The Influence of Intravenous L-Tryptophan on Plasma Melatonin and Sleep in Men

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Summary

The sleep-inducing mechanisms of L-Tryptophan (L-Trp) are generally thought to be mediated by a central serotonergic activation. Evidence is presented that some effects of L-Trp on sleep may be mediated by melatonin, a Trp-metabolite with sedative properties. Trp effects on vigilance, sleep, and plasma-melatonin concentrations were measured after double-blind application of 0, 1, 3, and 5 g L-Trp in nine and five healthy probands during daytime and nighttime, respectively. – A significant sleep-inducing effect was observed after L-Trp administration during daytime and nighttime. The infusions of L-Trp caused a massive elevation of plasma melatonin levels. This effect was significant both during the night and the day, indicating that the increment of circulating melatonin may be of extrapineal origin.

Die Wirkung von L-Tryptophan-Infusionen auf Plasma-Melatonin und Schlaf beim Menschen

Die Wirkungen von L-Tryptophan (L-Trp) auf den Schlaf wurden bisher ausschließlich zentralnervösen serotoninergen Mechanismen zugeschrieben. Diese Studie sollte klären, ob neben dem Serotonin (5-HT) andere Stoffwechsel-Metaboliten des L-Trp mit sedativer Potenz, wie z. B. das Melatonin, die Wirkung von L-Trp auf den Schlaf vermitteln. Vigilanz, Schlaf und Hormonprofil des Plasma-Melatonins wurden nach doppelblinder Gabe von 0, 1, 3, und 5 g L-Trp bei 9 gesunden Probanden am Tage und bei 5 Probanden in der Nacht untersucht. – L-Trp zeigte sowohl nachts als auch am Tage einen schlafanstoßenden Effekt. Der Melatonin-Plasma-Spiegel wurde nicht nur in der Nacht, sondern auch am Tage signifikant erhöht. Diese Ergebnisse stützen die Hypothese einer Melatonin-vermittelten Wirkung von L-Trp auf Vigilanz und Schlaf. Hohe Anstiege des Melatonin-Plasma-Spiegels am Tage zeigen, daß L-Trp die tageslichtbedingte pineale Suppression der Melatonin-Synthese durchbricht. Dies beruht möglicherweise auf einer extrapinealen Produktion oder Freisetzung des Melatonin.

Introduction

Until the publication of side-effects (*MMWR*, 1989), the serotonin (5-HT) precursor L-Tryptophan (L-Trp) was widely used as a drug and food additive for the natural support of central serotonergic transmission. A major field of its application was the treatment of sleep disorders. The underlying therapeutical concept was supported by the serotonergic theory of sleep (*Jouvet*, 1984). Trp was found to stimulate the release of pituitary hormones, like prolactin, by a mechanism which seemed to involve an activation of the serotonergic system (*Cowen*, 1987). The concept of the 5-HT mediated effect of L-Trp on sleep was therefore readily accepted. However, the existing neuroendocrine challenge tests of central serotonergic activity are not sufficiently selective or reliable (*van Praag*, 1987). Furthermore, clinical trials in normal subjects and insomniacs showed a shoertening of sleep latency as the most common effect (*Hartmann* and *Greenwald*, 1984; *Körner* et al., 1986; *Schneider-Helmert* and *Spinweber*, 1986; *George* et al., 1989). L-Trp effects on other sleep parameters were contradictory. These contradictions may be explained by effects of L-Trp on other, non serotonergic-mediated mechanisms of sleep regulation. The influence of L-Trp on metabolites further down the 5-HT pathway, for instance, has not been investigated in much detail. This is surprising, because at least one of these metabolites, the pineal hormone melatonin, is well known for its sedating, hypnotic, and sleep-inducing effects (*Liebermann*, 1986; *Mirmian* and *Pevet*, 1986; *Waldhauser* et al., 1990).

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The identification of high-affinity binding sites for melatonin in the brain (see *Stankov* and *Reiter*, 1990) and reports on influences of light on both 5-HT and melatonin plasma levels (*Rao* et al., 1990) have stimulated interest in the physiological role of this pineal hormone. Using a highly specific radio-immunoassay to measure plasma melatonin, the authors proved the dose-dependent reaction of circulating melatonin in men after L-Trp infusions during daytime and night-time.

Methods

A low-dose intravenous (i. v.) L-Trp challenge test was performed with 14 healthy male probands (24 – 33 years of age) during 36 day (nine subjects) and 20 night (five subjects) sessions. Each subject performed four trials, with weekly intervals between the sessions, which were conducted from 8 a. m. to 2 p. m. and 10 p. m. to 7 a. m. respectively. A standardized xanthine- and tyramine-free, protein- and carbohydrate-restricted diet was given over a period beginning 24 hours prior to the start of the test and lasting until the end of each test. Infusions containing 0.0 (placebo), 1.0, 3.0, and 5.0 g L-Trp diluted in 500 ml of 0.9 % NaCl solution were applied double-blind over a 40-minute period. Blood samples were obtained by an i. v. catheter from an antebrachial vein. Plasma was rapidly separated and stored at -70 °C. L-Trp and Trp metabolites were measured by high pressure liquid chromatography (HPLC) on a C_{18}-reversed phase column with online fluorescence and electrochemical detection. Plasma melatonin was measured by direct radioimmunoassay (RIA) (*Fraser* et al., 1983) with antibodies (GS 704-6483) from Guilthay Antisera (*Guilford*, U. K.). This very specific and sensitive RIA had a recovery rate of 93% and a detection limit of 1 pg/ml. Intra-assay and inter-assay variance was less than 6% and 12% respectively. The area under the curve (AUC) was calculated for the first two hours after the commencement of L-Trp infusion.

Status of vigilance was continuously measured by polysomnography, including electroencephalogram, electrooculogram, submental electromyogram, and electrocardiogram. Sleep parameters were evaluated manually according to the criteria of *Rechtschaffen* and *Kales* (1968). Within-dose differences in AUC and single measurement values and sleep parameters were analyzed using the Wilcoxon matched-pairs signed-rank test.

Results

Plasma melatonin like immunoreactivity rose immediately after L-Trp infusion during both daytime and night-time.

During daytime, plasma melatonin rose from 15 ± 5 pg/ml (AUC = 27 ± 10 pg/ml × h) to 138 ± 56 pg/ml, $p \leq 0.005$ (AUC = 164 ± 67 pg/ml × h, $p \leq 0.005$) and 284 ± 80 pg/ml, $p \leq 0.005$ (AUC = 343 ± 126 pg/ml × h, $p \leq 0.005$) following 3.0 and 5.0 g L-Trp respectively. A dose of 1.0 g L-Trp did not affect daytime melatonin significantly (Fig. 1).

During night-time, plasma melatonin increased from 10 ± 5 pg/ml (AUC = 40 ± 12 pg/ml × h) to 167 ± 105 pg/ml, $p \leq 0.05$ (AUC = 210 ± 73 pg/ml × h, $p \leq 0.05$) and 454 ± 131 pg/ml, $p \leq 0.05$ (AUC = 525 ± 124 pg/ml × h, $p \leq 0.05$) one hour after the beginning of 3.0 g and 5.0 g L-Trp infusions respectively. A slight but not significant increase in plasma melatonin was observed for 1.0 g L-Trp (Fig. 2).

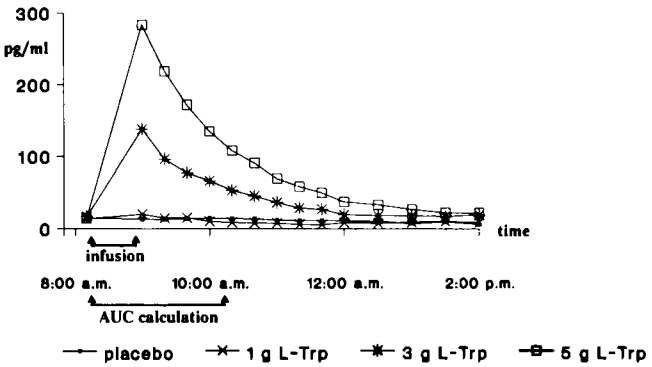

Fig. 1 Melatonin plasma concentration after L-Trp infusion during daytime in nine healthy volunteers

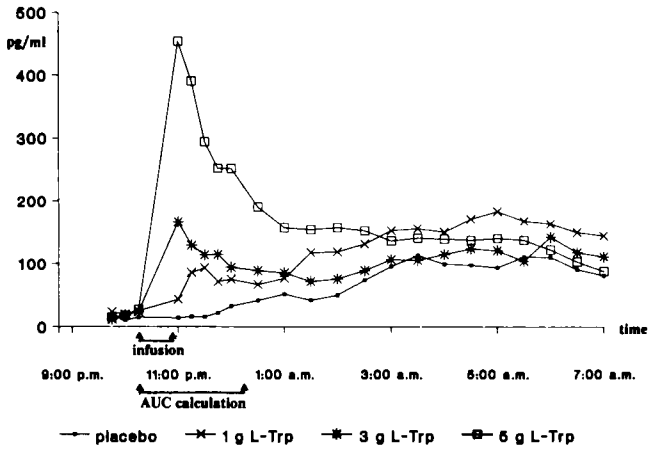

Fig. 2 Melatonin plasma concentration after L-Trp infusion during night-time in five healthy volunteers.

The effect of L-Trp infusions on plasma concentrations of a number of indolic compounds (5-hydroxyindole acetic acid, 5-hydroxytryptophol, N-acetyl-serotonin, 5-hydroxy-Trp, serotonin, N-methyl-serotonin, indole lactic acid, N-acetyl-Trp, tryptophol, indole acetic acid, 5-methoxyindole acetic acid, Trp, 5-methoxy-Trp, indole proprionic acid, tryptamine, 5-methoxytryptamine) was measured by HPLC. Only Trp and 5-methoxy-Trp exhibited a time- and dose-dependent profile comparable to the one seen for melatonin in the RIA. However, the addition of neither compound to control plasma at peak concentrations resulted in a significant cross-reactivity with the melatonin RIA.

During daytime, the polysomnographic recordings showed a dose-dependent increase in the percentages of sleep stage I and II for 3.0 g ($p \leq 0.05$) and 5.0 g ($p \leq 0.05$) compared to placebo (Fig. 3). For night sleep, latency stage I and stage II decreased after 1.0 g ($p \leq 0.05$) and 5.0 g LTP ($p = 0.05$) compared to placebo. Sleep efficiency increased in all doses compared to placebo ($p = 0.05$) (Fig. 4).

Discussion

The light-induced suppression of pineal melatonin synthesis is thought to be exclusively responsible for circadian fluctuations in circulating melatonin levels (*Brainard* et al., 1988). The results of the present study show that the infu-

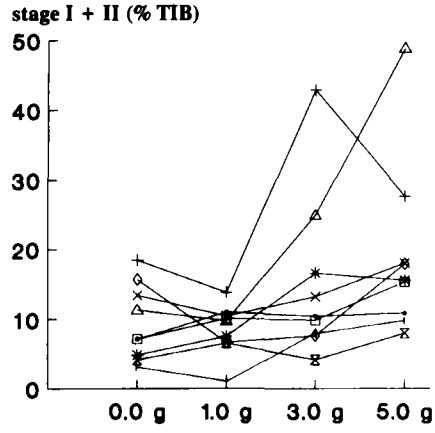

Fig. 3 Sleep parameter after L-Trp infusion during daytime in nine healthy volunteers

sion of as little as 1.0 g L-Trp (which raises plasma Trp about sixfold) during the night causes a slight elevation in plasma melatonin. Significant changes in plasma melatonin levels were seen after 3.0 g L-Trp (causing an approximately tenfold increase in plasma Trp) and 5.0 g L-Trp (causing an approximately 15fold increase in plasma Trp). During daytime, the light-induced suppression of melatonin synthesis and/or release is actually overridden by the infusion of 3.0 and 5.0 g L-Trp. Aside from the direct administration of melatonin (*Waldhauser* et al., 1990), administration of L-Trp seems to be a much more potent means of modulating circadian melatonin rhythms than other forms of treatment, such as the administration of drugs (*Srinivasan*, 1989) or exposure to bright light (*Brainard* et al., 1988).

The observed stimulation of melatonin release in daylight raises the question as to whether or not the Trp-induced increase in plasma melatonin is indeed of pineal origin. In rats, intraperitoneal injection of L-Trp has been shown to cause only a slight elevation of pineal melatonin content (*Young* and *Anderson*, 1982). A possible role of extrapineal sites of synthesis in regulating plasma levels has been suggested (*Vakkuri* et al., 1985). The persistence of circadian rhythms of melatonin observed in the serum of rats after pinealectomy (*Yu* et al., 1981) confirms the importance of extrapineal melatonin sources. Preliminary data obtained in animal experiments point to the enterochromaffine cells of the gastrointestinal tract as the major source of elevated plasma melatonin after L-Trp administration (*Huether* et al., 1991).

Melatonin passes the blood-brain barrier (*Cardinali*, 1981). If relatively moderate doses of L-Trp cause substantial increases in circulating melatonin, the central sleep-inducing effects seen after L-Trp administration may therefore actually be caused by the action of this increased plasma melatonin and the activation of central melatonin receptors rather than by the stimulation of serotonin synthesis in serotonergic nerve terminals. Distinct sedative and hypnotic effects of L-Trp infusions were noted in the present study. Almost identical sleep-inducing effects have also been observed after direct melatonin administration in humans (*Liebermann*, 1986; *Waldhauser* et al., 1990) and animals (*Mirmian* and *Pevet*, 1986).

The massive increase in plasma melatonin levels after L-Trp may also be related to the side-effects observed after consumption of Trp preparations which were most probably contaminated by bacterial endotoxins (*Medsger*, 1990). Melatonin is a stimulator of the immune response, either directly or by its suppressive action on the adrenocortical axis (*Maestroni* et al., 1987). The Trp-induced elevation of melatonin may thus have boosted the immune response to the bacterial endotoxins in a way which ultimately resulted in the occurrence of the observed eosinophilia-myalgia syndrome.

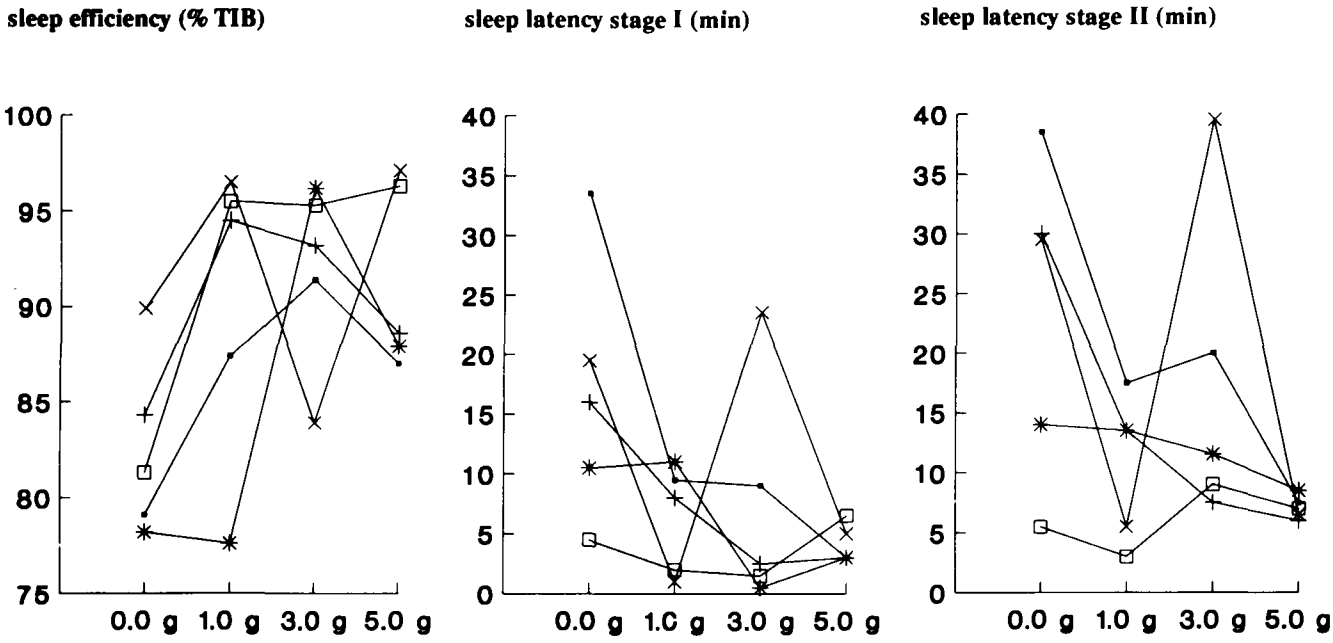

Fig. 4 Sleep parameter after L-Trp infusion during night-time in five healthy volunteers

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