sites could be attributed to several factors, including the occupancy of the binding sites by endogenous tryptophan or to mutations in the ligand binding region of the protein in the hepatoma. As a consequence of these possibilities, the binding protein could reveal decreased binding to ^{3}H-tryptophan.

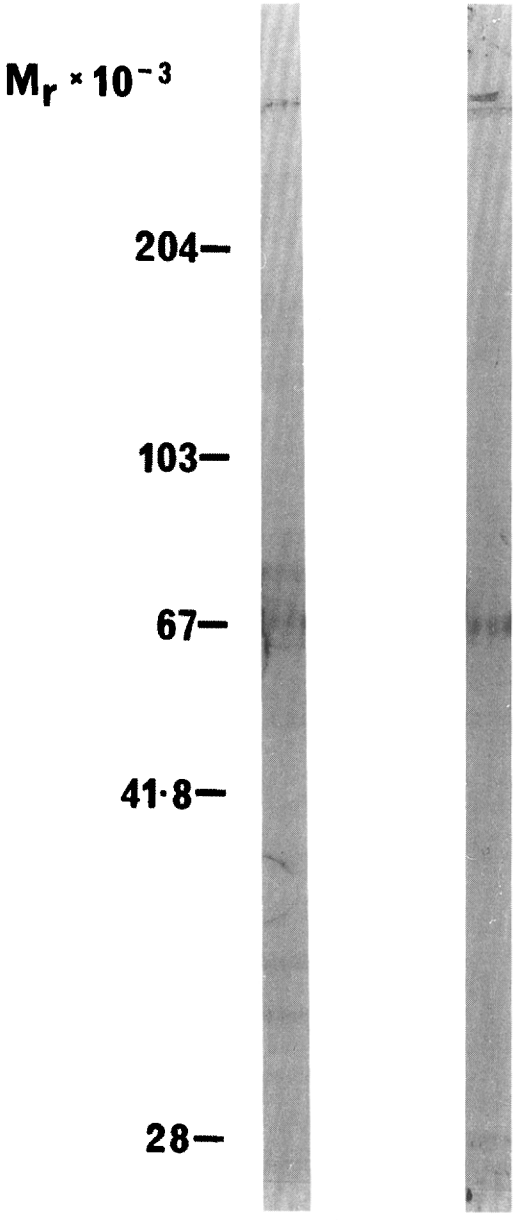

Figure 1 Immunoblot of nuclei of normal liver and of rat hepatoma 5123. Nuclear proteins were separated on a polyacrylamide gel under denaturing conditions and the proteins were electrophoretically transferred to a nitrocellulose sheet. The polyclonal antiserum was diluted 1,000 fold. (Left lane) Normal liver nuclei. (Right lane) Hepatoma 5123 nuclei.

References

1 Sidransky, H. (1985). Tryptophan: Unique actions by an essential amino acid. In *Nutritional Pathology. Pathobiology of Dietary Imbalances*. (H. Sidransky, ed.), p. 1–62, Marcel Dekker, New York, NY USA

2 Kurl, R.N., Verney, E., and Sidransky, H. (1987). Tryptophan-binding sites on nuclear envelopes of rat liver. *Nutr. Rep. Int.* **36,** 669–677

3 Kurl R.N., Verney, E., and Sidransky, H. (1988). Identification and immunohistochemical localization of a tryptophan binding protein in nuclear envelopes of rat liver. *Arch. Biochem. Biophys.* **265,** 286–293

4 Schroder, H.C., Wenger, R., Gerner, H., Reuter, P., Kuchino, Y., Sladic, D., and Muller, W.E.G. (1989). Suppression of the modulatory effects of the antileukemic and anti-human immunodeficiency virus compound avarol on gene expression by tryptophan. *Cancer Res.* **49,** 2069–2076

5 Sidransky, H. and Verney, E. (1979). Effect of nutritional alterations on protein synthesis in transplantable hepatomas and host livers of rats. *Cancer Res.* **39,** 1995–2000

6 Sidransky, H., Verney, E., and Murty, C.N. (1981). Effect of tryptophan on hepatoma and host liver of rats. Influence after treatment with hypertonic sodium chloride and carbon tetrachloride. *Exp. Mol. Path.* **35,** 124–136

7 Morris, H.P., Sidransky, H., Wagner, B.P., and Dyer, H.M. (1960). Some characteristics of transplantable rat hepatoma No. 5123 induced by ingestion of N-(2-fluorenyl)phthalamic acid. *Cancer Res.* **20,** 1252–1257

8 Sidransky, H. (1962). A transplantable rat hepatocellular carcinoma induced by ethionine. *J. Natl. Cancer Inst.* **28,** 1425–1435

9 Blobel, G. and Potter, V.R. (1966). Nuclei from rat liver: isolation method that combines purity with high yield. *Science* **154,** 1662–1665

10 Kurl, R.N., Holmes, S.C., Verney, E., and Sidransky, H. (1988). Poly(A)polymerase activity in murine serum. Elevation in animals with proliferative changes. *J. Natl. Cancer Inst.* **80,** 1060–1065

11 Kurl, R.N., Holmes, S.C., Verney, E., and Sidransky, H. (1988). Nuclear envelope glycoprotein with poly(A)polymerase activity of rat liver: isolation, characterization and immunohistochemical localization. *Biochemistry* **27,** 8974–8980

12 Jacob, S.T., Roe, F.J., and Rose, K.M. (1976). Chromatin-bound and free forms of poly(adenylic acid)polymerase in rat hepatic nuclei. *Biochem. J.* **1530,** 733–735

13 Denckla, W.D. and Dewey, H.K. (1967). The determination of tryptophan in plasma, liver, and urine. *J. Lab. Clin. Med.* **69,** 160–169

14 Lowry, O.H., Rosebrough, M.R., Farr, A.L., Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

15 Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods, p. 215–236, Iowa State University Press, Ames, IA USA

16 Kurl, R.N., Barsoum, A.L., and Sidransky, H. (1992). Association of poly(A)polymerase with tryptophan receptor in rat hepatic nuclei. *J. Nutr. Biochem.* **3,** 366–372

17 Sidransky, H., Verney, E., and Kurl, R. (1990). Comparison of effects of L-tryptophan and a tryptophan analog, D, L-β-(1-naphthyl)alanine, on processes relating to hepatic protein synthesis in rats. *J. Nutr.* **120,** 1157–1162

18 Sidransky, H., Verney, E., and Cosgrove, J.M. (1992). Competitive studies relating to tryptophan binding to rat hepatic nuclear envelopes as a sensitive assay for unknown compounds. *Toxicology* **76,** 89–100

19 Varga, J., Uitto, J., and Jimenez, S.A. (1992). The cause and pathogenesis of the eosinophilia-myalgia syndrome. *Ann. Int. Med.* **116,** 140–147

20 Sidransky, H., Verney, E., Cosgrove, J.M., and Schwartz, A.M.

(1992). Effect of benzodiazepoxides on tryptophan binding to rat hepatic nuclei. *Toxicol. Pathol.* **20,** 350–356

21 Sidransky, H., Verney, E., Cosgrove, J.M., Latham, P., and Schwartz, A.M. (1994). Indolic compounds affect tryptophan binding to rat hepatic nuclei. *J. Nutr.* **124,** 779–788

22 Sidransky, H. and Verney, E. (1994). Comparative studies on tryptophan binding to hepatic nuclear envelopes in Sprague-Dawley and Lewis rats. *Amer. J. Physiol.* **267** *Regulatory Integrative Comp. Physiol.* **36,** R502–R507

23 Felig, P. (1975). Amino acid metabolism in man. *Annu. Rev. Biochem* **44,** 933–955

24 DeWis, W.D. (1981) Nutritional abnormalities in cancer. In: *Nutrition and Metabolism in Cancer* (R. Kluthe and G.W. Lohr, eds.), p. 8–16, Georg Thieme Verlag, Stuttgart, Germany

25 Knox, W.E. (1967). The enzymatic pattern of neoplastic tissue. *Adv. Cancer Res.* **10,** 117–161

26 Snell, K. (1979). Alanine as a gluconeogenic carrier. *Trends Biochem. Sci.* **4,** 124–128

27 Lundholm, K., Byland, A.C., Holm, J., and Schersten, T. (1976). Skeletal muscle metabolism in patients with malignant tumor. *Eur. J. Cancer* **12,** 465–473

28 Goodlad, G.A.J. (1964). Protein metabolism and tumor growth. In: *Mammalian Protein Metabolism* (H.N. Monroe and J.B. Allison, eds.), vol. 2, p. 415–444. Academic Press, New York, NY USA

29 Marquez, J. and Nunez de Castro, I. (1991). Mouse liver free amino acids during the development of Ehrlich ascites tumor. *Cancer Lett.* **58,** 221–224

30 Marquez, J., Sanchez-Jimenez, F., Medina, M.A., Quesada, A.R., and Nunez de Castro, I. (1989). Nitrogen metabolism in tumor bearing mice. *Arch. Biochem. Biophys.* **268,** 667–675

31 Feigelson, P. and DeLap, L.W. (1977). Control of specific messenger RNA species in liver and hepatoma. *Adv. Exp. Med. Biol.* **92,** 307–330

32 Ramanarayanan-Murthy, L., Colman, P.D., Morris, H.P., and Feigelson, P. (1976). Pre-translational control of tryptophan oxygenase levels in Morris hepatoma and host liver. *Cancer Res.* **36,** 3594–3599

33 Wagle, S.R., Morris, H.P., and Weber, G. (1963). Comparative biochemistry of hepatomas: V. Studies on amino acid incorporation in liver tumor of different growth rates. *Cancer Res.* **23,** 1003–1007

34 Sidransky, H., Verney, E., Cosgrove, J.M., and Latham, P.S. (1994). Effect of 3-phenylamino-L-alanine on tryptophan binding to rat hepatic nuclear envelope. *Toxicology* **86,** 135–145

35 Sidransky, H., Verney, E., Cosgrove, J.M., Latham, P.S., and Mayeno, A.N. (1994). Studies with 1,1′-ethylidenebis(tryptophan), a contaminant associated with L-tryptophan implicated in the eosinophilia-myalgia syndrome. *Toxicol. Appl. Pharm.* **126,** 108–113

36 Sidransky, H., Murty, C.N., and Verney, E. (1978). Turnover of messenger RNA in transplantable hepatomas and host livers of rats. *Cancer Res.* **38,** 1645–1653

37 Verney E. and Sidransky, H. (1978). Further enhancement by tryptophan of hepatic protein synthesis stimulated by phenobarbital or cortisone acetate. *Proc. Soc. Exp. Biol. Med.* **158,** 245–249

38 Kurl, R.N., Verney, E., and Sidransky, H. (1993). Effect of tryptophan on rat hepatic nuclear poly(A)polymerase activity. *Amino Acids* **5,** 263–271