The Effect of L-Tryptophan on Daytime Sleep Latency in Normals: Correlation with Blood Levels

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Summary: L-Tryptophan, an essential amino acid, is readily converted to serotonin, which is thought to be important for expression of slow wave sleep and possibly rapid eye movement (REM) sleep. A vast but often confusing literature exists on L-tryptophan effects on inducing, maintaining, or altering sleep. In this study we measured the effects of L-tryptophan on objective (multiple sleep latency) and subjective [Stanford Sleepiness Scale (SSS)] measures of sleepiness and examined their relationship to blood L-tryptophan levels. Ten healthy volunteers (eight men and two women; mean ± SD age 34 ± 10 years) received placebo or 1.2 or 2.4 g of L-tryptophan on separate days in random double-blind fashion. Sleep latency and SSS were measured initially and at 60 and 120 min after ingestion. Blood and urine were collected at regular intervals. Compared with placebo both L-tryptophan doses reduced sleep latency at 1 h, with the reduction persisting at 2 h for the 2.4-g dose only (p < 0.05). There was a positive correlation between subjective and objective sleepiness measures but only with the 2.4-g dose (r_s = 0.76, p < 0.01). There was a highly significant correlation between blood L-tryptophan and sleep latency at 0, 60, and 120 min in all subjects for all drug conditions (r = 0.276, df = 79, p = 0.013). Very small amounts of free L-tryptophan or its metabolites were found in the urine, with the exception of kynurenic acid. We conclude that L-tryptophan consistently reduced sleep latency in normals and that this correlates with blood levels. Increased urinary excretion of kynurenic acid suggests that blood kynurenic acid was similarly elevated and that kynurenic acid, given its inhibitory effects on excitatory neurotransmission, may possibly be involved with inducing sleep. Key Words: L-Tryptophan—Sleep latency—Sleepiness—Kynurenic acid.

Since Jouvet's early work, it has been suggested that serotonin and its congeners are in some way responsible for slow-wave sleep (SWS) and for the priming of paradoxical or rapid eye movement (REM) sleep (1,2). L-Tryptophan is an essential amino acid that

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is readily converted to 5-hydroxytryptamine or serotonin. Thus, a vast literature has emerged on L-tryptophan effects on sleep induction, sleep maintenance, and sleep architecture. A wide range of dosages has been used in a wide variety of subjects, including normals, insomniacs, and patients with psychiatric illnesses. Unfortunately, the results have not been consistent and so it is not clear how effective L-tryptophan is for inducing or maintaining sleep or altering the distribution of sleep stages. For sleep architecture changes with L-tryptophan, some authors show no effect on SWS or REM with low doses (<5 g) (3–7), a modest increase in SWS and/or decrease in REM with an intermediate dose (5–9 g) (8–11), and paradoxical results, i.e., decreased SWS and increased REM with higher dosages (10–15 g) (8,9). In examining the effects on sleep latency, the nocturnal administration of L-tryptophan is compounded by the usually shortened sleep latency at bedtime. It has been suggested that L-tryptophan simply lowers the arousal threshold during the waking state to permit more rapid sleep onset (7). It is also possible that the effect of L-tryptophan on sleep is not related to serotonin at all. Some other L-tryptophan metabolic byproduct may have an effect on sleep. For example, one of the major metabolic end products of L-tryptophan metabolism is kynurenic acid. This agent has the effect of broad-spectrum inhibition of excitatory amino acid neurotransmission at the N-methyl-D-aspartate (NMDA) and non-NMDA receptors (12).

Most if not all studies have measured sleep latency and subsequent sleep architecture after L-tryptophan dosing without interrupting the sleep. As such, there is little or no information on L-tryptophan effects on sleepiness as objectively measured with the Multiple Sleep Latency Test (MSLT). Accordingly, we wanted to examine the effects of L-tryptophan, if any, on sleepiness as measured by a modified MSLT during the morning hours, when sleepiness is at a minimum. In addition, we measured blood L-tryptophan to examine its relationship to objective (sleep latency) and subjective (Stanford Sleepiness Scale) (SSS) measures of sleepiness.

L-Tryptophan is readily available in many countries from "health food stores." Since the dosage used is usually lower than reported in the literature, we studied dosages likely to be used by patients.

METHODS

We recruited 10 healthy volunteers (eight men and two women; mean ± SD age 34 ± 3.3 years) who gave no history of sleep disorders. Polysomnography or daytime MSLT testing was not performed prior to the study, but a sleep history was obtained and subjects maintained their usual sleep-wake cycle for the duration of the study. One subject reported his normal sleep period time as only 5 h. However, he denied any daytime somnolence, so was considered at the outset to be a short sleeper.

In a random double-blind fashion, each subject received placebo or 1.2 or 2.4 g of L-tryptophan in tablet form on separate days after an overnight fast. Subjects reported to the sleep laboratory at 0800 h after their usual night’s sleep at home. The only intake permitted was water until all samples were obtained (1600 h). Surface electrodes were applied to measure electroencephalogram (EEG) (C3-A2, C3-O2), submental electromyogram (EMG), and electrooculogram (EOG) and a heparin-locked intravenous line was established to allow repeated blood sampling. Measurement of sleep latency time (SLT) was recorded at 0900, 1000, and 1100 h according to standard protocol (13). Sleep was not permitted to accumulate between nap times. Subjects ingested placebo or drug
with water just prior to the first nap and subjective ratings of sleepiness were recorded before each nap using the short SSS (14).

To determine the absorption, distribution, and excretion of nonmetabolized L-tryptophan, blood and urine were collected over the course of each test day. Blood was collected just prior to ingestion of placebo or drug (time 0) and 30, 60, 80, 95, 105, 120, 150, 180, 240, and 360 min thereafter. Urine was collected at 60, 240, and 480 min, the volumes measured and rounded to the nearest 10 ml. Serum and urinary L-tryptophan levels and urinary metabolites of L-tryptophan were measured by high pressure liquid chromatography using a modification of the method of Pfeifer et al. (15). Serum L-tryptophan measurements were obtained after filtration to remove residual protein. Accordingly serum measurements are for free L-tryptophan.

Statistical analysis included analysis of variance for effects of subject, dosage, and time on SLT, while nonparametric correlation assessed the relationship between blood level of L-tryptophan, SLT, and SSS.

RESULTS

Sleepiness

The individual results of the SLTs and the SSS are shown in Table 1. One subject had a sleep latency consistently <5 min, which is in the pathological range, and he was excluded from the analysis. This subject (a physician) would regularly allow himself only 5 h of sleep per night. While we initially thought he might just be a short sleeper, his short sleep latencies were attributed to sleep deprivation.

Figure 1 shows the mean SLTs for placebo and two doses of tryptophan. Both doses (1.2 and 2.4 g) produced a significant reduction in sleep latency compared with placebo at 1 h, with the reduction persisting at 2 h for the 2.4-g dose only (p < 0.05). The relationship between subjective and objective measures of sleepiness is shown in Fig. 2. Here we are looking at the change in SLT between naps and the corresponding change in SSS. For SSS, an increasing score (from 1 to 7) relates to increasing subjective sleepiness; thus, a negative change in SSS between naps indicates increasing subjective sleepiness. Conversely, for SLT a decreasing value (from 20 to 1) indicates increasing sleepiness and will be reflected by a positive change in SLT between naps.

Grouping all three drug conditions together, there was an overall positive correlation between subjective and objective measures of sleepiness ($r_s = 0.44$, $p < 0.05$). However, taking each condition separately, this was statistically significant only for the 2.4-g dose ($r_s = 0.76$, $p < 0.01$) (Fig. 2), despite a similar reduction in sleep latency with the 1.2-g dose ($r_s = 0.37$, $p = NS$) (Fig. 1).

There were no adverse effects from the tryptophan although four subjects reported a mild transient headache with the 2.4-g dose.

Blood levels

Blood levels are shown in Fig. 3. After ingestion of 1.2 g, peak level was at 60 min. Peak blood level for the 2.4-g dose was at 105 min. Interestingly, the blood level at 60 min was virtually the same for both dosages. Our results are consistent with those of Yuwiler et al. (16) who used a dose of 50 mg/kg of tryptophan.

We examined the correlation between L-tryptophan blood level and sleep latency at 0, 60, and 120 min in all subjects for all conditions. This relationship was highly significant ($r = -0.2761$, df = 79, $p = 0.0126$). However, only 8% of the variability of SLT was explainable by the variability in absolute tryptophan blood level. Thus, we
also examined the relationship between the change in tryptophan level from the preceding nap versus the change in sleep latency from the preceding nap. Thus, for each subject for each condition, we had the following two sets of data points: (a) L-tryptophan level (60 min - 0 min) sleep latency (60 min - 0 min) and (b) tryptophan level (120 min - 60 min) sleep latency (120 min - 60 min). The relationship between the change in tryptophan level was significantly correlated with the change in sleep latency (r = -0.284, df = 54, p = 0.037). Very large increases in L-tryptophan levels between naps did not appear to further reduce sleep latency. Indeed, if one excludes the larger internap tryptophan changes (exceeding 50 mg/L) from analysis, then a much better correlation exists (r = -0.434, df = 41, p = 0.0036).

**Urine levels**

Very little free tryptophan appeared in the urine in the 8 h after ingestion: 12.9 ± 4.2 mg after placebo, 27.0 ± 10.1 mg after 1.2 g (representing 2.3% of the ingested dose),
and 27.5 mg after 2.4 g (representing 1.1% of the ingested dose). With the exception of kynurenic acid, there were very small amounts of other L-tryptophan metabolites in the urine (Table 2). Urinary 5-hydroxyindoleacetic acid increased with dose of L-tryptophan, but the levels were all low and within the accepted normal range of 1–5 mg/24 h. We found a marked increase in urinary excretion of kynurenic acid in the 8 h after ingestion of L-tryptophan (total excretion: 1.89 ± 2.96 mg after placebo, 17.89 ± 15.68 mg after 1.2 g, and 37.08 ± 15.24 mg after 2.4 g). The total urinary excretion of both L-tryptophan and kynurenic acid was highly significantly correlated with peak blood L-tryptophan during the study period \( r = 0.5865, df = 28, p = 0.0006 \) for tryptophan; \( r = 0.5476, df = 28, p = 0.017 \) for kynurenic acid). There was also a very high correlation between urinary excretion of tryptophan and kynurenic acid \( r = 0.5568, df = 28, p = 0.0014 \). Urinary 5-hydroxyindoleacetic acid, the main final metabolic byproduct of serotonin, was not correlated with either peak L-tryptophan blood level \( r = 0.27, df = 28, p = \text{NS} \) or total urinary excretion of L-tryptophan \( r = 0.18, df = 28, p = \text{NS} \). A detailed examination of the pharmacokinetics of the L-tryptophan metabolic products is being conducted.

**DISCUSSION**

Daytime sleepiness is normally minimal during the morning hours. By administering L-tryptophan in the morning and subsequently measuring sleep latency, we felt that any effect of L-tryptophan would be easily discernible at that time. The results show that sleep latency was consistently reduced with L-tryptophan in this short-term experiment. This reduction coincides with a rise in serum tryptophan, and as the serum level
falls, the sleep latency returns toward baseline. These results are consistent with the hypothesis that L-tryptophan mediates increases in serotonin and thus facilitates sleep onset. If this were the case, the previous vast literature should all be consistent. However, most previous studies with L-tryptophan have not measured serum or plasma levels and only a few have measured urinary excretion of metabolites. Accordingly, variations in absorption and metabolism of L-tryptophan might give widely varying blood levels and account for a good deal of the variation in the previous literature. By measuring blood levels frequently between naps, we could establish a relationship between serum L-tryptophan and sleep latency. As well, most studies with ingestion of L-tryptophan at bedtime have not been conducted after 8 h of fasting. L-Tryptophan ingestion in the fasting state, as in our case, may well be important in determining the amount of tryptophan available for metabolism and neurotransmitter synthesis in the brain.

Extensive work by Wurtman and colleagues (17–20) has shown that the composition of food intake influences amino acid transport across the blood–brain barrier. For large neutral amino acids (such as tryptophan, tyrosine, phenylalanine, leucine, isoleucine, and valine), there is a single carrier molecule for which each amino acid must compete. Since there is much less tryptophan in most proteins than the other large neutral amino acids, a high-protein meal will reduce the ratio of tryptophan to competing amino acids and less tryptophan will cross to the brain for subsequent metabolism. Conversely, a
**L-TRYPTOPHAN AND DAYTIME SLEEP LATENCY**

FIG. 3. Mean blood tryptophan (TRYPT) level changes with time for placebo and both doses of L-tryptophan (symbols same as in Fig. 1).

A high carbohydrate meal has the opposite effect because the insulin secreted in response to carbohydrate reduces the plasma level of competing amino acids more than it does tryptophan. While we did not measure the serum concentrations of the competing large neutral amino acids, it is quite possible that the ratio of tryptophan to other amino acids was increased by ingesting L-tryptophan in the fasting state. This would have facilitated increased blood-brain transport and increased metabolism to serotonin and other neurotransmitters.

Besides being readily metabolized to serotonin, L-tryptophan can undergo complete

<table>
<thead>
<tr>
<th>Compound</th>
<th>Placebo</th>
<th>L-Tryptophan 1.2 mg</th>
<th>L-Tryptophan 2.4 mg</th>
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<tr>
<td>5-Hydroxytryptophan</td>
<td>0</td>
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<td>2.6 (3.9)</td>
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<td>0</td>
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<tr>
<td>Indole-3-acetic acid</td>
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<td>2.3 (3.3)</td>
<td>2.9 (5.9)</td>
</tr>
<tr>
<td>Indole-3-proprionic acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3-Hydroxyacetic acid</td>
<td>0</td>
<td>0.3 (0.2)</td>
<td>0.7 (1.4)</td>
</tr>
<tr>
<td>5-Hydroxyindoleacetic acid</td>
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<td>0.5 (0.5)</td>
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</tr>
<tr>
<td>3-Hydroxykynurenine</td>
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<td>Kynurenic acid</td>
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<td>17.9 (15.7)</td>
<td>37.1 (15.2)</td>
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<td>Kynurenine</td>
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<td>0.2 (0.4)</td>
<td>2.8 (3.3)</td>
</tr>
<tr>
<td>Xanthic acid</td>
<td>0.3 (0.5)</td>
<td>0.9 (1.1)</td>
<td>4.1 (3.9)</td>
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Values are means (SD).
catabolism via the enzyme tryptophan pyrrolase and the kynurenine pathway. In peripheral tissues, the metabolism of tryptophan by this pathway is about half as much as the rate of tryptophan to serotonin (21), while in the pineal gland, kynurenine accounts for 32% of all tryptophan metabolites (22). Although at the outset we did not think to measure serum kynurenine levels (or other serum intermediate metabolites of the kynurenine pathway), we did note a marked increase in urinary kynurenic acid with L-tryptophan ingestion. This suggests that the blood kynurenine levels were similarly increased and we might expect brain levels to show an increase. A brain kynurenine increase is possible either by directly crossing the blood–brain barrier (as it does in animals) (21) or by increased metabolism of tryptophan since cerebral tryptophan pyrrolase can be induced by a tryptophan load. Since kynurenic acid is a broad-spectrum inhibitor of excitatory amino acid neurotransmission, it might possibly be having an effect on sleep. We acknowledge, however, that this is speculation since we do not have any serum or brain kynurenine levels. Our goal was not to address the complex neurotransmitter interaction involved in sleep onset and the expression of sleep stages. Instead we chose to examine short-term effects on sleep latency and concomitant changes in serum L-tryptophan.

Subjective measures of sleepiness (SSS) paralleled the objective measures (SLT), but only with the 2.4-g L-tryptophan dose. Despite similar blood levels and reductions in sleep latency at 60 min post L-tryptophan, only four subjects rated themselves more sleepy with 1.2 g L-tryptophan while eight subjects gave a higher SSS at 60 min with 2.4 g L-tryptophan. Although there was a highly significant relationship between absolute L-tryptophan blood level and sleep latency, only 8% of the variability in sleep latency was explainable by the variability in absolute L-tryptophan level. This suggests that factors other than absolute L-tryptophan blood level are important contributors to sleepiness. The design of the experiment does not permit identification of these factors.

In summary, L-tryptophan given to normals consistently reduces sleep latency and this correlates with the blood level at the time. Change in subjective sleepiness mirrors the reduction in sleep latency but consistently only with the larger dose despite similar blood levels. Increased urinary excretion of kynurenic acid suggests that blood levels were similarly elevated. Given the known inhibitory effects of kynurenic acid on excitatory amino acid neurotransmission, it is possible that it is involved in sleep onset. Further work is necessary to determine if kynurenic acid is involved in any way with sleep. In normals, L-tryptophan may be useful for sleep induction.

REFERENCES