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Effect of amino acid imbalances on the stimulatory effect of L-tryptophan on hepatic protein synthesis*

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Summary. Earlier it was reported that mice or rats tube-fed a single feeding of L-tryptophan (TRP) demonstrated a stimulation of hepatic protein synthesis. The present study was concerned with whether dietary imbalances induced by tube-feeding different ratios of L-alanine (ALA) or L-leucine (LEU) in relation to TRP would affect TRP's stimulatory effect on hepatic protein synthesis. Male Swiss mice, food-deprived overnight, were tube-fed one feeding of solution keeping TRP constant and varing ratios of ALA/TRP of 0.4, 2.1, or 4 or ratios of LEU/TRP of 4.8, 7.2, or 9.6. After 1 h, mice were killed and protein synthesis (^{14}C-leucine incorporation into proteins in vitro using microsomes of livers) was measured. TRP alone stimulated hepatic protein synthesis by 83% while ALA/TRP ratios of 2.1 or 4 but not of 0.4 and LEU/TRP ratios of 9.6 but not of 4.8 or 7.2 caused significant decreases in the stimulation of hepatic protein synthesis. Measurements of serum and hepatic free TRP concentrations in the experimental groups were similar in all groups tube-fed TRP alone or in combinations.

Keywords: Amino acids – L-Tryptophan – L-Alanine – L- Leucine – Protein synthesis – Liver – Mice

Introduction

In earlier studies (Sidransky et al., 1968, 1971) we have reported that the administration of L-tryptophan to mice or rats rapidly stimulates hepatic protein synthesis. This effect has been considered to be related to a sequence of events (Sidransky, 1985; Sidransky and Verney, 1996), one of which involves the ability of L-tryptophan to rapidly bind to a specific

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hepatic nuclear receptor (Kurl et al., 1987, 1988). Evidence that supports the latter explanation is based upon the following findings: 1) $NZBWF_1$ mice have been reported to have a low binding affinity of hepatic nuclei for L-tryptophan and demonstrate little or no stimulation of hepatic protein synthesis after being tube-fed L-tryptophan in contrast to the stimulatory effect observed in Swiss and other strains of mice that have high hepatic nuclear binding affinities (Sidransky and Verney, 1997); 2 L-alanine competes with L-tryptophan for hepatic nuclear receptor binding of L-tryptophan (Sidransky et al., 1992), yet does not itself stimulate hepatic protein synthesis in rats (Sidransky and Verney, 1996) but it will negate the stimulatory effect of hepatic protein synthesis due to L-tryptophan when administered at high levels before or together with L-tryptophan (Sidransky and Verney, 1996; Sidransky et al., 1992).

In this study we have investigated whether imbalances in the oral intake of L-alanine or L-leucine in relation to L-tryptophan would affect the experimentally-induced stimulation of hepatic protein synthesis due to L-tryptophan alone. Using ratios of L-alanine or L-leucine to L-tryptophan as derived from guidelines on adequate dietary proteins or amino acid mixtures (Fleck et al., 1965; Reeves et al., 1993) as well as other modified radios, we have been able to determine which levels of L-alanine or L-leucine (in relation to a constant level of L-tryptophan) can affect the stimulatory action of L-tryptophan. Our findings appear to substantiate that the ingestion of certain dietary levels of L-alanine or L-leucine may alter or inhibit the effect of L-tryptophan on hepatic protein synthesis.

Materials and methods

Animals

Male mice of the Swiss strain (Hilltop Lab Animals, Inc., Scottsdale, PA) average weight 40 g (range 35–42 g) were used in the experiments. The animals were fed Purina laboratory chow (no. 5001; Purina, St. Louis, MO) and maintained in a temperature-controlled room with a 12:12-h light-dark cycle. Before the experiments were begun, the animals were adapted to their quarters for at least 1 wk and then were deprived of food overnight but had free access to water. Mice were killed by decapitation. The protocol for these studies was reviewed and approved by the institutional animal care and use committee.

Chemicals

The [^{3}H]tryptophan used in the experiments was L-[5-^{3}H]tryptophan, 1.15 TBq/mmol, and L-[U-^{14}C]leucine, 12.9 GBq/mmol, obtained from Amersham/Searle (Arlington Heights, IL). L-tryptophan was obtained from US Biochemical (Cleveland, OH). L-alanine and L-leucine were from Life Technologies (Grand Island, NY.)

Tube-feeding of mice

In this study we selected the level of L-tryptophan (10 mg/100 g body weight) for tube-feeding of mice to enhance hepatic protein synthesis based upon data derived from earlier reports (Sidransky et al., 1968). Thus, using Swiss mice that on the average weighed 40 g,

we tube-fed 4 mg L-tryptophan per mouse to mice (food removed overnight) 1 h before killing. Based upon the formulated AIN purified diets for laboratory rodents (Reeves et al., 1993), we calculated the dietary ratio of L-alanine to L-tryptophan to be 2.1 and of L-leucine to L-tryptophan to be 7.2. These values were used as baselines and also one lower and one higher ratio for each amino acid while keeping the level of L-tryptophan (4 mg/40 g mouse) constant. In one experiment a complete amino acid mixture (Fleck et al., 1965; Sidransky et al., 1968) at 100 mg/mouse alone or with added L-leucine (19.2 mg/mouse) was tube-fed 1 h before killing.

In vitro protein synthesis

Microsomes prepared from postmitochondrial supernatants of livers of control and experimental animals were used for studies on incorporation in vitro as described earlier (Sidransky et al., 1968). In all assays, cytosols of livers of control (distilled water-treated) animals were used. 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). The protein was determined as described by Lowry et al. (1951).

Tryptophan levels

Tryptophan concentrations were determined spectrofluorometrically by the method of Denckla and Dewey (1967). Total (protein-free) tryptophan levels were assayed using liver homogenates after precipitation of proteins and using sera after precipitation of proteins.

Results

Table 1 summarizes the results of experiments in which mice, food-deprived overnight, were tube-fed in the morning 1 h before killing either water,

Table 1. In vitro ^{14}C-leucine incorporation into hepatic protein of Swiss mice tube-fed, L-tryptophan (TRP), L-alanine (ALA), or L-leucine (LEU) or combinations

Treatment[a]			Ratio: (ALA or LEU to TRP)	No of experiment	^{14}C-leucine incorporation into hepatic protein cpm/mg RNA
TRP mg/mouse	ALA	LEU			
–	–	–		7	100
4	–	–		7	182.8 ± 15.0[b,c]
–	16	–		5	107.3 ± 11.6[d]
–	–	38.4		5	99.7 ± 7.1[d]
4	1.4	–	0.4	5	140.0 ± 20.7
4	8.4	–	2.1	5	117.5 ± 15.3[e]
4	16	–	4.0	5	122.7 ± 23.9[d]
4	–	19.2	4.8	6	139.7 ± 18.1
4	–	28.8	7.2	5	145.1 ± 17.5
4	–	38.4	9.6	5	116.6 ± 14.1[d]

[a] Mice (2 per group) were tube-fed indicated compounds (mg/mouse) in 1 ml volume 1 h before killing; [b] Means ± SEM; [c] $P < 0.01$, compared with water group; [d] $P < 0.001$, compared with TRP group; [e] $0.05 > P > 0.01$, compared with TRP group.

Table 2. Serum and hepatic free tryptophan concentrations of Swiss mice tube-fed L-tryptophan (TRP), L-alanine (ALA), L-leucine (LEU) or combinations

Treatment[a]			Ratio (ALA or LEU to TRP)	No. of Experiments	Free TRP Concentrations[b]	
TRP mg/mouse	ALA	LEU			Serum μg/L	Liver μg/g
–	–	–		3	49.9 ± 9.3	12.1 ± 0.9
4	–	–		3	113.1 ± 14.4[c]	15.9 ± 4.1
4	1.4	–	0.4	2	114.7 ± 30.9	9.2 ± 1.6
4	8.4	–	2.1	2	106.3 ± 38.8	12.6 ± 3.3
4	16	–	4.0	3	98.6 ± 26.2	9.7 ± 1.0
4	–	19.2	4.8	2	104.3 ± 28.1	12.7 ± 4.3
4	–	28.8	7.2	2	93.9 ± 21.3	13.5 ± 4.8
4	–	38.4	9.6	2	117.7 ± 26.5	13.8 ± 1.7
–	16	–		3	71.1 ± 31.1	12.0 ± 1.6
–	–	38.4		3	75.1 ± 22.5	11.9 ± 1.0

[a] Mice (2 per group), food deprived overnight, were tube-fed indicated compounds (mg/mouse) in 1 ml volume 1 h before killing; [b] Values are means ± SEM; [c] $P < 0.05$.

L-tryptophan, L-alanine, L-leucine or combinations in a volume of 1 ml. Using mice that were tube-fed water as controls, mice tube-fed L-tryptophan (4 mg/mouse) revealed an 83% increase in hepatic protein synthesis (in vitro ^{14}C-leucine incorporation into hepatic proteins). Mice tube-fed L-alanine (16 mg/mouse) or L-leucine (38.4 mg/mouse) revealed no changes in hepatic protein synthesis. However, additions of L-alanine (8.4 mg or 16 mg/mouse) or of L-leucine (38.4 mg/mouse) to L-tryptophan (4 mg/mouse) caused a significant decrease in hepatic protein synthesis when compared to the group that received L-tryptophan alone. Lower levels of L-alanine (1.4 mg/mouse) or of L-leucine (19.2 mg or 28.8 mg/mouse) when added to L-tryptophan (4 mg/mouse) induced only minor (insignificant) decreases in hepatic protein synthesis compared to the L-tryptophan alone group.

In one experiment, overnight food-deprived mice were tube-fed water, a complete amino acid mixture (Fleck et al., 1965; Sidransky et al., 1968) (100 mg/mouse) (control), or the complete amino acid mixture plus 19.2 mg L-leucine (119.2 mg/mouse) (experimental) 1 h before killing. The ratios of L-leucine to L-tryptophan in the two diet feedings were: control, 2.4; and experimental, 7.2. In vitro hepatic protein synthesis was increased +50.3% in control group and 34.0% in experimental group in comparison to the water tube-fed group.

In several experiments serum and liver free L-tryptophan levels were assayed. Table 2 summarizes these findings. Mice tube-fed L-tryptophan 1 h before killing had a significant increase (+126.7%) in serum free tryptophan levels. On the other hand, rats tube-fed L-tryptophan plus L-leucine or L-alanine (varying levels) had variable increased levels of serum free tryptophan which were not statistically different than that due to L-tryptophan alone. Tube-feeding of high levels of L-leucine or L-alanine alone caused a small

insignificant increase in serum free tryptophan levels. Liver free L-tryptophan levels appeared to be similar in all groups.

Discussion

In an early study from our laboratory (Sidransky et al., 1968), we reported that tube-feeding of a complete amino acid mixture to overnight food-deprived mice for 1 h induced a significant increase in hepatic protein synthesis over that in controls (no tube-feeding). Tube-feeding the complete amino acid mixture devoid of L-tryptophan but not devoid of other single amino acids (L-threonine, L-isoleucine or L-methionine) negated the stimulatory effect due to the complete amino acid mixture on hepatic protein synthesis. Tube-feeding L-tryptophan alone (at the level present in the complete amino acid mixture) stimulated hepatic protein synthesis as occurred with the complete amino acid mixture. On the other hand, tube-feeding other single amino acids (L-threonine, L-isoleucine or L-methionine) at concentrations present in the complete amino acid mixture failed to stimulate hepatic protein synthesis. These findings initiated our continuing interest in L-tryptophan as having unique properties in regard to regulating hepatic protein synthesis (Sidransky, 1985). As background for our present study concerned with the effect or influence of other amino acids on the action of L-tryptophan on hepatic protein synthesis, we calculated the ratios of L-alanine and of L-leucine to L-tryptophan in our earlier complete amino acid mixture and the ratios of the two, respectively, were 0.4 and 2.4. These ratios did not affect or negate the stimulatory effect of L-tryptophan (Sidransky and Verney, 1968). Next, the guidelines for purified diets for laboratory rodents, specifically the AIN 93G diet (Reeves et al., 1993), was reviewed and the ratios of L-alanine and of L-leucine to L-tryptophan on calculation were, respectively, 2.1 and 7.2. Using the above information, we designed experiments using low, average (AIN-93G diet levels), and high ratios of L-alanine or L-leucine to L-tryptophan in the present study concerned with the possible effects of imbalances of selected amino acids. Our results (Table 1) demonstrate that certain high ratios do influence (inhibit) the stimulatory hepatic protein synthesis response due to L-tryptophan alone.

In this study we selected L-alanine and L-leucine for each one's effect on L-tryptophan-induced stimulation of hepatic protein synthesis since both have been found to affect the hepatic nuclear receptor binding affinity for L-tryptophan when assayed in vitro. L-alanine competes with L-tryptophan for a hepatic nuclear receptor involved in specific binding with L-tryptophan (Sidransky and Verney, 1996; Sidransky et al., 1992). L-leucine alone does not directly compete with L-tryptophan binding to hepatic nuclei (Sidransky et al., 1992) yet when added together with unlabeled L-tryptophan it inhibits ^{3}H-tryptophan binding to hepatic nuclei (unreported findings). Earlier L-alanine was reported to inhibit L-tryptophan-induced stimulation of hepatic protein synthesis when tube-fed at high levels (13, 50 or 90 mg/100 g body weight) together with L-tryptophan (5 mg/100 g

body weight)(Sidransky et al., 1992; Sidransky and Verney, 1996). In those studies the L-alanine to L-tryptophan ratios were, respectively, 2.6, 10 and 18.

In attempting to explain how certain amino acid imbalance may act, we have focused our attention upon how two amino acids, L-alanine and L-leucine, may affect another amino acid's (L-tryptophan) action on hepatic protein synthesis. Clearly, high ratios of L-alanine or L-leucine to L-tryptophan diminish the stimulatory effect of L-tryptophan alone on hepatic protein synthesis (Table 1). The selection of L-alanine and of L-leucine was based upon the ability of each to inhibit the specific binding of L-tryptophan to hepatic nuclei in vitro, a process which has been speculated to be involved in affecting hepatic protein synthesis (Kurl et al., 1988; Sidransky and Verney, 1996, 1997). Such an effect may indeed be one route by which ingested imbalances may act. Another possibility is that the imbalances may affect the regulatory and molecular aspects of mammalian amino acid transport. It appears that two groups (the aromatic amino acids (i.e. L-tryptophan) and the branched chain amino acids (i.e. L-leucine)) utilize the same major amino acid transport system (McGiven and Paster-Anglada, 1994). Thus, if the two groups are transported on a common carrier and this is the only major route of transport, then the transport of each amino acid may be competitively inhibited by the other. That such factors may play a role in our present study is difficult to conclude.

In an attempt to determine whether the amino acid imbalances induced in our experiments may possibly cause altered absorption and transport of L-tryptophan, we measured free L-tryptophan concentrations in sera and livers of mice at time of killing (1 h after each tube-feeding). The results (Table 2) indicated that the elevated serum free tryptophan levels were similar whether L-tryptophan was tube-fed alone or together with L-leucine or L-alanine. Also, the hepatic free tryptophan levels were similar in all groups. These findings suggest that major alterations in intestinal absorption and transport of L-tryptophan to the liver at least at 1 h after tube-feeding, are not apparent. Thus, it suggests that such a mechanism does not influence the results pertaining to hepatic protein synthesis.

Amino acid imbalances have been investigated in many experimental studies and have been described as affecting a variety of nutritional and metabolic effects (Harper et al., 1970). In our laboratory we have previously investigated amino acid imbalances induced rapidly (within days) by tube-feeding purified diets devoid of single, indispensable amino acids (Sidransky, 1972) or within minutes by the administration of complete amino acids devoid of single, indispensable amino acids (Sidransky et al., 1968) or of L-tryptophan alone (Sidransky et al., 1968; 1971). In the present study, we investigated imbalanced states by tube-feeding L-alanine or L-leucine alone or together with a constant level of L-tryptophan and at different ratios of each amino acid to L-tryptophan. Our findings that the levels of L-alanine or L-leucine in relation to L-tryptophan are of vital importance on the effect of L-tryptophan-induced stimulation of hepatic protein synthesis. At ratios such as L-alanine to L-tryptophan of less than 2.1 or as L-leucine to

L-tryptophan of 7.2 or less, the stimulatory effect of L-tryptophan is minimally reduced. However, at higher ratios of the above, respectively, the effects are those of significantly reduced stimulation of hepatic protein synthesis. The results of our controlled experiments stress the importance of imbalances that can be produced by altered concentrations of certain amino acids on the actions of another amino acid. Thus, when interpreting the stimulation of hepatic protein synthesis by one amino acid, such as L-tryptophan, when ingested alone, it is important to realize that when ingested with other amino acids, especially others at certain concentrations, the response due to the first one may become modified. Since L-tryptophan has been widely used for many years as a natural agent for possible relief of many conditions (depression, pain, insomnia, hyperactivity and eating disorders), it is of special interest to determine how it acts when administered alone as well as when it is ingested along with combinations of other amino acids, specifically as related to specific concentrations of the other amino acids.

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