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Changes in plasma amino acid and subjective sleepiness ratings in humans after consuming *L*-tryptophan/maltodextrin mixes

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Summary. In animals, there is some evidence that increasing brain tryptophan (TRP) levels can increase brain serotonin (5-HT) synthesis and facilitate sleep onset. The plasma ratio of TRP to the other large neutral amino acids (TRP/LNAA ratio) must at least triple before detectable increases in brain 5-HT occur. In young men, consumption of 500 mg TRP combined with a carbohydrate (CHO) load will triple this ratio. In a study on 72 volunteers with mild insomnia, using subjective ratings of sleep onset and quality, this combination significantly decreased sleep latency. We noticed, however, that young women seemed to be particularly responsive to the sleepiness-inducing effects of TRP/CHO mixes. The present study was designed to examine more closely the effects of TRP/CHO mixes on subjectively rated sedation in young women and to check if their plasma amino acid response differs from that of men. On three evenings, nine healthy young women consumed 0, 500, or 1000 mg TRP combined with 30 g of maltodextrin. Blood samples were collected at 0, 30, 60, 90 and 120 minutes. Sleepiness was rated during the evening. The study was carried out double-blind, and each woman received all 3 treatments balanced across days. Plasma TRP/LNAA ratios tripled after 500 mg and quadrupled after 1000 mg of TRP. All women reported a marked increase in sleepiness with TRP. We conclude that young women do seem to be more sensitive than men to the sedating effect of TRP/CHO mixes but their plasma amino acid responses are similar.

Keywords: Tryptophan – Maltodextrin – Sleep

Introduction

Serotonin (5-Hydroxytryntamine, 5-HT) in the brain is synthesized from the essential amino acid tryptophan (TRP). This amino acid is carried across the blood-brain barrier by the large neutral amino acid (LNAA) transport system, so

it must compete with valine (VAL), leucine (LEU), isoleucine (ILE), tyrosine (TYR), phenylalanine (PHE) and methionine (MET) for access to the carrier-binding site [1]. In rats, the plasma TRP/LNAA ratio correlates positively with brain levels of TRP, and large increases in the ratio can lead to changes in brain 5-HT and 5-HIAA (5-hydroxyindoleacetic acid; a metabolite of 5-HT) [2, 3]. A three to four-fold rise in the plasma TRP/LNAA ratio about doubles brain TRP, which in turn produces a 10 to 20% increase in brain 5-HT + 5-HIAA [2]. Increases in 5-HT of this order have been reported to facilitate sleepiness or sedation, probably by influencing brain functions linked to serotoninergic neurotransmission [4, 5, 6]. Lowering 5-HT (either by destruction of the raphé system or by blocking tryptophan hydroxylase by treatment with *p*-chlorophenylalanine) can lead to prolonged insomnia.

Current evidence suggests that the characteristics of blood-brain barrier transport are similar in rats, monkeys and humans [7, 8]. It is possible therefore that tripling or quadrupling the plasma TRP/LNAA ratio might raise brain TRP and 5-HT enough to influence sleep onset in man. We have followed changes in this ratio in healthy young men after high-carbohydrate, protein-free and high-protein evening meals, with or without addition of tryptophan [9]. The meals alone had little or no effect on the TRP/LNAA ratio. After administration of 400 mg TRP combined with a carbohydrate meal, the ratio doubled after 1h and tripled at 2 hours. As we have demonstrated in earlier experiments with other putative mild sedatives, questionnaire studies using a large population are very effective for establishing whether people actually experience a given treatment as being sedative, detecting which aspects of sleep are influenced, identifying groups of people who are "responders", and detecting side effects [10]. Using this approach, we tested on 72 volunteers the effects of 500 mg TRP combined with bitter-sweet chocolate, a vehicle which efficiently disguises the slightly bitter taste of tryptophan, and which provides a small carbohydrate load [11]. A double-blind cross-over design with five TRP and five placebo nights for each volunteer was used. After tryptophan administration, perceived sleep latency was significantly shorter, ratings for sleep depth increased, and TRP-containing samples were perceived as having a calming effect. When volunteers were separated into four subgroups (men/women; under 40/40 and over), it emerged that the young women appeared to be more responsive than the other groups to the sedative effects of tryptophan [3]. Since this observation (ie., the particular sensitivity of young women to TRP/CHO mixes) had not been predicted, it was important to check if the same finding occurred on repetition. In addition, since the initial biochemical studies were carried out on young men, it was also important to check if the plasma amino acid responses were similar in young women. The present experiment was designed to verify these two points.

Experimental procedures

Subjects

Nine healthy women, aged 22 to 38 years with Body Mass Indices (BMI) between 19 and 25 were recruited from the staff at the Nestlé Research Laboratories. A medical examination was given before enrollment, the nature of the study was described in detail to each volunteer as a study of the kinetics of plasma amino acid changes after consumption of tryptophan-containing drinks. No mention was made concerning possible sleep-inducing (or stimulating) effects of the treatments. Each woman signed a consent form and agreed to follow the instructions laid out in the experimental protocol. The protocol was approved by the Nestlé Ethical Committee for Human Experimentation.

Diets

Standard meals were fed for breakfast, lunch and an early evening snack on each of the three experimental days. The breakfast contained 710kcal and 28g protein; lunch, 620kcal, 27g protein; the early evening snack, 200kcal, 7g protein.

Test samples

Pharmaceutical grade L-tryptophan was bought from Hakko Kogyo Co. Ltd., Tokyo, and sealed into gelatine capsules (bicolor, opaque, size #0). Each capsule contained 250 mg L-TRP. The placebo capsules contained an equivalent volume of Celite. Filling and sealing of capsules was carried out by Schaller Pharmaceutical, CH-Lausanne. The test samples were taken with 200 ml orange juice supplemented with 309 maltodextrin (MD05).

Experimental design

The treatments were given at weekly intervals using a multiple latin square design so that each subject received all treatments, and treatments were balanced across days. The study was carried out using two repetitions with 4 women in the first group and 5 in the second. The women were asked to avoid excessive or abnormal meals, alcohol, or vigorous exercise during the day preceding each treatment. On each test day, they were given breakfast at 08h00, a lunch at 12h00, and the snack at 17h00. During this period they were allowed to drink water, or decafeinated, unsweetened coffee or tea. At 20h00 a catheter was placed in the anticubital vein in each volunteer, and a 10 ml sample of venous blood taken (time 0). The woman then received a drink (200 ml orange juice containing 30 g CHO) and 4 capsules containing either placebo, 500 mg TRP or 1000 mg TRP. Every 30 minutes and until 22h00, blood samples were collected.

Amino acids analysis

Blood was collected into EDTA-treated tubes, refrigerated on ice and centrifuged at 3000 rpm for 15 min. Aliquots (1 ml) of plasma were transferred into tubes and frozen at $-80°C$ until assayed. Plasma amino acids were measured using HPLC with on-line derivatisation [12], plasma total and free TRP by equilibrium dialysis followed by fluorimetry [13], triglycerides and free fatty acids by Cobas (Hoffman LaRoche Ltd. Basle, Switzerland).

Subjectives parameters

Sleepiness during the evening test was rated using a 3-point sleepiness questionnaire [11].

Statistical methods

Statistical analyses were performed using canonical analysis and analysis of variance [14, 15].

Results

The treatments were well tolerated. The only side-effects reported were 4 cases of mild headache (three with the placebo, one with 500 mg TRP and none with 1000 mg). Four subjects reported "intense sleepiness" about 90 min after consuming 1 g of TRP. The meals were considered adequate. The results for the two repetitions were practically identical and so were combined for data analysis.

Total and free tryptophan

After 30 g carbohydrate, plasma total tryptophan levels fell by 17%. With 500 mg TRP added, they doubled at 2 h, and after 1000 mg tripled at about 1.5 h (Fig. 1). Plasma free tryptophan fell by nearly 25% after carbohydrate alone, tripled after 500 mg TRP, and rose 6-fold after 1000 mg.

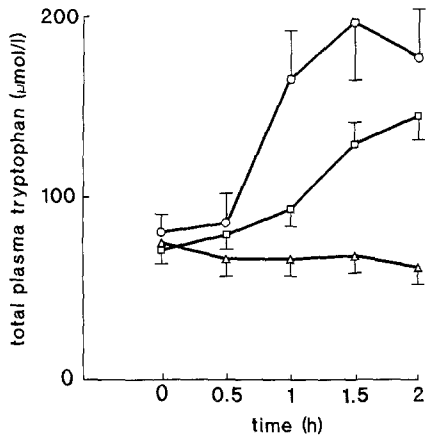

Fig. 1. Plasma total tryptophan levels in young women during the 2 h following ingestion of 0 (open triangles), 500 (open squares), or 1000 mg (open circles) of TRP combined with 30 g of maltodextrin

Plasma tryptophan/Large neutral amino acid ratios

As in earlier studies [9], the TRP/LNAA ratio was unchanged by carbohydrate alone. It tripled after administration of 500 mg TRP combined with a carbohydrate load. With 1000 mg, it rose 4-fold (Fig. 2).

Other large neutral amino acids

Plasma levels of all the large neutral amino acids fell by similar amounts with all three treatments, suggesting that the carbohydrate load influenced their

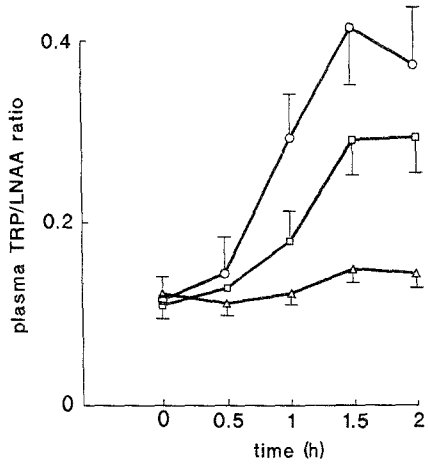

Fig. 2. Mean plasma tryptophan/large neutral amino acid ie.:

(TRP/TYR + PHE + LEU + ILE + VAL)

ratios in young women during the 2 h following ingestion of 0 (open triangles), 500 (open squares), or 1000 mg (open circles) of TRP combined with 30 g of maltodextrin

concentrations but TRP did not. Tyrosine, valine, phenylalanine and methionine decreased by 15–20%, leucine and isoleucine by about 30% (Table 1).

Glucose, triglycerides and free fatty acids

Plasma glucose rose by 60%, peaked between 30 and 60 minutes, and fell to starting values at 2 hours. The response patterns were practically identical for the three treatments (Table 2). Plasma triglycerides ranged from 6 to 8 mmol/l and were unaffected by tryptophan or maltodextrin, while free fatty acids fell from initial values of 20–40 micromole/l to almost undetectable levels after 1 h.

Behavioural responses

At the end of each evening, the women were also asked if they thought that the treatment they had received had been sedative, stimulating or without effect. All reported the placebo to be without effect. With the lower dose of TRP, seven reported the treatment to be sedating. With the higher dose, all found the treatment sedating. None reported a treatment to be stimulating. The TRP/CHO mixes also increased sleepiness scores (rated immediately after taking the last blood sample) in all subjects and in a dose-dependent manner (Table 3). The peak of sleepiness occurred about 90 min after consumption of TRP.

Discussion

The rationale for this series of studies was based on the following experimental observations: (A) In some experimental situations, increasing brain 5-HT by

Table 1. Mean plasma large neutral amino acid levels and TRP/LNAA ratios (see heading of Fig. 2 for details) in young women during the 2 h following ingestion of 0, 500, or 1000 mg of TRP combined with 30 g of maltodextrin

Treatment	Time after consumption of TRP/CHO mix (hours)				
	0	0.5	1.0	1.5	2
Tyrosine					
0	67	63	56	56	54
0.5 g TRP	74	68	56	50	57
1.0 g TRP	78	70	63	55	54
Valine					
0	245	245	214	225	210
0.5 g TRP	254	260	219	192	211
1.0 g TRP	266	249	238	217	213
Leucine					
0	141	129	110	112	100
0.5 g TRP	140	135	111	92	101
1.0 g TRP	155	131	119	106	105
Isoleucine					
0	78	76	62	65	55
0.5 g TRP	80	76	59	49	54
1.0 g TRP	87	71	59	51	54
Phenylalanine					
0	65	63	56	58	58
0.5 g TRP	66	63	53	51	55
1.0 g TRP	73	63	61	54	55
Tryptophan					
0	75	65	64	68	62
0.5 g TRP	70	82	94	129	144
1.0 g TRP	79	85	165	204	180
TRP/LNAA ratio					
0	0.12	0.11	0.12	0.13	0.12
0.5 g TRP	0.11	0.13	0.18	0.29	0.29
1.0 g TRP	0.12	0.14	0.29	0.42	0.37

small amounts can facilitate sleep onset [3, 4, 5, 6], with a change of about 10–20% being required to see a significant effect [5]. (B) Increases in the plasma TRP/LNAA ratio of three- to four-fold can increase brain 5-HT (and/or 5-HIAA) by 10–20% [2, 3].

We first showed, in young men, that 500 mg TRP combined with a small carbohydrate load will increase the plasma TRP/LNAA ratio 3-fold [9], and then noted a significant increase in sleepiness and a decrease in perceived sleep latency with a similar mixture [11, 16]. In this last experiment [11] we also found that

Table 2. Mean plasma glucose levels (±SEM) in young women during the 2 h following ingestion of 0, 500, or 1000 mg of TRP combined with 30 g of maltodextrin

Treatment	Time after consumption of TRP/CHO mix (hours)				
	0	0.5	1.0	1.5	2
Glucose (mmol/l)					
0	5.1 ± 0.2	8.0 ± 0.3	7.9 ± 0.4	6.7 ± 0.4	5.3 ± 0.3
0.5 g TRP	4.9 ± 0.1	7.5 ± 0.4	8.1 ± 0.5	7.2 ± 0.3	5.7 ± 0.3
1.0 g TRP	4.8 ± 0.2	8.2 ± 0.3	7.9 ± 0.4	6.5 ± 0.4	5.7 ± 0.3

Table 3. Distribution of sleepiness and treatment effect ratings in young women 2 h following ingestion of 0, 500, or 1000 mg of TRP combined with 30 g of maltodextrin

	SLEEPINESS RATINGS		
	NOT SLEEPY	SOMEWHAT SLEEPY	EXTREMELY SLEEPY
PLACEBO	7	2	0
0.5g TRP	3	6	0
1.0g TRP	0	1	8

young women appeared to be more responsive to the TRP/CHO mix than men or older women [3]. This study was designed to check: first, if young women were consistently responsive to TRP/CHO, and second, if their plasma amino acid responses were similar to those in men. The reason for the experimental design in which tryptophan was combined with a carbohydrate load and separated by several hours from any meal containing significant amounts of protein was that the influx of large neutral amino acids from concurrently eaten protein would be expected to increase the denominator of the plasma TRP/LNAA ratio, diminish the ratio and hence slow the rate of TRP transport into the brain [7].

The results suggest that the young women without particular sleep problems were more responsive to the sedative effects of TRP. Seven of the nine thought that the 500 mg dose of TRP was sedating, and all of them found 1000 mg sedating. Similarly, four reported increased sleepiness with the lower dose of TRP, and all reported this effect with the higher dose. In our earlier study, few of the men or older women reported increased sleepiness with 500 mg TRP [3].

As for the second question, the results show that, for equivalent ingestion of TRP/maltodextrin mixes, the changes in the plasma TRP/LNAA ratio in young men and young women were remarkably similar. With 500 mg TRP, in young men the ratio rose from 0.11 ± 0.01 to 0.29 ± 0.02 [9]. In this study, they rose from 0.11 ± 0.01 to 0.29 ± 0.025. Thus the greater responsiveness of young women to TRP seen earlier [3] does not seem to be due to a more marked increase in the plasma TRP/LNAA ratio.

At the moment we do not have any clear explanation for this difference in tryptophan's effects on men and women. We can speculate that perhaps there is no absolute difference but women are better able to detect the subtle changes in sleepiness produced by small doses of tryptophan combined with maltodextrin. An alternative explanation is that differences in metabolism between men and women may lead to diminished binding of TRP to plasma albumin in women as compared to men. This could in turn lead to a more efficient transport of TRP into brain [17].

These results illustrate several other points. First, they suggest that 500 mg is the limit at which effects of TRP on arousal can be detected in young women and that 1000 mg of TRP provides more consistent results. This conclusion is in agreement with that drawn by Hartman and Greenwald [18] in a survey of 43 different studies on the sedative effects of TRP. This fits well with our own more recent observations in non-human primates [19] where tripling the plasma TRP/LNAA ratio failed to produce significant changes in brain stem 5-HT or 5-HIAA. Higher ratios did increase brain indoleamines.

These observations are important for another reason. There has been considerable speculation that even very small changes in the ratio might influence brain TRP, as well as brain serotonin metabolism and function. The results of behavioural studies aimed at testing this idea have usually been negative, although there have been one or two provocative findings [20]. The confirmation that rather large changes in plasma TRP/LNAA ratios are needed before subjectively detectable sleepiness can be detected casts further doubt on these speculations. In addition, this experiment shows that a small carbohydrate load, taken in the evening, lowers plasma levels of all the LNAA (including TRP) but has no effect at all on the TRP/LNAA ratio (see Fig. 2).

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