Serotonin Function and the Mechanism of Antidepressant Action

Reversal of Antidepressant-Induced Remission by Rapid Depletion of Plasma Tryptophan

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• **Brain serotonin content is dependent on plasma levels of the essential amino acid tryptophan. We investigated the behavioral effects of rapid tryptophan depletion in patients in antidepressant-induced remission. Twenty-one patients who were depressed by *DSM-III-R* criteria received a 24-hour, 160-mg/d, low-tryptophan diet followed the next morning by a 16–amino acid drink, in a double-blind, placebo-controlled (acute tryptophan depletion and control testing), crossover fashion. Total and free tryptophan levels decreased 87% and 91%, respectively, during acute tryptophan depletion. Fourteen of the 21 remitted depressed patients receiving antidepressants experienced a depressive relapse after the tryptophan-free amino acid drink, with gradual (24 to 48 hours) return to the remitted state on return to regular food intake. Control testing produced no significant behavioral effects. Free plasma tryptophan level was negatively correlated with depression score during acute tryptophan depletion. The therapeutic effects of some antidepressant drugs may be dependent on serotonin availability.**

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The involvement of central nervous system serotonin (5-HT) function in the pathogenesis and treatment of affective disorders has been a subject of intensive research during the past 20 years.[1-11] Recent studies with the use of 5-HT precursors and agonists as pharmacologic probes and measurements of cerebrospinal fluid (CSF) monoamine metabolite levels indicate that alterations in central nervous system 5-HT function may be involved in the pathophysiology of depression[1-10] and impulsive suicide.[11] For example, prolactin responses to intravenous tryptophan[12,13] and oral fenfluramine hydrochloride[14] are blunted in patients with major depression. The CSF levels of the 5-HT metabolite 5-hydroxyindoleacetic acid are reduced in a subgroup of patients with major depression who attempt suicide by violent means.[11] The density of brain 5-HT_2 receptors may also be elevated in suicide victims.[15,16]

Investigations of the mechanism of action of antidepressant drugs suggest that most antidepressants enhance neurotransmission across 5-HT synapses after long-term but not short-term administration.[8,9] Despite such studies, consensus does not exist regarding the mechanism by which various drugs relieve depression,[2,5,6,10] and the precise role of central nervous system 5-HT function in depression remains controversial.[17] This situation exists in part because of the lack of specificity of clinically available pharmacologic probes and the limitations of the neuroendocrine measures used in clinically assessing brain 5-HT function.[18]

The synthesis of 5-HT depends on dietary intake of its precursor, the essential amino acid tryptophan (TRP).[19-29] Data now exist that suggest dietary TRP depletion may specifically reduce brain 5-HT function.[24-29] Both increases[28,29] and decreases[19-21,26,27,29] in dietary TRP intake lead to corresponding changes in brain TRP and 5-HT levels in laboratory animals. Ingestion of TRP-free amino acid mixtures in vervet monkeys decreases plasma TRP and CSF TRP and 5-hydroxyindoleacetic acid levels with no change in CSF tyrosine, homovanillic acid, or 3-methoxy-4-hydroxyphenylglycol levels.[26] Moreover, ingestion of TRP-free amino acid mixtures in laboratory animals leads to extremely rapid changes in plasma TRP and brain 5-HT levels, with maximal reductions of brain 5-HT level occurring within 2 hours of ingestion of the TRP-free mixture.[26,27] Dietary TRP depletion alters behavioral indexes of 5-HT function, increasing pain sensitivity,[30,31] acoustic startle,[32] and muricidal behavior,[33,34] reducing rapid eye movement sleep,[35] and enhancing the prolactin response to 5-hydroxytryptophan infusion[36] in laboratory animals. The effects of TRP depletion on pain sensitivity, acoustic startle, muricidal behavior, and rapid eye movement sleep are reversed by TRP repletion, probably through alterations in central 5-HT function.[30-35] Two important clinical studies published in the 1970s reported that the 5-HT synthesis inhibitor parachlorophenylalanine appeared to reverse the antidepressant effects of both imipramine hydrochloride[37] and tranylcypromine sulfate[38] within 24 hours in patients with major depression.

Diets free of or low in TRP diets administered to healthy humans cause reductions of plasma TRP levels.[39-41] Maintenance on such diets for up to 1 month has been reported without serious medical or psychological consequences.[42] Diets that reduce the plasma ratio of TRP to large neutral amino acids increase the competition of large neutral amino acids with TRP for passage across the blood-brain barrier, resulting in decreased levels of CSF 5-hydroxyindoleacetic acid.[43] A 200-mg/d, low-TRP diet for 8 days enhanced the prolactin response to intravenous tryptophan, possibly suggesting the development of postsynaptic 5-HT receptor supersensitivity.[41] Reduction in plasma TRP level of up to 80% can also be accomplished in 3 to 5 hours by administering an oral TRP-free amino acid

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Table 1.—Patient Characteristics

Patient/ Sex/Age, y	Diagnosis*	Current Antidepressant Medication/Dose, mg/d†	Previous Unsuccessful Antidepressant Trials	No. of Previous Episodes‡	Hamilton Depression Scale Score		
					Admission	Tryptophan Depletion§	
						Prediet	Peak
1/F/35	MD	Desipramine/400	0	0	36	10	10
2/F/24	MD	Desipramine/200	1	1	34	2	25
3/F/37	MD	Desipramine/150	1	0	22	6	8
4/M/39	MD	Desipramine/100	0	0	25	4	6
5/M/34	MD (M)	Desipramine/300; lithium/900	4	4	31	14	23
6/F/42	MD	Desipramine/150; lithium/600	0	0	33	11	8
7/M/69	MD (M)	Imipramine/150	6	5	32	13	27
8/F/43	MD	Fluvoxamine/300	6	4	33	7	18
9/M/29	MD	Fluvoxamine/300	3	2	23	10	25
10/M/33	MD	Fluvoxamine/300	1	1	33	5	7
11/F/62	MD (M)	Fluvoxamine/200	2	0	36	11	35
12/F/25	MD (M)	Fluvoxamine/300	4	0	35	11	30
13/F/37	MD	Fluvoxamine/300	1	0	41	19	23
14/F/30	BP (M)	Fluvoxamine/300; lithium/900	6	5	23	4	35
15/M/45	BP (M)	Fluvoxamine/300; lithium/900	1	0	42	1	6
16/F/39	MD (M) (P)	Fluvoxamine/300; lithium/900	1	2	49	11	29
17/F/46	MD (M)	Phenelzine/30	4	1	25	13	34
18/M/47	BP (M)	Phenelzine/45	3	3	25	10	21
19/M/31	BP (M)	Phenelzine/45	2	4	25	2	17
20/F/35	MD (M)	Tranylcypromine/10	3	1	35	7	25
21/F/50	BP (M)	Tranylcypromine/60	3	4	31	5	17
Mean/. . ./39.6	. . .	. . .	2.5	1.8	31.9	8.4	20.4

*MD indicates major depressive episode; (M), with melancholia; (P), with psychosis; and BP, bipolar, depressed.

†Desipramine was given as desipramine hydrochloride; lithium, as lithium carbonate; imipramine, as imipramine hydrochloride; phenelzine, as phenelzine sulfate; and tranylcypromine, as tranylcypromine sulfate.

‡Number of previous episodes of major depression.

§Prediet indicates baseline rating score before low-tryptophan diet; peak, highest depression rating after tryptophan depletion.

solution, which induces hepatic protein synthesis and thereby depletes available plasma TRP.[44] This procedure leads to mild impairment in attentive performance and increased subjective reports of negative mood, without clinical depression, in healthy male subjects.[45,46]

Depletion of TRP through dietary methods may be useful in evaluating the role of 5-HT function in depression and the therapeutic mechanism of action of antidepressant treatments. The purpose of the present investigation was to determine if rapid TRP depletion induced a return of depression in antidepressant-maintained, recently remitted depressed patients. Depletion of TRP was produced by administration of a TRP-free amino acid drink immediately after a 24-hour low-TRP diet.

PATIENTS AND METHODS

Patients

Twenty-one previously depressed patients in clinical remission after antidepressant treatment (8 inpatients and 13 outpatients; 13 women and 8 men; mean ± SD age, 39 ± 11 years; age range, 24 to 62 years) gave written informed consent to participate in a research study of the behavioral effects of acute TRP depletion. Nine patients had also given written informed consent to participate in a trial of the investigational drug fluvoxamine. Patients were informed that acute TRP depletion might lead to a change in their mood, possibly causing a return of their depressive symptoms. Complete medical and neurologic evaluations revealed each patient to be free of medical or neurologic illness. Patients with a history of significant substance abuse were excluded from the study. During the period of the investigation (November 1987 through August 1988), consecutive patients who had demonstrated clinical remission from depressive symptoms during treatment on the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center, New Haven, were asked to participate. No patient declined, and all patients enrolled completed the 2-week study.

Remission was defined as (1) sufficient improvement in global functioning, by consensus of patient, treating staff, and family, to warrant discharge from the acute treatment setting and (2) a 50% or greater improvement on the modified 25-item Hamilton Depression Rating Scale (HDRS)[47] after at least 4 weeks of antidepressant treatment. Testing was performed after the patient had remained clinically stable for at least 2 weeks (mean ± SD, 3.6 ± 1.6 weeks).

Diagnoses were made on admission to the CNRU (before antidepressant treatment) by our consensus with the use of *DSM-III-R* criteria.[48] Diagnoses were based on direct interview with the Yale Depression Inventory[47] and review of medical records. Of the 21 patients, 9 met *DSM-III-R* criteria for major depressive episode, 6 for major depressive episode with melancholia, 1 for major depressive episode with melancholia and psychotic features, 2 for bipolar disorder, depressed, and 3 for bipolar disorder not otherwise specified (because of a history of previous hypomania) (Table 1). All bipolar patients also met *DSM-III-R* criteria for melancholia.

Six patients were currently taking the investigational drug fluvoxamine (283 ± 41 mg/d), 3 fluvoxamine (300 mg/d) plus lithium carbonate (900 mg/d), 4 desipramine hydrochloride (213 ± 132 mg/d), 2 desipramine hydrochloride (225 ± 106 mg/d) plus lithium carbonate (750 ± 212 mg/d), 2 tranylcypromine sulfate (35 ± 35 mg/d), 3 phenelzine sulfate (40 ± 9 mg/d), and 1 imipramine hydrochloride (150 mg/d).

Procedure

We used an experimental design in which 1 day of a low-TRP diet was followed by a second day on which a TRP-free amino acid drink was given to lower plasma TRP levels further. This design reflected our concern that behavioral changes might not be evident after gradu-

Table 2.—Schedule for Test Sessions

Time	Event
	Day 1
8:30 AM	Arrive at research unit (outpatients); behavioral ratings,* plasma tryptophan (TRP) (10 mL); obtain 3 blue placebo or 500-mg tryptophan capsules†; pick up food and leave hospital (outpatients)
	Day 2
8:30 AM	Arrive at research unit (outpatients); behavioral ratings, plasma TRP (10 mL)
8:45 AM	Ingestion of methionine, cysteine, and arginine capsules
9 AM	Administration of amino acid drink‡
10 AM	Behavioral ratings, plasma TRP (10 mL)
11 AM	Behavioral ratings, plasma TRP (10 mL)
Noon	Behavioral ratings, plasma TRP (10 mL)
2 PM	Behavioral ratings, plasma TRP (10 mL)
4 PM	Behavioral ratings, plasma TRP (10 mL); return to unrestricted food intake
	Day 3
11 AM–noon	Behavioral ratings

*Hamilton Depression Rating Scale,[47] Hamilton Anxiety Scale,[49] and Symptom Checklist.[50]

†Capsules assigned as placebo or L-TRP based on assignment to control test or TRP depletion test.

‡Twelve amino acids with or without 2.3 gm L-TRP based on assignment to control test or TRP depletion test.

al dietary TRP restriction because of (1) its modest effects on plasma TRP[41] and (2) the possibility that adaptive changes in central nervous system 5-HT function might occur during a gradual period of TRP depletion. The experiment was conducted in a double-blind, placebo-controlled, balanced crossover fashion in 18 of the 21 patients. The first three patients were tested single blind with no placebo control. The acute TRP depletion test consisted of a 24-hour, 160-mg/d TRP diet (day 1) with blue placebo capsules given three times, followed the next morning by a TRP-free, 15–amino acid drink (day 2). Control testing consisted of a 24-hour, 160-mg/d TRP diet supplemented with blue capsules containing 500 mg of tryptophan given three times, followed the next morning by a 16–amino acid drink containing 2.3 g of tryptophan. The acute TRP depletion and control tests were separated by 2 to 7 days (mean ± SD, 6 ± 3 days). Patients continued to take the regular dose of their medication throughout the testing, except that medications were not given on the morning and afternoon of the amino acid drink (day 2) (Table 2).

The low-TRP diet consisted of food chosen in consultation with the research dieticians of Yale–New Haven (Conn) Hospital to provide an adequate protein (48 g/d) and energy (10 500 kJ/d) intake but containing the minimum possible amount of tryptophan (160 mg/d). The diet meals were as follows: breakfast: puffed rice, peaches, nondairy creamer, Knox gelatin, and sugar; lunch: graham crackers, cream cheese, boiled potatoes and butter, green beans, and Knox gelatin; dinner: salad (lettuce, tomatoes, celery, bacon, and oil/vinegar), apple, and Knox gelatin; and miscellaneous: four low-protein cookies and Kool-aid. The low-TRP diet food was considered to be bland but was consumed without difficulty by the patients, with no patients refusing to eat the meals. The dinner salad was unanimously described as being the tastiest part of the diet. Inpatients ate the low-TRP diet food at their regular mealtimes (8 AM, noon, and 5 PM), and outpatients were allowed to take the diet food home with them. Patients were instructed to eat all of the food and were allowed to drink water, apple juice, coffee without milk or cream, and carbonated soft drinks. Although records of the exact amount of the diet consumed were not kept, all patients ate the majority of the food given, with the gelatin being the most commonly uneaten portion.

The amino acid drink was composed of 15 amino acids with or without tryptophan (Table 3), as described by Young et al.[45] Because of the unpleasant taste of methionine, cysteine, and arginine, these amino acids were encapsulated, and patients took the capsules 15 minutes before consuming the remaining amino acids in drink form. A drink was prepared by mixing the remaining amino acid powder with water at room temperature to a final volume of 300 mL. The amino acid solution, which was grainy in texture, was flavored with chocolate syrup. Patients drank the solution quickly through a straw. All patients were able to accomplish this easily. Patients returned to

Table 3.—Amino Acid Drink*

Amino Acid	Amount, g
L-Alanine	5.5
L-Arginine	4.9
L-Cysteine	2.7
Glycine	3.2
L-Histidine	3.2
L-Isoleucine	8.0
L-Leucine	13.5
L-Lysine monohydrochloride	11.0
L-Methionine	3.0
L-Phenylalanine	5.7
L-Proline	12.2
L-Serine	6.9
L-Threonine	6.9
L-Tyrosine	6.9
L-Valine	8.9
L-Tryptophan	2.3

*L-Methionine, L-cysteine, and L-arginine were encapsulated and consumed separately. For the tryptophan depletion test, the drink contained no L-tryptophan; for the control test, L-tryptophan was included.

normal dietary intake immediately after the completion of test day 2 (at 4 to 5 PM).

Patients were rated and plasma for free and total TRP measurement was obtained at 9 AM before starting the diet (test day 1) and 15 minutes before and 1, 2, 3, 5, and 7 hours after the drink (test day 2). Ratings were obtained again between 11 AM and 1 PM the day after each amino acid drink (test day 3). Ratings were obtained by one of us (P.L.D.) or by a trained nurse clinician. Raters had established reliability on the rating instruments used and were blind to the sequence of placebo or active tryptophan supplementation. Ratings were obtained by the same rater throughout the testing whenever possible.

The first patient to complete the acute TRP depletion testing was given 1000 mg of tryptophan at 5 PM after the amino acid drink (day 2), but this resulted in abdominal distress, nausea, hyperreflexia with three beats of ankle clonus bilaterally, and breast swelling. These effects were not sustained and were not present on the day after the amino acid drink (day 3). Subsequent patients were not given tryptophan after the amino acid drink.

Behavioral ratings consisted of the 25-item, modified HDRS,[47] the Hamilton Anxiety Scale,[49] and the Symptom Checklist.[50] The Symptom Checklist is a self-rated scale consisting of 23 items, each scored from 0 ("not at all") to 3 ("severe"). Items included headache, constipation, poor memory, nausea or vomiting, feeling drowsy or sleepy, blurred vision, increased appetite, difficulty starting urination, trouble concentrating, nightmares, difficulty sitting still, heartbeat irregular or pounding, diarrhea, frequent need to urinate, dry mouth, decreased appetite, tremors or shakiness, rash, ringing in the ears, sweating, faintness or lightheadedness, poor coordination, and stiffness in muscles.

Biochemical Methods

Total plasma TRP was assayed by high-performance liquid chromatography with flourometric detection (HPLC-F).[51] Free plasma TRP was assayed by obtaining the ultrafiltrate of plasma from cellulose-based filters (30 000 molecular weight cutoff) (1000*g*) at room temperature and subjecting the ultrafiltrate to the high-performance liquid chromatography with flourometric detection method.[51]

Data Analysis

Change in plasma free and total TRP levels was calculated by comparing the fasting plasma TRP value from day 1 with the values 15 minutes before and 60, 120, 180, 240, 300, and 420 minutes after the amino acid drink (day 2). These analyses used paired *t* tests.

Since the first three patients were tested in a single-blind fashion, their data have not been included in the following data analysis. Results from behavioral ratings were analyzed by means of analysis of variance (ANOVA) with repeated measures. This allowed an assessment of the main effects of drug (control vs acute TRP depletion) and time (changes over the time points sampled). The interaction of drug × time indicates the effect of the acute TRP depletion in the sample as a whole. Significant interactions revealed by ANOVA were

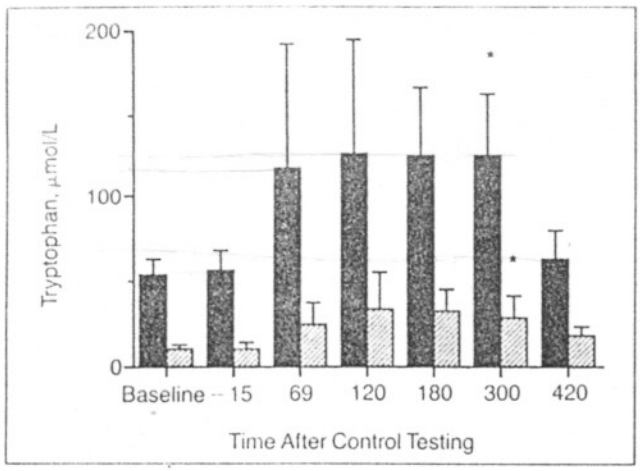

Fig 1.—Plasma total (darker bars) and free (lighter bars) tryptophan (TRP) levels before (baseline) a 500-mg tryptophan (three times daily)–supplemented low-TRP diet, and before (−15) and 60, 120, 180, 300, and 420 minutes after a TRP-containing, 16–amino acid drink. Baseline represents fasting baseline plasma TRP values. Asterisk indicates $P<.001$ compared with baseline (paired t test, two tailed).

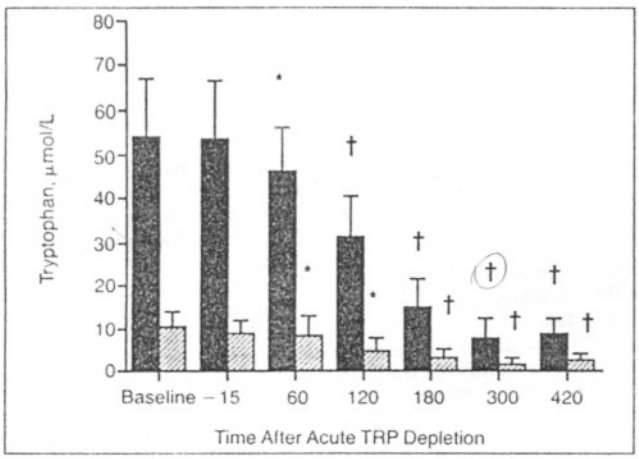

Fig 2.—Plasma total (darker bars) and free (lighter bars) tryptophan (TRP) levels before (baseline) a 160-mg/d low-TRP diet, and before (−15) and 60, 120, 180, 300, and 420 minutes after a TRP-free, 15–amino acid drink. Baseline represents fasting baseline plasma TRP values. Asterisk indicates $P<.01$ and dagger, $P<.001$, compared with baseline (paired t test, two tailed).

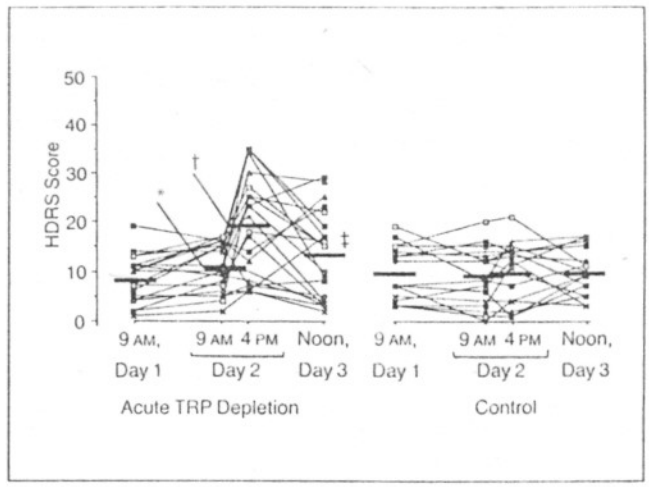

Fig 3.—Scores on the 25-item Hamilton Depression Rating Scale[46] (HDRS) for 21 remitted depressed patients taking various antidepressant medications before (9 AM, day 1) acute tryptophan (TRP) depletion or control testing and the day after testing (day 3). Acute TRP depletion represents a 24-hour, 160-mg/d low-TRP diet starting after the 9 AM point on day 1, followed the next morning (9 AM, day 2) by a TRP-free, 15–amino acid drink; control represents a 500-mg tryptophan (three times daily)–supplemented low-TRP diet starting after the 9 AM point on day 1 followed the next morning (9 AM, day 2) by a 2.3-g TRP-containing 16–amino acid drink. Unrestricted food intake began at 5 PM, day 2, on both tests. Acute TRP depletion and control tests were separated by 2 to 7 days, and the sequence of testing was randomly assigned in a double-blind fashion. Asterisk indicates $P<.003$, 9 AM, day 2, compared with day 1; dagger, $P<.0001$, 4 PM, day 2, compared with day 1; and double dagger, $P<.04$, day 3 compared with day 1 (all paired t test, two tailed).

further examined with paired t tests to determine when significant effects occurred. The ANOVAs of the HDRS and Hamilton Anxiety Scale total scores were used to assess the behavioral effects of acute TRP depletion. When significant effects on ANOVA were identified, then ANOVA of each of the individual items on that rating scale was performed to identify which items were changing. The ANOVAs of the individual items on the Symptom Checklist were analyzed to assess specific side effects associated with control or acute TRP depletion tests. As described above, when significant drug × time interactions on the HDRS, Hamilton Anxiety Scale, or individual items of these scales were identified, t tests were used to determine the time points at which these effects were occurring and the magnitude of the changes.

Pearson's correlations were calculated to evaluate the relationship between plasma TRP and behavioral changes. Because of the small sample size, Fisher's Exact Tests were used to identify clinical variables associated with relapse. Relapse was defined as an increase in depressive symptoms so that the HDRS total score had increased by at least 50% and was greater than or equal to 17. The number of previously unsuccessful antidepressant trials was determined by our consensus based on patient report, review of medical records, and direct contact with previous treaters. A previously unsuccessful antidepressant trial was defined as (1) failure to return to the premorbid level of functioning, (2) insufficient improvement to permit discharge from the acute treatment setting, or (3) need for a major medication change within 4 weeks of discharge from acute treatment after at least 4 weeks of treatment with either a therapeutic blood level (Task Force on the Use of Laboratory Tests in Psychiatry, 1985) or an adequate dose (≥300 mg/d of imipramine hydrochloride or its equivalent) of an antidepressant.

Results were considered significant when $P<.05$, but trends of $P<.10$ were also reported. All tests were two tailed. Data analysis and graphic presentation used SAS,[52] BMDP,[53] Statworks, Cricket Graph, and MacDraw II programs.

RESULTS

Changes in Plasma TRP Level

During control testing, plasma free and total TRP levels increased in all patients (Fig 1), as would be expected with normal food intake. Free TRP level was 9.8 ± 4.4 μmol/L before the diet and increased to 35.3 ± 22.5 μmol/L 5 hours after the TRP-supplemented drink ($P<.001$). Total TRP level was 44.4 ± 19.6 μmol/L before the diet and increased to 99.0 ± 43.1 μmol/L 5 hours after the TRP-supplemented drink ($P<.02$).

Acute TRP depletion dramatically decreased plasma TRP level (Fig 2). Baseline free TRP values were 10.3 ± 8.8 μmol/L before the diet (day 1) and decreased to 1.0 ± 1.5 μmol/L (91% decrease) 5 hours after the TRP-free amino acid drink on day 2 ($P<.0001$). Total TRP values were 54.4 ± 13.2 μmol/L before the diet and decreased to 6.7 ± 2.9 μmol/L (87% decrease) 5 hours after the TRP-free drink ($P<.0001$).

Behavioral Effects of Acute TRP Depletion in Remitted Patients Taking Antidepressants

The ANOVA assessing the change in depressive symptoms (reflected in HDRS total score) during acute TRP depletion and control

Table 4.—Individual Hamilton Depression Rating Scale Items Found Significant on Analysis of Variance During Acute Tryptophan Depletion

Item	Score			
		Day 2†		
	Day 1*	−15 min	+420 min	Day 3‡
Depressed mood	0.5 ± 0.5	0.8 ± 0.6§	1.2 ± 1.0‖	1.0 ± 0.8
Psychic anxiety	0.6 ± 0.8	0.2 ± 0.5§	0.3 ± 0.7	0.5 ± 0.9
Terminal insomnia	0.2 ± 0.4	0.8 ± 0.8‖	0.7 ± 0.8§	0.7 ± 1.0
Decreased appetite	0.1 ± 0.5	0.1 ± 0.3	0.8 ± 1.0§	0.7 ± 1.0‖
Loss of energy	0.4 ± 0.5	0.9 ± 0.9	1.8 ± 1.0¶	1.3 ± 1.2‖
Loss of interest	0.6 ± 0.2	0.6 ± 0.8	1.6 ± 1.3‖	1.1 ± 1.0§
Loss of pleasure	0.7 ± 0.8	0.5 ± 0.8	1.4 ± 1.3§	1.1 ± 1.0
Decreased concentration	0.6 ± 0.8	0.9 ± 0.8	1.5 ± 0.9¶	0.9 ± 0.9
Ruminative thinking	0.7 ± 0.9	0.8 ± 0.8	1.1 ± 1.1§	0.8 ± 0.9
Worthlessness/failure	0.4 ± 0.6	0.6 ± 0.7	0.8 ± 0.9§	0.4 ± 0.7

*At 9 AM before low-tryptophan diet.
†Day of tryptophan-free amino acid drink; −15 min indicates 15 minutes before the drink; +420 min, 7 hours after the drink.
‡Day after the tryptophan-free amino acid drink (noon).
§$P<.05$ vs day 1, paired t test, two tailed.
‖$P<.01$ vs day 1, paired t test, two tailed.
¶$P<.001$ vs day 1, paired t test, two tailed.

testing in remitted depressed patients revealed significant main effects of drug ($F=3.90$, $df=1,15$, $P<.07$) and time ($F=4.64$, $df=3,45$, $P<.007$). The interaction of drug (acute TRP depletion vs control testing) and time of sampling was also significant ($F=6.58$, $df=3,45$, $P<.0009$). This interaction reflects the difference in depressive symptoms elicited by acute TRP depletion and control testing over the time points sampled. Paired t tests revealed significant differences from baseline HDRS scores obtained before the diet (day 1) compared with before the amino acid drink (−15 minutes, day 2), the end of test day 2 (+420 minutes, day 2), and at noon the day after the test (day 3) (Fig 3) during acute TRP depletion but not during control testing. The greatest increase in HDRS score for most patients occurred at the +420-minute point after the TRP-free amino acid drink, at which time 11 of the 21 patients had relapsed, with 3 additional patients relapsing by the following day. Depressive symptoms did not appear sooner than 2 hours after ingestion of the TRP-free amino acid drink, and most patients did not become fully symptomatic until 5 to 7 hours after ingestion of the TRP-free amino acid drink. No patient met the relapse criteria during control testing.

To identify which symptoms on the HDRS were responsible for the change in total score, ANOVA was performed on each of the individual HDRS items. Significant drug × time interactions were identified for depressed mood ($F=6.20$, $df=3,45$, $P<.002$), psychic anxiety ($F=3.56$, $df=3,45$, $P<.03$), decreased energy ($F=5.10$, $df=3,45$, $P<.004$), decreased interest ($F=4.14$, $df=3,45$, $P<.02$), and decreased ability to experience pleasure ($F=4.45$, $df=3,45$, $P<.008$). There were trends toward significance on drug × time interactions for decreased concentration ($F=2.60$, $df=3,45$, $P<.07$), increased worry ($F=2.26$, $df=3,45$, $P<.1$), decreased self-esteem ($F=2.61$, $df=3,45$, $P<.07$), decreased appetite ($F=2.68$, $df=3,45$, $P<.06$), and terminal insomnia ($F=2.37$, $df=3,45$, $P<.09$). Table 4 presents the results of paired t tests used to identify the time of peak effect and magnitude of change for these items. There was a significant increase in reporting of terminal insomnia the night after the low-TRP diet and a statistically significant, but clinically insignificant, increase in reporting of depressed mood the day after the low-TRP diet. The reported levels of psychic anxiety were significantly decreased the day after the low-TRP diet; however, these effects were not evident after the ingestion of the TRP-free amino acid mixture (Table 4). Increases in reporting of depressed mood, decreased appetite, loss of energy, loss of interest, loss of pleasure, decreased concentration, ruminative thinking, and worthlessness/failure were maximal at the +420 point after ingestion of the TRP-free amino acid mixture (Table 4).

The ANOVA assessing the change in anxiety symptoms, as reflected in Hamilton Anxiety Scale total score, over each 3-day test period revealed a significant main effect of drug ($F=5.28$, $df=1,15$, $P<.04$), but no significant effect of time or drug × time interaction.

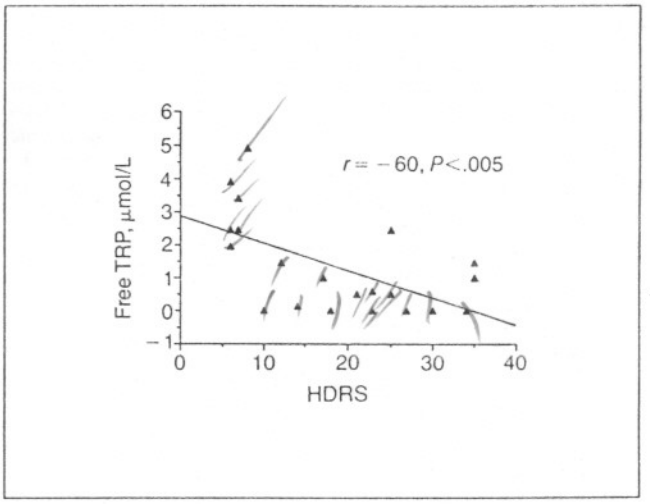

Fig 4.—Free plasma tryptophan (TRP) levels 5 hours after a TRP-free, 15–amino acid drink vs 25-item Hamilton Depression Rating Scale[46] (HDRS) scores 7 hours after the TRP-free amino acid drink in 21 depressed patients in remission with antidepressant treatment.

Assessment of side effects associated with acute TRP depletion or control testing was based on responses to the Symptom Checklist obtained before and 5 and 7 hours after each amino acid drink. An ANOVA revealed significant drug × time interactions only for decreased appetite ($F=3.86$, $df=2,18$, $P<.04$), with acute TRP depletion leading to an increase and control testing leading to a decrease in reporting of this item. Consistent with a nonspecific effect of the testing (significant main effect of time, but no significance in the drug × time interactions) with no difference between control testing and acute TRP depletion, there were significant main effects of time for increased nausea or vomiting ($F=5.33$, $df=2,18$, $P<.02$), increased feeling drowsy or sleepy ($F=8.07$, $df=2,18$, $P<.004$), increased sweating ($F=6.28$, $df=2,18$, $P<.009$), and increased faintness or lightheadedness ($F=3.62$, $df=2,18$, $P<.05$). There were trends toward significance on ANOVA main effects of time for increased headache ($F=3.16$, $df=2,18$, $P<.07$), decreased blurred vision ($F=2.74$, $df=2,18$, $P<.10$), and decreased ringing in the ears ($F=2.74$, $df=2,18$, $P<.10$).

Factors Associated With Return of Depression

To identify factors possibly related to the return of depressive symptoms during acute TRP depletion, correlations were calculated between minimum total and free plasma TRP values (at +300 minutes after the TRP-free amino acid drink) and maximum HDRS score (at +420 minutes after the TRP-free amino acid drink). This analysis failed to identify any relationship between HDRS and total plasma TRP, but it did disclose a highly significant negative correlation between the minimum free plasma TRP value and the maximum HDRS score ($r = -.60$, $P < .005$) (Fig 4).

Other factors were also examined, but the relatively small number of patients in each subgroup requires that these findings be interpreted with caution. The presence of melancholia was related to the return of depressive symptoms, with 11 of 13 melancholic patients but only 3 of 8 nonmelancholic patients experiencing a depressive relapse ($P = .04$, Fisher's Exact Test). The number of unsuccessful previous antidepressant treatments was also related to risk of relapse, with patients who had failed to respond to more than one previous antidepressant trial (12 of 12) being more likely to relapse than those with one or less previously unsuccessful antidepressant trials (2 of 9) ($P = .0003$, Fisher's Exact Test). Type of current antidepressant drug may also have been related to return of depression, with only 1 of 4 patients taking desipramine alone but 10 of 12 patients taking another type of antidepressant alone relapsing ($P = .005$, Fisher's Exact Test).

Among the most striking observations during the study was that TRP depletion often resulted in the reappearance of specific depressive ideation or content present before treatment, in addition to the reappearance of more general depressive symptoms. The following three case reports illustrate the effects of the TRP depletion on remitted depressed patients.

REPORT OF CASES

CASE 1.—A 62-year-old widow was admitted to the CNRU after a 2-year period of dysphoria, somatic and psychic anxiety, loss of appetite, midnocturnal awakening, severe decreased concentration, loss of energy and interest, anhedonia, and diurnal variation in mood with morning worsening. Particularly prominent were guilty ruminations that the patient might have prevented the death of her husband 4 years earlier if she had been with him at the hospital the day he died. Before this inpatient admission, she had failed to respond to a 6-week trial of desipramine hydrochloride, 200 mg/d, and a subsequent 3-week trial of combined lithium carbonate (600 mg/d) and desipramine hydrochloride (200 mg/d). The patient's HDRS score 1 week after admission was 36. After 4 weeks of treatment with fluvoxamine, 200 mg/d, she awoke one morning feeling "as if a cloud had lifted" from her head. She described increased interest, energy, and hope for the future, with an HDRS score of 11. During the next 2 weeks, she became more involved in ward activities and decided to return to school to become a mental health worker and "help other people like me." Her sleep improved and she was no longer anxious. She stopped ruminating about her husband's death and said that she felt as though she had been "hard" on herself. She acknowledged that she was not responsible for his death and that she needed to get on with her life and not live in the past.

After 2 weeks of clinical remission, the patient underwent acute TRP depletion. Within 2 hours of the TRP-free amino acid drink, she began to lament her husband's death and the fact that she had "had no male children to carry on the family name." Within 1 more hour she was crying uncontrollably, stating that she had been a failure and should have been present on the day her husband died. She became agitated and once again complained of not being able to see a future for herself. She also noted severe decreased concentration and energy (HDRS score, 35). She continued in this state during much of the night and slept little. The next morning she appeared shaken but was no longer agitated. She continued to cry sporadically but was able to regain control quickly, with an HDRS score of 17. By the evening of that day she was once again feeling in control and was no longer having tearful episodes. By the following morning she was once more optimistic about the future and was no longer ruminating about her husband's death. She stated that the past 2 days had been very confusing for her and that she did not know why she had started to think of her husband, but that she was once again ready to get on with her life.

CASE 2.—A 45-year-old woman employed as a banking executive was admitted to the CNRU inpatient service for a trial of fluvoxamine. She had a 15-year history of depressive symptoms and anxiety previously refractory to imipramine therapy but responsive to phenelzine therapy. Her symptoms had returned after the recent discontinuation of phenelzine. She complained of moderate levels of anxiety, depressed mood, difficulty falling asleep with frequent awakenings, loss of appetite, decreased concentration and energy, preoccupation with an "electrical" feeling along both forearms, an acute sense of loss of control and helplessness, and a sense of failure in her profession and marriage. Her HDRS on admission was 25. She was treated for 6 weeks with fluvoxamine, 300 mg/d, with no response, followed by augmentation with lithium carbonate, 900 mg/d, for an additional 4 weeks, with no response. Subsequently therapy was restarted with phenelzine sulfate, 45 mg/d, with rapid improvement during the first 3 weeks. She felt energetic, enthusiastic, and self-confident, and the "electrical" feelings in her arms disappeared. Her HDRS decreased to 13.

After 2 weeks of continued remission of depressive symptoms, she was scheduled for TRP depletion testing. The control test led to no significant change in her state. On the second day of the active test, however, approximately 4 hours after the TRP-free amino acid drink, she began to note a return of the "electrical" feelings in her forearms. Within 30 minutes of this she began to cry inconsolably and described her emotions as being "out of control." She said that she did not know why she was crying but could not stop. She also described psychic anxiety, difficulty concentrating, loss of energy, loss of self-confidence, and a sense that nothing was worthwhile. She felt as if all the gains she had made over the past few weeks had "evaporated," and her HDRS increased to 34. After eating a regular dinner that evening she began to feel more in control. She said that she felt "shaky" but that the "electrical" feelings had gone again and that she felt more optimistic. By the following morning she said that she felt "back to herself," with an HDRS score of 9. She commented that the previous day had been a "nightmare" but that she had learned that the depression was not her "fault." She also noted that, although she would not want to repeat the test, it had been worthwhile because of what she had learned about her illness.

CASE 3.—A 31-year-old man with a history of multiple episodes of depression and three previous episodes of hypomania was admitted to the CNRU outpatient clinic for treatment of a current depressive episode. His illness had begun 6 years earlier and had failed to respond to trials of desipramine hydrochloride, 300 mg/d, alone and augmented with lithium carbonate (900 mg/d). On admission he complained of "lack of feeling," irritability, mild psychic anxiety, hypersomnia, severely decreased energy, decreased concentration with inability to read, increased appetite with 4.5-kg weight gain, anhedonia, hopelessness, and a sense of helplessness. He said that he no longer wanted to be around other people and would spend the entire day inside his house. His HDRS score was 25. He was treated with phenelzine sulfate, 45 mg/d, and by the third week he noted sufficient improvement in energy and interest to start a new job. By the fourth week he said that he felt "good" again, that his concentration had improved, and that he was able to resume his hobby of writing. He felt that he was at 90% of his best functioning, except for mild difficulty falling asleep and complete anorgasmia consistent with side effects of the phenelzine. His HDRS score was 2. After 3 weeks in remission he was tested, showing no change with the control test. On the second day of the active test sequence, about 5 hours after the TRP-free amino acid drink, he was noted to be extremely quiet and withdrawn. He had stopped reading and complained of inability to concentrate. He described a loss of interest and a wish to be "alone." He said that he had very little energy and if left to himself would just "go to bed." His HDRS score was 17. He left the CNRU in the care of his wife. That evening he began to feel "better" at about 9 PM. By the next morning he said that he felt "good" again, with an HDRS score of 3. His concentration and energy had improved, and he said that the evening after the test he had had his "first orgasm in over 1 month."

COMMENT

In this group of recently remitted depressed patients maintained with various antidepressant medications, acute TRP depletion led to a clinically significant return of depressive symptoms in 14 (67%) of the 21 patients tested. Control testing, indistinguishable from the TRP depletion testing

except for its failure to deplete plasma TRP, produced no significant depressive symptoms (Fig 3). Both control testing and acute TRP depletion induced some unpleasant side effects, including mild headache, nausea, drowsiness, sweating, and faintness. However, only acute TRP depletion evoked such core symptoms of depression as depressed mood, terminal insomnia, decreased appetite, loss of energy, loss of interest, anhedonia, decreased concentration, ruminative thinking, and a sense of worthlessness and failure (Table 4). Moreover, the symptom complex and depressive cognitive content induced by acute TRP depletion often closely resembled the unique clinical features manifested by the patient before successful treatment, as illustrated in the case reports.

Interpretation of these findings is contingent on the extent to which the reduction in plasma TRP reflects decreases in brain 5-HT function. Preclinical studies in laboratory animals have repeatedly shown that brain TRP and 5-HT levels are dependent on plasma levels of TRP.[19-21,26,27,31] Similarly, both TRP-free diets and TRP-free amino acid drinks have been shown to reduce brain TRP and 5-HT levels rapidly (within 2 hours)[26,27] and to alter various behaviors dependent on 5-HT.[30-36]

In the present study, acute TRP depletion led to a 91% decrease in plasma free TRP level and an 87% decrease in plasma total TRP level within 5 hours after the TRP-free amino acid drink. In laboratory animals, this degree of plasma TRP depletion leads to a 60% to 70% decrease in brain 5-HT level in a similar period,[21,26,27] and the decrease in brain 5-HT level correlated best with the pool of free plasma TRP available.[26,27] Therefore, if a similar relationship between plasma TRP and brain 5-HT levels exists in humans, it is possible that the behavioral changes observed in the depressed patients were due to decreases in brain 5-HT availability. Consistent with this, the degree of depletion of plasma free TRP level was significantly correlated with the HDRS score 7 hours after the TRP-free amino acid drink (Fig 4).

The current findings suggest that a melancholic diagnosis and a history of refractoriness to antidepressant drugs may have increased the risk of relapse after acute TRP depletion. There is also suggestive evidence that remitted patients maintained with selective 5-HT reuptake inhibitors or monoamine oxidase inhibitors were more prone to relapse. However, the patients in this study were not randomly assigned to different antidepressant drugs but were treated on the basis of their previous responses to treatment. Patients who had never had antidepressant treatment or had never had an adequate trial of an antidepressant drug[54] were routinely treated with desipramine. Patients refractory to previous antidepressant trials with an available antidepressant were routinely treated with the investigational drug fluvoxamine, a selective 5-HT reuptake inhibitor. Patients refractory to fluvoxamine alone were routinely treated with lithium carbonate augmentation of fluvoxamine. Patients refractory to lithium carbonate augmentation of fluvoxamine usually were treated with a monoamine oxidase inhibitor. The resulting confounding factor of refractoriness and current drug treatment make it impossible to determine whether risk of relapse was increased more by current treatment with 5-HT reuptake inhibitors or monoamine oxidase inhibitors or by the presence of a more refractory form of depressive illness. Additional studies using random drug assignment are needed to determine if the dependence on 5-HT availability to maintain antidepressant efficacy is more characteristic of some antidepressant drugs than others.

Relationship to Mechanism of Action of Antidepressant Treatments

The results of this investigation suggest that the functional integrity of the 5-HT system may be necessary for the maintenance of remission induced by at least some antidepressant drugs. The data are consistent with a large body of preclinical evidence suggesting that most antidepressant drugs enhance 5-HT neurotransmission.[6,8,10] The findings represent an important replication of earlier uncontrolled studies that found the 5-HT synthesis inhibitor parachlorophenylalanine capable of producing depressive relapses in remitted patients maintained with either imipramine or tranylcypromine.

Many reviews of preclinical and clinical studies of antidepressant action have concluded that alterations in a single neurotransmitter system cannot account for the therapeutic mechanism of all, or even most, antidepressants.[5,10,17] For example, it has been hypothesized that antidepressant efficacy may depend on functional interactions between noradrenergic and serotonergic systems.[55] More recently, it has been suggested that antidepressant properties may depend on effects on second- and third-messenger pathways involving G-proteins, protein kinases, phosphoproteins, and phospholipids.[10,56] There is also interest in the relationship between neuropeptide function and depression; somatostatin[57-60] and corticotropin-releasing factor[61] have been implicated in the pathophysiology of depression, and corticotropin-releasing factor antagonists may have antidepressantlike effects in laboratory animals.

In this context, it is possible that factors other than reduction of brain 5-HT content were related to the depressive relapses observed in the present investigation. Acute TRP depletion may have affected brain peptides, second messengers, receptor synthesis, or some other nonspecific metabolic aspect of brain function. Similarly, TRP depletion and consequent reduction of 5-HT may have altered some balance between 5-HT and other neurotransmitter systems, such as the noradrenergic or dopaminergic systems. However, in vitro studies suggest that brain 5-HT neurons will use up available 5-HT stores in about 1 to 2 hours after depletion of TRP,[62] and TRP-free amino acid mixtures reduce brain 5-HT in rats within 2 hours.[26] The rapidity of the return of depressive symptoms during the TRP-depleted state in the current study, therefore, argues against some nonspecific reduction in protein synthesis secondarily affecting receptors or peptides. However, a nonspecific metabolic effect cannot be ruled out with the present data.

One of the tasks of future studies is to exclude such nonspecific effects of acute TRP depletion, such as by depletion of an essential amino acid other than TRP. Measurement of CSF levels of the monoamine metabolites 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenylglycol, and homovanillic acid might prove useful in assessing noradrenergic and dopaminergic function and in verifying a reduction in brain 5-HT level. Rapid reversal of the acute TRP depletion–induced depressive relapse by tryptophan infusion or by administration of a direct 5-HT agonist might also help to confirm relationship of the clinical change to changes in brain 5-HT function.

Several of the questions posed by the results of the present investigation may lead to an enhanced understanding of the mechanism of action of antidepressants not only in depression, but in other disorders where antidepressants are also useful. Studies attempting to answer some of these questions are ongoing.

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