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Effect of Tryptophan on Nuclear Envelope Nucleoside Triphosphatase Activity in Rat Liver[1] (40739)

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In earlier studies we reported that normal animals (fasted or fed) receiving a single administration of tryptophan responded rapidly with a shift in hepatic polyribosomes toward heavier aggregation and with an increase in hepatic protein synthesis (1). It appears that tryptophan can act independently of new RNA synthesis, as demonstrated in experiments with tryptophan following actinomycin D treatment (1–3), and without new poly(A) synthesis, as demonstrated in experiments with tryptophan following cordycepin treatment (4). Tryptophan increases the availability of mRNA and poly(A)–mRNA in the cytoplasm of the liver (5) by stimulating the rate of translocation of nuclear poly(A)–mRNA into the cytoplasm, as demonstrated by *in vivo* and *in vitro* experiments (6).

In other studies, we reported that tryptophan administration was associated with an improvement in hepatic polyribosomes and in protein synthesis in animals having been treated previously or simultaneously with ethionine (7), actinomycin D (3), puromycin (8), hypertonic sodium chloride (9), and carbon tetrachloride (10). Also, we reported that tryptophan stimulated the availability of cytoplasmic poly(A)–mRNA after administering hypertonic sodium chloride (9) or carbon tetrachloride (10) treatment.

Based upon our earlier findings, we have concluded that one of the actions of tryptophan upon the liver is to enhance nucleocytoplasmic translocation of mRNA (6). Our present study was undertaken to learn more about the mechanism whereby tryptophan influences nucleocytoplasmic transport of mRNA.

Several investigators have described a nucleoside triphosphatase (Mg^{2+}-dependent adenosine triphosphatase, EC 3.6.1.3) in mammalian liver nuclear envelopes (11) and have recently presented evidence to suggest that this enzyme is involved in nucleocytoplasmic translocation of RNA (12). The projected role for this enzyme *in vivo* is the provision of energy for the transport of RNA from nucleus to cytoplasm. This proposed action by the enzyme is supported by the experimental findings that ATP is required for the transport of messenger RNA from isolated hepatic nuclei (13, 14). However, other studies with HeLa cells' nuclei (15) and with rat liver nuclei (16) have stressed that ATP may act to deprive divalent cations in the reaction mixture as a chelating agent. Although the precise role of this enzyme in the control of nucleocytoplasmic exchange of macromolecules is far from clear, we present evidence in this paper that the administration of tryptophan stimulated the levels of activity of Mg^{2+}-dependent nucleoside triphosphatase of liver nuclear envelopes. This occurs along with a concomitant increase in the release of poly(A)–mRNA from isolated hepatic nuclei (6, 17).

Materials and methods. Animals. Female rats of the Sprague–Dawley strain (Sprague–Dawley, Madison, Wisc.) 6 weeks old, and weighing an average of 70 g, were used. The rats were maintained in a temperature controlled room with alternating 12-hr cycles of light and dark. Before each experiment, the animals were fasted overnight but had free access to water. The following morning, the animals were divided into groups, each of which contained two to five animals. Rats were tube-fed either distilled water (3 ml/100 g of body wt)

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alone or distilled water containing L-tryptophan (30 mg in 3 ml water/100 g of body wt). Puromycin (4 mg/100 g of body wt), actinomycin D (0.25 mg/100 g of body wt), [6-^{14}C]orotic acid (16 μCi/100 g body wt) were injected intraperitoneally.

Chemicals. [6-^{14}C]Orotic acid (60.8 mCi/mmole) was obtained from Amersham/Searle, Des Plaines, Illinois, adenosine-5′-triphosphate from Sigma Chemical Company, St. Louis, Missouri, actinomycin D, from Merck, Sharp and Dohme Research Laboratories, West Point, Pennsylvania, and deoxyribonuclease I and yeast RNA (RNAse-free) were purchased from Worthington Biochemical Corporation, Freehold, New Jersey.

Isolation of nuclei. Nuclei were isolated from the livers of control and tryptophan-treated rats by the method of Muramatsu and Busch (18) with minor modifications as described by Schumm and Webb (14). Livers were minced and homogenized in 2.3 M sucrose (1:15, w/v) containing 0.003 M calcium acetate, and filtered through cheesecloth. The filtrate was centrifuged at 105,000 rpm for 1 hr. The nuclear pellet was resuspended in 1.0 M sucrose (1.5 ml/g liver) containing 0.001 M calcium acetate and then centrifuged at 2000 rpm for 5 min to give a pellet of purified nuclei.

Isolation of nuclear envelopes. Nuclear envelopes were isolated from livers of control and experimental rats by the method of Harris and Milne (19). The nuclei obtained from 5 g liver were resuspended in 40 ml of 1 mM $NaHCO_3$ (pH 7.2.) by shaking and then allowed to equilibrate for 5 min before centrifuging at 12,000 rpm for 10 min in a Beckman JA-20 fixed-angle rotor in a J-21 centrifuge. The pellet of slightly swollen nuclei was again resuspended in 1 mM $NaHCO_3$ and recentrifuged as above after a further equilibration period of 5 min. A very swollen nuclear pellet was dispersed in 1 mM $NaHCO_3$ containing DNAse I to give a 40-ml suspension with an enzyme concentration of 10 μg/ml. The suspension was then incubated at 20°C for 15–20 min. The chromatin escaping from the bursted nuclei was degraded by the DNAse, thus allowing the nuclear envelopes to become freely dispersed. The nuclear envelopes were washed six times in 1 mM $NaHCO_3$ and finally resuspended in 2 ml of 1 mM $NaHCO_3$ and centrifuged at 2000 rpm for 5 min. The white supernatant was layered over a discontinuous sucrose gradient made up of 10 ml of 2.0 M sucrose, 10 ml of 1.8 M sucrose, 10 ml of 1.5 M sucrose, and 6 ml of 0.25 M sucrose (all solutions made up in 10 mM Tris–HCl, pH 7.4). The gradients were then centrifuged for 90 min in a Beckman SW 27.1 rotor at 25,000 rpm in an L2-65B ultracentrifuge. A major band of purified nuclear envelopes formed at the 1.5 M/1.8 M sucrose interface was removed with a Pasteur pipet and nuclear envelopes were washed free of sucrose by washing twice with 1 mM $NaHCO_3$ at 12,000 rpm for 10 min. The purified nuclear envelopes were resuspended in 25 mM Tris–HCl, pH 8.0, for enzyme assay.

Assay for nucleoside triphosphatase activity in the nuclear envelopes. The enzyme was assayed according to the method of Agutter *et al.* (12). The assay depends on determination of the P_i released from the substrate during the incubation for 30 min at 35°C (20).

Treatment of nuclei with Triton X-100. To some suspensions of isolated nuclei in 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.5), 25 mM KCl and 5 mM $MgCl_2$ at an optical density of 25 A_{260} per milliliter, a solution of 5% (v/v) Triton X-100 was added to give a final concentration of 0.5% detergent (21). Controls received an equivalent amount of buffer. After incubation at 0°C for 10 min, the treated and control samples were centrifuged for 10 min at 500 g and these nuclear pellets were used to study *in vitro* release of RNA and for the isolation of nuclear envelopes.

In vitro release of RNA from isolated hepatic nuclei. The release of prelabeled RNA from isolated nuclei was studied by the method of Schumm and Webb (14) and described earlier by us (6). The incubation medium contained the following components in a volume of 5 ml: 5×10^6 nuclei/ml; 50 mM Tris–HCl, pH 7.5; 25 mM KCl; 2.5 mM $MgCl_2$; 0.5 mM $CaCl_2$; 0.3 mM $MnCl_2$; 5 mM NaCl; 2.5 mM Na_2HPO_4; 5 mM spermidine; 2 mM dithiothreitol; 2.0 mM ATP; 2.5 mM phosphoenol pyruvate; 35 units

pyruvate kinase; 500 μg/ml yeast RNA; and dialyzed cell sap (12 mg protein/ml). The mixture was incubated at 30°C for 30 min. The RNA released from the nuclei was precipitated from the nuclei-free incubation medium with ice-cold 10% (w/v) trichloroacetic acid. The precipitate was washed twice with 5% trichloroacetic acid, twice with ethanol, then solubilized and assayed for radioactivity in liquid scintillant.

Chemical determinations. Protein was assayed using the modification by Maddy and Spooner (22) of the method of Lowry *et al.* (23). DNA was determined by the method of Fleck and Munro (24).

Results. In the first series of experiments the kinetics of nucleoside triphosphatase activity in the nuclear envelopes isolated from the livers of control and tryptophan-treated rats were investigated. Figure 1A shows the effect of the concentration of $MgATP^{2-}$ as substrate on nucleoside triphosphatase activity. Without added substrate, very little activity of this enzyme can be detected in the nuclear envelopes of either control or tryptophan-treated rats. This indicates that hepatic nuclear envelopes of control rats contain endogenous ATP only in trace amounts and further that the endogenous ATP levels within the nuclear envelopes are not significantly altered due to tryptophan administration. With increasing concentrations of the exogenous substrate, there is an increase in the activity of enzyme, reaching maximum levels with an optimal substrate concentration at approximately 0.5 mM. It is apparent that there is a higher activity of nucleoside triphosphatase in the nuclear envelopes of liver of tryptophan-treated rats compared to nuclear envelopes of liver of control rats. In subsequent studies we employed a substrate concentration of 1 mM.

The protein concentration dependence curve shown in Fig. 1B indicates that the rate of phosphate release from $MgATP^{2-}$ is linear with increasing amounts of nuclear envelopes up to 20 μg of protein. Figure 1C shows the time course of release of phosphate from $MgATP^{2-}$ by the liver nuclear envelopes of control and tryptophan-treated rats. The results indicated that the rate of hydrolysis of $MgATP^{2-}$ catalyzed by nuclear envelopes is linear up to incubation periods of 1 hr and further this rate of hydrolysis catalyzed by liver nuclear envelopes is higher using preparations of tryptophan-treated rats than when using those of control rats.

The effect of administration of tryptophan *in vivo* for 10, 30 and 60 min on hepatic nuclear-envelope nucleoside triphosphatase activity was studied in several experiments. The results summarized in Table I reveal that the administration of trypto-

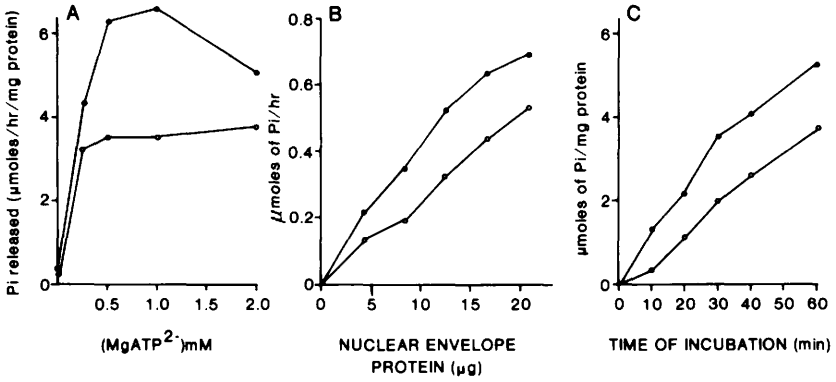

FIG. 1. Kinetics of nuclear envelope nucleoside triphosphatase activity of livers of control (○) and tryptophan-treated (10 min) (●) rats. Data represent three separate experiments. In each experiment livers from three or four rats of each group were pooled. (A) Effect of the concentration of the substrate $MgATP^{2-}$. (B) Effect of varying amounts of nuclear envelope protein on phosphate release from $MgATP^{2-}$. (C) Time course of $MgATP^{2-}$ hydrolysis catalyzed by nuclear envelope nucleoside triphosphatase.

TABLE I. EFFECT OF TRYPTOPHAN ON HEPATIC NUCLEAR-ENVELOPE NUCLEOSIDE TRIPHOSPHATASE ACTIVITY

No. of experiments	Time of administration (min)	Nuclear-envelope nucleoside triphosphatase activity[a] Control (μmole P_i/hr/mg protein)	Tryptophan (μmole P_i/hr/mg protein)	Percentage change[b]
11	10	14.9	24.1	+54.7 ± 14.5[c]
6	30	15.4	22.7	+40.0 ± 13.1[d]
7	60	13.3	21.7	+66.0 ± 10.0[c]

[a] Nuclear-envelopes were isolated from hepatic nuclei of rats tube-fed water (control) or L-tryptophan 10, 30, and 60 min before killing. In each experiment livers from three or four rats in each group were pooled. The enzyme in the nuclear envelope preparations was assayed as described under Materials and Methods.

[b] Means ± SEM of differences for each experiment.

[c] $P < 0.01$.

[d] $0.05 > P > 0.01$.

phan rapidly caused significant increases in the levels of nucleoside triphosphatase activity of the liver nuclear envelopes. Using a cell-free system, we recently demonstrated that following the administration of tryptophan to fasted rats, there was increased release of poly(A)–mRNA into the medium from isolated hepatic nuclei compared to control nuclei (6).

In previous studies we observed that, in animals pretreated with some selected inhibitors of RNA and protein synthesis, tryptophan administration was still able to induce a stimulatory effect on hepatic protein synthesis and increased levels of cytoplasmic mRNA (9, 10). Therefore, in the present study we investigated whether the stimulatory effect of tryptophan is associated with alterations in the nucleoside triphophatase activity of the nuclear envelopes in animals pretreated with selected inhibitors. Table II shows the effect of the administration of tryptophan on nuclear-envelope nucleoside triphosphatase activity and on *in vitro* release of labeled RNA from hepatic nuclei of rats pretreated *in vivo* with puromycin. While puromycin treatment alone did not appreciably influence the levels of the phosphatase activity, administration of tryptophan was able to stimulate the nucleoside triphosphatase activity in the rats pretreated with puromycin, similar to the increases in the control rats that received tryptophan alone. Concomitant with the increase in the enzyme activity, hepatic nuclei of rats that received tryptophan following puromycin also exhibited greater release of labeled RNA into the medium in comparison to control or puromycin-treated liver nuclei (Table II).

In two experiments the effect of tryptophan on nucleoside triphosphatase activity of hepatic nuclear envelopes of rats pretreated with another inhibitor, actinomycin D, was investigated. The results revealed that while actinomycin D treatment alone

TABLE II. EFFECT OF TRYPTOPHAN ON HEPATIC NUCLEAR-ENVELOPE NUCLEOSIDE TRIPHOSPHATASE ACTIVITY AND ON *IN VITRO* RELEASE OF LABELED RNA FROM HEPATIC NUCLEI OF RATS PRETREATED *IN VIVO* WITH PUROMYCIN

Group[a]	Nucleoside triphosphatase activity (% of control)[b]	Labeled nuclear RNA released[c] (% of control)[b]
Control	100	100
Tryptophan	173.8 ± 12.8[d]	198 ± 24.8[f]
Puromycin	115.9 ± 23.9	91 ± 9.1
Puromycin and tryptophan	177.7 ± 26.3[e]	188 ± 17.6[f]

[a] Rats received the following treatments per 100 g of body wt: 16 μCi of [6-^{14}C]orotic acid at zero time; 4 mg puromycin at 10 min; 30 mg tryptophan or water at 20 min and rats were killed at 30 min. In each experiment livers from four rats of each group were pooled.

[b] Mean ± SEM of the differences for each of the five experiments. The average values for enzyme activity and nuclear RNA release for the control group were 18.7 μmol P_i/hr/mg protein and 2746 cpm/ml incubation medium, respectively.

[c] *In vitro* RNA release from isolated hepatic nuclei of control and experimental groups was studied earlier using control liver cell sap in the incubation medium (17).

[d] $P < 0.01$.

[e] $P < 0.05$.

[f] $P < 0.02$.

had no influence there was a 68% increase in the nuclear-envelope enzyme activity in rats treated with actinomycin D and tryptophan.

Influence of Triton X-100 treatment on isolated nuclei. Ultrastructural studies indicate that the intact nuclear envelope consists of inner and outer nuclear membranes and nuclear pore complexes (21). Recently several workers (21, 25) have reported that treatment of nuclei with nonionic detergents completely removed the outer membrane, leaving intact nuclei with preservation of nuclear pore complexes. Further, it was demonstrated, using isolated myeloma nuclei, that *in vitro* RNA transport from detergent-treated nuclei is similar to that from untreated control nuclei (25), suggesting that nuclear pore complexes are mainly involved in the regulation of nucleocytoplasmic RNA transport.

In the present study the effect of Triton X-100 treatment of isolated hepatic nuclei of control and tryptophan-treated rats on their capacity to transport RNA *in vitro* was examined. We also studied the levels of nucleoside triphosphatase activity in the nuclear envelopes isolated from untreated and Triton X-100-treated nuclei. The results of these studies are summarized in Table III. Triton X-100 treatment of isolated hepatic nuclei caused little loss (less than 10%) of nuclei based on determination of nuclear DNA. As observed in our earlier studies, isolated hepatic nuclei of tryptophan-treated rats exhibited greater release of labeled RNA into the incubation medium than did these from control nuclei (6). Further, the data in Table III indicated that following the treatment of hepatic nuclei from control and experimental rats with Triton X-100, similar differences in the release of RNA between control and experimental nuclei persisted as compared with untreated nuclei. Similar findings were observed with nuclear envelope nucleoside triphosphatase activity. We observed that the enzyme activity in the nuclear envelopes isolated from Triton X-100 treated nuclei was decreased by 66.9% in the control and 73.3% in the experimental groups compared to untreated nuclei. This suggests that the enzyme is localized in the outer nuclear-membrane of the nuclear envelopes as well as in the nuclear pore complexes. However, untreated and Triton X-100 treated nuclei of livers of tryptophan-treated rats exhibited increases

TABLE III. EFFECT OF TRITON X-100 TREATMENT OF ISOLATED HEPATIC NUCLEI OF CONTROL OR TRYPTOPHAN-TREATED RATS ON THEIR CAPACITY FOR *IN VITRO* RNA RELEASE AND ON NUCLEAR-ENVELOPE NUCLEOSIDE TRIPHOSPHATASE ACTIVITY

Group[a]	Triton X-100 treatment	Nucleoside triphosphatase activity (% of control)[b]	Labeled nuclear RNA released (% of control)[b]
Control	–	100	100
Control	+	33.1 ± 12.0[c]	185.4 ± 11.4[d]
Tryptophan	–	234.3 ± 25.9[c]	150.2 ± 9.2[c]
Tryptophan	+	62.6 ± 13.9[e]	284.1 ± 19.6[f]

[a] Nuclei were isolated from livers of rats tube-fed water (control) or L-tryptophan 10 min before killing. To study *in vitro* RNA release from isolated nuclei, nuclear RNA was prelabeled *in vivo* with [6-^{14}C]orotic acid 20 min before tube-feeding water or tryptophan. Isolated nuclei were than treated with Triton X-100 as described under Materials and Methods. Labeled nuclear RNA release and isolation of nuclear envelopes from untreated and Triton X-100-treated nuclei were carried as described under Materials and Methods. In each experiment livers from three or four animals in each group were pooled.

[b] Means ± SEM of the differences for each of the three to four experiments. The average values for enzyme activity and nuclear RNA release for the control group were 6.1 μmole P_i/hr/mg protein and 3662 cpm/ml incubation medium, respectively.

[c] $0.05 > P > 0.01$, compared with control group.

[d] $P < 0.01$, compared with control group.

[e] $P < 0.01$, compared with tryptophan control.

[f] $P < 0.01$, compared with three other groups.

in nuclear envelope nucleoside triphosphatase activity in comparison to those of controls (Table III).

Discussion. The results of this study suggest that the increased rate of nucleocytoplasmic translocation of poly(A)−mRNA observed in the livers of rats tube-fed tryptophan (6, 17) may be related to alterations in the levels of nucleoside triphosphatase activity of hepatic nuclear envelopes. The present data add further support to our proposed mechanism by which the stimulatory effects of tryptophan on hepatic protein synthesis has been explained. Based upon evidence obtained so far from experimental studies, it appears that tryptophan can stimulate hepatic polyribosomes and protein synthesis in one or both of two ways: (a) increased synthesis of mRNA and (b) increased nucleocytoplasmic translocation of mRNA. In normal animals tryptophan may act by both mechanisms, while in animals treated with inhibitors of RNA or protein synthesis it probably acts by the second mechanism alone. In normal animals, there is evidence suggesting that tryptophan may act at the transcriptional level of control of hepatic protein synthesis. Tryptophan administration has been reported to cause an increase in DNA-dependent RNA polymerase activity (26), nuclear RNA synthesis (27), and polyribosomal RNA synthesis (28). In addition, our studies have shown that tryptophan administration to normal animals resulted in elevated levels of cytoplasmic mRNA (5), poly(A), and poly(A)−mRNA (4). More recent experiments from our laboratory have demonstrated that in normal animals, in particular during short exposure to tryptophan (within 10 min), and also in animals pretreated with agents which inhibit RNA or protein synthesis, tryptophan may act at the post-transcriptional level of control of hepatic protein synthesis, via enhanced nucleocytoplasmic translocation of mRNA (17). The present results suggest that this second mechanism may involve, at least in part, an enzyme, nucleoside triphosphatase, of nuclear membrane which may regulate or influence nucleocytoplasmic flow of mRNA. The parallel increases in both nucleoside triphosphatase activity of nuclear envelopes and RNA release from isolated nuclei following the administration of tryptophan suggest that these two processes may be associated with or related to one another. Although this enzyme has been characterized with respect to its substrate specificity and its kinetic behavior, little is known concerning the synthesis and turnover of the enzyme. Since there is already marked stimulation of hepatic protein synthesis in the nucleus and cytoplasm within 10 min following the administration of tryptophan (unreported data), it is possible that this may be related to the rapid induction in the synthesis of one or several specific regulatory proteins, such as nucleoside triphosphatase of the nuclear membrane, resulting in greater outpouring of nuclear RNA into cytoplasm. Although the question of nuclear protein synthesis has yet to be definitively established, some investigators have implicated the nucleus as a site of protein synthesis and also indicated that the reported nuclear protein synthesis is inhibited to a lesser extent by puromycin and cycloheximide than is cytoplasmic protein synthesis (29). Indeed, our data indicate that neither nuclear envelope nucleoside triphosphatase activity nor *in vitro* RNA release from isolated nuclei are affected by puromycin, an inhibitor of cytoplasmic protein synthesis. Thus, it is possible that regulatory proteins, possibly such as nucleoside triphosphatase or some other proteins, stimulated by tryptophan may still be involved in the alteration of hepatic nuclei even in animals which have been treated with selected inhibitors of protein synthesis.

To our knowledge, this study is the first demonstration that nuclear envelope nucleoside triphosphatase activity is stimulated with a concurrent increase in the rate of nucleocytoplasmic translocation of RNA. Furthermore, our studies along with those of Agutter *et al.* (12) suggest that nuclear envelope nucleoside triphosphatase may constitute an essential part of the mechanism of nucleocytoplasmic RNA translocation. We are currently investigating further as to how tryptophan may influence the activity of nuclear envelope nucleoside triphosphatase and how this may be

related to the stimulatory effect of tryptophan on hepatic protein synthesis.

Summary. The activity of nuclear envelope nucleoside triphosphatase, an enzyme implicated to be involved in nucleocytoplasmic translocation of mRNA, was investigated in the livers of rats that received a single tube-feeding of tryptophan. Tryptophan administered to fasted rats or to rats pretreated with puromycin or actinomycin D caused significant increases in the activity of Mg^{2+}-dependent nucleoside triphosphatase in the hepatic nuclear envelopes over that in controls. Concomitant with this rapid (10 min) increase in the enzyme activity, there was more release of RNA from isolated nuclei of livers of tryptophan-treated rats than from those of control rats.

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