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Influence of L-alanine on effects induced by L-tryptophan on rat liver

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This study was designed to determine if a nuclear tryptophan receptor-ligand relationship plays a role in the stimulatory effects of L-tryptophan administration on rat liver. Two compounds, L-alanine and DL-β-(1-naphthyl)alanine(β-NA), which competed for in vitro ^{3}H-tryptophan binding to hepatic nuclei similar to that of L-tryptophan but which when administered alone in vivo did not stimulate hepatic protein synthesis as did L-tryptophan, were used in this study. Rats treated with L-alanine or β-NA together or before L-tryptophan failed to demonstrate the stimulatory effects in the liver (protein synthesis, nuclear poly(A)polymerase activity, and nuclear RNA efflux) as observed in rats treated with L-tryptophan alone. The findings indicate that the enhanced hepatic protein synthesis that occurs following the in vivo administration of L-tryptophan appears to be related to L-tryptophan's ability to bind to a specific nuclear tryptophan receptor. (J. Nutr. Biochem. 7:200–206, 1996.)

Keywords: L-alanine; L-tryptophan; nuclear binding; protein synthesis

Introduction

For many years our laboratory has been concerned with the unique effect of L-tryptophan on hepatic protein synthesis.[1] We reported that the administration of L-tryptophan alone rapidly elicited a response of hepatic polyribosomes toward heavier aggregation as well as an enhancement of hepatic protein synthesis in mice and rats as measured in vivo or in vitro.[1–3] Tryptophan administrated alone, but not a single administration of other single essential amino acids, elicited a stimulatory hepatic response that was similar to that obtained with a complete amino acid mixture.[3] Others have subsequently confirmed the stimulatory effect of tryptophan on hepatic protein synthesis.[4–10]

A number of studies have been concerned with the mechanism responsible for the tryptophan-induced stimulation of hepatic protein synthesis.[1] A brief review of the significant findings of these studies follows. Tryptophan administration rapidly increases the mRNA(poly-(A)mRNA) present in the cytoplasm of rat liver.[11,12] An enhanced rate of translocation of mRNA from nuclei into the cytoplasm (demonstrated in in vivo and in vitro experiments) appears to account for this effect.[13,14] Intracytoplasmic translocation of poly(A)-mRNA from informosomal pools is not responsible.[15] Tryptophan stimulates the activities of enzymes of the nuclear envelope that influence the phosphorylation and dephosphorylation processes (those considered to be involved in the regulation of nucleocytoplasmic translocation of mRNA).[16] Also nuclear poly-(A)polymerase was stimulated.[17] In addition, tryptophan has been found to bind rapidly to protein of the nuclei and this binding appears to be correlated with the increased in vitro release of hepatic nuclear RNA[18] and with the enhanced activity of hepatic nuclear poly(A)polymerase activity.[19] Pretreatment of isolated hepatic nuclei with concanavalin A prevented the increases in binding and in nuclear RNA release, suggesting that glycoprotein(s) may be involved in the process whereby tryptophan acts.[18] Indeed, tryptophan has been found to increase ^{14}C-glucosamine incorporation into proteins of the subcellular fractions, particularly that of nuclear membrane.[20]

Recent studies have focused on the ability of tryptophan to bind to a nuclear envelope tryptophan receptor protein (glycoprotein).[21,22] This tryptophan binding protein of hepatic nuclear envelopes has been purified to apparent ho-

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mogeneity using either concanavalin A-agarose or tryptophan-agarose. The receptor has an M_R of 34,000 (64,000 when using several protease inhibitors).[22] The hepatic nuclear envelope protein contains two binding components for ^{3}H-tryptophan as revealed by Scatchard analysis. One of the components has a high affinity for tryptophan (K_D = 0.67 nM and B_{max} = 21.3 fmol/mg protein) whereas the low affinity binder has both a higher K_D (18.1 nM) and concentration (B_{max} = 327.3 fmol/mg protein).[21] Schroder et al.[23] have described a tryptophan binding protein of nuclear envelopes of mouse lymphoma (L5178g) cells that has similar characteristics as reported earlier in our laboratory for rat hepatic nuclear envelopes.

A number of analogs, metabolites, or related compounds of tryptophan have been tested to determine if they compete with in vitro ^{3}H-tryptophan binding to hepatic nuclear envelopes.[24–26] Some of the compounds that were observed to compete were DL-β-(1-naphthyl)alanine(β-NA), 5-fluorotryptophan, 7-azatryptophan, 5-hydroxtryptophan, L-alanine, L-phenylalanine, L-tyrosine, L-cysteine and L-cystine.[24,25] Based on the studies mentioned, we were able to map out which portion of the tryptophan molecule is crucial for binding of tryptophan to hepatic nuclear envelopes.[25] Of special interest is the finding that compounds that compete with ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes generally contain the α-amino-propionic acid (L-alanine) structure.[25]

To determine if the nuclear tryptophan receptor-ligand relationship plays a role in the subsequent biological or physiological events after the administration of L-tryptophan as described, we have undertaken in this study experiments designed to determine the possible association between the two. Earlier data revealed that certain compounds, though they competed with tryptophan binding to hepatic nuclei in vitro, did not themselves when administered in vivo stimulate hepatic protein synthesis.[24,25] Before beginning this study, we reviewed some of the pertinent data (some of which was of a preliminary nature) from earlier reports that are summarized in *Table 1*. In view of these data we conducted experiments in which we investigated if treatment with two selected compounds, L-alanine and β-NA, which competed with tryptophan binding in vitro,[24,25] would when administered in vivo alter the biological responses to L-tryptophan. Conceptionally, we considered that if the effect of L-tryptophan may be primarily via binding to hepatic nuclei (nuclear envelopes), then interruption (diminution) of such binding by an active competitor, such as L-alanine or β-NA, would disrupt or interfere with the usual effect (stimulatory) of L-tryptophan alone on hepatic protein synthesis. Our findings indicate that the administration of L-alanine or β-NA, compounds that compete for the nuclear receptor sites for L-tryptophan, appears to negate L-tryptophan's ability to stimulate hepatic protein synthesis.

Methods and materials

Animals

Female Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA, USA), average weight 250 g (range 225–300 g), were used in the experiments. The rats were maintained in a temperature-controlled room with a 12-hr light:dark cycle. Before the experiments were begun, the animals were adapted to their quarters and to the diet (Purina Lab Chow #5001, Purina, St. Louis, MO, USA) for 1 week or more; rats were then deprived of food overnight but had free access to water. Rats were killed by decapitation. These stud-

Table 1 Summary of earlier data pertaining to the effects of L-tryptophan and selected other compounds on inhibition of in vitro ^{3}H-tryptophan binding to nuclei, on in vitro protein synthesis, on in vitro nuclear RNA efflux, and on selected enzyme activities of liver

Compounds tested	Inhibition of ^{3}H-tryptophan binding %	In vitro protein synthesis	In vitro nuclear RNA efflux	PAP[a] activity	NTPase[b] activity
		% change			
L-Trp	53.3[c]	+86[d]	+98[e]	+46[f]	+74[e]
D-Trp	−5.0[c]	−2[d]			
[g]L-Trp	69.8	+71.2; +43.2	+156		+127
L-Ala	51.7	−8.1; +0.2	−1		+13
D-Ala	15.1				
L-Trp+ LAla		+17.2			
L-Ala → L-Trp		+15.0			
[h]L-Trp	59.4	+68.3	+28	+54	+24
β-NA[i]	52.9	−7.5	−2	+95	+21

[a]PAP; poly(A)polymerase
[b]NTPase; nucleoside triphosphatase
[c]Reference 21
[d]Reference 3
[e]Reference 14
[f]Reference 17
[g]Reference 25
[h]Reference 24
[i]β-NA; D,L-β-(1-naphthyl)alanine

ies were approved by the institutional animal care and use committee.

Chemicals

The radioactive compounds used in the experiments were L-5-^{3}H-tryptophan, radiochemical purity 98.5%, 1.13 TBq/mmol, and L-(U-^{14}C)-leucine ((radiochemical purity 99%, 12.9 GBq/mmol), obtained from Amersham/Searle Arlington Height, IL, USA; (6-^{14}C) orotic acid (radiochemical purity >99%, 1.48 GBq/mmol) was obtained from NEN Research Products, Du Pont, Boston, MA, USA; and L-alanine(2,3-^{3}H) (radiochemical purity >97%, 1.85 or 2.22 TBq/mmol) was obtained from ICN Biochemicals, Inc., Irvine, CA, USA. L-tryptophan and DL-β-(1-naphthyl)alanine from U.S. Biochemical (Cleveland, OH, USA) were used. L-alanine was from Life Technologies (Grand Island, NY, USA).

Preparation of nuclei

Immediately after the rats were killed, the livers were removed and placed on ice until homogenization was begun (within 15 min). Purified hepatic nuclei were prepared as described by Blobel and Potter.[27]

Binding of ^{3}H-tryptophan to nuclei

Rat hepatic nuclei were incubated with L-5-^{3}H-tryptophan (containing 5.6 kBq and 0.005 nmol L-tryptophan/assay) in the absence or presence of an excess of tryptophan (10^{-4} M) or test compound (10^{-4} M) at room temperature for 2 hr. These conditions were selected based on our earlier findings.[22] The nuclei were then washed three times with buffer A (0.05 M Tris·HCl, pH 7.5, 0.025 M KCl, 0.005 M $MgCl_2$, 0.0001 M phenylmethylsulfonyl fluoride, 0.0002 M dithiothretal and 0.25 M sucrose, to remove free and loosely bound radioactivity. After the final wash, the nuclei were suspended in buffer A and radioactivity was then measured after the addition of a scintillation mixture (Opti Fluor, Packard Instrument, Downers Grove, IL, USA). Binding of ^{3}H-tryptophan to hepatic nuclei was expressed as becquerels per unit protein. Specific binding was derived from total binding (in absence of unlabeled L-tryptophan) minus nonspecific binding (in presence of an excess of unlabeled L-tryptophan). Such binding was compared with values derived from total binding (in absence of unlabeled L-tryptophan or test compound) minus binding in presence of an excess of test compound. Values of test compounds were then compared with values obtained using unlabeled L-tryptophan (control group).

Preparation of microsomes

Postmitochondrial supernatants were prepared from homogenates of livers of rats of each group and were used to prepare microsomes.[3]

In vitro protein synthesis

In all assays, microsomes of livers of different groups of rats and cytosols of livers of control (water-treated) rats were used.[3] L-(U-^{14}C-leucine (18.5 kBq) was added to each incubation tube. Radioactivity in protein (trichloracetic acid-precipitable, washed with unlabeled carrier) was measured using a liquid scintillation spectrometer (Beckman Instruments, Palo Alto, CA, USA). The protein was determined as described by Lowry et al.[28]

In vitro release of RNA from isolated hepatic nuclei

The release of RNA from isolated nuclei was studied using the method of Schumm and Webb.[29] The incubation medium contained the following components in a volume of 2.5 mL: 5×10^{6} nuclei per 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; 2.0 mM dithiothreitol; 2.0 mM ATP; 2.5 mM phosphoenol-pyruvate; 35 units of pyruvate kinase; 500 μg/mL of yeast RNA, and dialyzed cell sap (12 mg protein per milliliter). The cell saps were prepared (one part liver and two and-a-half parts buffer) from livers of control rats and were dialyzed for 18 hr at 4°C against two changes of buffer (0.05 M Tris, pH 7.6, 0.025 M KCl and 0.0025 M $MgCl_2$). The mixture was incubated at 30°C, and additional phosphoenol-pyruvate (1.0 mM) was added at each 10-min interval of incubation. Aliquots of reaction mixture were removed after incubation for 30 min and were centrifuged at 460 × g for 10 min. The released RNA in the nuclei free medium was precipitated with ice-cold 10% trichloroacetic acid and after 15 min in the cold was filtered through fiberglass filters.

The precipitate was washed three times with cold 5% trichloroacetic acid and once with cold 95% alcohol. Each filter was dried and counted in a toluene scintillation mixture.

Enzyme assay

Poly(A)polymerase (EC 2.4.3.30) activity was measured as described by Jacob et al.[30]

Statistics

Data was analyzed by Student's *t*-test.[31]

Results

The first series of experiments were designed to determine if L-alanine would affect hepatic protein synthesis. *Table 2*

Table 2 Effect on L-alanine treatment on L-tryptophan-induced stimulation of in vitro ^{14}C-leucine incorporation into proteins of rat liver

Treatment			^{14}C-leucine incorporation into protein[a]
0	30 min	60 min	%
Water	0	Kill	(5) 100
L-tryptophan (5 mg/100g body weight)	0	Kill	(5) 143.2 ± 7.8[b,c]
L-alanine (50 mg/100g body weight)	0	Kill	(3) 108.0 ± 2.1[d]
L-tryptophan + L-alanine	0	Kill	(3) 116.3 ± 2.1[e]
L-alanine	L-tryptophan	Kill	(3) 99.5 ± 1.7[d]

[a]Mean absolute value (cpm/mg RNA) for control (water) groups were 4,241. Other values are expressed as % of controls in each experiment.
[b]Number of experiments in parentheses. Means ± SEM.
[c]$P < 0.01$, compared with water group.
[d]$P < 0.01$, compared with L-tryptophan group.
[e]$0.05 > P > 0.01$, compared with L-tryptophan group.

reveals that in vivo treatment (1 hr) of rats with L-alanine alone caused no change in hepatic protein synthesis. However, L-alanine together with or before L-tryptophan had an inhibitory effect on protein synthesis (in vitro assay of ^{14}C-leucine incorporation into protein) compared with that by treatment with L-tryptophan alone. Whereas L-tryptophan (5 mg/100 g body weight) tube-fed 1 hr before killing induced a significant increase in in vitro protein synthesis (^{14}C-leucine incorporation into hepatic protein), the administration of L-alanine (50 mg/100 g body weight) together with L-tryptophan or L-alanine tube-fed ½ hr before the L-tryptophan significantly reduced the increase due to L-tryptophan alone. In one experiment, we also used different levels of L-alanine (13 mg or 90 mg/100 g body weight) of L-alanine along with L-tryptophan (5 mg/100 g body weight) (treatments similar to those conducted with L-alanine (50 mg/100 g body weight) as given in *Table 2*) and the results revealed that such levels of L-alanine likewise diminished the stimulation of hepatic protein synthesis due to L-tryptophan alone. In one experiment, we also used D-alanine (50 mg/100 g body weight) and found that it did not induce the effect of L-alanine. D-alanine had been reported earlier not to compete, as did L-alanine, with L-tryptophan binding in vitro to hepatic nuclei.[25]

Next, we investigated the effects of administering L-alanine alone or together with L-tryptophan on hepatic nuclear poly(A)polymerase (PAP) activity. Rats were tube-fed L-tryptophan (5 mg/100 g body weight), L-alanine (13 or 50 mg/100 body weight), both together or L-alanine 30 min before L-tryptophan and all rats were killed after 60 min. The results for nuclear PAP activities (bound and free) are summarized in *Table 3*. Whereas L-tryptophan administration increased hepatic nuclear PAP activities, L-alanine alone had little effect. The combination of L-tryptophan and L-alanine revealed an increase in PAP activity similar to that of L-tryptophan alone. Next, we conducted two experiments in which the duration was decreased from 60 min to 10 min. The hepatic nuclear PAP activities (bound and free) for each group was summarized in *Table 3*. Under these conditions, there were appreciable decreases in PAP activity in the group that received L-alanine and L-tryptophan in comparison with the group that received L-tryptophan alone.

Next we investigated the effects of tube-feeding L-tryptophan, L-alanine or both on nuclear RNA efflux measured in vitro. The effect of L-tryptophan tube-feeding on in vivo ^{14}C-orotate labeled RNA efflux measured in vitro from hepatic nuclei was increased 137% over controls (water-treated) (*Table 4*). On the other hand, tube-feeding L-alanine plus L-tryptophan induced only a 14.8% increase in nuclear RNA efflux. Thus, L-alanine administration appears to inhibit the increase induced by L-tryptophan.

To investigate whether there may be a specific hepatic nuclear receptor for L-alanine, we measured the in vitro binding of L-alanine(2,3-^{3}H) to rat hepatic nuclei. Using concentrations, 0.35 to 160 nM (0.1 to 40 μCi), of ^{3}H-alanine in our in vitro assay system, we observed no saturable level of binding of ^{3}H-alanine to rat hepatic nuclei. Thus we conclude that under our assay conditions there appears to be no specific nuclear receptor binding for L-alanine, unlike that reported for L-tryptophan.

In an earlier study it was reported that treatment with β-NA did not stimulate hepatic nuclear RNA efflux (measured in vitro) as occurs with L-tryptophan treatment (*Table 1*).[24] However, because β-NA competed with ^{3}H-tryptophan binding to hepatic nuclei as did L-tryptophan (*Table 1*),[24] it was decided to investigate if β-NA pretreatment would alter the subsequent L-tryptophan effect on in vitro nuclear RNA efflux. *Table 5* summarizes three experiments conducted to determine whether pretreatments (one to three) of β-NA before treatment with L-tryptophan of rats would affect nuclear RNA efflux measured in vitro. In control rats receiving L-tryptophan (30 mg/100 g body weight) 10 min before killing, the in vitro hepatic nuclear RNA

Table 3 Effects of L-tryptophan (L-Trp), L-alanine (L-Ala) or both on rat hepatic poly(A)polymerase (PAP) activity

Treatment	No. of experiments	Nuclear PAP Activity		
		Bound form[a]	%	Free Form[b]
Water[c]	4	100		100
L-Trp[c]	4	136.6 ± 16.9[e]		204.8 ± 31.7[e]
L-Ala[c]	4	98.6 ± 23.0		121.7 ± 35.9
L-Trp + L-Ala[c]	3	150.1 ± 33.8[f]		168.1 ± 35.4[f]
L-Ala → L-Trp[d]	3	138.2 ± 26.2[f]		187.2 ± 55.9
Water[g]	2	100		100
L-Trp[g]	2	143.6		220.0
L-Ala[g]	2	94.5		106.7
L-Trp + L-Ala[g]	2	113.5		126.3

[a]100% corresponds to cmp of ^{3}H-AMP incorporated/mg nuclear protein for each experiment. Values are means ± SEM.
[b]100% corresponds to cmp of ^{3}H-AMP incorporated/mg nuclear protein for each experiment.
[c]Rats received intragastrically either the vehicle (water), L-tryptophan (5 mg/100 g body weight), L-alanine (13 of 50 mg/100 g body weight) or both at above doses for 60 min.
[d]Rats received intragastrically L-alanine (50 mg/100 g body weight) at 0 time and L-tryptophan (5 mg/100 body weight) at 30 min.
[e]$P < 0.001$
[f]$0.05 > P > 0.01$
[g]Rats received intragastrically either the vehicle (water), L-tryptophan (30 mg/100 g body weight) L-alanine (50 mg/100 g body weight) or both at above doses for 10 min.

Table 4 In vitro ^{14}C-orotate-labeled nuclear RNA release in livers of rats tube-fed water or L-alanine before tube-feeding water or L-tryptophan

Groups[a]		^{14}C-orotate-labeled nuclear RNA release
Tube-fed	Addition of L-tryptophan	%
Water	–	(5) 100
Water	+	(5) 237.0 ± 39.4[b,c]
L-alanine	–	(3) 76.8 ± 13.6[d]
L-alanine	+	(2) 114.8 ± 49.9

[a]Two rats were used for each group and livers of each group were pooled in each experiment. Rats were tube-fed water or L-alanine (13.4 mg/100 g body weight) at 0 and 70 min. At 60 min all rats received ^{14}C-orotic acid intraperitoneally. At 80 min each group of rats were tube-fed water or L-tryptophan (30 mg/100 g body weight) 10 min before killing.
[b]Number of experiments in parentheses. Means ± SEM.
[c]$P < 0.01$, compared with water group.
[d]$0.05 > P$ 0.01, compared with water group.

efflux was increased by 61.7 to 285.8% in comparison with control (water-treated) rats. On the other hand, β-NA pretreatment (one to three times) before L-tryptophan treatment given 10 min before killing caused much less nuclear RNA efflux increase (33.1 to 102%) compared with control (water-treated) groups. Thus, β-NA pretreatment caused a 47% decrease on the average in nuclear RNA efflux due to L-tryptophan compared with controls.

Discussion

Our present results indicate that the enhanced hepatic protein synthesis induced by the in vivo administration of L-tryptophan appears to be related to L-tryptophan's ability to bind to a hepatic nuclear receptor. By using L-alanine and β-NA, compounds that compete with L-tryptophan binding to hepatic nuclei but do not alone stimulate hepatic protein synthesis,[24,25] we have found that each (L-alanine, *Table 2;* β-NA *Table 1*)[24] when added along with L-tryptophan inhibits the induced increase in hepatic protein synthesis due to L-tryptophan alone. Also, such combinations inhibited the elevations due to L-tryptophan alone of other rapidly induced metabolic reactions as nuclear RNA efflux (nuclear-cytoplasmic translocation of mRNA) and nuclear poly(A)polymerase activity. These two stimulatory responses have previously been considered to be involved in the process of elevated hepatic protein synthesis due to L-tryptophan.[13,14,17]

In evaluating the inhibitory effects of L-alanine and β-NA on L-tryptophan-induced hepatic protein synthesis (*Tables 1* and *2*), nuclear PAP activity (*Table 3*), and nuclear RNA efflux (*Tables 4* and *5*), it is noteworthy that the two agents were added in our experimental studies at higher dose levels than that of L-tryptophan. Findings from an earlier study[24] suggested that higher levels of β-NA than that of L-tryptophan were needed to have an inhibitory effect on actions induced by L-tryptophan. This is not surprising because many amino acids, including L-alanine, are normally present in the blood at much higher levels than that of L-tryptophan. Therefore, one would expect that similar relationships might be needed to affect the stimulatory effects of administering L-tryptophan.

The concept of amino acid imbalances has been considered to be of importance in explaining a variety of nutritional alterations.[32] Such imbalances may be induced by manipulating dietary intake or by inducing internal metabolic alterations. Our laboratory has investigated amino acid imbalances induced rapidly (within days) by tube-feeding purified diets devoid of single essential amino acids[33] or induced within minutes by the administration of L-tryptophan.[1] Now we find that alterations in an amino acid-imbalanced state, such as induced by administering L-alanine in addition to L-tryptophan, can within minutes negate stimulatory effects induced by L-tryptophan alone. Thus, the effects induced by one dietary component (L-tryptophan) may be affected by its interrelationship with another dietary component (L-alanine). Indeed, numerous checks and balances may be present at the subcellular level, such as at a nuclear envelope receptor in the case of L-tryptophan, which act to maintain balanced metabolic states.

This study has dealt with two compounds, L-alanine (a normal amino acid) and β-NA (a tryptophan analog),[34] each of which compete with L-tryptophan for hepatic nuclear receptor binding and are thereby able at certain concentrations to inhibit the stimulatory effect of L-tryptophan alone on hepatic protein synthesis. However, other compounds, such as branched-chain amino acids (L-leucine, L-isoleucine, and L-valine) that do not alone directly compete with L-tryptophan nuclear binding[25] have also been demonstrated to inhibit the stimulatory effect of L-tryptophan on hepatic protein synthesis (unreported findings). The latter effect appears to be unrelated to active competition of binding at L-tryptophan's nuclear receptor. Nonetheless, L-tryptophan becomes unable to increase hepatic protein synthesis. Thus it appears that the effect of L-tryptophan on hepatic protein synthesis may be modulated in a number of ways. Selected amino acid imbalances, such as experimentally induced by combinations of L-alanine and L-tryptophan or of L-leucine and L-tryptophan, can affect, even though by different mechanisms, important regulatory steps involved in L-tryptophan's ability alone to affect hepatic protein synthesis.

The ability of L-tryptophan administration to stimulate hepatic nuclear receptor binding, increased nuclear poly(A)polymerase activity, enhanced nuclear RNA efflux, and increased protein synthesis suggests that these processes may be interrelated and possibly be sequentially involved. Activation of certain steps does not necessarily trigger other effects. Whereas L-tryptophan affects all of the above processes, L-alanine affects only nuclear tryptophan receptor binding and β-NA affects nuclear tryptophan receptor binding and also, increases nuclear poly(A)polymerase activity (*Table 1*).[24] The ability of L-alanine or of β-NA, which compete for nuclear tryptophan receptor binding, to act to negate L-tryptophan's other actions indicated above, suggests that receptor binding is an early crucial event that probably triggers the others leading to enhanced protein synthesis.

Perez-Sala et al.[35] have reported that the administration

Table 5 Influence of pretreatment with D,L-β-(1-naphthyl)alanine (β-NA) before L-tryptophan (L-Trp) treatment on in vitro nuclear RNA efflux

Treatment								In vitro nuclear RNA efflux	
0	20 min	30 min	40 min	60 min	70 min	80 min	90 min	%	Changes due to L-Trp
Water[a]				^{14}C-OA[b]		Water	Kill	100[c]	
Water				^{14}C-OA		L-Trp[d]	Kill	181.0	+81.0
β-NA[e]				^{14}C-OA		Water	Kill	125.2	
β-NA[e]				^{14}C-OA		L-Trp	Kill	185.3	+47.9
Water				^{14}C-OA		Water	Kill	100	
Water				^{14}C-OA		L-Trp	Kill	385.8	+285.8
β-NA[f]				^{14}C-OA		Water	Kill	94.8	
β-NA[f]				^{14}C-OA		L-Trp	Kill	191.7	+102.0
Water		Water		^{14}C-OA		Water	Kill	111.1	
Water		Water		^{14}C-OA		L-Trp	Kill	388.4	+249.6
β-NA[e]		β-NA[e]		^{14}C-OA		Water	Kill	100.6	
β-NA[e]		β-NA[e]		^{14}C-OA		L-Trp	Kill	194.0	+92.7
Water				^{14}C-OA	Water	Water	Kill	100	
Water				^{14}C-OA	Water	L-Trp	Kill	228.0	+128.0
β-NA[e]				^{14}C-OA	β-NA[e]	Water	Kill	133.0	
β-NA[e]				^{14}C-OA	β-NA[e]	Trp	Kill	235.3	+76.9
Water	Water		Water	^{14}C-OA		Water	Kill	100	
Water	Water		Water	^{14}C-OA		L-Trp	Kill	161.7	+61.7
β-NA[e]	β-NA[e]		β-NA[e]	^{14}C-OA		Water	Kill	91.0	-9.0
β-NA[e]	β-NA[e]		β-NA[e]	^{14}C-OA		L-Trp	Kill	121.2	+33.2

[a]Results are the means of two experiments.
[b]^{14}C-OA, (6-^{14}C) orotic acid (16 μCi/100 g body weight), administered intraperitoneally.
[c]Values (cpm/L incubation medium) are ^{14}C-OA labeled RNA released. In each experiment control values were set at 100%.
[d]L-trp (30 mg/100 g body weight) tube-fed in 3 ml water/100 g body weight.
[e]β-NA (32 mg/100 g body weight) tube-fed in 2 ml water/100 g body weight.
[f]β-NA (64 mg/100 body weight) tube-fed in 3 ml water/100 g body weight)

intraperitoneally of L-alanine (approximately 45 mg/100 g body weight) to fasted rats rapidly (10 min) increased hepatic polyribosomal aggregation and protein synthesis. Also, using perfused livers they reported that the alanine-induced stimulation of hepatic protein synthesis was due to rate changes in ion fluxes accompanying the transport of alanine (uptake of alanine increased the intracellular content of K^+ and the cell volume).[36] They concluded that the alanine-induced cell swelling was similar to that induced by hypotonic buffer and could be sufficient to stimulate protein synthesis. These latter findings agree with our earlier findings that changes of the osmotic pressure of portal blood affect hepatic protein synthesis: hypertonic NaCl diminished whereas distilled water increased protein synthesis compared with isotonic NaCl.[37] In vivo administration of L-tryptophan appears to act independently of the tonicity effect because L-tryptophan rapidly stimulates protein synthesis compared with that in rats receiving distilled water alone. Also, L-tryptophan increases hepatic protein synthesis when administered before or after hypertonic NaCl when compared to that of comparable controls receiving the hypertonic NaCl alone.[38] In the present study tube-feeding of 50 mg L-alanine/100 g body weight for 30 min did not stimulate ^{14}C-leucine incorporation into protein using hepatic microsomes in comparison with controls (*Table 2*). The differences in our results with L-alanine and those of Perez et al.[35] may be related to the duration, route of administration, and assays used.

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