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Influence of L-leucine on L-tryptophan binding to rat hepatic nuclei

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This study was concerned with the effect of L-leucine on L-tryptophan's actions (nuclear receptor binding and protein synthesis) on rat liver. Earlier studies dealing with ^{3}H-tryptophan binding to rat hepatic nuclei in vitro revealed that some amino acids, such as L-alanine, L-phenylalanine, and L-tyrosine, but not others, such as L-leucine, competed for such binding. This study evaluated the influence of L-leucine together with unlabeled L-tryptophan on ^{3}H-tryptophan binding to hepatic nuclei in vitro. Whereas L-leucine alone had little effect, L-leucine together with unlabeled L-tryptophan (excess, 10^{-4}M) caused significantly less inhibition of ^{3}H-tryptophan binding to hepatic nuclei than did unlabeled L-tryptophan alone (9% vs. 68% inhibition, respectively). L-leucine (10^{-10} to 10^{-4}M), when added together with unlabeled L-tryptophan (10^{-7} to 10^{-4}M), abrogated the inhibition of binding because of the unlabeled L tryptophan alone. Other in vitro ^{3}H-tryptophan binding effects were: 1) L-isoleucine and L-valine revealed binding effects similar to that with L-leucine; 2) the dipeptide, TRP · LEU, acted similarly (but somewhat less) to that of L-leucine; and 3) L-alanine and sodium selenite, each of which was inhibitory to ^{3}H-tryptophan binding, showed less inhibition when L-leucine was also added. In vivo effects were investigated after tube-feeding water, L-leucine or/and L-tryptophan to rats 10 min before killing and revealed the following results: 1) using isolated hepatic nuclei for in vitro ^{3}H-tryptophan binding revealed less specific binding to nuclei of rats tube-fed L-tryptophan alone than to nuclei of those tube-fed L-tryptophan plus L-leucine, suggesting an in vivo inhibitory effect of L-leucine; and 2) using hepatic microsomes for in vitro ^{14}C-leucine incorporation into protein revealed greater stimulation by L-tryptophan alone (+61%) than by L-tryptophan plus L-leucine (+8%) over control (water alone). Thus, the addition of L-leucine appears to have an inhibitory effect on actions attributable to L-tryptophan alone. (J. Nutr. Biochem. 8:592–602, 1997)

Keywords: L-tryptophan; L-leucine; L-isoleucine; L-valine; nuclear binding; rats

Introduction

For many years our laboratory has been concerned with the unique action of L-tryptophan on hepatic protein synthesis in experimental animals.[1] In earlier studies we reported that the administration of L-tryptophan rapidly induced a number of changes in the livers of mice and rats: a shift of polyribosomes toward heavier aggregation,[2–4] increased protein synthesis (measured in vivo or in vitro,[2–4] increased cytoplasmic poly(A)mRNA,[5,6] enhanced nuclear mRNA efflux,[7,8,9] increased activities of certain nuclear enzymes (poly(A)polymerase,[10] nucleoside triphosphatase,[11] and RNA polymerase I and II,[12] and the existence of specific L-tryptophan binding to a nuclear envelope receptor.[13,14]

Interest in our laboratory has recently centered on studies concerned with the hepatic nuclear envelope receptor for L-tryptophan.[1–19] In one study[17] that dealt with selected compounds that did or did not compete with in vitro ^{3}H-tryptophan binding to hepatic nuclear envelopes, we observed that compounds that competed with L-tryptophan binding contained the α-amino-propionic acid structure (compounds such as alanine, phenylalanine, or tyrosine). However, other single amino acids (such as leucine, isoleucine, valine, or threonine) did not compete. Our in vitro assay system contained isolated hepatic nuclei incubated with additions of water (control), unlabeled L-tryptophan

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(excess, 10^{-4}M), or test compound along with ^{3}H-tryptophan (constant level, 278 Kbq, 0.245 nmol per assay, added last).

Because of our past interest in nutritional imbalances, especially amino acid imbalances,[20,21] we recently decided to test whether certain amino acids (some that did or did not compete for ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes on in vitro assay) when added together with unlabeled L-tryptophan (excess, 10^{-4}M) would influence the ^{3}H-tryptophan binding response as well as tryptophan's effects on other parameters. In one study[22] dealing with L-alanine, which had been reported to compete with ^{3}H-tryptophan binding to hepatic nuclei in vitro,[17] we observed that the administration of L-alanine along with L-tryptophan in vivo had an inhibitory effect on the stimulation of hepatic protein synthesis induced by L-tryptophan alone. In this study we investigated whether branched-chain amino acids (L-leucine, L-isoleucine, and L-valine), which themselves did not compete with ^{3}H-tryptophan binding to hepatic nuclei in vitro,[17] would influence the effects attributable to L-tryptophan alone on hepatic nuclear binding and protein synthesis. In other experimental studies, an adverse response between the branched-chain amino acids and L-tryptophan had been reported.[23,24] Addition of each of the branched-chain amino acids to a nicotinic acid-free, low-protein diet was found to decrease the conversion of tryptophan to nicotinamide. Also, the ratios of branched-chain amino acids to aromatic amino acids have been shown to affect both albumin synthesis and secretion in cultured rat hepatocytes; high ratios induce degradation of albumin, whereas low ratios reduce biosynthesis of albumin.[25]

Our present study revealed that L-leucine abrogated the inhibitory effect of unlabeled L-tryptophan (at high levels) to decrease ^{3}H-tryptophan binding to hepatic nuclei in vitro. Also, in vivo administration of L-leucine along with L-tryptophan diminished 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 of 250 g (range, 225 to 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 No. 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 studies 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), L-leucine (2,3,4,5-^{3}H) (radiochemical purity >99%, 4.9 TBq/mmol), and L-(U-^{14}C)-leucine (radiochemical purity 99%, 12.9 GBq/mmol), obtained from Amersham/Searle, Arlington Heights, 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). In one experiment a mixture of 15 pure (>98%) L-amino acids(^{3}H) (9.4 GBq/mg) obtained from ICN Radiochemicals (Irvine, CA USA) was used. The test compounds were obtained from Sigma Chemical (St Louis, MO USA) and from Aldrich Chemical Co. (Milwaukee, WI USA). L-tryptophan, was obtained from U.S. Biochemicals (Cleveland, OH USA).

Preparation of nuclei and nuclear envelopes

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.[26] Nuclear envelopes of hepatic nuclei were isolated with the procedure of Harris and Milne[27] as modified by Agutter and Gleed,[28] and as routinely used in this laboratory.[13,14] Purified hepatic nuclei were treated with 0.001 M $NaHCO_3$, digested with DNase I (10 mg/L) and centrifuged on a step-wise sucrose gradient (up to 2 M sucrose); the nuclear envelope band at interface (1.5 to 1.8 M sucrose) was then removed.

Binding of ^{3}H-tryptophan to nuclei or nuclear envelopes

Rat hepatic nuclei or nuclear envelopes were incubated with L-5-^{3}H-tryptophan (containing 278 kBq, 0.245 nmol L-tryptophan/assay, added last) in the absence or presence of a 2,000-fold excess of unlabeled L-tryptophan (10^{-4}M) or test compound (10^{-4}M) in 5 mL at room temperature for 2 h. These conditions were selected based on our earlier findings.[13] The nuclei were incubated in and then washed three times with buffer (0.05 M Tris·HCl, pH 7.5; 0.0025 M KCl; 0.005 M $MgCl_2$; 0.0001 M phenylmethylsulfonyl fluoride; 0.0002 M dithiothreitol, and 0.25 M sucrose), and the nuclear envelopes were incubated in and then washed two times with buffer (0.05 M Tris · HCl, pH 7.5; 0.002 M EDTA, 10% (v/v) glycerol; 0.001 M phenylmethylsulfonyl-fluoride; and 0.002 M β-mercaptoethanol). After the final wash, the nuclei or nuclear envelopes were suspended in the appropriate buffer and radioactivity was then measured after the addition of a scintillation mixture (Opti Fluor, Packard Instruments, Downers Grove, IL USA). Binding of ^{3}H-tryptophan to hepatic nuclei or nuclear envelopes was expressed as cpm per unit of protein (total binding in absence of unlabeled L-tryptophan or test compound). This total binding was then compared with the binding in the presence of excess unlabeled L-tryptophan or unlabeled test compound or of both. When specific binding as compared using hepatic nuclei of in vivo treated rats, specific binding for each group consisted of total binding (in the absence of unlabeled L-tryptophan) minus nonspecific binding (in the presence of an excess (10^{-4}M) of unlabeled L-tryptophan).

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 (trichloroacetic acid-precipitable and 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.[29]

Table 1 Inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei using excess (10^{-4}M) of test compounds

Unlabeled test compounds	Number of experiments	Total binding[a]	Inhibition due to test compounds	Change relative to L-tryptophan
Water		100	%	
L-tryptophan	20	31.6	68.4 ± 0.17[b]	
L-leucine	14	96.1	3.9 ± 1.40[c]	−94.3
L-isoleucine	4	93.5	6.5 ± 2.61[c]	−90.5
L-valine	6	90.6	9.4 ± 1.91[c]	−86.3
L-tryptophan + L-leucine	8	91.0	9.0 ± 4.17[c]	−86.8
L-tryptophan + L-isoleucine	4	91.5	8.5 ± 4.70[c]	−87.6
L-tryptophan + L-valine	6	63.7	36.3 ± 9.07[c]	−46.9

[a]In each experiment, total binding (water control) was set at 100% and effects of test compounds were expressed as relative to control. The mean value ± SEM for total binding of control (water-treated group) was 16,615 ± 597.
[b]Means ± SEM
[c]$P < 0.01$, compared with L-tryptophan group.

Enzyme assay

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

Statistics

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

Results

In the first series of experiments we investigated whether each of the branched-chain amino acids (10^{-4}M) when added together with unlabeled L-tryptophan (10^{-4}M) would affect ^{3}H-tryptophan (278 kBg and 0.245 nmol per assay) binding to hepatic nuclei in vitro. The data are summarized in *Table 1*. Whereas unlabeled L-tryptophan alone inhibited ^{3}H-tryptophan binding to hepatic nuclei by 68.4%, L-leucine alone caused 3.9% inhibition, L-isoleucine alone caused 6.5% inhibition, and L-valine alone caused 9.4% inhibition. These results are similar to those reported earlier.[17] However, when each of the branched-chain amino acids was added with unlabeled L-tryptophan, the inhibition of ^{3}H-tryptophan binding was as follows: addition of L-leucine, 9%; of L-isoleucine, 8.5%; and of L-valine, 36.3%. Thus, under the above in vitro assay conditions, the addition of each of the branched-chain amino acids together with L-tryptophan antagonized the inhibitory binding effect that occurred with unlabeled L-tryptophan alone upon ^{3}H-tryptophan binding to hepatic nuclei in vitro.

Next, we investigated which concentrations of L-leucine were able to affect unlabeled L-tryptophan's inhibition of in vitro ^{3}H-tryptophan binding to hepatic nuclei. First, we calculated the ratio of L-leucine to that of L-tryptophan (^{3}H-tryptophan at 5×10^{-8}M) used in the preceding experiments (*Table 1*). In these in vitro binding assays, the addition of L-leucine (10^{-4}M) to L-tryptophan (^{3}H-tryptophan at 5×10^{-8}M), ratio of 2000:1, did not affect total ^{3}H-tryptophan binding to hepatic nuclei (3.9% inhibition). The results of the additional experiments are summarized in *Figure 1*. Using additions of unlabeled L-tryptophan (10^{-10} to 10^{-4}M) to constant ^{3}H-tryptophan levels, the inhibition of ^{3}H-tryptophan binding to hepatic nuclei ranged from 25.4% (for 10^{-10}M) to 69.2% (for 10^{-4}M) compared to the binding in the absence of unlabeled L-tryptophan. Next, different levels of L-leucine (10^{-10} to 10^{-4}M) were added to the different concentrations of unlabeled L-tryptophan (10^{-10} to 10^{-4}M) and the effects on ^{3}H-tryptophan (constant levels) binding to hepatic nuclei were assayed (*Figure 1*). It is apparent that the addition of 10^{-4}M L-leucine to varying levels of unlabeled L-tryptophan caused increased ^{3}H-tryptophan binding (diminished inhibition of binding) compared to that of the unlabeled L-tryptophan alone. The addition of low levels of L-leucine (10^{-10}M) abrogated to a considerable extent, the binding of ^{3}H-tryptophan by the unlabeled L-tryptophan (10^{-8} to 10^{-4}M). The abrogative effect of L-leucine seems to exhibit multiphase kinetics. For example, at 10^{-10}M L-leucine, there is a strong abrogative effect, then there seems to be a plateau up to 10^{-7}M and then again an abrogative effect at higher concentrations (10^{-6} to 10^{-4}M).

Of concern was whether the concentrations of unlabeled L-tryptophan used in the in vitro binding assays were similar to that which exists in vivo within the rat liver. According to data from earlier reports, the free tryptophan level in livers of overnight, food-deprived rats or mice was 0.24×10^{-4}M.[15,18,32] Free tryptophan levels in the intracellular fractions of liver were found to be at the following average concentrations (M) in the livers of overnight, food-deprived animals: nuclei, 0.28×10^{-5}M; cytosol, 0.5×10^{-4}M; and microsomes, 0.7×10^{-4}M. Thus, the effects of using L-tryptophan levels of 10^{-6} to 10^{-4}M in our vitro binding assay system seemed to be in the physiological range. The results in *Figure 1* demonstrate that, at unlabeled L-tryptophan levels of 10^{-6}, 10^{-5}, and 10^{-4}M, the additions of L-leucine at different concentrations (particularly at 10^{-10}M or at 10^{-6} to 10^{-5}M) had abrogative effect on in vitro ^{3}H-tryptophan binding as compared with that of using unlabeled L-tryptophan alone.

Next, we investigated whether the time (0 or ½ hr) of addition of excess (10^{-4}M) of either unlabeled L-tryptophan or L-leucine during the 2-hr incubation would influence ^{3}H-tryptophan (added at 1 hr) binding to hepatic nuclei in vitro. The results are summarized in *Table 2*. When L-leucine was added alone at 0 time, it caused little (1.1 to 5.4%) inhibition of ^{3}H-tryptophan binding to nuclei. When

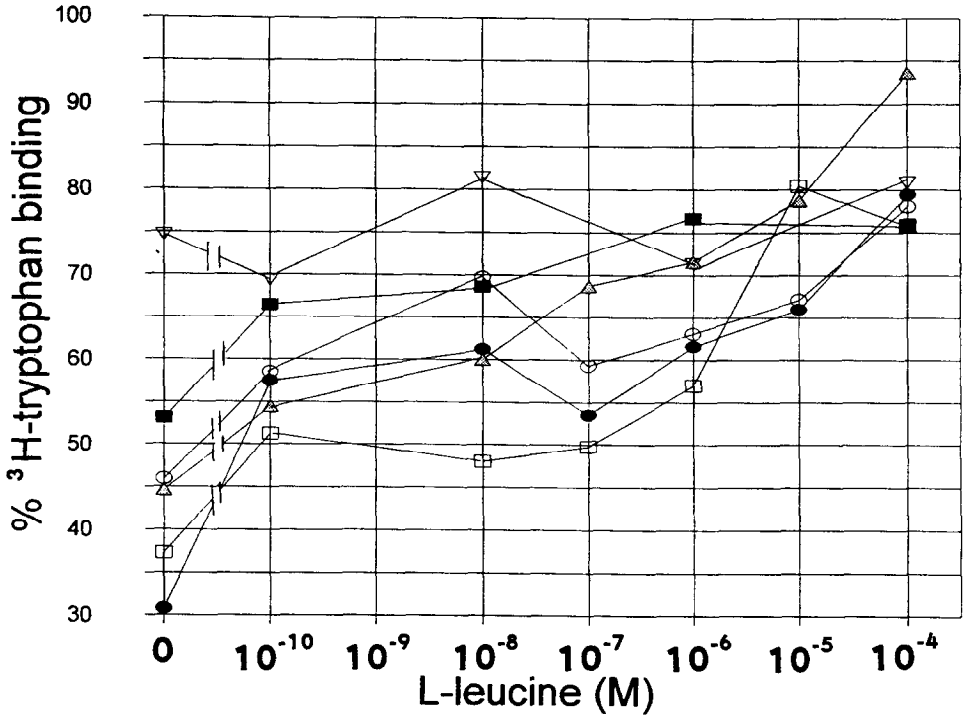

Figure 1 Effect of increasing concentrations of L-leucine (10^{-10} to 10^{-4}M) together with varying levels of unlabeled L-tryptophan (10^{-10} to 10^{-4}M) on ^{3}H-tryptophan binding to rat hepatic nuclei in vitro. Curves represent the following concentrations of L-tryptophan: 10^{-10}M, ▽—▽; 10^{-8}M, ■—■; 10^{-7}M, ○—○; 10^{-6}M, △—△; 10^{-5}M, □—□; and 10^{-4}M, ●—●. Each point represents mean of values of 3 to 14 experiments.

unlabeled L-tryptophan was added alone at 0 time, it caused between 56.9 to 59.2% inhibition of binding. When L-leucine and L-tryptophan were added together at 0 time, the inhibition of binding was 14.9% whereas the addition of L-leucine ½ hr after or before L-tryptophan, caused 24.5% or 40.1% inhibition, respectively, of ^{3}H-tryptophan binding. Next, we investigated in three experiments whether the effect of L-leucine treatment was persistent or reversible. Here hepatic nuclei were incubated for ½ hr after the addition of L-tryptophan, L-leucine, or L-tryptophan plus L-leucine (all at 10^{-4}M). Then hepatic nuclei were spun down and washed twice with buffer and then resuspended in buffer and treated with water, L-tryptophan or L-leucine for 1½ hr as indicated in *Table 2*. The results revealed that such L-leucine pretreatment affected the subsequent addition of unlabeled L-tryptophan on ^{3}H-tryptophan binding to hepatic nuclei (32.8% inhibition versus 67.2% inhibition (L-tryptophan without pretreatment with L-leucine) (*Table 2*). Such pretreatment with L-tryptophan did not appear to affect the subsequent action of L-leucine on ^{3}H-tryptophan binding (78 vs. 73%). In one experiment, we used isolated nuclear envelopes rather than nuclei to determine whether pretreatment (½ hr) would affect subsequent incubation and ^{3}H-tryptophan binding. The results were identical with nuclear envelopes as with nuclei indicating that nuclear contents, including internal nuclear enzymes, were probably not important in relation to the effect of L-leucine.

The saturation isotherm for ^{3}H-tryptophan binding using L-tryptophan (10^{-4}M) with or without added L-leucine (10^{-6}M) is indicated in *Figure 2*. It is apparent that, at all of the concentrations of added ^{3}H-tryptophan, the added L-leucine (10^{-6}M) to the L-tryptophan (10^{-4}M) diminished the specific binding to hepatic nuclei.

In view of the above described experimental effects in vitro, we investigated whether the administration of L-leucine in vivo would affect the subsequent ^{3}H-tryptophan binding to hepatic nuclei in vitro. Groups of rats were treated by stomach tube with water, L-tryptophan (30 mg/100 g body weight), L-leucine, (19.2 mg/100 g body weight) or both 10 min before killing. The isolated hepatic nuclei of each group were used for in vitro assays (*Table 3*). The hepatic nuclei from L-tryptophan-treated rats demonstrated a significant decrease (50.6%) in specific binding (total binding minus nonspecific binding (in presence of excess (10^{-4}M) unlabeled L-tryptophan)) compared with that of control rats. Whereas liver nuclei of rats treated with L-leucine showed essentially no change (0.9% increase) in specific binding compared with controls, the hepatic nuclei of rats treated with L-tryptophan plus L-leucine showed a 34.4% decrease in specific binding compared with that of controls or of the L-leucine group and less inhibition (34.4 vs 50.6%) than that of the L-tryptophan group. These findings suggest that the tube-feeding of L-tryptophan alone causes much specific binding to hepatic nuclei in vivo, which is reflected by a significant decrease in specific binding subsequently measured in vitro in comparison to controls. On the other hand, the tube-feeding of L-leucine plus L-tryptophan causes less specific binding in vivo, which is then reflected in less decrease in specific binding measured subsequently in vitro.

In the next series of experiments, we determined whether compounds related to L-leucine would act similarly to that of L-leucine in disturbing unlabeled L-tryptophan's ability to inhibit ^{3}H-tryptophan binding to hepatic nuclei in vitro. *Table 4* summarizes these data. D-leucine acted similarly to L-leucine. Whereas α-ketoisocaproic acid acted similarly to that of L-leucine when added alone, α-ketoisocaproic acid together with L-tryptophan did not act as did L-leucine

Table 2 Effects of additions of L-tryptophan (Trp), L-leucine (Leu), or both at 10^{-4}M concentrations on in vitro ^{3}H-tryptophan binding to hepatic nuclei

Incubation conditions				^{3}H-tryptophan binding to hepatic nuclei (%)
0	1/2 hr	1 hr	2 hr	
Water	0	^{3}H-Trp	Stop	(4) 100[a]
Trp	0	^{3}H-Trp	Stop	(3) 40.8 ± 6.5[b]
Leu	0	^{3}H-Trp	Stop	(4) 94.6 ± 5.4[c]
Trp + Leu	0	^{3}H-Trp	Stop	(4) 85.1 ± 5.5[c]
Water	Water	^{3}H-Trp	Stop	(4) 100.8 ± 1.0
Water	Leu	^{3}H-Trp	Stop	(3) 90.6 ± 9.2[d]
Trp	Water	^{3}H-Trp	Stop	(4) 43.1 ± 11.1[b]
Trp	Leu	^{3}H-Trp	Stop	(4) 75.5 ± 1.7[d]
Leu	Water	^{3}H-Trp	Stop	(4) 98.9 ± 3.1
Leu	Trp	^{3}H-Trp	Stop	(4) 59.9 ± 5.0
Water[e]	Trp	^{3}H-Trp	Stop	(4) 32.8 ± 2.6[b,f]
Leu[g]	Water	^{3}H-Trp	Stop	(3) 95.6 ± 6.3
Trp[g]	Water	^{3}H-Trp	Stop	(2) 73.0 ± 3.8[h]
Trp + Leu[g]	Water	^{3}H-Trp	Stop	(3) 85.8 ± 4.4
Leu[g]	Trp	^{3}H-Trp	Stop	(3) 67.2 ± 12.9[i]
Trp[g]	Leu	^{3}H-Trp	Stop	(3) 78.0 ± 13.3

[a]Number of experiments in parentheses. Means ± SEM. The mean value ± SEM for total binding of control (water-treated group) in cpm/mg nuclear protein was 9,713 ± 3,339.
[b]$P < 0.01$, compared with water control groups (water or water + water).
[c]$P < 0.01$, compared with Trp group.
[d]$0.05 > P > 0.01$, compared with Trp + water group.
[e]The results with this group were the same as when incubated with water for 1/2 hr and then nuclear pellet was washed × 2, resuspended and incubated further as indicated. Therefore, results were combined.
[f]$P < 0.01$, compared with Leu + Trp group.
[g]Incubated with indicated compounds for 1/2 hr and then nuclear pellet was washed × 2. At 1/2 hr nuclear pellet was resuspended and incubated further as indicated.
[h]$P < 0.01$ compared with water + Trp group.
[i]$0.05 > P > 0.01$, compared with water + Trp group.

together with L-tryptophan but revealed an appreciable inhibitory effect as did L-tryptophan alone. L-tert-leucine and norleucine, whereas revealing a 16.8% and 17.9% inhibition, respectively, when added alone, showed an inhibitory effect of 63.6% and 70.1% respectively when added together with L-tryptophan (similar to the 71.0% inhibition with L-tryptophan alone). D- or L-leucinol alone competed moderately (36.7% and 20.3%) with L-tryptophan binding, while each when added together with tryptophan showed moderate inhibition of binding (32.0% and 26.5%, respectively). Esters (methyl, ethyl, butyl, allyl, or benzyl) of L-leucine alone essentially did not compete with L-tryptophan binding, but when added together with L-tryptophan showed only a 25% decrease (average, 45.7% versus 71.0%) of binding inhibition compared with that of L-tryptophan alone. Somewhat similar effects to those with the esters were observed with L-leucinamide HCl or L-leucine-*p*-nitroanilide.

Table 5 summarizes the data from experiments where other branched-chain(isoleucine and valine)-related compounds were studied. It is apparent that L-isoleucine and L-valine acted similarly to L-leucine (*Table 1*) on inhibition of ^{3}H-tryptophan binding to hepatic nuclei when added alone. L-valine when combined with L-tryptophan had somewhat less effect than did L-leucine (*Table 1*) or L-isoleucine when combined with L-tryptophan. D-isoleucine, unlike L-isoleucine, when added to L-tryptophan had only a small inhibitory effect on ^{3}H-tryptophan binding. However, D-valine alone or together with L-tryptophan acted similarly to that of L-valine. The addition of other compounds (L-allo-isoleucine, D-allo-isoleucine, DL-α-keto-β-methyl-*N*-valeric acid, (S) · (+) · isoleucinol, L-norvaline, D-norvaline, α-ketoisovaleric acid, or L-valinol) together with L-tryptophan did not appear to appreciably alter the binding effect due to L-tryptophan alone (*Table 5*).

To determine whether the terminal methyl groups in L-leucine per se may be of importance, we investigated whether L-methionine or choline, compounds having terminal methyl groups, would act similarly or differently compared with that of L-leucine on binding (*Table 5*). Whereas L-methionine or choline alone caused a 26.8% or 42.5% inhibition of tryptophan binding, respectively, when each was added together with L-tryptophan the inhibition was 51.1% or 60.6%, respectively, compared with 71.0% for L-tryptophan alone.

Because L-leucine together with L-tryptophan acted as described above, we next investigated how dipeptides consisting of L-leucine or L-valine with L-tryptophan would affect ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes in vitro. The results of these experiments are summarized in *Table 6*. Whereas Trp · Leu (10^{-4}M) alone caused little (8.6% or 6.4%) inhibition of in vitro ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes respectively, its addition together with unlabeled L-tryptophan (10^{-4}M) caused a 48.9% inhibition of ^{3}H-tryptophan binding to hepatic nuclei, less than L-tryptophan alone (69.1%) but more than Trp · Leu alone (8.6%). Whereas Leu · Trp (10^{-4}M) alone caused moderate (44.2 or 35.4%)

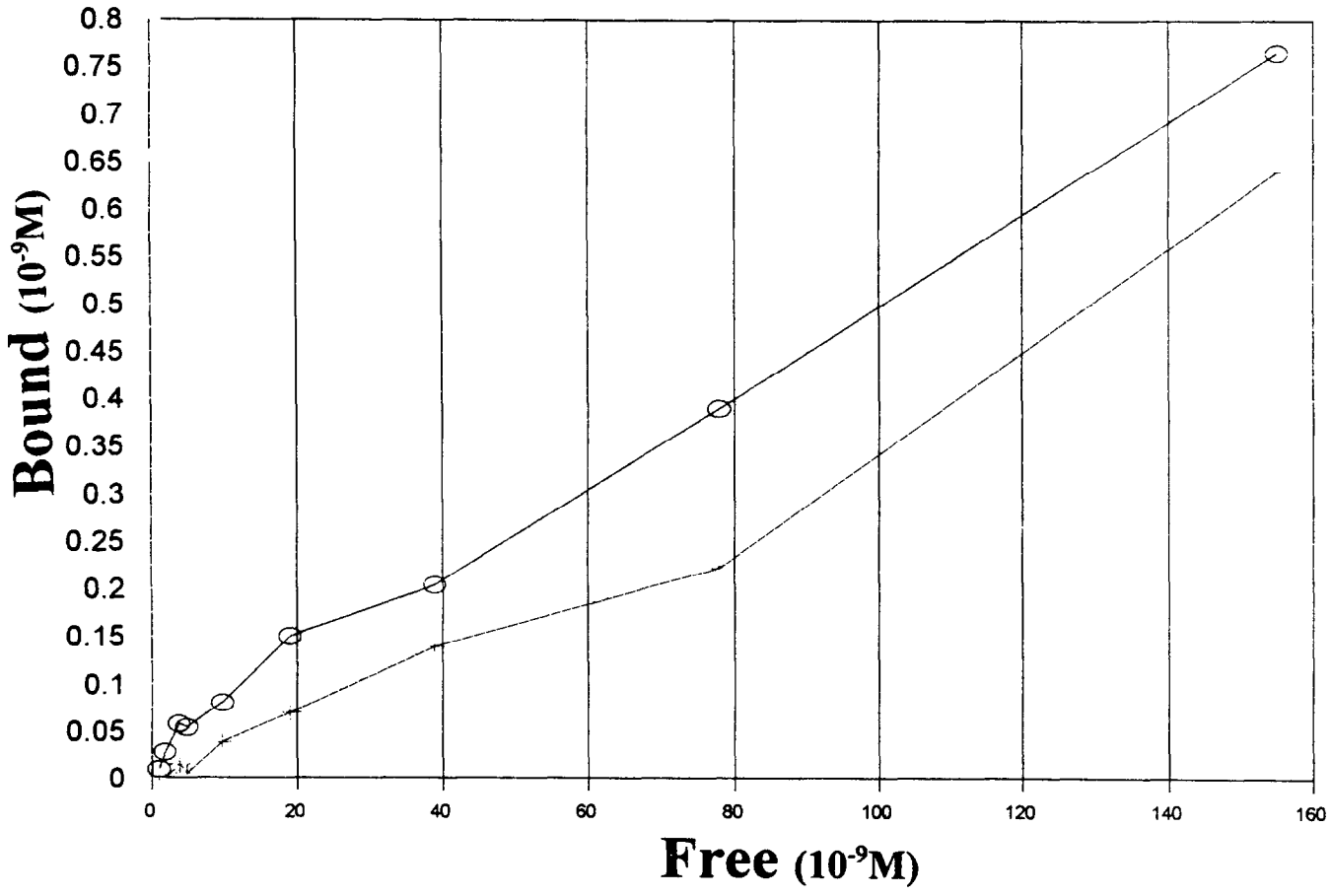

Figure 2 Saturation isotherm for ^{3}H-tryptophan binding to rat hepatic nuclei in vitro using L-tryptophan (10^{-4}M) without (0-0) or with added L-leucine (10^{-6}M) (+-+). Each point represents mean of values of two experiments. ^{3}H-tryptophan used ranged from 0.19 to 30 nM.

inhibition of ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes respectively, its addition together with unlabeled L-tryptophan caused a 35.6% inhibition of ^{3}H-tryptophan binding to hepatic nuclei, less than that with L-tryptophan alone (69.1%). Thus, Trp · Leu was less effective than Leu · Trp in inhibiting ^{3}H-tryptophan binding when each was added alone (8.6 ± 1.5% versus 44.2 ± 4.6%) but when added together with L-tryptophan the inhibitory effects were less different (48.9 ± 5.1 and 35.6 ± 8.0%, respectively). Thus, the free LEU of the TRP · LEU is capable of acting similarly, but somewhat less than L-leucine. Trp · Val and Val · Trp when added alone (10^{-4}M) caused moderate inhibition of ^{3}H-tryptophan binding similar to that of LEU · TRP (*Table 6*).

A recent report[33] described that sodium selenite inhibited ^{3}H-tryptophan binding to rat hepatic nuclei in vitro. Because dithiothreitol (DTE), a protective agent for SH groups, negated the effect of sodium selenite, it was deduced that thiol groups within the nuclear receptor for L-tryptophan were intimately involved in the binding. Therefore, we now checked whether L-leucine would affect the selenite-inhibitory effect on ^{3}H-tryptophan binding. *Table 7* summarizes the data. The addition of L-leucine to sodium selenite decreased the inhibition of ^{3}H-tryptophan binding to hepatic nuclei in vitro induced by sodium selenite alone by 36% (from 49.1 to 31.4%). Also, the addition of L-leucine to L-tryptophan plus sodium selenite decreased the inhibition of binding induced by L-tryptophan plus sodium selenite by 30.5% (from 58.0 to 40.3%). The differences in magnitude of the binding responses to L-leucine by L-tryptophan (−92%) and by sodium selenite (−36%) suggest that other effects in addition to those involving thiol groups (action of selenite) are applicable in regard to L-tryptophan.

Because L-alanine competed for ^{3}H-tryptophan binding to hepatic nuclei similar to that of unlabeled L-tryptophan,[17,22] we investigated whether L-leucine would affect the action of L-alanine on in vitro ^{3}H-tryptophan binding to hepatic nuclei. *Table 7* summarizes the data. The addition of L-leucine (10^{-4}M) to L-alanine (10^{-4}M) negated the inhibiting binding effect of L-alanine alone, similar to the effect of L-leucine added to L-tryptophan. Addition of L-leucine to L-alanine plus sodium selenite decreased the inhibition of binding induced by L-alanine plus sodium selenite by 32.5% (from 39.0% to 26.3%). Thus, L-leucine affects (negates) the inhibitory effect of L-alanine as well as that of L-tryptophan.

In the preceding experiments the in vitro ^{3}H-tryptophan binding assays were conducted predominantly with hepatic nuclei. Because hepatic nuclei contain many enzymes, one or more of which may act on or in relation to L-leucine

Table 3 In vitro ^{3}H-tryptophan binding to hepatic nuclei of rats treated with L-tryptophan, L-leucine, or both 10 min before killing

Groups[a]	Specific binding of hepatic nuclei[b] (%)
Water	(5) 100[c]
LTrp	(5) 49.4 ± 2.9[d,e]
L-Leu	(4) 100.9 ± 3.5[f]
L-Trp + L-Leu	(4) 65.6 ± 4.7[g]

[a]Rats were tube-fed L-tryptophan (30 mg/100 g body weight), L-leucine (19.2 mg/100 g body weight), or both (same doses) 10 min before killing. Hepatic nuclei were prepared from each group.
[b]Specific binding was derived from total binding minus nonspecific binding (in presence of 10^{-4}M unlabeled L-tryptophan).
[c]In each experiment, specific binding of each control group was set at 100%. The mean values ± SEM for total binding and for specific binding of control (water-treated group) in cpm/mg nuclear protein were 13,924 ± 1,117 and 10,162 ± 826, respectively.
[d]Number of experiments in parentheses. Means ± SEM.
[e]$P < 0.01$, compared with water group.
[f]$P < 0.01$, compared with L-Trp group.
[g]$0.05 > P > 0.01$, compared with L-Trp group.

Table 4 Inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei using excess (10^{-4}M) of L-leucine related compounds alone or with unlabeled L-tryptophan

Unlabeled test compound	Inhibition attributable to: Test compound (%)	Test compound plus L-tryptophan
L-tryptophan	(11) 71.0 ± 1.1	
L-leucine	(4) +7.4 ± 5.9[b]	(3) 8.1 ± 3.7[b,c]
D-leucine	(4) 12.7 ± 2.4[b]	(4) 19.7 ± 2.3[b,d]
α-Ketoisocaproic	(3) 0.4 ± 5.0[b]	(3) 62.2 ± 1.3[b,d]
L-tert-leucine	(3) 16.8 ± 6.7[b]	(3) 63.6 ± 2.1[b,d]
L-Norleucine	(3) 17.9 ± 6.2	(3) 70.1 ± 7.3[b,d]
L-leucinol	(2) 36.7 ± 1.3[b]	(3) 32.0 ± 4.6[b,c]
D-leucinol	(2) 20.3 ± 3.2[b]	(3) 26.5 ± 2.6[b,c]
L-leucine methyl ester	(2) 7.1 ± 1.3[b]	(3) 41.0 ± 0.7[b,d]
L-leucine ethyl ester	(2) 17.4 ± 5.2[b]	(3) 42.9 ± 1.1[b,d]
L-leucine butyl ester	(2) 10.3 ± 2.4	(3) 47.3 ± 0.5[b,d]
L-leucine allyl ester	(2)+6.0 ± 0.9[b]	(2) 43.9 ± 1.6[b,d]
L-leucine benzyl ester	(2) 7.4 ± 1.6[b]	(3) 53.5 ± 0.4[b,d]
L-leucinamide HCl	(2) 9.9 ± 4.7[b]	(3) 30.8 ± 1.4[b,d]
L-leucine-*p*-nitroanilide	(2) 29.6 ± 3.3[b]	(3) 35.0 ± 2.3[b,d]

[a]Number of experiments in parentheses. Means ± SEM. The mean value ± SEM for total binding of control (water-treated group) in cpm/mg nuclear protein was 28,746 ± 4,540.
[b]$P < 0.01$, compared with L-tryptophan group.
[c]$0.05 > P > 0.01$, compared with L-leucine group.
[d]$P < 0.01$, compared with L-leucine group.

and/or L-tryptophan at higher concentrations, the effect of L-leucine was tested also on hepatic nuclear envelopes as well as on hepatic nuclei. The results of three experiments indicated the following effects on ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes respectively when incubated in the presence of indicated unlabeled compounds: L-tryptophan (10^{-4}M) 73.1%, 76.9%; L-leucine (10^{-4}M) 17.5%, 2.7%; L-tryptophan plus L-leucine 22.9%, 11.8%; L-alanine (10^{-4}M) 69.8%, 67.0%; L-alanine plus L-leucine 29.9%, 21.3%. Thus, the effects of L-leucine plus L-tryptophan or L-alanine were similar with nuclei and nuclear envelopes.

In view of the effects of L-leucine on the abrogative effect of unlabeled L-tryptophan on ^{3}H-tryptophan binding to hepatic nuclei in vitro, it was important to determine whether L-leucine itself may bind to hepatic nuclei. In several experiments, we investigated whether L-leucine(2,3,4,5-^{3}H) would specifically bind to hepatic nuclei in vitro. Using the same in vitro assay system as that used for ^{3}H-tryptophan binding to hepatic nuclei, we observed in four experiments that the specific binding (total ^{3}H-leucine binding minus nonspecific binding (binding in the presence of excess, 3,000-fold, unlabeled L-leucine (10^{-4}M)) was 36.8 ± 2.3% (much lower relatively than that for specific ^{3}H-tryptophan binding (68.4 to 71.0%). On calculation, the specific ^{3}H-leucine binding to hepatic nuclei was determined to be 6.16 pmol/mg liver. Addition of excess, unlabeled L-tryptophan (10^{-4}M) essentially did not affect (6.6 ± 2.7%) the ^{3}H-leucine binding to hepatic nuclei.

To determine whether the in vivo administration of L-leucine could influence the stimulatory effect of L-tryptophan on hepatic protein synthesis as previously reported,[3] we investigated whether simultaneous treatment (tube-feeding) with both amino acids or pretreatment with L-leucine before L-tryptophan would alter the responses to that of L-tryptophan treatment alone. *Table 8* summarizes the results. In experiments in which each or both compounds were tube-fed 10 min before killing, ^{14}C-leucine incorporation into protein in vitro using hepatic microsomes revealed a 61.3% increase with L-tryptophan, a 1.5% decrease with L-leucine, and only a 8.1% increase with combined L-tryptophan and L-leucine. In one experiment of longer duration (120 min rather than 10 min), rats were tube-fed water or L-leucine (19.2 mg/100 g body weight) at 0 time and water, L-tryptophan (30 mg/100 g body weight), L-leucine (19.2 mg/100 g body weight) or both at 30 min and all rats were killed at 120 min. The results dealing with protein synthesis in vitro are summarized in *Table 8* and reveal effects similar to those found at 10 min. In one 10 min tube-feeding experiment, we used a mixture of 15 L-amino acids, ^{3}H-labeled instead of ^{14}C-leucine, with microsomes to measure incorporation into proteins in vitro. The hepatic microsomes of the L-tryptophan group incorporated 29% more radioactivity into protein than did the hepatic microsomes of the L-tryptophan plus L-leucine group.

In three experiments we investigated the effect of tube-feeding of L-tryptophan, L-leucine or both, as conducted above for protein synthesis, on hepatic nuclear poly(A)polymerase activity. Within 10 min after administration, the activities (means) for hepatic nuclear poly(A)polymerase (engaged and free forms) expressed as per cent were respectively as follows: control (water) group; 100, 100%; L-tryptophan group, 161.5 ± 14.1, 202.3 ± 69.1%, L-leucine group, 103.4 ± 10.9, 117.6 ± 9.2%, L-tryptophan plus L-leucine, 117.3 ± 10.9, 150.1 ± 3.3%. Under these conditions, the addition of L-leucine to L-tryptophan seemed to cause some decrease, but not a statistically significant one, in the stimulatory response because of L-tryptophan.

Table 5 Inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei using excess (10^{-4}M) of other branched-chain (isoleucine and valine) related compounds or of methionine and choline alone or with unlabeled L-tryptophan

Unlabeled test compound	Inhibition due to Test compound (%)	Test compound plus L-tryptophan (%)
L-tryptophan	(11) 71.0 ± 1.1[a]	
L-isoleucine	(4) 3.9 ± 2.6[b]	(4) 8.5 ± 4.7[b]
D-isoleucine	(3) 0.1 ± 4.4[b]	(2) 52.4 ± 0.9[b]
L-allo-isoleucine	(3) 17.3 ± 11.3[b]	(3) 69.7 ± 3.7[c]
D-allo-isoleucine	(3) +10.4 ± 9.8[b]	(3) 71.6 ± 2.8[c]
DL-α-keto-β-methyl-*n*-valeric acid	(3) +1.7 ± 4.2[b]	(3) 48.8 ± 10.6[c]
(S) · (+) · isoleucinol	(3) 27.5 ± 3.2[b]	(3) 57.2 ± 4.6[c,d]
L-valine	(6) 9.8 ± 1.8[b]	(6) 33.6 ± 9.3[b,c]
D-valine	(3) +12.5 ± 9.9[b]	(2) 30.4 ± 10.4[b,e]
L-norvaline	(5) 52.1 ± 6.9[d]	(4) 67.0 ± 5.2
D-norvaline	(3) 17.8 ± 3.0[b]	(2) 71.1 ± 0.8[c]
α-ketoisovaleric acid	(3) +4.9 ± 5.3[b]	(3) 57.7 ± 1.3[b,c]
L-valinol	(3) +23.3 ± 14.8[b]	(3) 48.1 ± 2.5[b]
L-methionine	(3) 26.8 ± 3.3[b]	(3) 51.1 ± 1.5[b,c]
Choline	(3) 42.5 ± 4.7[b]	(3) 60.6 ± 1.3[b,e]

[a]Number of experiments in parentheses. Means ± SEM. The mean value ± SEM for total binding of control (water-treated group) in cpm/mg nuclear protein was 28,690 ± 4,546.
[b]$P < 0.01$, compared with L-tryptophan group.
[c]$P < 0.01$, compared with specific unlabeled test compound.
[d]$0.05 > P > 0.01$, compared with L-tryptophan group.
[e]$0.05 > P > 0.01$, compared with specific unlabeled test compounds.

Discussion

This study investigated whether L-leucine would influence the effects of L-tryptophan on hepatic protein metabolism. In regard to protein synthesis, the administration of L-tryptophan to animals has been demonstrated to rapidly enhance hepatic protein synthesis.[2–4] Whereas the administration of L-leucine did not affect hepatic protein synthesis, when it was administered together with L-tryptophan it inhibited the stimulation attributable to L-tryptophan alone (*Table 8*). In regard to nuclear L-tryptophan receptor binding, our in vitro assay of ^{3}H-tryptophan (5×10^{-8}M) binding to hepatic nuclei revealed that L-leucine itself did not essentially influence binding. However, when L-leucine was added together with unlabeled L-tryptophan at varying levels, it abrogated the inhibitory effect of the unlabeled L-tryptophan on binding. This effect occurred even at low concentrations of L-leucine and at levels of L-tryptophan considered to be within physiologic concentrations. These alterations in L-tryptophan binding to hepatic nuclei (in vitro as well as in vivo) attributable to L-leucine enabled us to speculate that this abrogation of L-tryptophan binding to nuclei by L-leucine may act to negate the stimulatory effect of L-tryptophan upon hepatic protein synthesis.

In attempting to gain some insight into the mechanism whereby L-leucine acts in an adversary manner to that of L-tryptophan, we have explored a number of possibilities. Although our findings have not revealed the mechanism/s by which L-leucine acts, we have been able to rule out a number of possibilities. First, L-leucine does not compete with L-tryptophan for ^{3}H-tryptophan binding to hepatic nuclei in vitro (*Table 1*). L-leucine (10^{-10}M to 10^{-4}M) added to ^{3}H-tryptophan (5×10^{-8}M) did not affect the binding to hepatic nuclei in vitro. However, L-leucine (10^{-10} to 10^{-4}M) did abrogate the inhibitor effect of unlabeled L-tryptophan (at concentrations of 10^{-7} to 10^{-4}M) on ^{3}H-binding to hepatic nuclei in vitro (*Figure 1*). This indicates that the effect of L-leucine can occur at physiologic concentrations of L-tryptophan. This type of reaction pattern seems to be compatible with a response of an allosteric nature. Second, L-leucine, at high levels of labeled L-leucine (L-(U-^{14}C)-leucine), revealed some specific binding to hepatic nuclei. The addition of L-tryptophan did not affect this specific binding of L-leucine to hepatic nuclei. High affinity binding of L-leucine to hepatic membranes of the chick has been reported.[34] Using L-4-5-^{3}H-leucine (58 Ci/mmol, 5×10^{-8}M), they reported that the specific binding to chick hepatic membranes in vitro was 2 pmol/10 mg tissue. Using hepatic nuclei, we observed that the specific L-leucine binding in vitro (61.6 pmol/10 mg tissue) was appreciably higher than that reported for chick hepatic membrane. The specific interactions between L-leucine and different hepatic organelles are of interest and their possible significance merits study. In regard to our own interest, one may speculate that L-leucine may possibly bind or react at structural sites adjacent to the L-tryptophan receptor on the nuclear envelope, which may then abrogate the inhibitory effect of the unlabeled tryptophan on the nuclear binding of labeled tryptophan. Third, disturbance in transport of L-tryptophan attributable to L-leucine does not seem to be influential. Because the aromatic and branched-chain amino acids use the same major amino acid transport system,[35] it was important to determine whether L-leucine might competitively inhibit the transport of L-tryptophan under our experimental conditions. In a recent report,[36] we determined that the serum and hepatic free tryptophan levels were similar after rats were tube-fed L-tryptophan alone or together with L-leucine under experimental conditions similar to those used in this study (*Table 3* and *8*). Also, in our

Table 6 Inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei using excess (10^{-4}M) of test dipeptides containing branched-chain amino acids

Unlabeled test compound	Inhibition attributable to test compounds Nuclei (%)	Nuclear envelopes (%)
Trp	(18) 69.1 ± 0.86[a]	(11) 66.5 ± 1.87
Trp · Leu	(8) 8.6 ± 1.49[b]	(7) 6.4 ± 2.26[b]
Leu · Trp	(5) 44.2 ± 4.60[b,c]	(7) 35.4 ± 4.53[b,c]
Trp + Trp Leu	(6) 48.9 ± 5.08[b,c]	
Trp + Leu Trp	(5) 35.6 ± 8.04[b]	
Trp · Val	(5) 34.9 ± 4.35[b]	(4) 52.0 ± 5.00[c,d]
Val · Trp	(4) 44.6 ± 5.28[b]	(6) 41.8 ± 5.58[b]

[a]Number of experiments in parentheses. Means ± SEM. The mean values ± SEM for total binding of control (water-treated groups) in cpm/mg nuclear protein were 17,298 ± 640 for nuclei and 5,045 ± 57 for nuclear envelopes.
[b]$P < 0.01$, compared with Trp group.
[c]$P < 0.01$, compared with Trp · Leu group.
[d]$0.05 > P > 0.01$, compared with Trp group.

Table 7 Inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei using test compounds (all to 10^{-4}M)

Test compound/s	Number of experiments	Inhibition attributable to test compound/s (%)	Changes relative to L-tryptophan or L-alanine
L-tryptophan	8	69.4 ± 0.79[a]	
L-leucine	7	5.4 ± 1.78[b]	−92.2
Na selenite	7	49.1 ± 1.48[b]	−29.3
L-tryptophan + L-leucine	5	9.1 ± 5.91[b]	−86.9
Na selenite + L-leucine	4	31.4 ± 4.02[c]	−54.8
L-tryptophan + Na selenite	6	58.0 ± 3.43[b]	−16.4
L-tryptophan + Na selenite + L-leucine	7	40.3 ± 3.29[d]	−41.9
L-alanine	8	64.4 ± 1.56	
L-alanine + L-leucine	6	8.1 ± 3.90[e]	−87.4
L-alanine + Na selenite	7	39.0 ± 7.62[e]	−39.4
L-alanine + Na selenite + L-leucine	7	26.3 ± 6.05	−59.2

[a]Mean ± SEM. The mean value ± SEM of control (water-treated group) in cpm/mg nuclear protein was 17,813 ± 1,522.
[b]$P < 0.01$, compared with L-tryptophan group.
[c]$0.05 > P > 0.01$, compared with Na selenite group.
[d]$0.05 > P > 0.01$, compared with L-tryptophan + Na selenite group.
[e]$P < 0.01$, compared with L-alanine group.

in vitro studies in which only isolated hepatic nuclei or nuclear envelopes were studied, alterations in tissue and cellular transport which could possibly occur in vivo should not have been involved and, therefore, would not influence the nuclear binding of L-tryptophan.

In consideration of what portion of the molecule of L-leucine or of the other branched-chain amino acids is involved in abrogating L-tryptophan's inhibition of ^{3}H-tryptophan binding to hepatic nuclei in vitro, we have investigated the effects of a number of compounds related to each of the branched-chain amino acids (*Tables 4* and *5*). This has enabled us to map which regions of L-leucine, L-isoleucine, or L-valine are involved based upon studies with the following related compounds: in relation to L-leucine; D-leucine, α-ketoisocaproic acid, L-tert-leucine, L-norleucine, D- or L-leucinol, and L-leucine esters; in relation to L-isoleucine; D-isoleucine, D- or L-allo-isoleucine, isoleucinol, and α-keto-β-methyl-*n*-valeric acid; and in relation to L-valine; D-valine, D- or L-norvaline, valinol, and α-ketoisovaleric acid. The amino group of D- or L-leucine, of L-isoleucine, and of D- or L-valine is vital; the CH_3 CH_3 > CH-terminal end of leucine and of valine and the CH_3-CH_2-$\overset{CH}{\underset{CH_3}{}}$-terminal group of isoleucine are necessary; and the esterified acid, the alcohol and the amide groups of leucine appear to diminish somewhat the effectiveness of leucine. Attachment to the amino group of L-leucine, such as in the peptide linkage with L-tryptophan (the dipeptide, Trp · Leu) causes only a small decrease in the inhibition of ^{3}H-tryptophan binding to hepatic nuclei in vitro when it is added to unlabeled L-tryptophan (*Table 6*). On the other hand, the dipeptide Leu · Trp itself or when added to unlabeled L-tryptophan causes a small inhibition of ^{3}H-tryptophan binding to hepatic nuclei (*Table 6*).

Although L-leucine does not stimulate hepatic protein synthesis, L-leucine has been reported to stimulate muscle protein synthesis.[37] Though evidence to date has established that leucine has a specific anabolic effect on the processes of protein synthesis and degradation in muscle, understanding of these processes remains largely at a phenomenological level. On the other hand, L-tryptophan has been found to stimulate liver protein synthesis.[1,3,38] Speculation as to how L-tryptophan affects steps relating to enhanced hepatic protein synthesis has been reviewed.[1,39] In the present study, the administration of L-leucine was observed to reduce the ability of L-tryptophan to stimulate hepatic protein synthesis. Because L-leucine seemed to exert an early inhibitory effect on L-tryptophan's binding to hepatic

Table 8 In vitro ^{14}C-leucine incorporation into proteins of liver of rats treated with L-leucine, L-tryptophan or both

Groups[a]	^{14}C-leucine incorporation into protein[a] (%)
10 min experiment[b]	
Water	(4) 100
L-tryptophan	(4) 161.3 ± 13.5[c]
L-leucine	(4) 98.5 ± 12.3[d]
L-tryptophan + L-leucine	(4) 108.1 ± 9.1
120 min experiment[e]	
Water-Water	100
Water-L-tryptophan	148.5
Water-L-leucine	112.6
Water-L-Tryptophan + L-leucine	127.7
L-leucine-Water	98.3
L-leucine-L-tryptophan	123.5

[a]Livers of 2 to 3 rats per group were pooled in three experiments. Mean absolute values (cpm/mg RNA (microsomal)) for control (water) group was 1,080 for 10 min experiment and 2,659 for 120 min experiment. Values as means ± SEM.
[b]Rats were tube-fed water, tryptophan (30 mg/100 g body weight), L-leucine (19.2 mg/100 g body weight), or both 10 min before killing.
[c]$P > 0.01$, compared with water group.
[d]$0.05 > P > 0.01$, compared with L-tryptophan group.
[e]Rats were tube-fed water or L-leucine at 0 time and water, L-tryptophan, L-leucine or both at 30 min and all rats were killed after 120 min.

nuclei, we have considered that this important action may affect the protein synthetic process. Thus, an imbalance, created by the addition of L-leucine, may establish an environment which becomes inhibitory for L-tryptophan's stimulatory effect on hepatic protein synthesis and thereby this effect is diminished. Understanding the interrelationship between the concentrations of specific essential amino acids as presented in this study may prove to be important in learning how the regulatory role of a specific amino acid, such as L-tryptophan, influences hepatic protein synthesis. The significance of such interrelationships may become understood only after one has established how single indispensable amino acids, such as L-tryptophan, act in a regulatory manner upon cells in the liver and/or other organs. Our recent studies with L-tryptophan have been concerned with this problem.

Of great interest are the findings that a branched-chain amino acid, such as L-leucine, may have a dual effect: it can stimulate muscle protein synthesis, while on the other hand, it may be inhibitory to hepatic protein synthesis (as in the inhibition of stimulation of hepatic protein synthesis induced by L-tryptophan (*Table 8*)). Conceptually, for L-leucine to stimulate muscle protein synthesis most effectively, it might be beneficial that it may simultaneously limit protein synthesis in other active organs, such as liver. Indeed, it is known that many checks and balances exist that serve to regulate protein synthesis in different organs.

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