ORIGINAL ARTICLE

Rapid Tryptophan Depletion, Sleep Electroencephalogram, and Mood in Men With Remitted Depression on Serotonin Reuptake Inhibitors

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Background: In previous studies, depletion of brain serotonin by administration of a tryptophan-free amino acid drink (TFD) (1) temporarily reversed the antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) in euthymic patients who had a history of major depression, and (2) enhanced rapid eye movement (REM) sleep in normal volunteers. In this study, we hypothesized that the TFD would not only increase depressive symptoms but also the propensity for REM sleep in euthymic patients treated with SSRIs.

Methods: Ten fully remitted, medicated male patients who had a history of major depressive episode ingested a 100-g TFD (the experimental dose) or a 25-g TFD (designed to be the control drink) in double-blind, random order on separate days. The effects were assessed with mood ratings, plasma tryptophan concentrations, and an all-night sleep electroencephalogram.

Results: The TFDs produced a dose-dependent reduction in plasma tryptophan concentrations, sleep latency, and REM latency, as well as increased REM percentage, REM minutes, REM density, and total sleep time. Neither strength of TFD altered mood to a clinically significant degree.

Conclusions: Although the TFD affected plasma tryptophan concentrations and various sleep measures, our study did not confirm previous reports that TFD temporarily reversed the antidepressant effects of SSRIs in euthymic patients. Our patients, however, had been treated for a longer period with SSRIs and were more fully remitted at the time of the study. Our results suggest that TFD-induced relapse in SSRI-treated patients in remission decreases as a function of treatment duration, degree of remission, or both.

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SEROTONIN (5-hydroxytryptamine, 5-HT) has been implicated in the pathophysiology of depression, in the mechanism of action of some types of antidepressants, and in sleep and neuroendocrine abnormalities associated with depression.[1]

Rapid reduction of plasma tryptophan by a tryptophan-free amino acid drink (TFD) seems to deplete central serotonin concentrations in humans[2] and in animals.[3-6] Delgado et al[7] reported that a TFD temporarily reversed antidepressant-induced clinical remission in euthymic patients treated with selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs), but not in patients treated with tricyclic antidepressants. Transient mood-lowering effects of the TFD have been seen in healthy male subjects as well.[8]

In terms of sleep, serotonergic neurotransmission apparently inhibits rapid eye movement (REM) sleep in humans and animals. Administration of a TFD reduces REM latency (RL) (elapsed time between sleep onset and the first REM period) and increases REM percentage in normal male volunteers.[9] Consistent with the serotonergic depletion hypothesis for depression, patients with moderate to severe depression often display short RL, increased REM percentage, and increased ocular activity during REM sleep (increased REM density [RD]) compared with normal controls. Furthermore, administration of SSRIs increases RL and reduces REM percentage in healthy controls[9] and in depressed patients.[10,11] In addition, "activation," insomnia, reduced total sleep time (TST), and sleep efficiency (SE) are common side effects seen in depressed patients treated with SSRIs.[12,13]

This study examined the effect of acute tryptophan depletion on mood measures and sleep in euthymic depressed patients currently treated with SSRIs. Based on the studies of Delgado et al,[7] we expected that the TFD—a 100-g mixture of amino acids—would significantly increase depressive symptoms. Further-

SUBJECTS AND METHODS

Ten euthymic white male patients (mean [± SD] age, 42.5 ± 12.1 years) who had a history of a major depressive episode completed this study (**Table 1**). Patients were recruited from outpatient clinics at the San Diego Veterans Affairs Medical Center, San Diego, Calif, and by public announcements on radio and television, and in the newspapers. They were paid for their participation, and gave written informed consent to participate in the study.

Inclusion criteria were the following: (1) a history of a major depressive episode in full remission for at least 2 months by the clinical judgment of the patient and a psychiatrist, (2) on-going treatment with only an SSRI and no other major psychotropic medications, (3) a score below 7 on the 17-item Hamilton Rating Scale for Depression (HRSD),[14] and (4) no other recent or current major comorbid medical, psychiatric, or substance abuse disorders. All subjects underwent a full medical and psychiatric diagnostic interview, physical examination, screening medical laboratory tests (chemistry panel, complete blood cell count, urinalysis, electrocardiogram, human immunodeficiency virus screen, and drug screen), a formal structured diagnostic psychiatric interview (Structured Clinical Interview for *DSM-III-R* [SCID]), and ratings of symptoms with the HRSD and Profile of Mood States (POMS).[15] Diagnostic interviewers and raters were trained and tested for reliability by the Diagnostic and Rating Core of the University of California, San Diego (UCSD) Mental Health Clinical Research Center (MHCRC).

As given in Table 1, patients were virtually euthymic. This was the first depressive episode for 3 patients; the second for 3 patients; the third for 2 patients; and the fourth and fifth depressive episode for the remaining 2 patients. Two of the 10 patients had prior psychiatric hospitalization. For 5 patients, this was the first trial with antidepressants; for 2 patients it was the second, and for 3 patients it was the third.

We excluded female patients because of increased vomiting compared with men with the 100% TFD (ie, 4/4 women, 2/20 men).

Patients received their usual dose of antidepressant throughout the protocol, including the day of the TFD challenge.

Patients ate a low-tryptophan diet starting at dinner the night before administration of the TFDs. The diet contained 9623 kJ/d (2300 kcal/d) and 48 g/d of protein, but only 160 mg/d of tryptophan. The TFD contains 15 amino acids in the same proportion as human milk, except that tryptophan, aspartic acid, and glutamic acid are omitted.[16] The 100%-strength TFD mixture weighed a total of 102.5 g, the 25-g or 25%-strength TFD was one quarter strength. Both TFDs were served with chocolate-flavored syrup in a 300-mL "slurry" with crushed ice. Methionine, cysteine, and arginine were given in blinded pill form (21 pills per TFD challenge) because of the bitterness and unpalatability they added to the drink. The blood samples for tryptophan were collected with EDTA, centrifuged, and frozen (–80°C) until analysis. Free plasma tryptophan and total plasma tryptophan concentrations were determined using a modified fluorometric method.[17]

Current mood was assessed by blinded raters ($\kappa = 0.82$), using a modified 19-item version of the HRSD, based on the 24-item HRSD excluding items 4 through 6 (pertaining to insomnia/sleep), 16 (weight change), and 18

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more, based on preclinical studies in animals and on our sleep studies in normal volunteers, we predicted the following: (1) that REM measures would be suppressed at baseline compared with a group of matched never-depressed comparison subjects and (2) that the TFD would shorten RL, increase REM percentage, and increase RD in these patients. When we designed this study, the Food and Drug Administration did not permit the administration of tryptophan because of the eosinophilia-myalgia syndrome that had been associated with synthetically made tryptophan. We chose a one quarter–strength dose of the TFD (25 g of amino acids) as our control drink based on both personal communication (Pedro Delgado, MD, and Norman Rosenthal, MD, oral communication) and previous publications indicating that the quarter-strength TFD did not affect plamsa concentrations of tryptophan.

RESULTS

PLASMA TRYPTOPHAN MEASURES

Eight hours after ingesting the 100% TFD, total and free plasma tryptophan concentrations decreased significantly ($P<.001$, 61.4% [from 48 ± 9 µmol/L to 19 ± 4 µmol/L], and 52% [from 5 ± 1 µmol/L to 2 ± 1 µmol/L], respectively). With the 25% TFD, these measures decreased but not to a statistically significant degree (4% and 5.6%, respectively).

SLEEP MEASURES

At baseline, the SSRI-treated patients showed prolonged SL and RL and decreased TST, REM sleep (in minutes and percentage), and RD relative to the comparison group (**Table 2**). Compared with baseline, the TFDs, in a dose-dependent manner, were associated with significantly reduced SL and RL and increased TST, REM sleep (in minutes and percentage), and RD. The patients on the 100% TFD were restored to normal limits for TST, and while both SL and sleep efficiency somewhat improved, both remained outside normal limits. The REM sleep measures, however, "overshot," relative to the comparison group: RL went from too long to too short, and REM sleep (in minutes and percentage) and RD went from too low to too high.

None of the General Sleep Questionnaire items were significantly different across conditions (baseline, 25% TFD, 100% TFD).

None of the correlations between the absolute sleep measures and plasma tryptophan levels (total or free) were statistically significant. In addition, the sleep change scores (baseline to TFD night) did not correlate with the plasma tryptophan concentration change scores (from pre-TFD to post-TFD).

(diurnal variation). Patients also completed the POMS and a side effects symptom checklist was also administered.

At 2:30 PM, mood ratings and 10 mL of blood for tryptophan levels were obtained. At 3 PM, either the 25% TFD or the 100% TFD was given, in double-blind, randomized, cross-over fashion. At 11 PM, mood ratings, a side effects scale, and a blood sample were obtained. Polysomnographic recordings were conducted from about 11:30 PM until about 6:30 AM on an adaptation night (including screening for sleep disorders), a baseline night, and 2 experimental nights following either the 25% TFD or the 100% TFD. Challenge nights were separated by at least 48 hours.

Electroencephalogram, bilateral electrooculogram, chin electromyogram, and electrocardiogram were recorded all night. Records were visually scored in 30-second epochs according to standard criteria[18] by well-trained sleep technicians (interrater reliability of >0.80) blind to experimental condition. Sleep variables are briefly defined as follows. Time in bed is the time in minutes from "lights out" until "lights on." Total sleep time is the time in bed minus any awake time. Sleep efficiency is the TST divided by the time in bed, expressed in percentage value. Sleep latency (SL) is the time in minutes from "lights out" until the first onset of stage 2 or REM sleep, with less than 1 minute of wakefulness within the first 10 minutes. Wakefulness after sleep onset is the time spent awake after sleep onset. Rapid eye movement minutes is the total number of minutes of REM sleep. Rapid eye movement percent is the time spent in REM sleep divided by TST. Rapid eye movement latency is the time in minutes from SL until first onset of REM sleep. Rapid eye movement latency corrected is the time from SL until first REM minus any intervening minutes of wake. Rapid eye movement density is a visually estimated measure of ocular activity during REM sleep, scored on a scale of 0 to 4 per epoch, but presented as 0 to 8 per minute of REM.

For the sleep analysis, a post hoc comparison group of 10 healthy, never-depressed men was culled from our medical record archives by matching for age (43.1 ± 11.5 years; range, 24-59 years), ethnicity, and time of year (± 2 months).

Each morning, a General Sleep Questionnaire was administered. General Sleep Questionnaire items included the following: (1) how long did it take you to fall asleep last night; (2) how much total sleep do you feel that you had last night; (3) how many times did you wake up during the night; (4) how long do you feel you were awake in the night, in minutes; (5) rate the quality of your sleep on a scale of 1 to 10 (1=poor and 10=excellent); (6) rate how well rested you feel this morning on a scale of 1 to 10 (1=poor and 10=excellent).

Although we initially assumed that the 25% TFD would be a placebo, it was physiologically active; in fact, its effects on sleep were similar to the 100% TFD, in both normal controls[19] and in study patients. Therefore we used a repeated measures analysis of variance for a within-subjects factor (baseline, 25% TFD, 100% TFD). Pairwise comparisons were done using Student *t* tests. Values less than .05 were considered statistically significant.

Change scores were calculated from pre-TFD to post-TFD for total and free plasma tryptophan at the 25%- and 100%-TFD strengths. Pearson product moment correlation coefficients were calculated between sleep electroencephalographic variables and plasma levels or between sleep and plasma change scores. All values were expressed as mean (± SE).

Table 1. Demographic, Diagnostic, and Baseline Mood Variables of the Subjects

Subject No./ Age, y/ Weight, kg	Secondary SCID Diagnoses*	SSRI, Dosage × Treatment Duration†	Remission Duration, mo	HRSD Score (24-Item)‡
1/48/90.0	ADFR, SocPh, SimPh, and PTSD	Fluoxetine, 40 × 3	2.5	7
2/58/59.4	ADFR	Fluoxetine, 40 × 6	4	3
3/35/103.3	None	Fluoxetine, 40 × 13	3.5	1
4/40/96.2	ADFR and A-SDFR	Fluoxetine, 40 × 5	4	0
5/51/77.3	ADFR	Fluoxetine, 60 × 12	8	1
6/49/70.5	None	Sertraline, 50 × 6	5	0
7/37/96.7	ADFR and CDFR	Paroxetine, 20 × 12	9	0
8/22/77.6	SimPh	Paroxetine, 20 × 10	6	0
9/28/72.0	None	Paroxetine, 20 × 5	4.5	6
10/57/79.5	None	Fluoxetine, 20 × 2.5	2	0

**The secondary Structured Clinical Interview for* DSM-III-R *(SCID) diagnoses are as follows: ADFR indicates alcohol dependence, full remission; A-SDFR, amphetamine or sympathomimetic dependence, full remission; CDFR, cannabis dependence, full remission; PTSD, posttraumatic stress disorder; SocPh, social phobia; and SimPh, simple phobia.*

†The dosage in milligrams times the treatment duration in months the subjects received a selective serotonin reuptake inhibitor (SSRI).

‡HRSD indicates Hamilton Rating Scale for Depression.

MOOD MEASURES

Although the mean 19-item HRSD score increased significantly about 8 hours after the 100% TFD compared with both baseline and the 25% TFD, the changes were small (<2 points total) and clinically insignificant. And, although the Depression subscale of the POMS did not change, other POMS subscales (Vigor, Elation, and Friendliness) significantly changed in a depressivelike direction following both the 25% and 100% TFDs (**Table 3**). In addition, the POMS subscale score of Confusion also increased significantly at the 100% TFD compared with the other 2 conditions.

Table 2. Results of All-Night Polysomnographic Measures*

Variable	Baseline	25% TFD	100% TFD	$F_{2,18}$	*P*	Comparison Group Values
TST, min	341 ± 19	360 ± 22	378 ± 17[a]	3.93	<.05	383 ± 15
SL	41 ± 10	30 ± 8	23 ± 4	3.88	<.05	14 ± 6
RL	151 ± 19	121 ± 24	64 ± 11[a,b]†	11.34	<.001	78 ± 10
REM minutes	56 ± 6	76 ± 12[a]†	107 ± 10[a,b]	18.86	<.001	80 ± 7
REM %	16.6 ± 2	20.1 ± 3	28.0 ± 2[a,b]	22.45	<.001	21 ± 2
RD	1.8 ± 0.2	2.5 ± 0.1	2.7 ± 0.2[a]	9.00	.005	2.0 ± 0.2
SWS minutes	37 ± 16	44 ± 12	35 ± 10	0.76	>.05	27 ± 11
SWS %	9 ± 4	11 ± 3	9 ± 2	0.95	>.05	6 ± 3
Sleep efficiency	80 ± 3.3	78 ± 3.2	83 ± 2.2[b]	1.88	>.05	90 ± 1.8
WASO	35 ± 6	56 ± 8[a]	45 ± 7	3.94	>.05	23 ± 3
Stage 1 minutes	38 ± 7	41 ± 6	41 ± 7	0.17	>.05	29 ± 4
Stage 1%	12 ± 3	12.5 ± 3	11.5 ± 2	0.13	>.05	8 ± 1
Stage 2 minutes	211 ± 14	200 ± 11	195 ± 9	1.30	>.05	248 ± 10
Stage 2%	62 ± 4	56 ± 3[a]	52 ± 2[a,b]	9.47	<.05	65 ± 2

**The effects of tryptophan-free amino acid drinks (TFDs) on subsequent sleep compared with baseline sleep, and with normal comparison sleep values. Values are expressed as mean (± SE) unless otherwise stated. TST indicates total sleep time; SL, sleep latency; RL, rapid eye movement (REM) latency; RD, REM density; SWS, slow-wave sleep (stages 3 and 4); and WASO, wakefulness after sleep onset.*

†a vs baseline, P<.05, Student t test; b, 25% TFD vs 100% TFD, P<.05, Student t test.

Table 3. Effects of Tryptophan-Free Amino Acid Drinks (TFDs) on Mood Measures*

Variable	Baseline	25% TFD	100% TFD	$F_{2,18}$	*P*
19-Item HRSD (at bedtime)	1.7 ± 0.9	1.4 ± 0.8	3.2 ± 1.1	3.88	<.05
POMS subscale					
Depression	3.0 ± 1.3	3.3 ± 1.6	2.7 ± 1.4	0.21	>.05
Vigor	12.0 ± 2.2	5.9 ± 1.5[a]†	6.7 ± 1.5[a,b]†	13.05	<.005
Confusion	3.9 ± 0.9	4.1 ± 0.9	5.4 ± 0.9[a,b]	2.00	<.05
Elation	9.5 ± 1.1	6.2 ± 1.0[a]	5.6 ± 0.7[a,b]	14.75	<.005
Friendliness	19.7 ± 1.7	15.2 ± 2.4	15.4 ± 1.9[a]	10.08	<.005

**HRSD indicates Hamilton Rating Scale for Depression; POMS, Profile of Mood States. Values are expressed as mean (± SE) unless otherwise stated.*

†a vs baseline, P<.05, Student t test; b, 25% TFD vs 100% TFD, P<.05, Student t test.

COMMENT

In our euthymic, fully remitted, male patients maintained on SSRIs, the TFD unexpectedly failed to reverse the antidepressant benefits of the SSRIs, previously reported by Delgado et al.[7,16] Nevertheless, both the 25% and the 100% doses of TFD significantly reversed the REM-suppressing effects of SSRIs in a dose-dependent fashion, increased reduced TST, and shortened SL. In addition, the 100% TFD, but not the 25% TFD, significantly reduced both free and total plasma concentrations of tryptophan.

Our failure to reverse the antidepressant effects of SSRIs may reflect pronounced differences of our patient sample compared with patient samples previously reported. First, our patients had been treated for 2 to 13 months vs 2 to 6 weeks at the time of the TFD challenge. Second, our patients were less depressed (mean 24-item HRSD score at baseline, 1.75; range, 1-6); compared with others (modified 25-item HRSD mean score, 8.4; range, 1-19). Third, we administered the TFD in the midafternoon (3 PM) in contrast with the 9 AM ingestion typically used by other groups. Absolute plasma tryptophan levels[20] as well as the ratio of tryptophan to other large neutral amino acids[21] vary in a circadian fashion, which may influence tryptophan availability and transport into brain.

In patients treated with MAOIs who were remitted for approximately 2 to 3 weeks, administration of the tryptophan hydroxylase inhibitor, *para*-chlorophenylalanine (PCPA), which depletes serotonin, induced a relapse of depressive mood.[22] These observations support the hypothesis that reversal of antidepressant treatment by any method of serotonin depletion is more likely in partially remitted patients than in fully remitted patients, and is more likely early rather than later in SSRI or MAOI treatment.

To an extent, the clinical studies with the TFD are consistent with this preclinical concept that different antidepressant treatments (tricyclic antidepressants, MAOIs, SSRIs, and electroshock therapy) induce a cascade of neurochemical changes that evolve with long-term treatment.[23] The SSRIs seem to down-regulate somatodendritic serotonin$_{1A}$ and terminal serotonin$_{1D}$ inhibitory autoreceptors, as well as postsynaptic serotonin$_{1A}$ and serotonin$_2$ receptors.[23-31] Postsynaptic serotonin$_{1A}$ receptors are especially prominent in limbic brain regions.[32] This tempts us to speculate that long-term SSRI treatment leads to serotonin$_{1A}$-receptor sensitivity changes preferentially in limbic regions, as compared with brainstem regions involved with initiating REM sleep, such as laterodorsal tegmental/pedunculopontine tegmental nuclei. Thus, REM

sleep may remain somewhat sensitive to the effects of acute tryptophan depletion, whereas mood may not.

Our results are consistent with the hypothesis that serotonin inhibits REM sleep. Not only were the patients receiving SSRIs REM suppressed at baseline sleep (pre-TFD challenge), but the TFD reversed the REM inhibition associated with SSRIs.

Our observations also suggest that serotonin itself is responsible for some of the "activating" side effects associated with SSRIs and MAOIs. The TFD reversed the prolonged SL and reduced TST of the patients. We have previously reported that ipsapirone, a serotonin$_{1A}$-receptor agonist, increased SL and reduced TST in both normal controls and depressed patients.[33] Furthermore, in an 8-week, double-blind trial of nefazodone (a so-called SARI, serotonin$_2$ antagonist/reuptake inhibitor, that combines serotonin reuptake blocking properties with antagonism at serotonin$_2$ receptors) and fluoxetine, SE fell in the group receiving fluoxetine but increased in the group receiving nefazodone.[13] Perhaps the activating effects of the SSRIs are mediated through the serotonin$_2$ receptor.

The experimental design reported in this study, in retrospect, was not optimal, because it was based on the assumption that the 25% TFD was a placebo. Order effects cannot be ruled out because the baseline night always occurred prior to the 2 TFD challenges. Second, the baseline night was not blinded in the same fashion as the experimental nights. Third, the subjects ate a low tryptophan diet and had blood samples drawn prior to bedtime on the TFD nights but not on the baseline night. Nevertheless, despite these unanticipated problems in research design, the sleep and mood data on the baseline nights fall within expected values for remitted patients currently treated with SSRIs. Second, the 25% and 100% TFDs were administered in random order, in a double-blind manner. Third, the 2 TFDs had the anticipated effects on plasma tryptophan concentrations and on polygraphic sleep measures.

The evidence that the TFD exerts its effects by reducing central serotonergic activity is circumstantial. By reducing plasma-free tryptophan levels, the TFD presumably limits synthesis of serotonin. Consistent with this hypothesis, brain serotonin neurons apparently use up available serotonin stores about 1 to 2 hours after tryptophan is depleted from superfusate in the slice preparation.[34] Furthermore, serotonin release is diminished (1) in the slice after superfusion with a tryptophan-free large neutral amino acid (LNAA)–supplemented medium[35]; and (2) in the anesthetized rat, raphe neurons 2 hours after intake of a tryptophan-free amino acid load.[36]

In vivo, however, tryptophan transport from plasma across the blood-brain barrier apparently depends not only on plasma levels of tryptophan but on plasma levels of other LNAAs. A reduced ratio of trytophan to LNAAs may decrease brain tryptophan levels even without significantly changing absolute plasma tryptophan. In a recent study with a few subjects, a 25% TFD administered in the midafternoon did not significantly change the tryptophan-LNAA ratio in plasma samples taken prior to bedtime.[37] In our study, the 25% TFD did not alter plasma concentrations of tryptophan though it had significant effects on some sleep and mood measures. If the 25% TFD has central effects without altering either the absolute plasma concentration of tryptophan or the tryptophan-LNAA ratio, the mechanism by which the TFD affects the brain would be in question.

In addition, continuous infusion of an amino acid mixture containing tryptophan in rats increased REM percentage and decreased non-REM percentage during the rest period.[38] While not strictly comparable to our study, these findings raise the possibility that large amounts of amino acids increase REM sleep.

In summary, we did not find that the TFD reversed the antidepressant effects of SSRIs in euthymic, fully remitted, male patients who had received antidepressants for 2 to 13 months, even though it significantly reduced plasma tryptophan concentrations (at the 100% dose) and significantly altered polygraphic sleep measures (at both 25% and 100% doses). The failure of the TFD to induce depression is in contrast to previous studies reporting that the TFD induced a transient relapse in moderately remitted patients with a history of mood disorder who had been treated for about 4 to 6 weeks. As a preliminary explanation for these discrepant findings, we hypothesize that the TFD reverses the antidepressant effects of SSRIs only early in the course of antidepressant therapy or only in patients who remain mildly depressed. These speculations are consistent with hypotheses that successful treatment with antidepressant medications depends on a time-dependent cascade of neuropharmacologically induced events at critical sites within the brain, eg, alterations in affinity and number of selected serotonin receptor subtypes.

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