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Effects of vitamins on hepatic nuclear binding of L-tryptophan

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Summary. This study investigated the in vitro effects of selected vitamins on nuclear L-tryptophan receptor binding of rat liver. Our results revealed that some fat-soluble vitamins, β-carotene, retinyl acetate, calciferol, α-tocopherol, and Trolox, as well as some water-soluble vitamins, thiamine and riboflavin, acted to inhibit in vitro ^{3}H-tryptophan binding to hepatic nuclei. On the other hand, pyridoxine had little or no effect. The addition of dithiothreitol, a protective agent for sulfhydryl groups, along with each vitamin decreased the vitamin's inhibitory effect on in vitro ^{3}H-tryptophan binding to nuclei, with the exception of riboflavin and calciferol. The addition of L-leucine, which alone had no inhibitory effect on in vitro ^{3}H-tryptophan binding to hepatic nuclei but when added with unlabeled L-tryptophan negated the effect of unlabeled L-tryptophan, caused a markedly diminished inhibitory binding effect due to each of the following vitamins, thiamine, β-carotene, retinyl acetate, and α-tocopherol and Trolox, but no effect on riboflavin and calciferol.

Keywords: Amino acids – Vitamins – L-Tryptophan – Hepatic nuclear binding – Rats

Introduction

Our experimental studies have been directed toward an understanding as to how certain essential nutrients may regulate metabolic events in vital organs. Specifically, we have been concerned with how an indispensable amino acid, L-tryptophan, acts to affect protein synthesis in the liver. Our studies have led us to conclude that L-tryptophan binds to a specific receptor which resides in the nuclear membrane (envelope) of liver cells. This binding, which is saturable, stereospecific and of high affinity (Kurl et al., 1987, 1988), appears to trigger a series of events which lead to stimulation of hepatic protein synthesis (Kurl et al., 1988; Sidransky et al., 1992; Sidransky and Verney, 1996a, 1997c). Recently, we have directed our attention as to how other compounds (dietary, hormonal, therapeutic (drugs) or toxic) may affect the

L-tryptophan-induced stimulatory response on hepatic protein synthesis (Sidransky et al., 1989, 1992a,b,c, 1994a,b,c; Sidransky and Verney, 1994, 1996a,b, 1997a,b, 1999a,b, 2000a). In approaching this problem, we have investigated whether each of these agents or components may individually affect the in vitro ^{3}H-tryptophan binding to hepatic nuclei or nuclear envelopes. The present experiments evaluate whether certain vitamins (water- or fat-soluble and with or without antioxident activities) affect the nuclear receptor binding to L-tryptophan. There is biological evidence that, at high levels, reactive oxygen species can be damaging to cells and thus contribute to cellular dysfunction and disease. Thus, antioxidant compounds in the diet have been considered to have a role with possible health benefits. Some vitamins are known to have antioxidant activities. Indeed, many substances, including some vitamins have been shown to have antioxident activity in vitro. Thus, a study using different vitamins was evaluated in terms of their effects on nuclear tyrptophan binding in vitro. Essentially all of the antioxidant vitamins (β-carotene, retinyl acetate, α-tocopherol and Trolox), as well as some that do not have this property (calciferol, thiamine and riboflavin), were observed to inhibit in vitro ^{3}H-tryptophan binding to hepatic nuclei. These findings are the subject of this report.

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 compound used in the experiments was L-5-^{3}H-tryptophan (radiochemical purity 98.5%, 1.13 TBq/mmol), obtained from Amersham/Searle, Arlington Heights, IL USA). 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 (1966). Nuclear envelopes of hepatic nuclei were isolated with the procedure of Harris and Milne (1974) as modified by Agutter and Gleed (1980) and as routinely used in this laboratory (Kurl et al., 1987; 1988). 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 (Kurl et al., 1987). 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 envelops 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. The protein was determined as described by Lowry et al. (1951).

Statistics

Data were analyzed by Student's paired t-test (Snedecor and Cochran, 1989).

Results

Water-soluble vitamins

In an earlier report (Sidransky and Verney, 2000b) we described that vitamin C (ascorbic acid) had an inhibitory effect on in vitro ^{3}H-tryptophan binding to hepatic nuclei and nuclear envelopes. On the other hand, niacin had not demonstrated such an effect in an earlier report (Kurl et al., 1987). We have now extended our study to include other water-soluble vitamins, thiamine, riboflavin, and pyridoxine. The results are summarized in Table 1. In our present experiments we investigated the effects of varying concentrations (10^{-12} to 10^{-4} M) of each vitamin on in vitro ^{3}H-tryptophain binding to hepatic nuclei. The normal levels of each vitamin in the blood as described in a handbook (Geigy Scientific Tables, 1970) have been used as rough baselines. Therefore, assuming that the blood concentration of each vitamin was similar to that present in hepatic cells, we used varying levels which were approximately within the physiological level of each vitamin. Based upon our calculations, the following concentration of each vitamin was considered to be approximately in the physiological range: thiamine, 10^{-7} M; riboflavin, 10^{-7} M; and pyridoxine, 10^{-7} M.

Table 1 reveals that the addition of thiamine induced a 32.8% inhibition at 10^{-4} M level but, at 10^{-6} to 10^{-8} M levels (closer to physiological), induced a

Table 1. Effect of thiamine, riboflavin or pyridoxine on inhibition of total in vitro ^{3}H-tryptophan binding to rat hepatic nuclei or nuclear envelopes

Compounds tested		Thiamine		Riboflavin		Pyridoxine	
		Nuclei	Nuclear envelopes	Nuclei	Nuclear envelopes	Nuclei	Nuclear envelopes
				% inhibition			
L-Tryptophan	10^{-4}M	(18) 68.1 ± 0.9[a]	(4) 70.5 ± 1.0	(12) 69.0 ± 0.7	(3) 69.6 ± 1.9	(6) 66.7 ± 0.9	(3) 68.8 ± 2.0
Vitamin	10^{-4}M	(13) 32.8 ± 5.5[b]	(4) 37.8 ± 2.9[b]	(8) 20.0 ± 3.2[b]	(3) 27.7 ± 8.4[b]	(6) 10.7 ± 2.4[b]	(3) 8.7 ± 6.7[b]
	10^{-6}M	(16) 23.0 ± 4.8		(10) 8.2 ± 2.3	(2) 20.4 ± 5.5	(4) 5.1 ± 1.9	(3) +17.7 ± 9.4
	10^{-8}M	(13) 20.0 ± 3.5	(4) 16.3 ± 6.1	(4) 11.5 ± 3.4	(3) 21.3 ± 9.0	(4) 5.1 ± 2.7	(3) 3.5 ± 1.4
	10^{-10}M	(13) 13.4 ± 3.1		(4) 4.9 ± 1.9	(2) 11.0 ± 4.6	(4) 2.3 ± 1.6	(2) 10.4 ± 14.7
	10^{-12}M	(5) 10.6 ± 3.6	(4) 2.8 ± 1.5	(3) 5.3 ± 2.9	(2) 10.7 ± 4.7		
L-Tryptophan	10^{-4}M plus						
Vitamin	10^{-4}M	(8) 56.6 ± 3.1[b]	(1) 59.2 ±	(5) 50.4 ± 0.5[b]		(3) 37.8 ± 5.9[b]	(2) 45.4 ± 2.9[b]
	10^{-6}M	(9) 55.8 ± 2.9		(2) 54.8 ± 0.5			
	10^{-8}M	(5) 51.8 ± 6.8		(2) 57.4 ± 1.6			
	10^{-10}M	(4) 51.4 ± 6.9		(2) 59.2 ± 0.9			
	10^{-12}M	(2) 54.1 ± 1.4		(1) 42.3			

[a] Number of experiments in parenthesis. Means ± SEM. [b] $P < 0.01$, compared with L-tryptophan group.

20–23% inhibition of in vitro ^{3}H-tryptophan binding to hepatic nuclei. Addition of riboflavin at 10^{-4}M induced a 20% inhibition in binding, but at 10^{-6} to 10^{-8}M levels (near physiological levels), it induced only an 8–11.5% inhibition. Addition of pyridoxine (10^{-4} to 10^{-10}M) had little or no inhibitory nuclear tryptophan binding effect.

Table 1 also summarizes data where each vitamin at different concentrations was added to a constant concentration (10^{-4}M) of unlabeled L-tryptophan and in vitro ^{3}H-tryptophan binding to hepatic nuclei was assayed. Each vitamin added caused some decrease in inhibition of binding compared to that of unlabeled L-tryptophan alone. Table 1 also summarizes the experiments in which the addition of vitamins were studied on their effects on in vitro ^{3}H-tryptophan binding to hepatic nuclear envelopes rather than to hepatic nuclei as described earlier. In general, the findings paralleled those with hepatic nuclei.

Fat-soluble vitamins

In our studies to evaluate the fat-soluble vitamins, we used the following: for vitamin A, β-carotene or retinyl acetate; for vitamin D, calciferol; and for vitamin E, α-tocopherol or Trolox (a water-soluble analogue of vitamin E). Each of the compounds was dissolved in an appropriate vehicle, ethanol, acetone or DMSO, and then added at varying concentrations (10^{-12} to 10^{-4}M) to the in vitro incubation medium. Each vehicle alone was also assayed and used as a blank. According to blood levels, the following concentrations were considered to be within physiological range: β-carotene, 10^{-6}M; and α-tocopherol, 10^{-5}M. Table 2 summarizes the findings when each of the above compounds was assayed in vitro using hepatic nuclei. β-carotene, retinyl acetate, calciferol, α-tocopherol and Trolox were each able to appreciably inhibit in vitro ^{3}H-tyrptophan binding to hepatic nuclei. Blanks alone revealed essentially no effects.

Effect of dithiothreitol (DTT) on vitamin effects

As reported earlier (Sidransky and Verney, 1996c), we observed that the addition of DTT, a protective agent for sulfhydryl groups, along with sodium selenite negated the inhibition of in vitro ^{3}H-tryptophan binding to hepatic nuclei caused by sodium selenite alone. Thus, DTT protection of the sulfhydryl groups of the nuclear tryptophan receptor was considered to involve the site affected by the sodium selenite, which was altered by its oxidation of sulfhydryl groups. In this way, sodium selenite had an inhibitory effect on nuclear ^{3}H-tryptophan binding. In the present study, we determined whether the addition of DTT would affect the action of each vitamin which had an in vitro inhibitory nuclear tryptophan binding effect. Table 3 summarizes the effects of adding DTT (10^{-4}M) on the in vitro ^{3}H-tryptophan binding to hepatic nuclei as influenced by each vitamin. It is apparent that of the vitamins which alone induced appreciable inhibition of in vitro ^{3}H-

Table 2. Effect of β-carotene, retinyl acetate, calciferol, α-tocopherol and trolox on inhibition of total in vitro ^{3}H-tryptophan binding of rat hepatic nuclei

Compounds tested		β-Carotene		Retinyl acetate		Calciferol	α-Tocopherol		Trolox
		DMSO	Acetone	DMSO	Ethanol		DMSO	Ethanol	
					% inhibition				
L-Tryptophan	10^{-4} M	(8) 69.3 ± 1.2[a]	(3) 66.3 ± 2.3	(8) 68.6 ± 1.1	(4) 66.6 ± 2.6	(10) 67.8 ± 1.1	(3) 67.6 ± 0.9	(5) 68.7 ± 1.7	(11) 68.7 ± 1.0
Vitamin	10^{-4} M	(8) 45.6 ± 5.7[b]	(3) 30.6 ± 6.9[b]	(8) 31.6 ± 2.9[b]	(4) 23.5 ± 1.3[b]	(10) 31.7 ± 4.4[b]	(3) 29.5 ± 2.3[b]	(5) 21.7 ± 5.2[b]	(11) 38.6 ± 2.4[b]
	10^{-6} M	(4) 44.9 ± 1.9	(3) 23.3 ± 7.2	(4) 22.4 ± 5.1	(3) 19.0 ± 8.0	(4) 42.4 ± 2.2		(4) 16.4 ± 3.1	(6) 31.9 ± 1.3
	10^{-8} M	(4) 37.2 ± 1.7	(3) 14.6 ± 6.0	(4) 14.9 ± 2.6	(3) 17.7 ± 2.9	(4) 27.7 ± 9.6		(4) 17.4 ± 1.5	(3) 26.6 ± 4.4
	10^{-10} M	(3) 15.6 ± 4.7	(3) 10.4 ± 3.2	(3) 11.3 ± 1.1	(3) 17.4 ± 6.3	(4) 3.6 ± 2.0		(4) 14.7 ± 2.8	(3) 23.8 ± 4.5
	10^{-12} M							(4) 13.9 ± 2.5	
L-Tryptophan	10^{-4} M								
plus vitamin	10^{-4} M	(2) 55.7 ± 0.4[b]				(3) 56.7 ± 10.6[b]		(3) 39.2 ± 8.6[c]	(3) 46.8 ± 0.0[b]
	10^{-6} M	(2) 56.0 ± 3.1				(3) 53.1 ± 7.8		(2) 43.7 ± 1.1	
	10^{-8} M	(2) 59.5 ± 3.4				(3) 43.5 ± 11.5		(2) 49.2 ± 1.6	
	10^{-10} M	(2) 63.3 ± 3.1				(3) 64.7 ± 2.1		(2) 50.0 ± 5.6	
	10^{-12} M					(3) 67.7 ± 1.4		(2) 59.9 ± 4.1	

[a] Number of experiments in parenthesis. Means ± SEM. [b] $P < 0.01$, compared with L-tryptophan group. [c] $0.05 > P > 0.001$, compared with L-tryptophan group.

Table 3. Effect of added dithiothreitol (DTT) or L-leucine to vitamins on inhibition of in vitro ^{3}H-tryptophan binding to hepatic nuclei

Vitamin tested	Vitamin alone	Vitamin plus DTT	Vitamin plus L-leucine
	% inhibition		
Thiamine	(13) 30.2 ± 0.9[a]	(4) 21.2 ± 1.7[b]	(4) 10.0 ± 2.7[b]
Riboflavin	(4) 25.0 ± 1.0	(4) 21.9 ± 2.9	(4) 20.6 ± 1.7
β-Carotene (DMSO)	(4) 35.5 ± 4.2	(4) 4.0 ± 1.3[b]	(3) 16.9 ± 9.0
Retinyl acetate (DMSO)	(4) 32.0 ± 7.8	(4) 17.0 ± 3.9	(3) 9.5 ± 5.5
Calciferol	(6) 24.6 ± 0.8	(6) 21.4 ± 3.3	(5) 20.8 ± 5.5
α-Tocopherol (DMSO)	(4) 27.7 ± 2.6	(4) 13.9 ± 0.8[b]	(3) 11.7 ± 2.6[b]
(Ethanol)	(3) 27.8 ± 2.2	(3) 20.5 ± 1.1[c]	(3) 20.1 ± 1.0
Trolox	(8) 37.4 ± 2.5	(7) 22.8 ± 4.3[c]	(4) 10.9 ± 2.7[b]

[a]Number of experiments in parenthesis. Means ± SEM. [b]$P < 0.01$, compared with vitamin alone. [c]$0.05 > P > 0.01$, compared with vitamin alone.

tryptophan binding to hepatic nuclei described in Tables 1 and 2, thiamine, β-carotene, retinyl acetate, α-tocopherol, and Trolox, showed appreciable decreases in the binding inhibition due to the added DTT. Riboflavin and calciferol were not affected by the added DTT.

Effect of L-leucine on vitamin effects

In an earlier report (Sidransky and Verney, 1997b) we observed that the addition of L-leucine (10^{-4} M) alone had no inhibitory effect on in vitro ^{3}H-tryptophan binding to hepatic nuclei. However, when L-leucine was added with unlabeled L-tryptophan, it negated the effect of the latter alone on binding. Therefore, we now investigated whether the addition of L-leucine would affect the inhibitory nuclear tryptophan binding effect due to certain vitamins. Table 3 summarizes the results and reveals that L-leucine exerted a markedly diminishing effect on the inhibition due to thiamine, β-carotene, retinyl acetate, α-tocopherol, and Trolox. Again, riboflavin and calciferol were not affected by the added L-leucine.

Discussion

Since vitamins considered to have antioxidant properties (ascorbic acid, as reported in an earlier study Sidransky and Verney (2000b), β-carotene, and α-tocopherol (Table 2) have been found to inhibit nuclear tryptophan binding in vitro, one may speculate that their antioxidant properties were possibly involved or of importance. However, other, non-antioxidant, vitamins, such as thiamine and riboflavin, have also been demonstrated to induce similar inhibitory nuclear tryptophan binding properties (Table 1). Thus, vitamin properties other than their anti-oxidant actions can be important and anti-oxidant properties alone are not responsible in all cases. In reports concerning

dietary antioxidants, many complicating factors are evident. Some compounds, at least in vitro, can be antioxidants under one condition and prooxidants under others. Antioxidants in foods are not interchangeable and may differ from one another both in their sites and mechanisms of action.

The redox state of receptors is regulated in vivo in order to maintain the receptor in a state that is neither fully oxidized nor fully reduced (Gozland and Bean Ari, 1995). Redox agents have been reported to elicit a wide variety of effects on the ligand affinity of ionotropic glutamate receptors (Abele et al., 1998). One such effect is the DTT-induced increase in agonist affinity of certain ionotropic glutamate receptors, presumably due to reduction of a disulfide bridge formed between cysteine residues. This disulfide is shown to exist in the ligand-binding domain of the alpha-amino-3-hydoxy-5-methyl-isoxazole-4-propionic acid receptor subunit.

Indeed, the availability and importance of thiol groups in receptor binding has been described in many receptors. Investigators have used DTT as a protective agent for sulfhydryl groups in many studies and were able to gain information regarding the importance of free thiol groups rather than the oxidized forms as disulfides in receptors. Such studies have been reported for receptors for retinoic acid (Brtko et al., 1998) glucocorticoids (Karle et al., 1989; Galigniana et al., 1999), thyroid hormone (Brtko et al., 1995) and aldosterone (Tashima et al., 1984). In our present study we have observed that the addition of DTT (10^{-4} M) was able to prevent or diminish the inhibitory binding effect of certain vitamins (thiamine, β-carotene, retinyl acetate, α-tocopherol, and Trolox) on ^{3}H-tryptophan binding to hepatic nuclei (Table 3). This suggests that these vitamins may act in part to affect the status of thiol groups in the nuclear receptor which may lead to alterations in receptor binding affinity.

While this study has considered the importance of the antioxidant activities of certain vitamins on hepatic nuclear receptor binding affinity for L-tryptophan, it is worthwhile to mention that L-tryptophan and some of its metabolites have themselves antioxidant properties (Christen et al., 1990). These antioxidant activities of tryptophan and some of its oxidative metabolites have been investigated (Christen et al., 1990). Enzyme activity involved or related to oxidative tryptophan metabolism has been considered to play a role in local antioxidant defense against some inflammatory diseases.

In consideration that certain vitamins can influence the metabolism of L-tryptophan, we searched the literature for such effects. Hoey and Butler (1984) reported that antioxidants, specifically α-tocopherol, could repair tryptophan radicals produced from the one-electron oxidation of the free tryptophan amino acid. Bisby et al. (1984) reported that free radicals derived from one-electron oxidation of tryptophan have been repaired by Trolox C. Conceivably, changes in the radical cation of L-tryptophan by vitamins may affect its reactivity and possibly also its overall binding affinity for the nuclear receptor under certain conditions.

In consideration that certain vitamins may bind to hepatic nuclei and that this could possibly influence hepatic nuclear tryptophan binding, we reviewed the literature. ^{3}H-alpha-tocopherol has been reported to become incorpo-

rated into isolated rat liver nuclei in a nonspecific manner by binding to chromatin nonhistone chromosomal protein (Patnaik and Nair, 1977). As for folate, rat liver nuclei contain receptors for a folate binding protein (daCosta et al., 1983). Specifically, a folate binding protein, purified from cytoplasm saturated with ^{3}H-pteroylglutamic acid, binds to rat liver nuclear fractions. How and whether such binding by selected vitamins could in any way impact on L-tryptophan binding to hepatic nuclei is not clear. It is of interest that Matsuuro et al. (1979) have reported that α-tocopherol inhibited the binding of ^{14}C-benzo(a) pyrene to rat liver nuclear macromolecules in the presence of phenobarbital-induced microsomes. Thus, some vitamins have the capacity to affect (directly or indirectly) binding reactions of other compounds.

In an earlier study (Sidransky and Verney, 1997b) we investigated the effect of L-leucine on in vitro ^{3}H-tryptophan binding to hepatic nuclei. We observed that L-leucine did not compete with the ^{3}H-tryptophan binding. However, it did abrogate the inhibitory effect of unlabeled L-tryptophan (10^{-7}M to 10^{-4}M) on ^{3}H-tryptophan binding to hepatic nuclei in vitro. The mechanism of this effect of the L-leucine is still not clear (Sidransky and Verney, 1997b). Nonetheless, we used the addition of L-leucine to explore whether it would affect the binding effects of certain vitamins. The findings in Table 3 indicate that L-leucine added to thiamine, β-carotene, retinyl acetate, α-tocopherol, or Trolox, but not to riboflavin or calciferol, diminished the inhibitory binding effect of ^{3}H-tryptophan to hepatic nuclei by the vitamin alone. This suggests that there are differences in mechanisms whereby the different vitamins act in our assay system in that L-leucine only influences the actions of some but not of others.

The findings of our earlier experiments, as well as of the present experiments, indicate that a variety of agents or conditions may influence how hepatic cells respond to the administration of L-tryptophan. It seems that the agents or conditions act in a variety of ways to influence the enhanced hepatic protein synthesis induced by L-tryptophan. In evaluating how each agent or condition affects hepatic nuclear tryptophan binding, considered a vital component in tryptophan's enhancement of protein sythesis, the following mechanisms have been considered. 1) Competition at the nuclear receptor binding site. Examples are a number of analogs, metabolites, or related compounds of tryptophan, such as DL-beta-(1-napththyl)-alanine, 5-fluoro-tryptophan, 7-azatryptophan, 5-hydroxytryptophan, L-alanine, L-phenylal-anine, L-tyrosine, L-cysteine and L-cystine (Sidransky et al., 1990, 1992a; 1996a). Studies with these and other compounds have enabled us to determine which portion of the tryptophan molecule is crucial for binding of tryptophan to hepatic nuclear envelopes (Sidransky et al., 1992a). Generally, such compounds contain the alpha-amino-propionic acid structure (Sidransky et al., 1992a). Also, 3,5,3′-triiodothyronine (T_3) had a competitive effect (Sidransky and Verney, 1999a). Likewise, demoxepam (Sidransky et al., 1992c) and cycloheximide (Sidransky and Verney, 2000a) demonstrated competitive inhibitory binding effects. 2) Effect on thiol groups within the nuclear receptor protein. Examples of compounds that have such an effect are sodium selenite (Sidransky and Verney, 1996c), sodium arsenite (Sidransky

and Verney, 1999b) and the vitamins, β-carotene, retinyl acetate and α-tocopherol of this study (Table 3). 3) Genetic differences in nuclear receptors. Hepatic nuclei of $NZBWF_1$ mice, frequently used as a model of systemic lupus erythematosus, have a markedly decreased affinity for L-tryptophan binding in vitro in comparison to that of other strains of mice (Swiss, BALB/c, DBA and $SJLF_1/J$) (Sidransky and Verney, 1997c). 4) Undetermined mechanisms. Examples are the effects with L-leucine (Sidransky and Verney, 1997b), lead acetate (Sidransky and Verney, 1999b) valproic acid (Sidransky and Verney, 1996b), and metryrapone (Verney and Sidransky, 1994).

Our current findings, in which we observed that certain vitamins affected the binding affinity of L-tryptophan for hepatic nuclei or nuclear envelopes in vitro, emphasize that checks and balances exist within cells which can be attributed to essential dietary components. Certain vitamins appear to affect the binding affinity of an indispensable amino acid, L-tryptophan, to hepatic nuclei. Although an understanding of how each vitamin acts on this process is not clear, it appears that differences in how each acts do exist. Thus, the interactions of vital components in the regulatory control of L-tryptophan on liver metabolism appear to be complex and need further elucidation.

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