# Hormonal Influences on Tryptophan Binding to Rat Hepatic Nuclei

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**This study evaluated whether selected hormones, 3,5,3′-triiodothyronine ($T_3$), hydrocortisone (HC), or insulin, would influence the binding of L-tryptophan to rat hepatic nuclei or nuclear envelopes. The first two hormones have nuclear receptors that belong to the same superfamily, while insulin belongs to a different unrelated superfamily of receptors. Previous reports have indicated that the binding of L-tryptophan to hepatic nuclear proteins was saturable, stereospecific, and of high affinity. Also, previous studies showed that administration of L-tryptophan rapidly stimulated hepatic protein synthesis. In this study, we investigated whether each hormone alone or together with unlabeled tryptophan would influence tryptophan binding to rat hepatic nuclei or nuclear envelopes as assayed by in vitro L-5-$^3$H-tryptophan binding. Our results indicate that $T_3$ $10^{-14}$ to $10^{-10}$ mol/L appreciably inhibited in vitro $^3$H-tryptophan binding to hepatic nuclei and $T_3$ $10^{-16}$ to $10^{-4}$ mol/L appreciably ameliorated the inhibitory effect of unlabeled tryptophan ($10^{-4}$ mol/L) on in vitro $^3$H-tryptophan binding. In vivo administration (1 hour) of tryptophan alone stimulated hepatic protein synthesis, but addition of $T_3$ negated such stimulation. Addition of HC $10^{-12}$ to $10^{-4}$ mol/L had no effect and addition of insulin $10^{-16}$ to $10^{-4}$ mol/L had only a small inhibitory effect on in vitro $^3$H-tryptophan binding to rat hepatic nuclei, but each ($10^{-12}$ to $10^{-4}$ mol/L), when added to unlabeled tryptophan ($10^{-4}$ mol/L), diminished the inhibitory binding effect of unlabeled tryptophan alone. Our study indicates that $T_3$ competes with tryptophan for hepatic nuclear tryptophan binding, and it also appears to negate tryptophan's stimulatory effect on hepatic protein synthesis.**


FOR MANY YEARS, our laboratory has been investigating the unique actions of L-tryptophan.[1] Administration of tryptophan to experimental animals rapidly stimulates hepatic protein synthesis.[2,3] This has led to investigations concerned with events that are considered to be involved in this stimulatory process.[1,4,5] One important finding is the ability of tryptophan to rapidly bind to a specific hepatic nuclear receptor.[6,7] Several reports have suggested that such specific binding is intimately involved in the stimulatory process.[4,5,8] For example, $NZBWF_1$ mice have been found to have a low binding affinity of hepatic nuclei for tryptophan and show little or no stimulation of hepatic protein synthesis due to tryptophan, in contrast to the stimulatory response induced by tryptophan in other strains of mice that have a high binding affinity of hepatic nuclei for tryptophan.[5] Also, L-alanine, which competes with L-tryptophan for hepatic nuclear tryptophan binding, negates the stimulatory effect on hepatic protein synthesis due to tryptophan.[4,8]

In view of these previous findings that stress the importance of L-tryptophan binding to a specific nuclear receptor in relation to stimulation of protein synthesis, we have recently turned our attention to whether other internal factors, such as certain hormones, could influence the receptor binding of tryptophan, as well as its stimulatory effect on protein synthesis. We selected to study 3,5,3′-triiodothyronine ($T_3$) and glucocorticoid, two hormones that are part of a group of nuclear proteins, "ligand-responsive transcription factors," that belong to the same protein superfamily as steroid receptors.[9,10] Others[11,12] have reported that selenite, a blocking agent for sulfhydryl groups, had a significant inhibitory effect on the binding characteristics of these hormones on receptors in rat liver. Likewise, we reported previously that selenite inhibited $^3$H-tryptophan binding to its hepatic nuclear receptor.[13] Thus, we concluded that the carbonyl group of L-tryptophan normally reacted with sulfhydryl groups of its receptor protein. This similar response to selenite on receptor binding by the above-mentioned hormones and by L-tryptophan encouraged us to conduct this study, where we investigated whether $T_3$ or HC would influence tryptophan binding to rat hepatic nuclei (in vitro $^3$H-tryptophan binding to nuclei) and, if so, the influence of the effect on tryptophan's ability to stimulate hepatic protein synthesis. Insulin has a different superfamily of receptors and was added as an unrelated additional hormone in this study.

## MATERIALS AND METHODS

### *Animals*

Female Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) with a mean weight of 250 g (range, 225 to 300) were used in the experiments. The rats were maintained in a temperature-controlled room with a 12-hour light/dark cycle. Before the experiments began, the animals were adapted to their quarters and to the diet (Purina Lab Chow #5001; Purina, St Louis, MO) for 1 week or more; rats were then deprived of food overnight but had free access to water. In one experiment, female thyroidectomized Sprague-Dawley rats (Hilltop Lab Animals) were used. In one experiment, male mice of the Swiss strain (Hilltop Lab Animals) and of the $MZBWF_1$/J (NZB/BINJ male × NZW/LacJ female) strain (Jackson Laboratory, Bar Harbor, ME; range, 30 to 40 g) were used. Animals were killed by decapitation. These studies were approved by the institutional animal care and use committee.

### *Chemicals*

The radioactive compound used in the experiments was L-5-$^3$H-tryptophan (radiochemical purity, 98.5%; 1.13 TBq/mmol). The test compounds were as follows: $T_3$ from Sigma Chemical (St Louis, MO), HC sodium succinate (Solu-Cortef) from Upjohn (Kalamazoo, MI), and insulin (Novolin R) regular human insulin injection (recombinant DNA origin) USP from Novo Nordisk Pharmaceuticals (Princeton, NJ). L-Tryptophan from US Biochemicals (Cleveland, OH) was used. Other chemicals were from Sigma Chemical. Radioisotopes, L-5-$^3$H-tryptophan and L-U-$^{14}$C-leucine, were from Amersham/Searle (Arlington Heights, IL).

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*Submitted December 8, 1997; accepted August 4, 1998.*

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*0026-0495/99/4802-0002$10.00/0*

### *Preparation of Nuclei and Nuclear Envelopes*

Immediately after the rats were killed, the liver was removed and placed on ice until homogenization (within 15 minutes). Purified hepatic nuclei were prepared as described by Blobel and Potter.[14] The relative purity of the nuclei was determined previously by light and electron microscopy.[6] Nuclear envelopes of hepatic nuclei were isolated with the procedure of Harris and Milne[15] as modified by Agutter and Gleed[16] and routinely used in this laboratory.[6,7] Purified hepatic nuclei were treated with 0.001 mol/L $NaHCO_3$, digested with DNase I (10 mg/L), and centrifuged on a stepwise sucrose gradient (≤2 mol/L sucrose); the nuclear envelope band at interface (1.5 to 1.8 mol/L sucrose) was then removed. The yield of isolated hepatic nuclei was 0.15 mg nuclear protein/g liver.

### *Binding of $^3$H-Tryptophan to Nuclei or Nuclear Envelopes*

Rat hepatic nuclei (0.15 mg protein) or nuclear envelopes (0.5 mg protein) were incubated with L-5-$^3$H-tryptophan (containing 278 kBq, 0.245 nmol tryptophan per assay, added last) in the absence or presence of a 2,000-fold excess of unlabeled tryptophan ($10^{-4}$ mol/L) or test compound (≤$10^{-4}$ mol/L) in 5 mL at room temperature for 2 hours. These conditions were selected based on our previous findings.[6] The nuclei were incubated in and then washed three times with buffer (0.05 mol/L Tris HCl, pH 7.5, 0.025 mol/L KCl, 0.005 mol/L $MgCl_2$, 0.0001 mol/L phenylmethylsulfonyl fluoride, 0.0002 mol/L dithiothreitol, and 0.25 mol/L sucrose), and the nuclear envelopes were incubated in and then washed two times with buffer (0.05 mol/L Tris HCl, pH 7.5, 0.002 mol/L EDTA, 10% vol/vol glycerol, 0.001 mol/L phenylmethylsulfonyl fluoride, and 0.002 mol/L β-mercaptoethanol). After the final wash, the nuclei or nuclear envelopes were suspended in the appropriate buffer, and radioactivity was measured after addition of a scintillation mixture (Opti Fluor; Packard Instruments, Downers Grove, IL). Binding of $^3$H-tryptophan to hepatic nuclei or nuclear envelopes was expressed as cpm per unit of protein. This total binding (binding in the absence of unlabeled tryptophan or test compound) was then compared with binding in the presence of various concentrations of unlabeled tryptophan, unlabeled test compound, or both.

### *Preparation of Microsomes*

Postmitochondrial supernatants were prepared from liver homogenates of rats from the control and experimental groups and used to prepare microsomes.[3]

### *In Vitro Protein Synthesis*

In all assays, liver microsomes of control and experimental rats and liver cytosol of control (water-treated) rats were used.[3] The incubation mixture in a total volume of 1 mL consisted of 0.2 mL microsomes in TKM (0.05 mol/L Tris HCl, pH 7.5, 0.025 mol/L KCl, and 0 005 mol/L $MgCl_2$), 0.3 mL cytosol prepared from the liver of control animals in 0.125 mol/L sucrose in TKM, and 0.5 mL of a solution containing 0.7 μmol ATP, 0.28 μmol GTP, 7.0 μmol phosphoenolpyruvic acid (tricyclohexyl ammonium salt), 1.5 μmol Tris, pH 7.4, 7.5 μmol $NH_4Cl$, 0.175 μmol $MgCl_2 \cdot 6H_2O$, 0.05 μmol L-[U-$^{14}$C]leucine (18.5 kBq), and 18 μg pyruvate kinase. Samples for in vitro incorporation were incubated for 60 minutes at 37°C in a water bath with mechanical shaking, and the reaction was stopped by addition of 0.1 mL KOH (1N) and kept on ice for 30 minutes. Aliquots (80 μL) were spotted on glass microfiber paper and washed with TCA (10%) three times (first wash contained 0.2% unlabeled L-leucine). The samples were dried, and after addition of a scintillation mixture (Opti Fluor) radioactivity was measured using a liquid scintillation spectrometer (Beckman Instruments, Palo Alto, CA). The protein content was determined as described by Lowry et al.[17]

### *Enzyme Assays*

Nucleoside triphosphatase ([NTPase] $Mg^{2+}$-dependent adenosine triphosphatase, EC 3.6.1.3) was assayed according to the method of Agutter et al.[18] The assay depends on determination of the inorganic phosphate released from the substrate (ATP) during incubation with hepatic nuclei for 30 minutes at 35°C. Poly-(A)polymerase ([PAP] EC 2.4.2.30) activity was measured as described by Jacob et al.[19]

### *Statistics*

Data were analyzed by Student's paired *t* test or ANOVA.[20]

## RESULTS

In the first series of experiments, we investigated whether the addition of $T_3$, HC, or insulin at $10^{-4}$ mol/L affected in vitro $^3$H-tryptophan binding to rat hepatic nuclei. This concentration was selected because it is usually used for the addition of unlabeled tryptophan (2,000-fold excess) to the in vitro $^3$H-tryptophan binding assay.[7] The results summarized in Tables 1 to 3 show that the addition of each hormone affected $^3$H-tryptophan binding to hepatic nuclei compared to that without addition as follows: $T_3$, −20.3%; HC, +0.9%; and insulin, −2.6%. On the other hand, the addition of unlabeled tryptophan ($10^{-4}$ mol/L) in these experiments caused a 67.9% to 69.4% inhibition, which indicates specific tryptophan binding (total binding minus nonspecific binding in the presence of 2,000-fold

**Table 1. Effects of $T_3$ Alone or Together With Unlabeled L-Tryptophan on In Vitro $^3$H-Tryptophan Binding to Hepatic Nuclei**

| Concentration (mol/L) | Inhibition of $^3$H-Trp Binding by Unlabeled Compounds (%) | | |
|---|---|---|---|
| | Trp | $T_3$ | Trp + $T_3$ |
| $10^{-4}$ | (16) 68 3 ± 0.64 | (10) 20.3 ± 4.02* | |
| $10^{-6}$ | | (7) 19.2 ± 3.88 | |
| $10^{-8}$ | | (11) 24.9 ± 3.38 | |
| $10^{-10}$ | | (9) 33.3 ± 3.20† | |
| $10^{-12}$ | | (10) 34.2 ± 4.28† | |
| $10^{-14}$ | | (5) 30.6 ± 7.96 | |
| $10^{-16}$ | | (6) 23.2 ± 8.64 | |
| $10^{-4} + 10^{-4}$ | | | (9) 36.9 ± 5.00* |
| $10^{-4} + 10^{-6}$ | | | (9) 34.4 ± 4.60 |
| $10^{-4} + 10^{-8}$ | | | (12) 42.2 ± 4.70 |
| $10^{-4} + 10^{-10}$ | | | (11) 47.2 ± 4.44 |
| $10^{-4} + 10^{-12}$ | | | (8) 42.0 ± 3.99 |
| $10^{-4} + 10^{-14}$ | | | (5) 43.3 ± 5.51 |
| $10^{-4} + 10^{-16}$ | | | (7) 50.0 ± 2.59‡ |
| $10^{-6} + 10^{-6}$ | | | (4) 18.0 ± 4.17 |
| $10^{-8} + 10^{-8}$ | | | (4) 26.5 ± 2.85 |
| $10^{-10} + 10^{-10}$ | | | (4) 32.7 ± 5.08 |
| $10^{-12} + 10^{-12}$ | | | (5) 40.1 ± 1.69 |
| $10^{-14} + 10^{-14}$ | | | (3) 22.7 ± 12.44 |
| $10^{-16} + 10^{-16}$ | | | (2) 10.7 ± 1.84 |

NOTE. The number of experiments is in parentheses. Values are the mean ± SEM. The mean value for the control (water-treated) group was 20,544 ± 478 cpm/mg nuclear protein and was set at 100% in each experiment ANOVA for the experimental groups yielded F = 11.48 and $P < .01$. Pairwise comparisons were also made using *t* tests, and significance levels were adjusted using the Bonferroni multiple-comparison procedure. $P \leq .01$ was considered highly significant.

*$P < .01$ *v* Trp $10^{-4}$ mol/L.

†$.05 > P > .01$ *v* $T_3$ $10^{-4}$ mol/L.

‡$.05 > P > .01$ *v* Trp $10^{-4}$ mol/L + $T_3$ $10^{-4}$ mol/L.

**Table 2. Effects of HC Alone or Together With Unlabeled L-Tryptophan on In Vitro $^3$H-Tryptophan Binding to Hepatic Nuclei**

| Concentration (mol/L) | Inhibition of $^3$H-Trp Binding by Unlabeled Compounds (%) | | |
|---|---|---|---|
| | Trp | HC | Trp + HC |
| $10^{-4}$ | (8) 67.9 ± 1.20 | (7) +0.9 ± 1.66* | |
| $10^{-6}$ | | (3) +4.2 ± 2.54 | |
| $10^{-8}$ | | (3) +5.9 ± 3.95 | |
| $10^{-10}$ | | (3) +12.6 ± 8.06 | |
| $10^{-12}$ | | (2) +5.0 ± 9.52 | |
| $10^{-4} + 10^{-4}$ | | | (7) 18.5 ± 3.05* |
| $10^{-4} + 10^{-6}$ | | | (4) 17.7 ± 6.58* |
| $10^{-4} + 10^{-8}$ | | | (6) 28.8 ± 5.35* |
| $10^{-4} + 10^{-10}$ | | | (4) 38.0 ± 11.21† |
| $10^{-4} + 10^{-12}$ | | | (4) 39.1 ± 1.55* |
| $10^{-4} + 10^{-14}$ | | | (2) 52.9 ± 4.17‡ |
| $10^{-4} + 10^{-16}$ | | | (2) 62.6 ± 4.88* |
| $10^{-6} + 10^{-6}$ | | | (3) 17.6 ± 8.58 |
| $10^{-8} + 10^{-8}$ | | | (3) 6.9 ± 2.90 |
| $10^{-10} + 10^{-10}$ | | | (3) 9.3 ± 3.22 |
| $10^{-12} + 10^{-12}$ | | | (3) 11.0 ± 7.85 |

NOTE. The number of experiments is in parentheses. Values are the mean ± SEM. The mean value for the control (water-treated) group was 21,489 ± 907 cpm/mg nuclear protein and was set at 100% in each experiment. ANOVA for the experimental groups yielded F = 21.4 and $P < .01$. Pairwise comparisons were also made using *t* tests, and significance levels were adjusted using the Bonferroni multiple-comparison procedure. $P \leq .006$ was considered highly significant.

*$P < .001$ *v* Trp $10^{-4}$ mol/L.

†$.05 > P > .01$ *v* Trp $10^{-4}$ mol/L.

‡$P < .01$ *v* Trp $10^{-4}$ mol/L + HC $10^{-4}$ mol/L.

excess of unlabeled tryptophan). Next, we investigated the effects of the addition of each hormone ($10^{-4}$ mol/L) combined with unlabeled tryptophan ($10^{-4}$ mol/L) to the in vitro binding assay. The results showed percent inhibition due to the combinations as follows (with tryptophan $10^{-4}$ mol/L): $T_3$, 36.9%; HC, 18.5%; insulin, 12.6%. These results indicate that the addition of each hormone at $10^{-4}$ mol/L diminishes to varying degree the inhibitory effect of unlabeled tryptophan ($10^{-4}$ mol/L) alone (67.9% to 69.4%) on in vitro $^3$H-tryptophan binding to rat hepatic nuclei.

Because it is known that hormones are effective biologically at low concentrations, it became necessary to quantify the effects of each hormone at levels less than $10^{-4}$ mol/L. These results are summarized in Tables 1 to 3. Results for varying concentrations of tryptophan alone are given in Fig 1. The findings with $T_3$ indicate that $T_3$ alone at $10^{-10}$ to $10^{-14}$ mol/L inhibited in vitro $^3$H-tryptophan binding to rat hepatic nuclei by 31% to 34%, a greater inhibition than obtained at $10^{-4}$ or $10^{-6}$ mol/L. When $T_3$ was added along with unlabeled tryptophan ($10^{-4}$ mol/L), the inhibition of $^3$H-tryptophan binding increased from 36.9% at $10^{-4}$ mol/L $T_3$ to 50.0% at $10^{-16}$ mol/L $T_3$. When both $T_3$ and unlabeled tryptophan were varied from $10^{-4}$ to $10^{-16}$ mol/L, a biphasic inhibitory response was observed in relation to binding inhibition (Fig 1). The findings with HC indicate that HC alone had essentially no inhibitory effect (+0.9% to +12.6%) at concentrations from $10^{-4}$ to $10^{-12}$ mol/L. When HC was added along with unlabeled tryptophan ($10^{-4}$ mol/L), the inhibition of $^3$H-tryptophan binding progressively increased due to HC ($10^{-4}$ to $10^{-16}$ mol/L) from 18.5% to 62.6%, approaching the level (67.9%) of inhibition due to unlabeled tryptophan ($10^{-4}$ mol/L) alone. When both HC and unlabeled tryptophan were varied from $10^{-4}$ to $10^{-12}$ mol/L, the inhibitory responses were similar (18.5% to 11.0%). The findings with insulin indicate that all concentrations ($10^{-4}$ to $10^{-16}$ mol/L) tested showed 2.6% to 24.2% inhibition (more at $10^{-6}$ to $10^{-10}$ mol/L *v* $10^{-4}$ or $10^{-12}$ and $10^{-16}$ mol/L). When insulin was added along with unlabeled tryptophan ($10^{-4}$ mol/L), the inhibition of $^3$H-tryptophan binding was essentially similar at $10^{-4}$ to $10^{-12}$ mol/L (12.6% to 16.9%), but at $10^{-16}$ mol/L it was 37.4%. When both insulin and unlabeled tryptophan were varied from $10^{-4}$ to $10^{-10}$ mol/L, the inhibitory responses were similar (from 11.5% to 12.5%).

Figure 1 summarizes graphically the results of varying concentrations of $T_3$ alone, unlabeled tryptophan alone, or $T_3$ together with unlabeled tryptophan on in vitro $^3$H-tryptophan binding to hepatic nuclei. When $T_3$ alone was tested, it caused moderate (19% to 34%) inhibition at $10^{-16}$ to $10^{-4}$ mol/L, with the greatest inhibition (31% to 34%) at $10^{-14}$ to $10^{-10}$ mol/L. When similar varying concentrations of $T_3$ together with varying concentrations of unlabeled tryptophan were added, $T_3$ plus unlabeled tryptophan caused moderate inhibition, similar to that of $T_3$ alone. When varying concentrations of $T_3$ together with a constant concentration of unlabeled tryptophan ($10^{-4}$ mol/L) were added, a marked inhibition persisted from $10^{-16}$ to $10^{-8}$ mol/L $T_3$, and then somewhat less at $10^{-6}$ to $10^{-4}$ mol/L $T_3$. Tryptophan alone showed progressively greater inhibition of binding with increasing concentrations of unlabeled tryptophan, with an $IC_{50}$ of $10^{-8}$ mol/L, similar to findings reported previously.[6,7] When comparing $T_3$ alone with unlabeled trypto-

**Table 3. Effects of Insulin Alone or Together With Unlabeled L-Tryptophan on In Vitro $^3$H-Tryptophan Binding to Hepatic Nuclei**

| Concentration (mol/L) | Inhibition of $^3$H-Trp Binding by Unlabeled Compounds (%) | | |
|---|---|---|---|
| | Trp | Insulin | Trp + Insulin |
| $10^{-4}$ | (8) 69.4 ± 0.92 | (7) 2.6 ± 3.04* | |
| $10^{-6}$ | | (5) 16.0 ± 7.98 | |
| $10^{-8}$ | | (7) 12.4 ± 5.82 | |
| $10^{-10}$ | | (6) 24.2 ± 8.36† | |
| $10^{-12}$ | | (6) 2.3 ± 2.22 | |
| $10^{-16}$ | | (3) 8.4 ± 0.11 | |
| $10^{-4} + 10^{-4}$ | | | (6) 12.6 ± 5.71* |
| $10^{-4} + 10^{-6}$ | | | (4) 12.7 ± 8.55 |
| $10^{-4} + 10^{-8}$ | | | (7) 15.4 ± 5.31 |
| $10^{-4} + 10^{-10}$ | | | (4) 16.9 ± 10.78 |
| $10^{-4} + 10^{-12}$ | | | (5) 16.1 ± 7.35 |
| $10^{-4} + 10^{-16}$ | | | (3) 37.4 ± 3.15‡ |
| $10^{-6} + 10^{-6}$ | | | (4) 12.5 ± 9.38 |
| $10^{-8} + 10^{-8}$ | | | (4) 11.5 ± 9.64 |
| $10^{-10} + 10^{-10}$ | | | (4) 12.3 ± 6.65 |

NOTE. The number of experiments is in parentheses. Values are the mean ± SEM. The mean value for the control (water-treated) group was 21,058 ± 775 cpm/mg nuclear protein and was set at 100% in each experiment. ANOVA for the experimental groups yielded F = 8.80 and $P < .01$. Pairwise comparisons were also made using *t* tests, and significance levels were adjusted using the Bonferroni multiple-comparison procedure. $P \leq .0125$ was considered highly significant.

*$P < .01$ *v* Trp $10^{-4}$ mol/L.

†$.05 > P > .01$ *v* Trp $10^{-4}$ mol/L.

‡$P < .01$ *v* Trp $10^{-4}$ mol/L + insulin $10^{-4}$ mol/L.

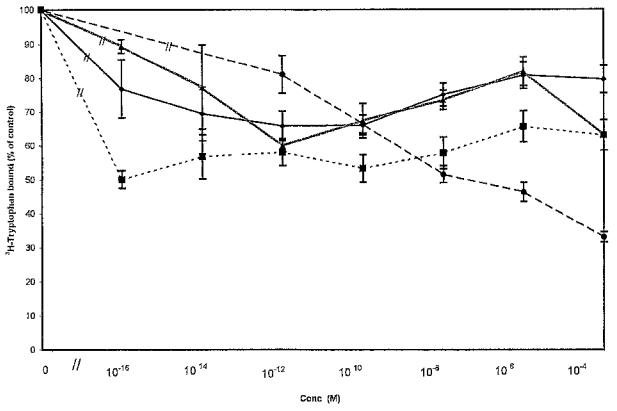


**Fig 1. Effects of various concentrations of L-tryptophan (●---●), 3,5,3′-triiodothyronine ($T_3$) (◆—◆) or both on $^3$H-tryptophan binding to rat hepatic nuclei. When both agents were used, in one case L-tryptophan was kept constant at $10^{-4}$ mol/L with variable levels of $T_3$ (■ · · · · · ■) and in the second case, both were equally and simultaneously varied from ($10^{-16}$ to $10^{-4}$ mol/L). (▲—▲).**

phan alone, $T_3$ at low concentrations ($10^{-12}$ mol/L or less) appeared to induce greater inhibition than unlabeled tryptophan alone at comparable concentrations, but $T_3$ alone at higher concentrations ($10^{-8}$ to $10^{-4}$ mol/L) caused less inhibition than unlabeled tryptophan alone at similar concentrations.

The saturation isotherm for $^3$H-tryptophan binding using L-tryptophan ($10^{-4}$ mol/L) with or without added $T_3$ ($10^{-10}$ mol/L) is indicated in Fig 2. It is apparent that at all concentrations of added $^3$H-tryptophan (0.19 to 15 μCi), the $T_3$ added ($10^{-10}$ mol/L) to L-tryptophan ($10^{-4}$ mol/L) diminished the specific binding to hepatic nuclei. Scatchard plots appear to indicate that the addition of $T_3$ to tryptophan decreased the $B_{max}$ value but did not change the $K_D$ value.

All of the preceding studies were performed with in vitro $^3$H-tryptophan binding to hepatic nuclei. It became of interest to determine whether similar effects would occur with nuclear

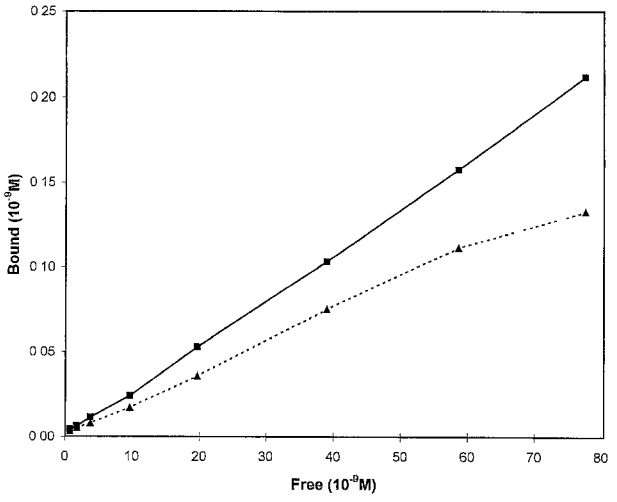


**Fig 2. Saturation isotherm for $^3$H-tryptophan binding to rat hepatic nuclei in vitro using unlabeled L-tryptophan ($10^{-4}$ mol/L) without (■—■) or with added $T_3$ ($10^{-10}$ M) (▲ · · · · · · ▲). Each point represents mean of values of 2 experiments. $^3$H-tryptophan used ranged from 1.25 to 100 nmol/L.**

envelopes instead of nuclei. The results of one to five experiments (Table 4) indicated that the findings with nuclear envelopes were essentially the same as with nuclei for experiments with $T_3$ (Table 1) and HC (Table 2). However, with insulin, the effects on nuclear envelopes were manyfold (two-fold to 10-fold) higher (in percent inhibition of $^3$H-tryptophan binding) than with nuclei when using $10^{-8}$ to $10^{-4}$ mol/L insulin (Table 3). Also, when insulin was added to unlabeled tryptophan ($10^{-4}$ mol/L), the percent inhibition was threefold to fourfold greater than with comparable assays with nuclei (Table 3).

To investigate further the effects of $T_3$ on $^3$H-tryptophan binding to hepatic nuclei, we investigated whether pretreatment with $T_3$ and posttreatment with $T_3$ affects $^3$H-tryptophan binding to hepatic nuclei. Table 5 summarizes these data. In the pretreatment experiments, incubation of nuclei with $T_3$ ($10^{-10}$ mol/L) before adding unlabeled tryptophan did appreciably negate the $^3$H-tryptophan binding effect of unlabeled tryptophan alone on nuclei (17.8% *v* 69.2% inhibition). However, the

**Table 4. Effects of $T_3$, HC, or Insulin Without or With L-Tryptophan on In Vitro $^3$H-Tryptophan Binding to Hepatic Nuclear Envelopes**

| | Inhibition of $^3$H-Trp Binding by Unlabeled Compounds Using Hormones (%) | | |
|---|---|---|---|
| Treatment (mol/L) | $T_3$ | HC | Insulin |
| Trp $10^{-4}$ alone | (5) 66.2 ± 1.8 | (3) 67.1 ± 1.2 | (5) 67.3 ± 0.7 |
| Hormone $10^{-4}$ | (3) 15.9 ± 11.6* | (3) 0.4 ± 1.4* | (5) 30.5 ± 2.0* |
| Hormone $10^{-6}$ | (2) 28.8 ± 2.6* | | (2) 31.2 ± 1.2* |
| Hormone $10^{-8}$ | (2) 41.2 ± 4.2* | (1) 13.3 | (5) 38.1 ± 0.8* |
| Hormone $10^{-10}$ | (3) 42.0 ± 3.0* | | (2) 22.5 ± 6.6* |
| Hormone $10^{-12}$ | (2) 23.2 ± 0.4* | (1) 17.2 | (3) 9.7 ± 0.9* |
| Hormone $10^{-14}$ | (2) 25.8 ± 9.6* | | |
| Hormone $10^{-16}$ | (2) 23.4 ± 0.5* | | |
| Trp $10^{-4}$ + hormone $10^{-4}$ | (3) 26.9 ± 6.9* | (3) 18.9 ± 2.6* | (5) 36.9 ± 3.5 |
| Trp $10^{-4}$ + hormone $10^{-6}$ | (1) 47.1 | | (2) 41.7 ± 1.6 |
| Trp $10^{-4}$ + hormone $10^{-8}$ | (1) 40.6 | | (5) 55.1 ± 4.0 |
| Trp $10^{-4}$ + hormone $10^{-10}$ | (2) 41.8 ± 5.0 | | (2) 58.0 ± 2.7 |
| Trp $10^{-4}$ + hormone $10^{-12}$ | (1) 34.5 | | (3) 59.8 ± 3.7 |
| Trp $10^{-4}$ + hormone $10^{-14}$ | (1) 57.4 | | |
| Trp $10^{-4}$ + hormone $10^{-16}$ | (1) 59.3 | | |
| Trp $10^{-6}$ + hormone $10^{-6}$ | (3) 48.7 ± 4.8 | | (2) 24.6 ± 1.5 |
| Trp $10^{-8}$ + hormone $10^{-8}$ | (3) 47.5 ± 0.9 | (1) 30.2 | (2) 37.8 ± 2.1 |
| Trp $10^{-10}$ + hormone $10^{-10}$ | (2) 40.3 ± 1.9 | | (2) 37.4 ± 0.6 |
| Trp $10^{-12}$ + hormone $10^{-12}$ | (2) 39.9 ± 1.6 | (1) 30.7 | (2) 30.9 ± 4.2 |
| Trp $10^{-14}$ + hormone $10^{-14}$ | (1) 24.6 | | |

NOTE. Values are the mean ± SEM. The number of experiments is in parentheses. The mean value for the control (water-treated) group was 5,984 ± 390 cpm/mg nuclear protein and was set at 100% in each experiment.

*$P < .01$ *v* Trp $10^{-4}$ mol/L.

**Table 5. Effects of $T_3$ Addition Before or After $^3$H-Tryptophan Binding to Rat Hepatic Nuclei**

| Incubation Conditions* | | | | $^3$H-Trp Binding to Hepatic Nuclei | |
|---|---|---|---|---|---|
| 0 | 0 5 h | 1 h | 2 h | cpm/mg Protein | % Change |
| Effects before binding | | | | | |
| $H_2O$ | $H_2O$ | $^3$H-Trp | Stop | (2) 22,334 ± 2,019 | |
| $H_2O$ | Trp | $^3$H-Trp | Stop | (2) 6,877 ± 566† | −69.2 |
| $H_2O$ | $T_3$ | $^3$H-Trp | Stop | (2) 17,527 ± 1,235 | −21.5 |
| Trp | $H_2O$ | $^3$H-Trp | Stop | (2) 8,063 ± 863† | −63.9 |
| Trp | $T_3$ | $^3$H-Trp | Stop | (2) 8,594 ± 108† | −61.5 |
| $T_3$ | $H_2O$ | $^3$H-Trp | Stop | (2) 18,130 ± 1,969 | −18.8 |
| $T_3$ | Trp | $^3$H-Trp | Stop | (2) 18,358 ± 2,290‡ | −17.8 |
| Trp + $T_3$ | $H_2O$ | $^3$H-Trp | Stop | (2) 18,409 ± 2,325 | −17.6 |

| 0 | 2 h§ | 2.25 h | Total | Nonspecific | Specific‖ |
|---|---|---|---|---|---|
| Effects after binding | | | | | |
| $H_2O$ | 0 | Stop | | | |
| Trp | 0 | Stop | (3) 21,930 ± 1,733 | 6,830 ± 483 | 15,101 ± 1,251 |
| $H_2O$ | $H_2O$ | Stop | | | |
| Trp | $H_2O$ | Stop | (4) 20,375 ± 674 | 7,659 ± 533 | 12,716 ± 697¶ |
| $H_2O$ | Trp | Stop | | | |
| Trp | Trp | Stop | (3) 9,751 ± 1,743# | 8,658 ± 89 | 916 ± 370# |
| $H_2O$ | $T_3$ | Stop | | | |
| Trp | $T_3$ | Stop | (4) 17,577 ± 767 | 9,165 ± 1,670 | 8,412 ± 1,471# |

NOTE. Results are the mean ± SEM. The number of experiments is in parentheses.

*Trp was added at $10^{-4}$ mol/L and $T_3$ was added at $10^{-10}$ mol/L.

† $.05 > P > .01$ *v* control ($H_2O$, $H_2O$) group.

‡ $.05 > P > .01$ *v* $H_2O$, $T_3$ group.

§Incubated for 2 h using regular incubation media with indicated additions plus $^3$H-Trp. Nuclear pellets were then centrifuged, washed 2 times, resuspended in incubation medium with indicated additions, and incubated for .25 h.

‖Total binding was obtained from control ($H_2O$) groups. Nonspecific binding was obtained from Trp groups. Specific binding was obtained by subtracting nonspecific binding from total binding of appropriate groups.

¶ $.05 > P > .01$ *v* control ($H_2O$) group.

# $P < .01$ *v* control ($H_2O$) group.

addition of $T_3$ 0.5 hours after unlabeled tryptophan did not have such an effect. Thus, the effect of $T_3$ was marked when added prior to unlabeled tryptophan. In experiments on the effect of $T_3$ on binding breakdown, addition of $T_3$ ($10^{-10}$ mol/L) for 15 minutes after 2 hours of binding incubation caused a decrease in specific binding (12,716 to 8,412, 34%), indicating enhancement of binding breakdown. This breakdown effect was significant, but was less than the effect obtained by adding unlabeled tryptophan ($10^{-4}$ mol/L) for 15 minutes after a 2-hour binding incubation. Thus, $T_3$ appears to affect tryptophan binding and the tryptophan binding breakdown of hepatic nuclei.

In view of prior reports[21,22] that the addition of certain protease inhibitors was inhibitory to the binding of $T_3$ to rat hepatic nuclei, we investigated the effects of each of two protease inhibitors on in vitro $^3$H-tryptophan binding to rat hepatic nuclei. The results of these experiments are summarized in Table 6. Using two protease inhibitors (inhibitors of chymotrypsin), *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and *N*-CBZ-L-phenylalanine chloromethyl ketone (ZPCK), at $10^{-4}$ mol/L, we observed that each inhibitor did not appreciably affect (8.2% and 9.4% inhibition, respectively) $^3$H-tryptophan binding to hepatic nuclei. However, when the protease inhibitors were added with unlabeled tryptophan ($10^{-4}$ mol/L), they diminished (29.8% or 25.7%, respectively) the inhibition compared with unlabeled tryptophan alone. While $T_3$ ($10^{-10}$ mol/L) itself caused 40.7% inhibition in $^3$H-tryptophan binding (compared with 67.5% inhibition due to unlabeled tryptophan $10^{-4}$ mol/L), the addition of each protease inhibitor to $T_3$ caused a diminution of the inhibition of $^3$H-tryptophan binding (−71.7% or −48.9%, respectively). The addition of $T_3$ to unlabeled tryptophan caused a 44.5% inhibition of binding, which in-

**Table 6. Effects of Protease Inhibitors, $T_3$, and/or Unlabeled L-Tryptophan on In Vitro $^3$H-Tryptophan Binding to Hepatic Nuclei**

| Group (mol/L) | Inhibition of $^3$H-Trp Binding by Unlabeled Compounds (%) |
|---|---|
| Trp $10^{-4}$ | (4) 67.5 ± 0.90 |
| TPCK $10^{-4}$ | (3) 8.2 ± 0.27* |
| ZPCK $10^{-4}$ | (2) 9.4 ± 4.88* |
| Trp $10^{-4}$ + TPCK $10^{-4}$ | (3) 47.4 ± 2.17* |
| Trp $10^{-4}$ + ZPCK $10^{-4}$ | (2) 50.1 ± 6.36 |
| $T_3$ $10^{-10}$ | (4) 40.7 ± 0.83 |
| $T^3$ $10^{-10}$ + TPCK $10^{-4}$ | (3) 11.5 ± 4.28† |
| $T_3$ $10^{-10}$ + ZPCK $10^{-4}$ | (2) 20.8 ± 5.16‡ |
| Trp $10^{-4}$ + $T_3$ $10^{-10}$ | (4) 44.5 ± 3.66* |
| Trp $10^{-4}$ + $T_3$ $10^{-10}$ + TPCK $10^{-4}$ | (3) 56.2 ± 1.93§ |
| Trp $10^{-4}$ + $T_3$ $10^{-10}$ + ZPCK $10^{-4}$ | (2) 55.7 ± 6.43 |

NOTE. Results are the mean ± SEM. The number of experiments is in parentheses. The mean value for the control (water-treated) group was 21,075 ± 1,042 cpm/mg nuclear protein and was set at 100% in each experiment.

*$P < .01$ *v* Trp $10^{-4}$ mol/L.

†$P < .01$ *v* $T_3$ $10^{-10}$ mol/L.

‡ $.05 > P > .01$ *v* $T_3$ $10^{-10}$ mol/L.

§ $.05 > P > .01$ *v* Trp $10^{-4}$ mol/L + $T_3$ $10^{-4}$ mol/L.

creased to 56.2% or 55.7% inhibition by adding the protease inhibitors. These findings indicate that protease inhibitors negate the effects predominantly due to $T_3$ as they relate to the inhibition of $^3$H-tryptophan binding to hepatic nuclei.

In one group of experiments, we investigated whether administration of $T_3$ to overnight-fasted rats (control or tryptophan-treated) affects hepatic nuclear tryptophan binding and protein synthesis assayed in vitro after killing the control and experimental (tryptophan-treated) rats. The results of these experiments are summarized in Table 7. Control (water-treated) rats were tested, and the values for in vitro $^{14}$C-leucine incorporation in hepatic proteins by microsomes and $^3$H-tryptophan binding to hepatic nuclei (total and specific (value obtained by subtracting nonspecific binding [binding in the presence of excess unlabeled tryptophan] from the total) were set as baseline (100% for protein synthesis and total binding and 65.0% for specific binding). For hepatic protein synthesis, tryptophan administration alone for 60 minutes caused a 76% enhancement and treatment with $T_3$ alone or together with tryptophan for 60 minutes showed essentially no change compared with controls. Tryptophan administered 30 minutes after $T_3$ caused a slight but insignificant increase of 17%. For $^3$H-tryptophan binding to hepatic nuclei, both total and specific binding showed appreciable decreases after tryptophan alone, but only moderate decreases in the other three groups (Table 7).

In two experiments, we investigated the effect of administering tryptophan, $T_3$, or both, as before for protein synthesis, on hepatic nuclear PAP activity. The results showed that at 60 minutes after administration, the activity (mean) for hepatic nuclear PAP (engaged and free forms) expressed as a percentage were respectively as follows: control (water), 100% and 100%; tryptophan, 183.8% ± 1.98% and 213.8% ± 19.87%; $T_3$, 101.7% ± 2.40% and 98.9% ± 1.48%; and tryptophan plus $T_3$, 85.2% ± 5.94% and 67.1% ± 3.46%. The mean value for nuclear PAP activity of the control (water) group for the engaged form was 18.9 μmol $P_i$/h/mg protein, and for the free form, 32.9 μmol $P_i$/h/mg protein. Under these conditions, the addition of $T_3$ to tryptophan seemed to cause a significant decrease ($P < .01$) in the stimulatory response due to tryptophan. Also, the findings for the activity (mean) of hepatic nuclear NTPase expressed as a percentage were respectively as follows: control (water), 100%; tryptophan, 157.2% ± 8.98%; $T_3$, 97.4% ± 3.68%; and tryptophan plus $T_3$, 136.1% ± 1.91%. The mean value for nuclear NTPase for the control (water) group was 12.5 μmol $P_i$/h/mg protein. The addition of $T_3$ to tryptophan seemed to cause a small but not statistically significant decrease, in the stimulatory activity of NTPase due to tryptophan.

Since the preceding experiments revealed the effects of administering $T_3$ to normal rats in relation to their response to tryptophan, it became of interest to investigate whether diminished $T_3$ levels as induced by thyroidectomy would have any effect. After thyroidectomy (2 to 5 weeks), the rats were killed and the binding affinity of their isolated nuclei were assayed along with those of control (normal) rats. The results of three experiments showed that the responses were similar in both groups. Results expressed as percent inhibition of $^3$H-tryptophan binding due to varying concentrations of unlabeled tryptophan or $T_3$ of hepatic nuclei of control and thyroidectomized rats were respectively as follows: $10^{-4}$ mol/L tryptophan, 66.9% and 64.8%: $10^{-8}$ mol/L tryptophan, 26.1% and 30.7%; $10^{-4}$ mol/L $T_3$, 9.3% and 13.8%; $10^{-8}$ mol/L $T_3$, 26.5% and 26.5%; $10^{-12}$ mol/L $T_3$, 45.2% and 45.9%; $10^{-4}$ mol/L tryptophan + $10^{-4}$ mol/L $T_3$, 28.8% and 22.0%; $10^{-4}$ mol/L tryptophan + $10^{-8}$ mol/L $T_3$, 43.3% and 48.0%; and $10^{-4}$ mol/L tryptophan + $10^{-12}$ mol/L $T_3$, 35.0% and 39.8%. In one experiment, we measured serum $T_3$ levels of control and thyroidectomized rats: the level was 2.25 μg/mL in controls and less than 1 μg/mL in experimental rats.

In one experiment, we determined how hepatic nuclei from Swiss and $NZBWF_1$ mice responded to in vitro $^3$H-tryptophan binding in the presence of $T_3$, HC, or insulin. The results expressed as percent inhibition of $^3$H-tryptophan binding due to different concentrations of unlabeled tryptophan or to $T_3$, HC, or insulin to hepatic nuclei of Swiss and $NZBWF_1$ mice were respectively as follows: $10^{-4}$ mol/L tryptophan, 72.9% and 37.9%; $10^{-4}$ mol/L $T_3$, 13.7% and 36.0%; $10^{-10}$ mol/L $T_3$, 34.2% and 31.1%; $10^{-12}$ mol/L $T_3$, 39.3% and 28.4%; $10^{-4}$ mol/L tryptophan + $10^{-10}$ mol/L $T_3$, 48.7% and 36.0%; $10^{-10}$ mol/L HC, 7.3% and 13.4%; $10^{-4}$ mol/L trp + $10^{-10}$ mol/L HC, 20.1% and 19.7%; $10^{-10}$ mol/L insulin, 18.3% and 25.5%; and $10^{-4}$ mol/L tryptophan + $10^{-10}$ mol/L insulin, 32.6% and 32.7%. The marked difference in tryptophan binding affinity for liver nuclei of Swiss and $NZBWF_1$ mice has been reported.[5] The

**Table 7. Effects of $T_3$ Treatment on L-Tryptophan–Induced Stimulation of In Vitro $^{14}$C–Leucine Incorporation Into Proteins of Rat Liver and on In Vitro $^3$H-Trp Binding to Hepatic Nuclei**

| Treatment* | | | $^{14}$C-Leucine Incorporation into Protein (%) | $^3$H-Trp Binding to Hepatic Nuclei (%) | |
|---|---|---|---|---|---|
| 0 | 30 min | 60 min | | Total | Specific |
| Water | 0 | Kill | 100† | 100‡ | 65.0 ± 1.8 |
| Trp (5 mg/100 g BW) | 0 | Kill | 176.1 ± 11.3§ | 45.3 ± 2.3‖ | 22.2 ± 1.2‖ |
| $T_3$ (50 ng/100 g BW) | 0 | Kill | 102.9 ± 6.7¶ | 65.0 ± 4.8§# | 40.6 ± 6.3§# |
| Trp + $T_3$ | 0 | Kill | 96.9 ± 7.2¶ | 56.8 ± 7.4 | 35.7 ± 5.2 |
| $T_3$ | Trp | Kill | 116 7 ± 8.6# | 66.7 ± 8.7 | 41.9 ± 7.7 |

NOTE. Results are the mean ± SEM.

*Rats were fasted overnight and treated as indicated in the morning. Water or Trp were tube-fed and $T_3$ was administered intraperitoneally.

†100% represents 29,098 ± 2,587 cpm/mg microsomal RNA of 3 experiments. This value represents 33 pmol leucine incorporated into protein.

‡100% represents 20,922 ± 1,232 cpm/mg nuclear protein of 3 experiments.

§$.05 > P > .01$ *v* control (water).

‖$P < .01$ *v* control (water).

¶$P < .01$ *v* Trp.

#$.05 > P > .01$ *v* Trp.

current findings indicate that the responses to the added hormones of $^3$H-tryptophan binding in hepatic nuclei are markedly diminished in $NZBWF_1$ mice compared with Swiss mice, which responded like rats.

## DISCUSSION

Nuclear receptors have been reported for a variety of compounds. The nuclear receptors for steroid hormones have been extensively investigated, and the complexity of the actions of these hormones on a high number of physiologic and pathologic processes is apparent.[23] Receptors for glucocorticoids and for $T_3$ are part of a group of nuclear proteins, ligand-responsive transcription factors, that belong to the same superfamily as steroid receptors.[9,10] In our laboratory, we have reported a specific nuclear receptor in liver for L-tryptophan[5,6] and attributed its action to the ability of L-tryptophan to stimulate the nuclear efflux of mRNA[24,25] and to the enhancement of protein synthesis.[2,3] In a recent study,[12] we observed that selenite, a catalyst of the oxidation of sulfhydryl groups, had an inhibitory effect on hepatic nuclear binding of tryptophan,[12] an effect similar to that reported previously for glucocorticoid[11] and $T_3$.[12] Thus, we were encouraged to determine whether commonalities may exist in relation to the binding affinity of L-tryptophan and glucocorticoid or $T_3$ to rat hepatic nuclei and also their actions. Since it has been considered that steroid and thyroid hormones may exert their effects through fundamentally similar mechanisms,[9,26] it became of interest to investigate whether the nuclear tryptophan receptor may also be similarly related to other nuclear receptors and whether possible commonalities between their actions exist. In the present study, we investigated whether $T_3$, HC, or insulin would compete with or inhibit the hepatic nuclear receptor binding of tryptophan. Of special interest is our observation that $T_3$ at low concentrations ($10^{-14}$ to $10^{-12}$ mol/L) did inhibit (compete for) in vitro $^3$H-tryptophan binding to hepatic nuclei, more so than similar concentrations of unlabeled tryptophan (Fig 1), but this inhibition decreased at higher concentrations ($10^{-8}$ to $10^{-4}$ mol/L) of $T_3$ (Table 1). Also, it appeared that $T_3$ administered in vivo had an effect on hepatic nuclear tryptophan binding when measured in vitro (Table 7). $T_3$ administration in vivo negated the stimulation of hepatic protein synthesis due to L-tryptophan (Table 7). Unlike the effects of $T_3$, HC and insulin did not appreciably inhibit in vitro $^3$H-tryptophan binding to hepatic nuclei but did appear to diminish the inhibitory effects of unlabeled tryptophan when both the hormone plus unlabeled tryptophan were added together (Tables 2 and 3), effects similar to those reported previously with L-leucine.[27]

In considering whether proper and adequate concentrations of $T_3$ were used in our assays, we found that Brtko et al,[21,22] assaying in vitro $^{125}$I-$T_3$ binding to hepatic nuclear receptors, used $1.15 \times 10^{-11}$ mol/L labeled $T_3$ to obtain total binding and added $3 \times 10^{-7}$ mol/L unlabeled $T_3$ to obtain nonspecific binding, which accounted for about 10% of total binding. Earlier, Eberhardt et al[28] indicated that the in vitro binding of $T_3$ to hepatic nuclei was linear over the concentration range of $5 \times 10^{-11}$ to $5 \times 10^{-9}$ mol/L. These studies indicate that in vitro $T_3$ binding to its nuclear receptor occurs effectively at very low concentrations of $T_3$, and the similar concentrations of $T_3$ used in the present study were able to affect tryptophan binding to nuclear receptors. However, the reason that higher concentrations of $T_3$ caused a lesser response is not clear. The failure to observe differences for in vitro $^3$H-tryptophan binding to hepatic nuclei of control and thyroidectomized rats in our experiments is not surprising, since others have reported that the binding capacity of $T_3$ in extracts of liver from hypothyroid animals was similar to that from euthyroid animals.[29]

Our laboratory first reported on the unique action of tryptophan on hepatic polyribosomes and protein synthesis many years ago.[2,3] Administration of tryptophan alone to mice or rats rapidly induced enhanced hepatic protein synthesis, whereas other single amino acids did not have such an effect.[2] Others have confirmed the stimulatory effect of tryptophan on hepatic protein synthesis.[30-37] Subsequently, studies concerned with the mechanism of the unique effect of tryptophan have revealed the following findings attributable to tryptophan: increased cytoplasmic poly(A)mRNA,[38,39] probably related to increased translocation of mRNA from nuclei to cytoplasm,[24,25] stimulation of the activity of enzymes associated with nuclear efflux of mRNA[40] and of nuclear poly(A)polymerase[41]; and the apparent relation of nuclear envelope trytophan binding to increased nuclear mRNA efflux[42] and poly(A)polymerase activity.[43] A number of subsequent studies have implicated the nuclear tryptophan receptor as having an important role in the sequence of events by which tryptophan acts to stimulate protein synthesis.[4,5,8] The present study indicates that $T_3$ influences the affinity of nuclear receptor tryptophan binding yet does not appear to stimulate hepatic protein synthesis as does tryptophan alone.

Our present results with tryptophan and $T_3$ substantiate our earlier findings with compounds such as L-alanine and β-naphthylalanine,[4,8] which also competed with tryptophan for binding to hepatic nuclei in that neither one alone stimulates hepatic protein synthesis[4,8] but each administered along with tryptophan inhibits the increase in hepatic protein synthesis due to tryptophan alone. $T_3$ alone at low levels competed with in vitro $^3$H-tryptophan binding to hepatic nuclei in vitro (Table 1), but when added to unlabeled tryptophan, it diminished the inhibition of $^3$H-tryptophan binding to hepatic nuclei due to unlabeled tryptophan (Table 1). Assays of in vitro binding of $^3$H-tryptophan to hepatic nuclei after in vivo treatment (1 hour) of rats with tryptophan, $T_3$, or both showed that $T_3$ diminished in vitro $^3$H-tryptophan binding (Table 7), probably reflecting the effect of $T_3$ in vivo. Also, our findings indicate that $T_3$ competed with tryptophan for tryptophan's stimulatory effect on certain hepatic nuclear enzymes, PAP and NTPase, especially in the case of the former. The probable relationship between these enzymes and enhanced nuclear mRNA efflux in the liver due to tryptophan has been considered previously.[40-43] Another possible consideration in our in vivo experiments in which $T_3$ and tryptophan were administered to rats is that $T_3$ may inhibit tryptophan transport to the liver. A mutual competitive inhibition between the transport of tryptophan (mediated by the aromatic amino acid transport system T) and $T_3$ has been reported.[44,45] Although system T transport activity has been studied mainly in erythrocytes, it is also expressed in hepatocytes.[46] Interactions between thyroid hormone and tryptophan transport in rat liver have been reported to be modulated by thyroid status.[47] In consideration of this possibility, we investigated in one experiment whether administration of $T_3$ and tryptophan under our experimental conditions would affect free tryptophan levels in the liver. The results showed that rats

tube-fed tryptophan and given $T_3$ intraperitoneally at time 0 and killed after 1 hour (Table 7) had the same increase (10%) in free tryptophan levels in the liver as rats tube-fed tryptophan alone. This suggests that at 1 hour, the liver in both groups received similar elevated levels of free tryptophan.

Numerous investigations have been directed toward gaining an understanding of the consequences of nuclear receptor ligand binding of hormones and of certain nutrients. A number of interrelationships have been reported such that certain commonalities seem to exist. The amino acid sequence similarities between thyroid and steroid receptors, especially in their DNA binding domains and in the organization of their functional domains, appear to correlate with similarities in their mechanism of action.[9,10,24] Unfortunately, the amino acid sequence for the nuclear receptor for tryptophan has not yet been determined. Protease inhibitors such as TPCK, ZPCK, and others have been reported to inhibit the binding of adrenal and sex steroids to their cognate receptors[48] and the binding of $T_3$ to the hepatic nuclear receptor.[22] In consideration of the mechanism by which protease inhibitors act to inhibit $T_3$ binding to rat hepatic nuclei, Brtko et al[22] have suggested that the $T_3$ receptor contains a nucleophilic site at or close to the hormone binding domain that is recognized and affected by protease inhibitors. Such a nucleophilic site has been described with receptors for aldosterone, dihydrotestosterone, estrogen, progesterone, and vitamin D.[22] In this study, we investigated whether TPCK or ZPCK would affect in vitro $^3$H-tryptophan binding to hepatic nuclei as well as when unlabeled tryptophan, $T_3$, HC, or insulin were added. Unlike the effect of protease inhibitors on receptor binding of the above-cited hormones, the addition of TPCK or ZPCK did not appreciably (8% to 9%) inhibit in vitro $^3$H-tryptophan binding to hepatic nuclei (Table 6). However, such additions appeared to diminish the inhibitory effect of $T_3$ (from 41% to 12% or 21%) and to increase the inhibitory effect by adding each protease inhibitor to $T_3$ plus unlabeled tryptophan (from 45% to 56%) (Table 6). Thus, the mechanism by which protease inhibitors act to inhibit $T_3$ binding to hepatic nuclei likewise seems to be involved in controlling how $T_3$ acts to affect in vitro $^3$H-tryptophan binding to hepatic nuclei. Our present findings suggest that tryptophan binding to its receptor may not be affected at a nucleophilic site as proposed by Brtko et al.[22] However, the effects of $T_3$ on tryptophan binding may be influenced by the added protease inhibitor through an effect on the nucleophilic site of the $T_3$ receptor.

Overall, much information is now available that may assist in unraveling the series of events triggered by ligand-receptor binding. Our current study raises questions about the possible interrelationships between the nuclear tryptophan receptor and $T_3$. Also, some different changes in the tryptophan receptor binding affinity may occur with the addition of HC or possibly insulin. These preliminary findings should serve as a stimulus for further investigation into the regulatory interrelationships between certain hormones and essential nutrients such as tryptophan at the level of ligand receptor activity.

## REFERENCES


1. Sidransky H: Tryptophan, unique action by an essential amino acid, in Sidransky H (ed): Nutritional Pathology. Pathobiochemistry of Dietary Imbalances. New York, NY, Dekker, 1985, pp 1-62
2. Sidransky H, Bongiorno M, Sarma DSR, et al: The influence of tryptophan on hepatic polyribosomes and protein synthesis in fasted mice. Biochem Biophys Res Commun 27:242-248, 1967
3. Sidransky H, Sarma DSR, Bongiorno M, et al: Effect of dietary tryptophan on hepatic polyribosomes and protein synthesis in fasted mice. J Biol Chem 243:1123-1132, 1968
4. Sidransky H, Verney E: Influence of L-alanine on effects induced by L-tryptophan on rat liver. J Nutr Biochem 7:200-206, 1996
5. Sidransky H, Verney E: Differences in tryptophan binding to hepatic nuclei of $NZBWF_1$ and Swiss mice: Insight into mechanism of tryptophan's effects. J Nutr 127:270-275, 1997
6. Kurl RN, Verney E, Sidransky H: Tryptophan binding sites on nuclear envelopes of rat liver. Nutr Rep Int 36:669-677, 1987
7. Kurl RN, Verney E, Sidransky H: Identification and immunohistochemical localization of a tryptophan binding protein in nuclear envelopes of rat liver. Arch Biochem Biophys 265:286-293, 1988
8. Sidransky H, Verney E, Cosgrove JW, et al: Studies with compounds that compete with tryptophan binding to rat hepatic nuclei. J Nutr 122:1085-1095, 1992
9. Weinberger C, Thompson CC, Ong ES, et al: The c-erb-A gene encodes a thyroid hormone receptor. Nature 324:641-646, 1986
10. Sap J, Munoz A, Damm K, et al: The c-erb-A protein is a high affinity receptor for thyroid hormone. Nature 324:635-640, 1986
11. Tashima Y, Terui M, Itoh H, et al: Effect of selenite on glucocorticoid receptor. J Biochem 105:358-361, 1989
12. Brtko J, Filipcik P: Effect of selenite and selenate on rat liver nuclear 3,5,3′-triiodothyronine ($T_3$) receptor. Biol Trace Elem Res 41:191-199, 1994
13. Sidransky H, Verney E: The presence of thiols in the hepatic nuclear binding site for L-tryptophan. Studies with selenite. Nutr Res 16:1023-1034, 1996
14. Blobel G, Potter VR: Nuclei from rat liver: Isolation method that combines purity with high yield. Science 154:1662-1665, 1966
15. Harris JR, Milne JF: Rapid procedure for isolation and purification of rat-liver nuclear envelope. Biochem Soc Trans 2:1251-1253, 1974
16. Agutter PS, Gleed CD: Variability of mammalian liver nuclear envelope preparations. Biochem J 192:85-89, 1980
17. Lowry OH, Rosebrough MR, Farr AL, et al: Protein measurement with Folin phenol reagent. J Biol Chem 193:265-275, 1951
18. Agutter PS, McArles HJ, McCaldin B: Evidence for involvement of nuclear envelope triphosphatase in nucleocytoplasmic translocation of ribonucleoprotein. Nature 263:165-167, 1976
19. Jacob ST, Roe FJ, Rose KM: Chromatin-bound and free forms of poly(adenylic acid) polymerase in rat hepatic nuclei. Biochem J 153:733-735, 1976
20. Snedecor GW, Cochran WG: Statistical Methods. Ames, IA, Iowa State University Press, 1980, pp 215-236
21. Brtko J, Knopp J, Filipcik P: Effect of protease inhibitors and substrates on 3,5,3′-triiodothyronine binding to rat liver nuclear receptors. Endocr Regul 26:127-131, 1992
22. Brtko J, Knopp J, Baker ME: Inhibition of 3,5,3′-triiodothyronine binding to its receptor by protease inhibitors and substrates. Mol Cell Endocrinol 93:81-86, 1993
23. Beato M, Herrlech P, Schutz G: Steroid hormone receptors: Many actors in search of a plot. Cell 83:851-857, 1995
24. Murty CN, Verney E, Sidransky H: The effect of tryptophan on nucleocytoplasmic translocation of RNA in rat liver. Biochim Biophys Acta 747:117-128, 1977
25. Murty CN, Verney E, Sidransky H: In vivo and in vitro studies on the effects of tryptophan on translocation of RNA from nuclei on rat liver. Biochem Med 22:98-109, 1979
26. Thompson CC, Evans RM: Transactivation by thyroid hormone receptors: Functional parallels with steroid hormone receptors. Proc Natl Acad Sci USA 86:3494-3498, 1989

27. Sidransky H, Verney E: Influence of L-leucine on L-tryptophan binding to rat hepatic nuclei. J Nutr Biochem 8:592-602, 1997

28. Eberhardt NL, Valcano T, Timiras PS: Triiodothyronine nuclear receptors: An in vitro comparison of the binding of triiodothyronine to nuclei of adult rat liver, cerebral hemisphere, and anterior pituitary. Endocrinology 102:556-561, 1978

29. Silva ES, Astier H, Thakare U, et al: Partial purification of the triiodothyronine receptor from rat liver nuclei. J Biol Chem 252:6799-6805, 1977

30. Pronezuk AW, Baligam BS, Triant JW, et al: Comparison of the effect of amino acid supply on hepatic polysome profiles in vivo and in vitro. Biochim Biophys Acta 157:204-206, 1968

31. Cammarano P, Chinali G, Gaeteni S, et al: Involvement of adrenal steroids in the changes of polysome organization during feeding of imbalanced amino acid diets. Biochim Biophys Acta 155:302-304, 1968

32. Rothschild MA, Oratz M, Mongelli J, et al: Amino acid regulation of albumin synthesis. J Nutr 98:395-403, 1969

33. Oravec M, Sourkes TL: Inhibition of hepatic protein synthesis by α-methyl-DL-tryptophan in vivo. Further studies on the glyconeogenic action of α-methyl-tryptophan. Biochemistry 9:4458-4464, 1970

34. Park OJ, Henderson M, Swan PB: Effects of the administration of single amino acids on ribosome aggregation in rat liver. Proc Soc Exp Biol Med 142:1023-1024, 1973

35. Jorgenson AJF, Majumdar APN: Bilateral adrenalectomy: Effect of a single tube-feeding of tryptophan on amino acid incorporation into plasma albumin and fibrinogen in vivo. Biochem Med 13:231-240, 1975

36 Majumdar APN: Tryptophan requirement for protein synthesis. A review. Nutr Rep Int 26:509-522, 1982

37. Ponter AA, Cortamira NO, Seve B, et al: The effects of energy source and tryptophan on the rates of protein synthesis and on hormones of the entero-insular axis in the piglet. Br J Nutr 71:661-674, 1994

38. Murty CN, Sidransky H: The effect of tryptophan on messenger RNA of the liver of fasted mice. Biochim Biophys Acta 262:328-335, 1972

39. Murty CN, Verney E, Sidransky H: Effect of tryptophan on polyriboadenylic acid and polyadenylic acid-messenger ribonucleic acid in rat liver. Lab Invest 34:77-85, 1976

40. Murty CN, Hornseth R, Verney E, et al: Effect of tryptophan on enzymes and proteins of hepatic nuclear envelopes of rats. Lab Invest 48:256-262, 1983

41. Kurl RN, Verney E, Sidransky H: Effect of tryptophan on rat hepatic nuclear poly(A)polymerase activity. Amino Acids 5:263-271, 1993

42. Sidransky H, Murty CN, Verney E: Nutritional control of protein synthesis. Studies relating to tryptophan-induced stimulation of nucleocytoplasmic translocation of mRNA in rat liver. Am J Pathol 117:298-309, 1984

43. Kurl RN, Barsoum AL, Sidransky H: Association of poly(A)polymerase with tryptophan receptor in rat hepatic nuclei. J Nutr Biochem 3:366-372, 1992

44. Zhou Y, Samson M, Francon J, et al: Thyroid hormone concentration uptake in rat erythrocytes. Biochem J 281:81-86, 1992

45. Zhou Y, Samson M, Osty J, et al: Evidence for close link between the thyroid hormone transport system and the aromatic amino acid transport system T in erythrocytes. J Biol Chem 262:17000-17004, 1990

46. Salter M, Knowles RG, Pogson CI: Transport of the aromatic amino acids into isolated liver cells. Biochem J 233:499-506, 1986

47. Kemp HF, Taylor PM: Interactions between thyroid hormone and tryptophan transport in rat liver are modulated by thyroid status. Am J Physiol 272:E809-E816, 1997

48. Hubbard JR, Kalimi M: Influence of proteinase inhibitors on glucocorticoid receptor properties: Recent progress and future perspectives. Mol Cell Biochem 66:101-109, 1985