Chapter

Studies on Tryptophan Metabolites in Patients of Major Monopolar Depression

Hiroi Tomioka, Junichi Masuda, Akikazu Takada and Akira Iwanami

Abstract

Plasma levels of tryptophan metabolites were compared between healthy volunteers and patients of major monopolar depression at various ages and genders. An ultrahigh-speed liquid chromatography/mass spectrometry has been used for analysis. There are significant gender and age differences in TRP metabolites of healthy volunteers. At the upper stream of metabolism, metabolites of young women and old men are higher, but at the lower stream of metabolism, their levels are higher in young men and old women. Such differences disappear in plasma of patients of major monopolar depression except for kynurenine (KYN). Daily variation of blood serotonin (5-HT) levels showed that 5-HT levels were low in the morning and increased toward evening, but blood levels of 5-HT were higher in healthy people than depressive people in the morning and decreased to ward evening. Significant age and gender differences of plasma levels of tryptophan metabolites in healthy volunteers disappear in patients of major monopolar depression. Blood levels of 5-HT were higher in healthy people than depressive patients.

Keywords: depression, monopolar depression, bipolar depression, tryptophan, serotonin, 5-hydroxyindoleacetic acid, kynurenine, 3-hydroxykynurenine, kynurenic acid, anthranilic acid, xanthurenic acid, indole-3-acetic acid, selective serotonin reuptake inhibitor (SSRI), serotonin norepinephrine reuptake inhibitor (SNRI), anxiolytic, antipsychotic, and circadian variation

1. Introduction

Recently, it has been shown that both the responses to placebo and antidepressant increased [1]. Kirsch has claimed that pharmaceutical companies did not include mildly and moderately depressed patients in trials of efficacy after finding that these patients did not benefit beyond placebo [2]. He consistently insists that antidepressants are not more effective than placebos in moderately depressed patients [3]. Drug-placebo differences are considered to be small in efficacy trials, and most of the response to antidepressants seems due to expectancy [3].

Major depressive disorder is one of the most common psychiatric disorders which is burdensome and costly worldwide in adults. Although pharmacological and non-pharmacological treatments are available, because of inadequate resources, antidepressants are used more frequently than psychological interventions.

By using a meta-analysis, all antidepressants were shown more efficacious than placebo in adults with major depressive disorder. Smaller differences between active drugs were found when placebo-controlled trials were included in the analysis [4].

Serotonin (5-HT) has been indicated to be involved in etiology of depression [5].

The roles of various metabolites of kynurenine (KYN) pathway are reviewed [6], so we do not discuss these roles in detail.

As to relationships between serotonin levels and depression, we analyzed plasma levels of TRP metabolites in patients of depression.

Although the concentration of 5-HT has been considered to be low in depressive patients [7], 5-HT concentration in the brains of suicide victims were not low [8]. Therefore, it is not known if 5-HT concentration is decreased in the brains of depressive patients.

We have recently succeeded in simultaneous measurements of TRP metabolites in plasma using an ultrahigh-speed liquid chromatography/mass spectrometry (LC/MS) [9–12].

We now report age and gender differences of various TRP metabolites in patients of major monopolar depression and healthy volunteers.

2. Results

2.1 Comparison of plasma levels of TRP metabolites between healthy people and patients of major depression

2.1.1 Healthy volunteers

The sample sizes and ages of participants are as follows. Old men (n = 25; age, 60.8 ± 9.9) and old women (n = 39; age, 67.4 ± 7.5) and young men (n = 49; age, 20.7 ± 1.5) and young women (n = 47; age, 21.2 ± 0.7). Characteristics of these people are described in **Table 1**.

Subjects	young men n=48 a	young women n=47 b	old men n=44 c	old women n=39 d
Age (years)	20.7±1.5	21.2±0.7	62.4±9.6	67.4±7.5
Height (m)	1.72±0.06	1.58±0.06	1.68±0.07	1.57±0.06
Weight (kg)	65.1±9.2	50.9±5.8	68.8±10.9	50.6±6.8
BMI	22.1±3.2	20.3±1.6	24.3±3.2	20.5±2.5

Table 1.
Background of healthy participants.

2.1.2 Patients

Outpatients of depression were recruited in this study. Fasting blood samples were taken early in the morning. Their severity of depression was checked by clinical global impression—severity scale (CGI-S), SRS, and Hamilton depression rating scale (HDRS). The history of prescriptions of drugs such as antidepressants, anxiolytics, mood stabilizers, and other drugs were asked.

Sample numbers are 55 (male, 15; female, 40; average age, 45.4 ± 11.9). The number of MDD is 38 and BD is 17. Further characteristics of patients are described below.

Plasma factors were measured after plasma was separated from blood (3000 rpm/min at 4°C). Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant.

2.1.3 The simultaneous measurements of TRP metabolites in plasma

An ultrahigh-speed liquid chromatography/spectrometry was used for the assay. Although detailed methodology was described elsewhere [5–9], the important improvement of the assay method is described here.

2.1.3.1 Reagents and instrumentation

The simultaneous analytical method developed can be adapted to major metabolites of TRP including melatonin in clinical sample.

The analytical targets of developed method are major metabolites, such as tryptophan (TRP), L-5-hydroxytryptophan (5-HTP), serotonin (5-HT), kynurenine (KYN), 5-hydroxy-tryptophol, tryptophol, 5-hydroxyindoleacetic acid (5-HIAA), indole-3-acetic acid, anthranilic acid (AA), kynurenic acid (KYNA), quinaldic acid, indole-3-butyric acid, 3-hydroxykynurenine (3-HKYN), 3-hydroxyanthranilic acid (3-HAA), xanthurenic acid (XA), melatonin, and quinolinic acid (QA). Each compound was purchased from major chemical regent manufacturers, such as FUJIFILM Wako chemical (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO, USA).

Metabolite analysis was performed by a liquid chromatograph tandem mass spectrometer, the LCMS-8060 quadrupole mass spectrometer combined with Nexera X2 liquid chromatograph system (Shimadzu Corporation, Kyoto, Japan).

The targets are separated by reversed-phase chromatography using C18 analytical column, L-Columns ODS2 (2.1 mm × 150 mm, CERI, Tokyo, Japan) with a gradient elution. Mobile phases were 0.1% formic acid solution and acetonitrile with the gradient elution by 5% concentration of acetonitrile in 3 min and then 5–95% in 6 min, followed by 5% in 3 min at a total flow rate of 0.4 mL/min. The temperature of the column was 40°C. Electrospray ionization (ESI) was used as mostly positive ionization with multi-reaction monitoring (MRM) detection.

Flow rate of the neutralizer and the drying gas were 2 L/min and 10 mL/min, respectively. Temperature of desolvation line (heated capitally tube) was 250°C. ESI interface was used at 400°C with 10 L/min of heating gas flow. Each MRM transition was optimized using each standard solution. Optimized results were shown in **Table 1**.

All mother solutions of 1 mg/mL had been stocked under −80°C, and standard samples for calibration curve were prepared prior to use as mixture solution by consideration of each range of measurement concentration.

2.1.3.2 Analysis of human plasma

Aliquot of 50 μL human plasma was used for each sample analysis. The procedure including deproteinization is shown in **Figure 1**.

TRP metabolic pathways are shown in **Figure 1**.

Figure 1 shows metabolic pathways of TRP. Metabolites were measured by an ultrahigh-speed liquid chromatography/mass spectrometry.

One-way ANOVA was used for evaluating statistical significance. A, b, c, and d indicate values of young and old men and women. Tukey's test was used for post hoc test.

Table 2 shows that there are significant gender and age differences in plasma levels of TRP of healthy volunteers.

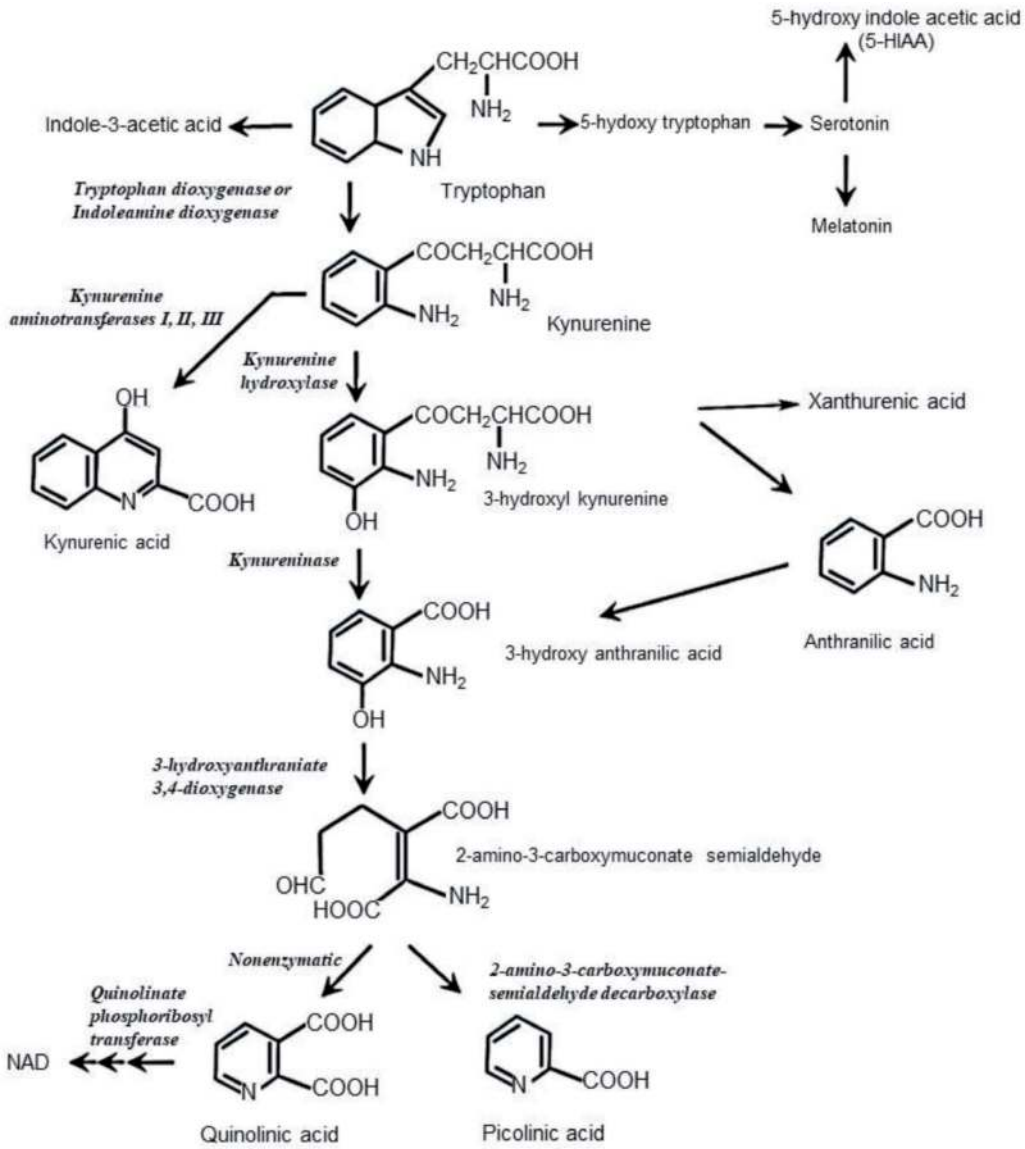

Figure 1.
Metabolic pathways of tryptophan.

Generally speaking, plasma levels of 5-hydroxyindoleacetic acid (5-HIAA), indole-3-acetic acid (IAA), KYN, and AA are higher in young women and old men than in young men and old women. Plasma levels of XA and 3HK are higher in young men and old women than in young women and old men.

One-way ANOVA was used for evaluating statistical significance. A, b, c, and d indicate values of young and old men and women. Tukey's test was used for post hoc test.

Table 3 shows that in contrast to cases in healthy people, age and gender differences disappeared in MMD except for KYN.

Sample name	young men n=25 a	young women n=10 b	old men n=20 c	old women n=20 d	significant differences
Tryptophan (μM)	63.92±10.83	67.79±9.30	68.89±13.32	47.68±7.19	a vs. d : p<0.01 b vs. d : p<0.01 c vs. d : p<0.01
L-5-Hydroxytryptophan (nM)	1.32±1.88	0.00±0.00	0.63±1.32	0.56±0.95	-
Serotonin (nM)	1.556±1.79	42.94±35.42	30.95±68.23	4.70±5.86	-
Kynurenine (nM)	693.9±177.8	1588±313	1696±1074	719.9±173.9	a vs. b : p<0.01 a vs. c : p<0.01 b vs. d : p<0.01 c vs. d : p<0.01
5-hydroxy-tryptophol (nM)	1.56±5.93	0.00±0.00	0.92±4.07	0.13±0.526	-
Tryptophol (nM)	0.09±0.38	4.96±6.59	1.19±2.95	0.10±0.42	-
5-Hydroxyindoleacetic acid (nM)	9.00±1.92	30.87±9.55	33.15±12.57	13.17±3.30	a vs. b : p<0.01 a vs. c : p<0.01 c vs. d : p<0.01
Indole-3-acetic acid (nM)	280.8±161.1	2446±1686	2433±854	262.8±142.4	a vs. b : p<0.01 a vs. c : p<0.01 b vs. d : p<0.01 c vs. d : p<0.01
Anthranilic acid (nM)	3.57±2.93	7.72±8.592	16.04±12.05	6.02±6.38	a vs. c : p<0.01 c vs. d : p<0.01
Kynurenic acid (nM)	73.64±23.74	53.20±16.66	64.03±21.65	65.40±26.12	-
Quinaldic acid (nM)	5.31±4.25	8.87±5.12	8.82±7.76	4.07±2.67	-
3-Indolebutyric acid (nM)	0.97±1.19	15.35±8.38	5.94±4.78	1.24±2.26	a vs. b : p<0.01 a vs. c : p<0.01 b vs. d : p<0.01 b vs. c : p<0.01 c vs. d : p<0.01
3-Hydroxykynurenine (nM)	10.31±2.24	4.42±1.27	2.60±2.24	12.80±3.80	a vs. b : p<0.01 a vs. c : p<0.01 a vs. d : p<0.05 b vs. d : p<0.01 c vs. d : p<0.01
3-hydroxyanthranilic acid (nM)	4.81±3.12	15.26±6.08	10.34±8.23	4.17±4.39	a vs. b : p<0.01 b vs. d : p<0.01
Xanthurenic acid (nM)	53.05±21.73	12.46±7.26	12.37±5.29	43.86±24.64	a vs. b : p<0.01 a vs. c : p<0.01 b vs. d : p<0.01 c vs. d : p<0.01

One-way ANOVA was used for evaluating statistical significance. A, b, c, d indicate values of young and old men and women. Tukey's test was used for post hoc test.

Table 2.
Measurements of plasma levels of TRP metabolites in healthy volunteers.

Diagnosis	MDD n=38				
Sample name	young men n=7 a	young women n=18 b	old men n=3 c	old women n=10 d	significant differences
Age (years)	36.00±9.256	40.00±7.004	60.00±14.73	59.40±7.306	a vs c: p<0.01 a vs d: p<0.01 b vs c: p<0.01 b vs d: p<0.01 b vs d: p<0.01
Height (m)	1.72±0.07	1.59±0.06	1.64±0.10	1.59±0.05	a vs b: p<0.01 a vs d: p<0.01
Weight (kg)	74.41±12.86	55.92±7.670	58.95±16.05	54.52±8.389	a vs b: p<0.01 a vs d: p<0.01
BMI	24.97±3.998	22.31±4.051	22.51±2.772	21.58±2.982	-
CGI-S	4.429±0.535	3.667±0.907	4.667±0.577	3.700±0.483	-
SDS	62.43±6.754	45.00±13.31	58.00±1.414	52.22±6.399	a vs b: p<0.01
HDR-S	21.29±3.817	16.94±5.150	29.67±5.033	18.30±4.398	b vs c: p<0.01 c vs d: p<0.01
Triptophan (μM)	64.09±13.77	58.48±10.40	59.11±2.026	51.24±11.01	-
L-5-Hydroxytryptophan (nM)	11.26±3.447	9.191±2.942	1.1800000	8.106±6.660	-
Serotonin (nM)	325.6±351.4	267.6±204.4	120.3±96.01	160.2±145.2	-
Kynurenine (nM)	2506.5±1042.9	1653.0±510.16	1014.2±418.25	1666.4±534.60	a vs b: p<0.05 a vs c: p<0.05
5-Hydroxytryptophol (nM)	-	10.50	25.11	2.20	-
Tryptophol (nM)	2.2950000	12.00±18.10	-	11.99±11.18	-
5-Hydroxyindoleacetic acid (nM)	28.04±22.07	35.00±28.99	22.82±28.11	48.68±58.22	-
Indole-3-acetic acid (nM)	2631.3±1666.0	2068.4±1525.8	583.0±266.4	2412.5±1026.1	-
Anthranilic acid (nM)	5.61	14.29±11.76	-	13.89±21.11	-
Kynurenic acid (nM)	67.07±39.75	32.09±15.37	26.47±12.76	36.57±14.38	-
Quinaldic acid (nM)	10.14±7.55	7.46±3.27	-	7.81±3.96	-
3-Indolebutyric acid (nM)	18.93±12.54	18.45±11.93	2.92±4.04	25.62±20.27	-
3-Hydroxykynurenine (nM)	19.03±14.17	20.55±11.06	29.96±8.09	13.98±5.98	-
3-hydroxyanthranilic acid (nM)	27.37±11.08	28.12±21.21	-	26.85±14.18	-
Xanthurenic acid (nM)	19.50±11.06	10.12±6.17	11.88±1.91	13.05±5.79	-
Melatonin (nM)	0.17	6.61±8.26	-	25.85±36.24	-
Quinolinic acid (positive) (nM)	776.1±1091.6	404.6±277.7	168.5±5.9	334.1±303.9	-

One-way ANOVA was used for evaluating statistical significance. A, b, c, d indicate values of young and old men and women. Tukey's test was used for post hoc test.

Table 3.
TRP metabolite levels of patients of MMD.

3. Discussion of part 1

The availability of endogenous 5-HT as a neurotransmitter is crucial in many physiological processes. Serotonergic neurons in the central nervous system are involved in regular behavioral states and physiological processes including arousal,

sleep, appetite, pain, releases of hormone, and mood. Dysfunction of 5-HT neurons may lead to depression and other mental disorders.

Many scientific research has been done to know roles of 5-HT in pathophysiology of depression.

Since pathological changes are investigated in the brain and cerebrospinal fluid of suicides, it is claimed that 5-HT neurotransmission is implicated in the causes of suicide [13, 14]. Low levels of 5-HIAA in cerebrospinal fluid were shown in suicide attempters of depression [15]. Although the brainstem of suicide attempters had less 5-HT and 5-HIAA, most postmortem studies report no differences in cortical 5-HT or 5-HIAA of suicides [16].

Furthermore [17] patients with MDD have been reported to have higher 5-HIAA in jugular venous blood and have been argued to reflect higher brain 5-HT neurotransmission and turnover [18].

So the roles of 5-HT in depression is still confusing.

We simultaneously analyzed plasma levels of TRP metabolites in healthy people and patients of MMD. As shown in **Tables 2** and **3**, significant age and gender differences disappear in patients of MMD.

It is difficult to speculate reasons of such changes in MMD. Probably, hormonal changes may be implicated.

These results suggest that much attention has to be paid to age and gender if we want to analyze TRP metabolites, especially 5-HT and 5-HIAA.

Statistical differences of TRP metabolites between MMD or BD and healthy people will be reported elsewhere.

3.1 The diurnal variation of 5-HT in the blood of patients of depression

As stated above, serotonin (5-HT) plays roles in a state of depression since selective inhibitors of the uptake of 5-HT and the blockers of 5-HT 1A receptors are effective in its treatment [19, 20].

There is some evidence indicating that in patients of affective disorders, the regulation of circadian rhythms is disturbed [21].

We have shown that plasma levels of 5-HT were very low in patients of depression, but the levels of 5-HIAA or KYN were not different from the levels of control persons suggesting that 5-HT was immediately converted to 5-HIAA in patients of depression [9]. Due to the presence of 5-HT transporter in platelet membranes, most of 5-HT are believed to be stored in platelets in the blood [22].

We have shown that whole blood 5-HT concentration showed marked changes throughout daytime, with maximum values in the evening and lowest values in the morning, whereas its metabolite 5-HIAA followed contrary [23].

So we wanted to measure 5-HT levels in the blood of patients of depression and controls.

We examined at five timepoints whole blood 5-HT levels in depressive patients of Hamamatsu University Hospital and control volunteers. The number of depressive patients was 18 and 30 volunteers.

Patients were in depressive states as confirmed by a mean score of 18.7 (range 12–24) on the 24-item scale of Hamilton depression rating scale [24]. None of them were administered with any drug except for small doses of benzodiazepines, for at least 10 days before blood was taken.

Blood levels of 5-HT were measured using HPLC as described by Anderson et al [25]. Analytical recoveries were 85% (SD 4.5%, CV 5.6%). Amount and response were nearly related.

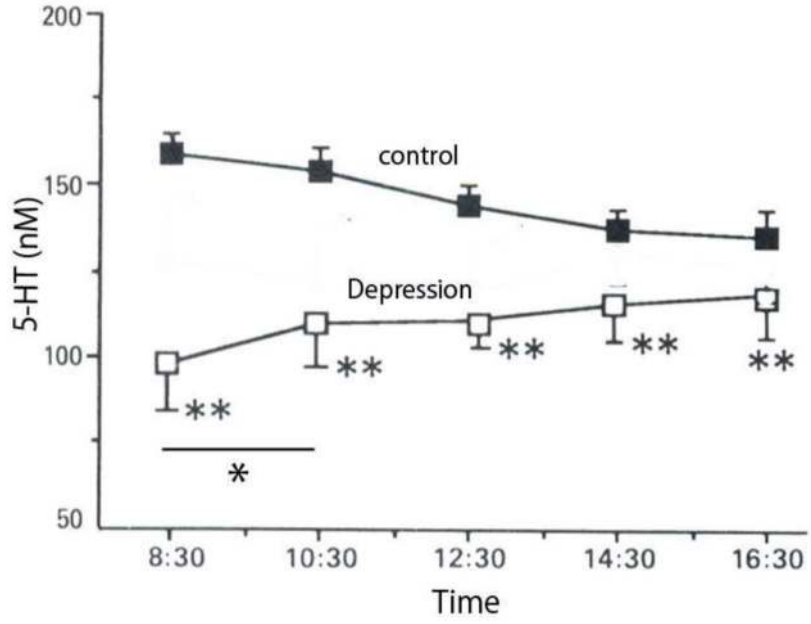

Figure 2.
*Diurnal variation of blood serotonin levels of healthy men and patients of major depression. Ordinate, blood levels of 5-HT; abscissa, time when blood was taken. **p < 0.01 control vs. depression, *0.05, levels at 8:30 vs. 10.30 of depressive patients.*

As shown in **Figure 2**, whole blood 5-HT levels were significantly lower in depressed patients at 8:30 10:30, 12:30, and 14:30. The blood concentration of 5-HT showed a circadian variation.

In the group of depression, the lowest value was shown at 8:30 and the level progressively increased to 14:30.

4. Discussion of part 2

Platelet 5-HT content is most likely regulated by the platelet transport activity.

Variations of 5-HT uptake in depressed patients have been reported by several groups [26–28].

Seasonal changes of serotonin (5-HT) uptake in blood platelets from depressed patients and normal controls were studied over a 2-year period to know if seasonal variations were present [26]. A measure of the number of 5-HT uptake sites in normal controls and depressed patients was significantly higher in fall and winter than in spring and summer. The number of 5-HT uptake in the depressed patients was lower than in normal controls throughout the year. Normal controls showed lower number in April and June. A similar trend was present in the depressed patients but the lowest values were found in the month of December.

Blood levels of melatonin, 5-HT, cortisol, prolactin, and serotonin uptake by platelets were measured at 08:00 to 08:00 hours of the following day in healthy men in age from 27 to 35 years [27]. The active transport of 5-HT by platelets was shown to be significantly correlated with melatonin blood levels. This finding suggests either a direct effect of melatonin on 5-HT active transport or the influence of the suprachiasmatic nucleus on serotonin uptake by platelets.

So far depressive disorders are considered to be associated with various neurobiological alterations like hyperactivity of the hypothalamic-pituitary-adrenal axis, altered neuroplasticity, and altered circadian rhythms. Unfortunately, the causal

connections between depressive disorders and disturbed circadian rhythms have not been completely clarified. Chronobiological therapy is based on these disturbed processes. For the treatment of the circadian symptoms, various scientifically tested chronotherapeutics are available with different effectiveness and evidence like light therapy or sleep deprivation. The successful treatment of depression also frequently leads to an improvement in altered circadian rhythm.

Further studies of circadian variation of 5-HT system may help to understand the control of serotonergic nervous system and the treatment of depression.

Abbreviations

TRP	tryptophan
5-HT	serotonin
5-HIAA	5-hydroxyindoleacetic acid
IAA	indole-3-acetic acid
KYN	kynurenine
XA	xanthurenic acid
AA	anthranilic acid
KNA	kynurenic acid
3-HKN	3-hydroxykynurenine
IDO	indoleamine dioxygenase
TDO	tryptophan dioxygenase
SSRI	selective serotonin uptake inhibitor
SNRI	serotonin epinephrine reuptake inhibitor
CGI-S	clinical global impression—severity scale
SDS	self-rating depression scale
HDRS	Hamilton depression rating scale

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