Provisional chapter

Measurement of Plasma Tryptophan Metabolites: Clinical and Experimental Application for Depression and Stress States Assessment

Akikazu Takada, Fumiko Shimizu and Junichi Masuda

Additional information is available at the end of the chapter

Abstract

There are three pathways in tryptophan (TRP) metabolism. Serotonin (5-hydroxytryptamine; 5-HT) pathway is important in mood, anxiety, memory, and cognition and is impaired in depression. Kynurenine (KYN) pathways are involved in immunity, inflammation, muscles movement, and mental health. We investigated changes in TRP metabolites in plasmas of stressed rats and in depressive patients. TRP metabolite levels in 5-HT and KYN pathways in various brain areas and plasma were increased soon after electric foot shock given to rats but returned to normal 24 h later. Plasma levels of 5-HT were very low or undetectable in patients of monopolar depression. 5-hydroxyindole acetic acid (5-HIAA)/TRP ratios or KYN/TRP ratios were not different between healthy controls and depressive patients, indicating 5-HT quickly being degraded into 5-HIAA in patients of depression but KYN levels were not changed in depression. These results indicate that TRP metabolism changes upon stress application and in patients of depression.

Keywords: tryptophan, serotonin, 5-hydroxyindole acetic acid, kynurenine, 3-hydroxykynurenine, kynurenic acid

1. Introduction

Tryptophan (TRP) is one of the essential amino acids, which must be taken as food. It is not only needed for protein synthesis but serves as a substrate for bioactive component with important physiological roles.

There are three pathways in TRP metabolism: serotonin (5-HT), kynurenine (KYN), and indole-3-acetic acid pathways.

Since 5-HT is known as an important neurotransmitter and derived from TRP, many people know about TRP to some extent. 5-HT is really involved in the adaptive responses in the central nervous system and considered to be related to mood, anxiety, or cognition [1].

5-HT is further converted in the pineal body and the retina to N-acetyl serotonin (NAS) and melatonin which controls circadian rhythm [2].

In mammals, most of the free TRP is converted to KYN and generates metabolites involved in inflammatory, immune, responses, and neurotransmission [3].

We have recently succeeded in simultaneous measurements of almost all TRP metabolites including melatonin by using an ultrahigh speed liquid chrom`atography and mass spectrometry (LC-MS) [4], which is the first time in the world.

In this chapter, we report about the precise methodology of the simultaneous measurements and some results obtained from stressed rats and depressive patients.

1.1. Measurements of TRP metabolites using LC-MS

1.1.1. Background

Since 5-HT and its derivatives have the strong fluorescence, high-sensitive analyses of some TRP metabolites can be performed by liquid chromatography with fluorescence detection. However, it is very limited to analyze the total metabolites including kynurenine by photometric detection although kynurenic acid (KYNA) can be detected as zinc chelate compound under zinc acetate solution.

In the past years, liquid chromatograph mass spectrometry (LC-MS) has been widely spread in many areas including clinical and biological analyses. Since LC-MS with electrospray ionization (ESI) is suitable for determination of metabolites of TRP, LC-MS is expected as a powerful tool for this type of analysis.

In addition, the tandem mass spectrometer also has widely used in recent years with its high sensitivity and selectivity.

There are several researches for the simultaneous analyses of TRP metabolites using LC-MS or LC-MS/MS methods for clinical samples such as human serum and plasma [5, 6]. In general, isotope-labeled internal standards are used in LC-MS/MS analysis to improve the accuracy, although isotope-labeled regents are expensive and limited availability [7, 8]. Even though less accurate, acceptable results can be obtained without internal standards for the screening purposes. In this chapter, the simultaneous determination of TRP metabolites in human plasma by LC-MS/MS technique combined with simple pretreatment procedure is described.

1.1.2. Regents and instrumentation

The simultaneous analytical method was developed for major metabolites of TRP including melatonin.

The targets are 17 of major metabolites, 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, 3-indolebutyric acid, 3-hydroxykynurenine (3-HKYN), 3-hydroxyanthranilic acid (3-HAA), xanthurenic acid (XA), melatonin, and quinolinic acid (QA). Each compound is commercially available from major chemical regent manufacturers, such as Fujifilm-Wako chemical (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO, USA).

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

The targets are separated by reversed phase mode using ODS analytical column, L-Columns ODS (2.1 mm x 150 mm, CERI, Tokyo, Japan) with a gradient elution. Mobile phases were 0.1% formic acid solution and acetonitrile with 5% concentration of acetonitrile to 3 min, then 5–95% in 6 min followed by 5% in 3 min (12 min analytical cycle) at total flow rate of 0.4 mL/min. The

			Qualification (m/z)		Qualification (m/z)	
Compound	**Molecular weight**	**Monoisotopic mass**	**Precursorion**	**Production**	**Precursorion**	**Production**
DL-Trypotophan	204.225	204.090	205.10	188.10	205.10	146.10
L-5-Hydroxytrypotophan	220.225	220.085	221.10	204.05	221.10	162.00
Serotonin	176.215	176.095	177.10	160.10	177.10	115.05
L-Kynurenine	208.214	208.085	209.10	192.00	209.10	94.05
5-Hydroxytrypotophol	177.200	177.079	178.10	160.10	178.10	115.05
Trypotophol	161.200	161.084	162.10	144.05	162.10	117.10
5-Hydroxyindole -3-acetic acid	190.176	190.051	192.10	146.05	192.10	110.00
Indole-3-acetic acid	175.184	175.063	176.10	130.05	176.10	77.05
Anthranilic acid	136.129	136.040	138.10	120.05	138.10	65.05
Kynurenic acid	189.167	189.043	190.10	144.05	190.10	89.10
Quinaldic acid	172.161	172.040	174.10	128.05	174.10	156.05
Indole-3-acetic acid	202.230	202.087	204.10	186.10	204.10	130.10
3-Hydroxykynurenine	224.213	224.080	225.15	208.20	225.15	162.15
Hydroxyanthranilic acid	152.128	152.035	154.15	136.20	154.15	80.15
Xanthurenic acid	205.167	205.038	206.15	160.20	206.15	132.20
Melatonin	232.279	232.121	232.20	174.10	232.20	130.05
Quinolinic acid	167.120	167.022	168.00	78.10	168.00	150.00

Table 1. MRM transition.

temperature of the column was 40°C. For LC-MS, electrospray ionization (ESI) was used with multi-reaction monitoring (MRM) mode.

Flow rate of the neutralizer and the drying gas were 2 L/min and 10 mL/min, respectively. The 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 are shown in **Table 1**.

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

1.1.3. 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**. The typical chromatograms of 17 major metabolites are shown in **Figure 2** as standard solution and in **Figure 3** as human plasma sample. These chromatograms demonstrate the usefulness of the developed method for simultaneous analysis of TRP metabolites.

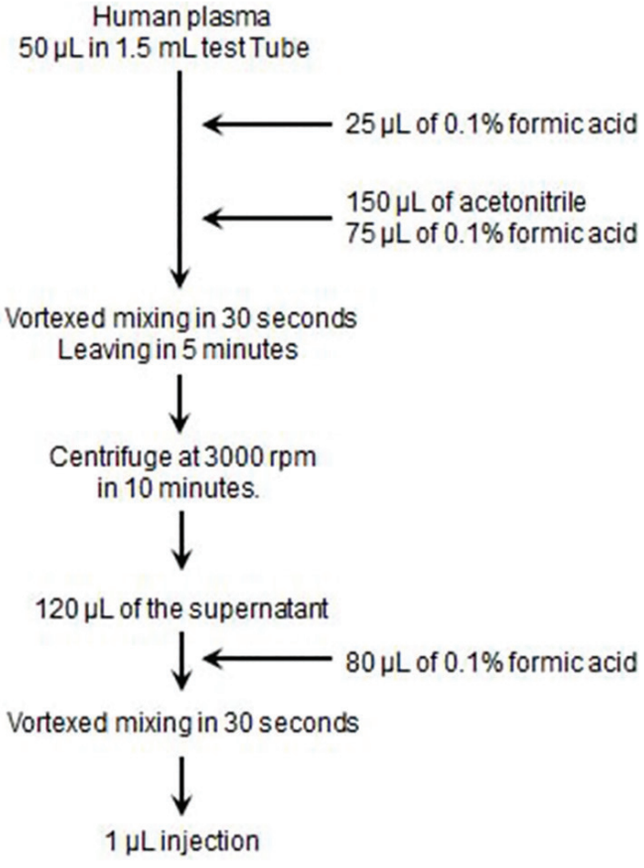

Figure 1. The procedure of deproteinization.

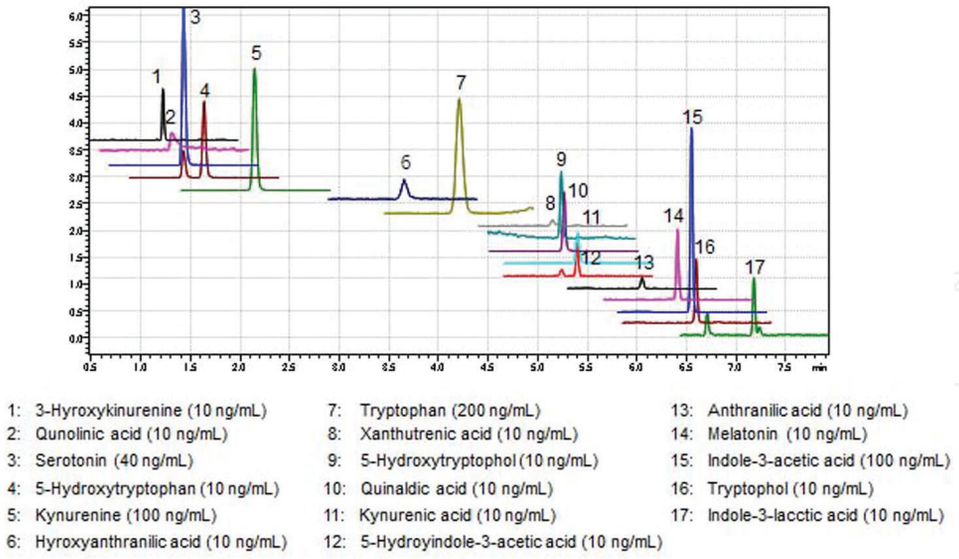

Figure 2. Chromatograms of 17 major metabolites of tryptophan.

1.2. Stress and TRP metabolism

Stress influences many functions related to mental and body health. We have shown that the application of electric shock increased plasma and brain TRP, 5-HT, and 5-HIAA levels [9, 10] and changed nicotine-induced release of 5-HT or dopamine in rats [11–14]. We also examined changes in serotonergic and kynurenic pathways in rats exposed to foot shock [15].

Stress induces a number of changes in the central system of neurotransmitters, particularly noradrenaline and 5-HT [16, 17].

A pathologic overabundance of endogenous excitotoxin, quinolinic acid, or hypofunction of KYNA has been hypothetically linked to the occurrence of seizures and nerve cell death [18, 19].

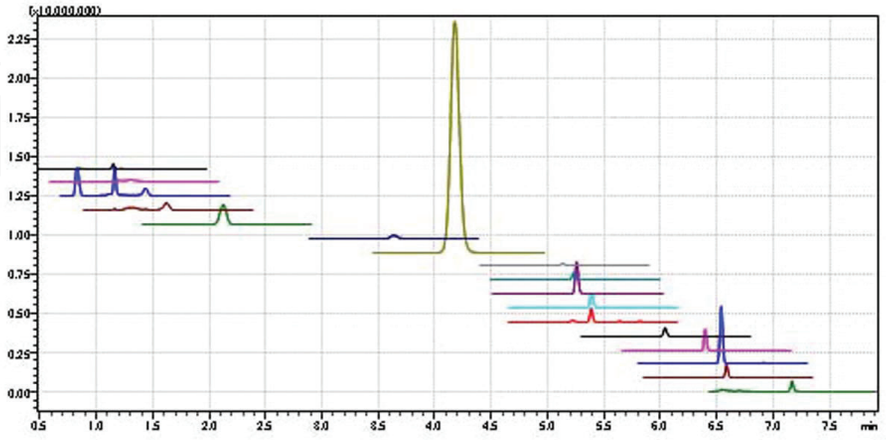

Figure 3. Chromatogram of human plasma sample.

1.2.1. Stress and TRP metabolites in the brain

Animals: Male Wistar rats (9 weeks old) were fed with standard laboratory foods and tap water *ad libitum*. Two weeks later, rats were randomly divided into two groups. One group received foot shock, which was given as a series of 10-s shock (o.19 mA) followed by 50 s intervals during a 60-min period. CT 110 cycle timer and NS-SG01 shock generator scrambler (Neuroscience Inc., Tokyo, Japan) were used. Samples of 10 rats were taken immediately after the foot shock and samples of another 10 rats were taken 24 h after the shock. Ten rats were used as controls.

Blood: Rats were anesthetized with pentobarbital, and blood was taken by heart puncture and put into a tube containing 3/13% sodium citrate. Plasma was obtained by centrifugation at 3000 rpm for 20 min.

Brain sampling: The brains were removed and chilled on ice. Eight regions (cerebellum, medulla, hypothalamus, striatum, midbrain, hippocampus, cortex, and frontal cortex) were dissected and samples were immediately frozen. Frozen brain samples were homogenized in 0.15 N perchloric acid containing 0.025% EDTA (pH 3.0). The samples were centrifuged at 14,000 rpm for 20 min at 4°C. After centrifugation, the supernatant was filtered (0.45 μm Millipore filter) and stored at −80°C until assayed.

Figure 4 shows TRP pathway and its metabolites.

Figure 5 shows that 5-HT levels increased significantly at hypothalamus and midbrain soon after the shock but returned normal 24 h later.

5-HT levels were not increased in cerebellum, medulla, striatum, hippocampus, cortex, and frontal cortex.

Figure 6 shows 5-HIAA levels of various brain areas after foot shock.

5-HIAA levels significantly increased in all the brain areas except striatum but returned normal 24 h later.

Figure 7 shows KYN levels in various brain areas after foot shock.

KYN levels significantly increased in all the brain areas after foot shock but returned to normal 24 h later.

Figure 8 shows plasma levels of TRP, 5-HT and 5-HIAA after foot shock.

Plasma levels of TRP, 5-HT, and 5-HIAA significantly increased after foot shock but returned to normal 24 h later.

Figure 9 shows plasma levels of KYN,3-HKYN, and KYNA after foot shock.

Plasma levels of KYN, 3-HKYN, and KYNA increased significantly after foot shock but returned to normal 24 h later.

1.3. TRP metabolites in plasma of patients of depression

We asked male and female acquaintances older than 50 years old and male and female college students to participate in the experiments. We checked their health carefully and

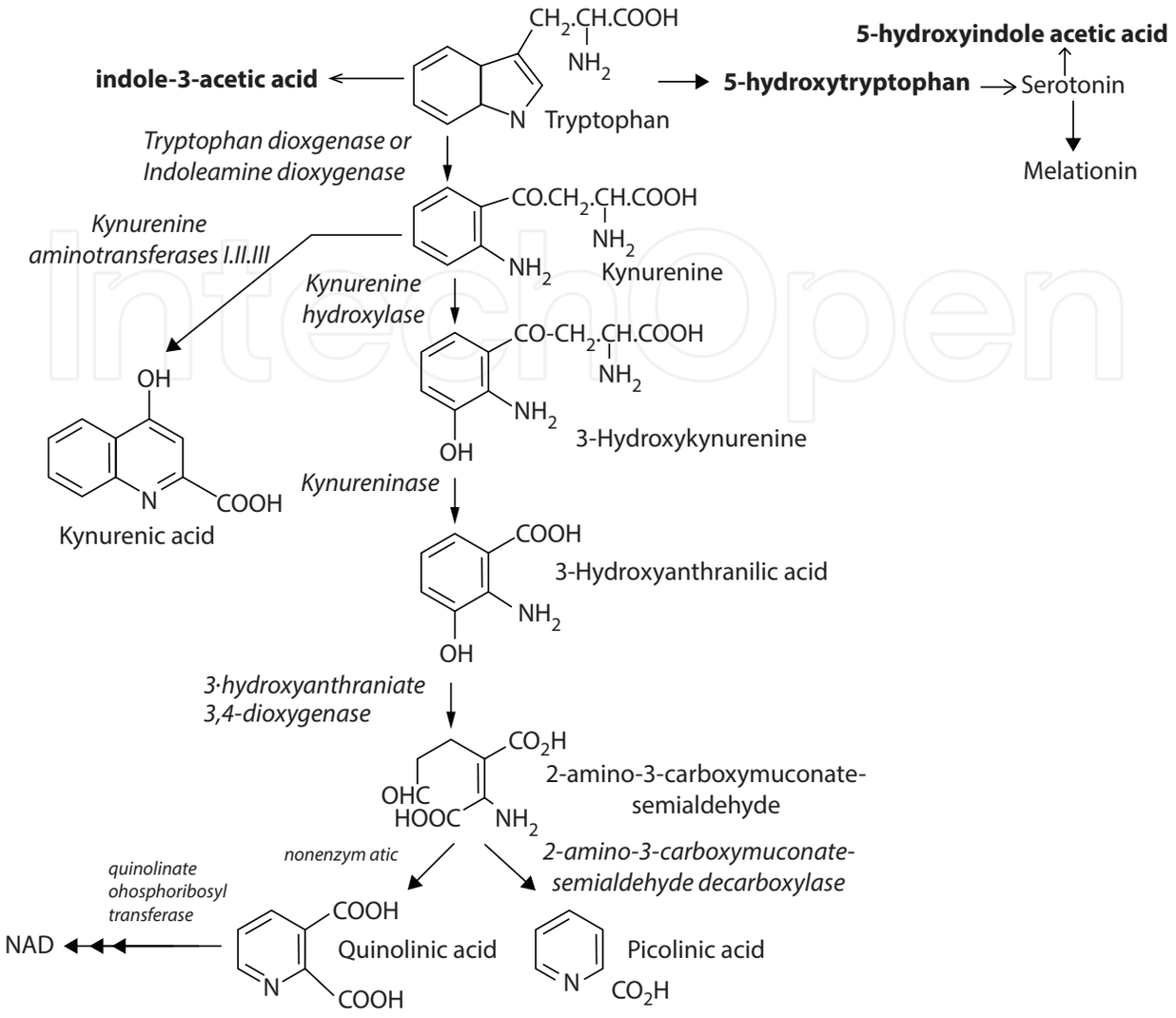

Figure 4. TRP pathways.

recruited them if there were no health problems such as diabetes, hypertension, or no serious diseases experienced in the past. They did not smoke in the past. We also excluded people who took drugs for dyslipidemia, hyperglycemia, or hypertension. We collected blood samples early morning. Participants were asked not to eat anything after 21.00 PM the previous evening. Plasma specimens were collected for assays of blood parameters. We obtained an informed consent prior to conducting the protocol which had been approved by the Ethical Committee of Showa Women's University and Yokohama North Hospital of Showa University.

Patients were diagnosed to be monopolar depression at the psychiatry clinic of Yokohama North Hospital of Showa University. The Zung Self-Rating Depression Scale [20] was used, which is a short self-administered survey to quantify the depressed status of a patient. There are 20 items on the scale that rate the affective, psychological, and somatic symptoms associated with depression. We took blood from five male patients (26, 30, 45, 47, and 56 years old) and four female patients (23, 25, 41, and 60 years old).

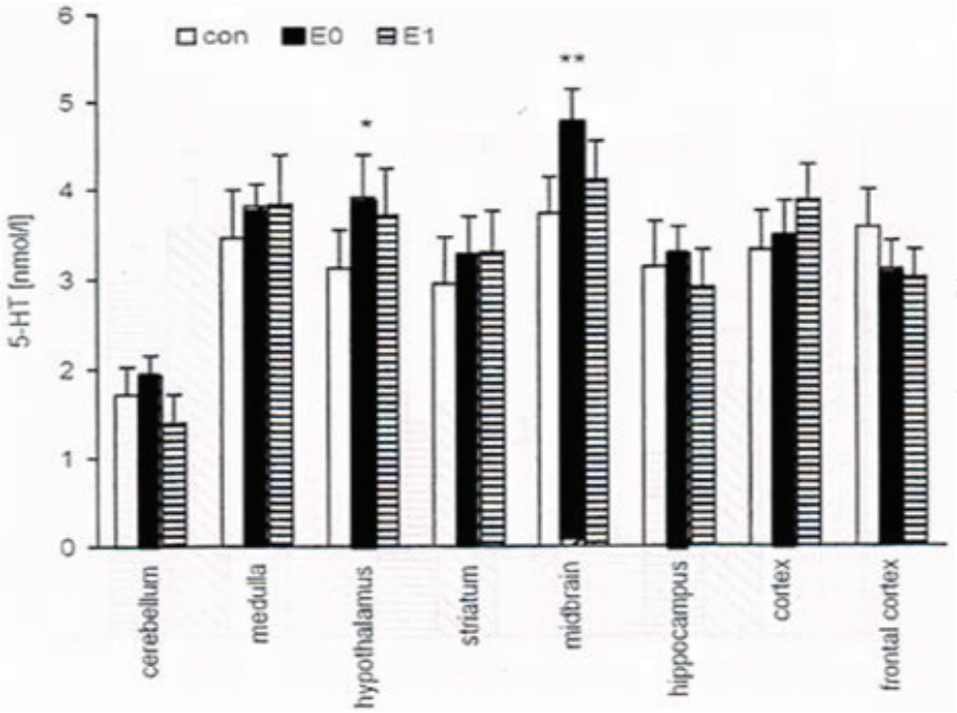

Figure 5. Serotonin (5-HT) levels in various brain areas after electric foot shock. con, control; E0, soon after the shock; E1, 24 h after the shock. *$p < 0.05$ and **$p < 0.01$.

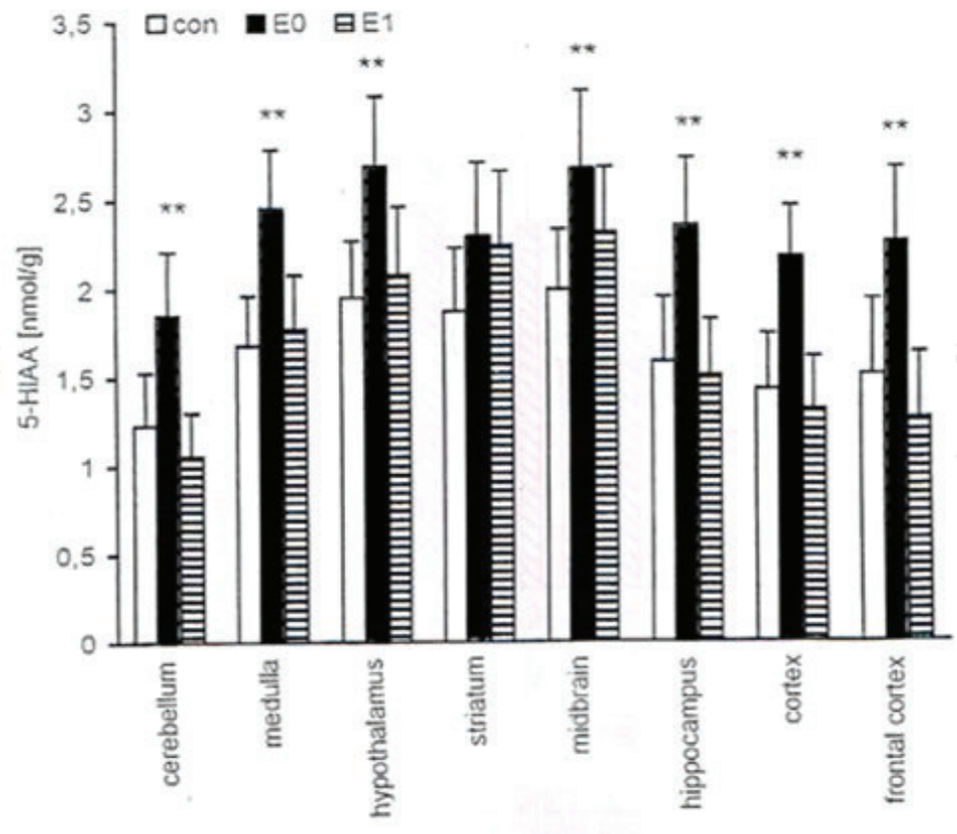

Figure 6. 5-HIAA levels in various brain areas after foot shock. con, control; E0, soon after the shock; E1, 24 h after the shock. *$p < 0.05$.

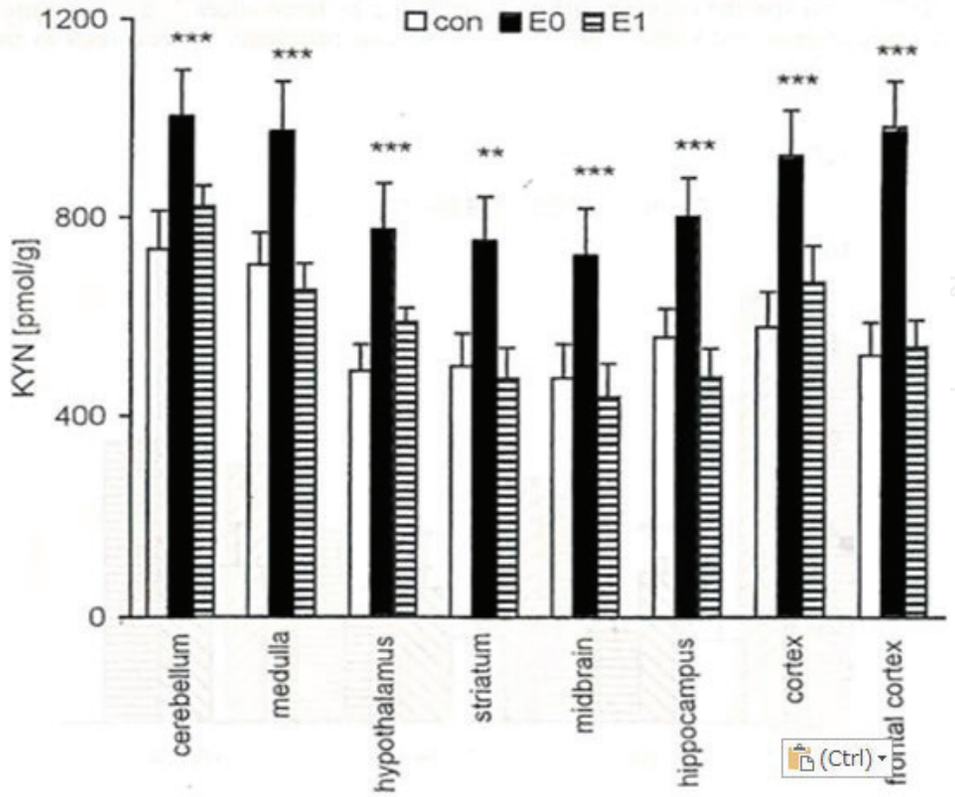

Figure 7. Kynurenine levels in various brain areas after foot shock. con, control; E0, soon after the shock; E1, 24 h after the shock. **p < 0.01 and ***p < 0.001.

5-HT was detected only in plasma of two patients. Both of them were young females. A 60-year-old woman took Jay Zoloft 25 mg and ethyl loflazepate 1 mg, and another 41-year-old woman took Cymbalta 20 mg and Luran 3 mg. 5-HT was not detected in plasmas of these women.

The number of healthy old men and young women were 20, and the number of depressive patients were 9.

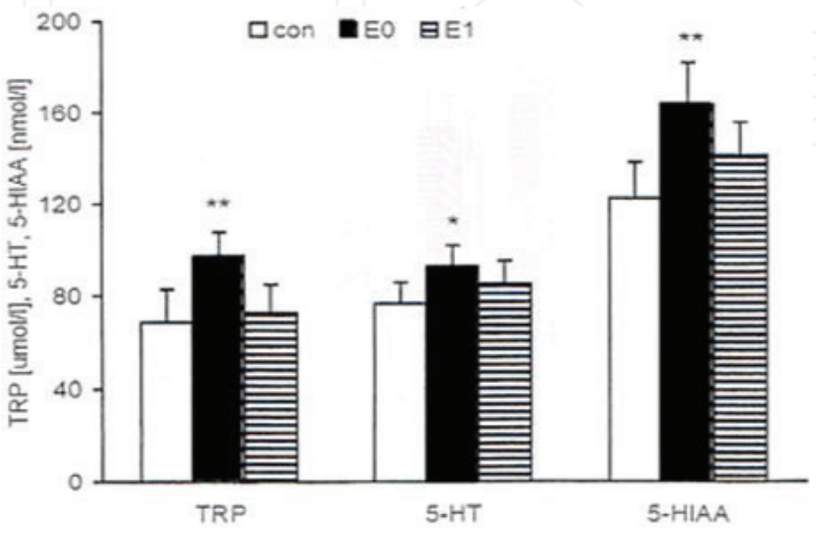

Figure 8. Plasma levels of TRP, 5-HT, and 5-HIAA after electric foot shock. con, control; E0, soon after the shock; E1, 24 h after the shock. *p < 0.05 and **p < 0.01.

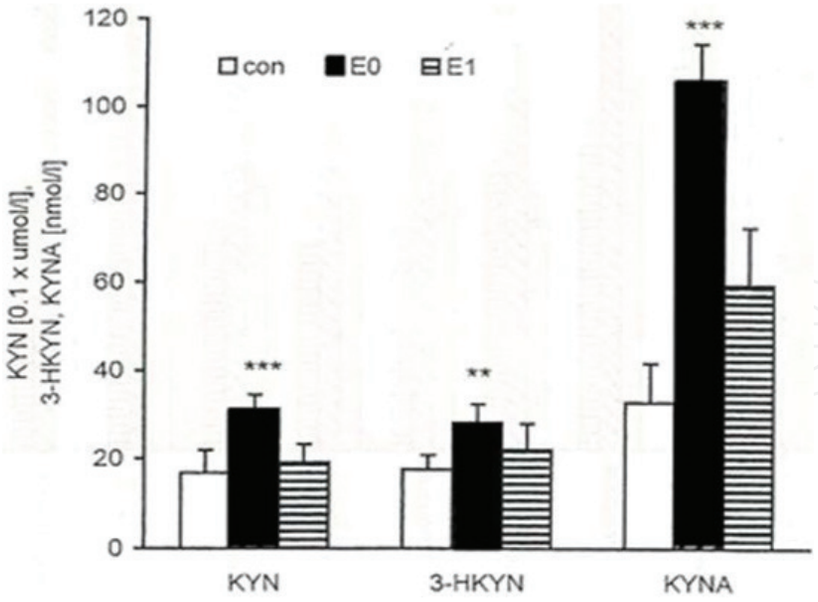

Figure 9. Plasma levels of KYN (kynurenine), 3-HKYN (3-hydroxykynurenine), and KYNA (kynurenic acid). con, control; E0, soon after the shock; E1, 24 h after the shock. **$p < 0.01$ and ***$p < 0.001$.

	Young men (n = 20)	Young women (n = 20)	Old men (n = 20)
Age	20.7 ± 1.5	21.2 ± 0.7	60.8 ± 9.9
Height (m)	1.72 ± 0.06	1.58 ± 0.05	1.69 ± 0.07
Weight (kg)	65.1 ± 8.9	51.4 ± 5.8	71.1 ± 13.1
BMI	22.1 ± 3.1	20.4 ± 1.6	24.9 ± 3.7

Table 2. Basic backgrounds of healthy participants.

Patients	Sex	Serotonin detection	Visit (age)	Medication
H1	Male	—	45	None
H2	Male	—	47	None
H3	Female	—	60	Jay Zoloft 25 mg, ethyl loflazepate1 mg
H4	Female	+	23	None
H5	Female	+	25	None
H6	Male	—	30	None
H7	Female	—	41	Cymbalta 20 mg, Luran 3 mg
H8	Male	—	56	None
H9	Male	—	26	None

Table 3. Various parameters of patients.

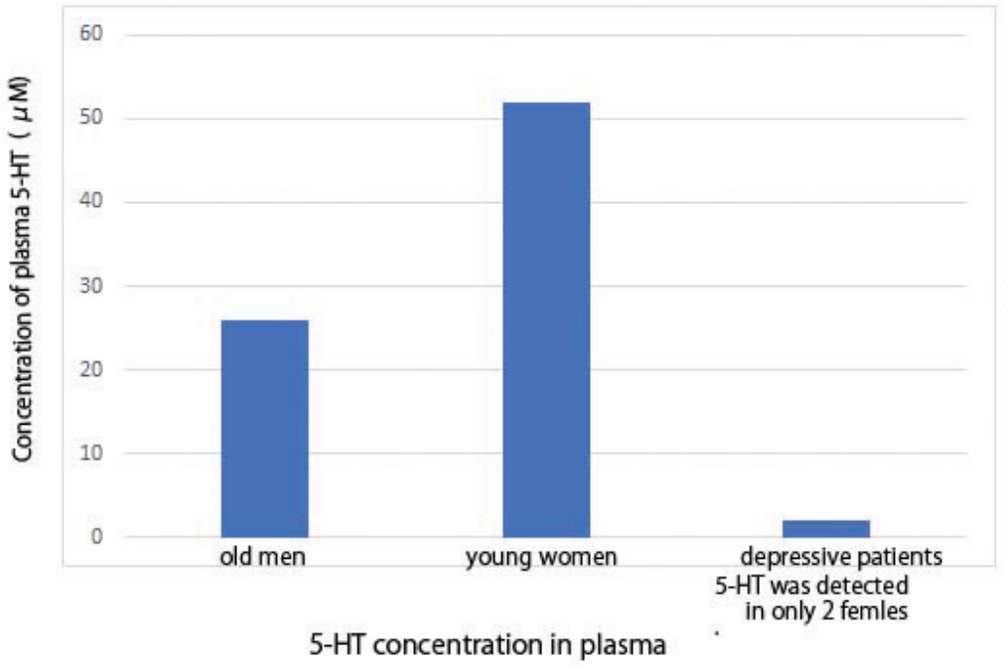

Figure 10. 5-HT levels in plasmas of depressive patients and controls.

Tables 2 and **3** show the background data of these patients.

Figure 10 shows plasma levels of 5-HT of healthy young women, old men and depressive patients.

Plasma levels of 5-HT in depressive patients were very low compared to those of old men and young women. 5-HT was detected in plasmas of only two young females.

Figure 11 shows 5-HT/TRP and 5-HIAA/TRP ratios.

Although 5-HT/TRP ratio of depressive patients was low compared to that of old men and young women, 5-HIAA/TRP ratio was almost the same as that of old men and young women.

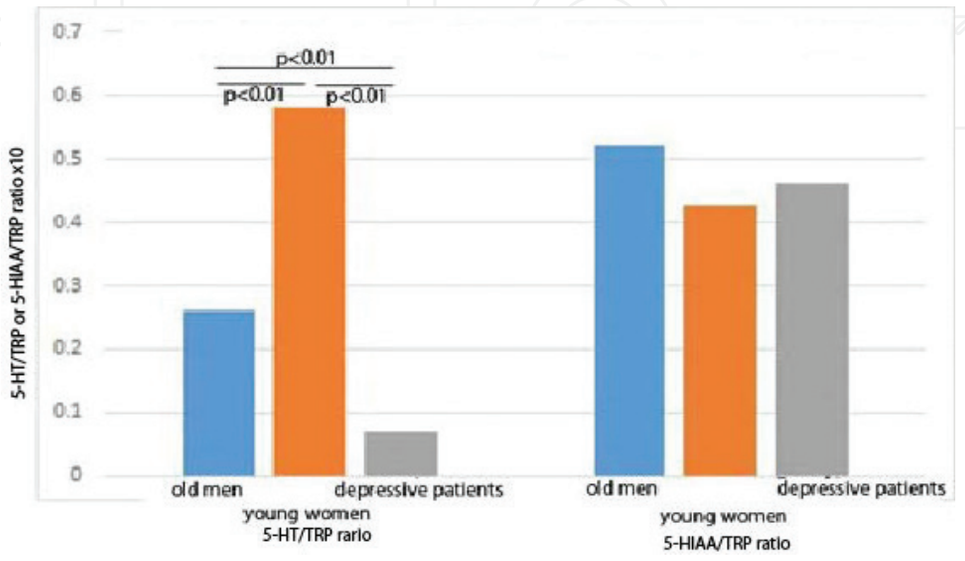

Figure 11. 5-HT or 5-HIAA/TRP ratio.

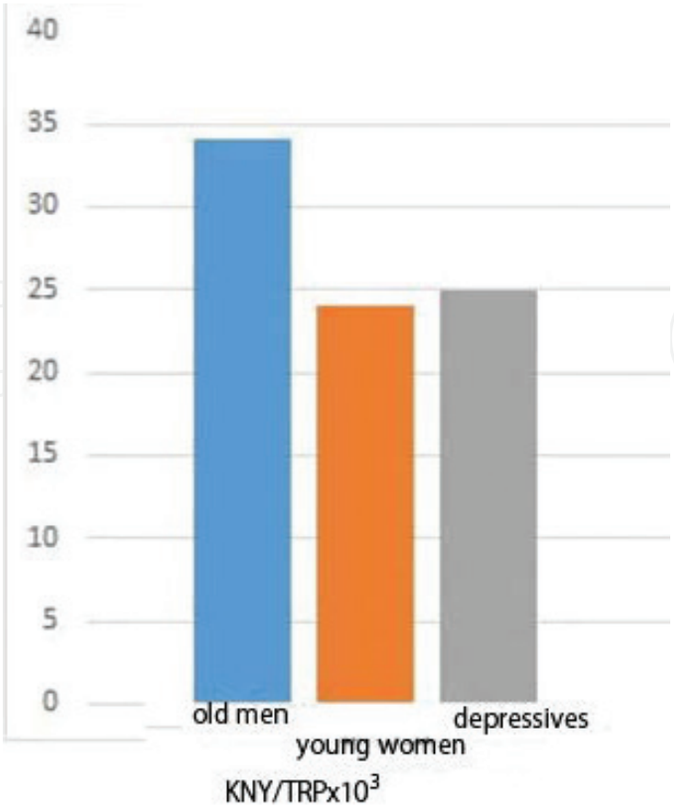

Figure 12. KYN/TRP ratio.

Figure 12 shows that there were no differences in KYN/TRP ratios between that of old men, young women, and depressive patients.

2. Discussion

As stated above, most of the metabolites of KYN pathway are found in the brain [1, 21–23]. Some metabolites of KYN pathway are neurotoxic and some are neuroprotective.

KYNA is an endogenous neuroprotective agent that is usually present in the brain at nanomolar concentration [24]. KYNA is an antagonist to quinolinic acid (QA) and acts on the glycine modulatory site of the NMDA receptor at low concentration [22] and at higher concentration at the glutamate site of the NMDA receptors and also on the a-amino3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors [25]. It also antagonizes the alpha 7 nicotinic acetylcholine receptors [26] and selectively activates a G-protein-coupled receptor, GPR35–48 [27]. Many neuroactive intermediates are shown in KYN pathways [28]. So, we decided to measure some of TRP metabolites in the brain and plasma in the stressed rats.

Our results show that 5-HT levels increased only in hypothalamus and midbrain, but its degradation product, 5-HIAA levels, increased in every part of the brain. These results may imply that 5-HT is quickly converted to 5-HIAA soon after shock, so that 5-HT levels apparently did not increase after shock. This finding is important in the explanation of plasma 5-HT and 5-HIAA levels in patients of depression.

Some of TRP metabolites were shown to be neuroexcitatory and convulsive, thus toxic [29]. One of such neuroexcitatory factors in KYN pathway is 3-HKYN [24]. Its synthesis is catalyzed by kynurenine 3-hydroxylase. Although data of brain analyses were not shown here, 3-HKYN levels increased in all the brain areas and plasma (**Figure 6**).

3-HKYN is said to be the most toxic substance in TRP metabolism [30]. So stress induces disturbances in the central nervous system by increasing levels of 3-HKYN (**Figures 4–9**).

KYN is usually hydroxylated to 3-HKYN and then further converted to 3-hydroxyanthranilic acid (3-HAA). 3-HAA is rapidly converted to QN by the non-enzymatic reaction and further to NAD^+.

The other pathway of KYN, the production of KYNA and xanthurenic acid (XA), is minor under normal conditions. KYNA is an endogenous antagonist of excitatory amino acid receptors and may serve as a modulator of excitatory nerve transmission. This study may suggest that stress induces the indirect modulation of excitatory amino acids in the central nervous system by increasing KYNA.

KYNA has an antagonist activity of the three ionotropic excitatory amino acid receptors [22, 31]. At low concentrations, KYNA blocks the glycine co-agonist site of N-methyl-D-aspartate receptor and may serve to prevent the overactivation of glutamic acid receptor. When brain kynurenic acid levels were increased experimentally, neuroprotection and seizure reduction have been reported [32].

The roles of various metabolites of KYN pathway are reviewed [28], 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 [33], 5-HT concentration in the brains of suicide victims was not low [34]. Therefore, it is not known if 5-HT concentration is decreased in the brains of depressive patients (**Table 2**).

We decided to measure TRP metabolites in patients of monopolar depression. **Table 3** shows five male patients participated in the experiments. The age is 44–61 years old (41.2 ± 11.3). Plasma serotonin levels were detected only in two young female patients. We could not measure 5-HT in plasma in seven persons, which is shown in **Figure 10**.

Although plasma serotonin levels and 5-HT/TRP ratio were low in depressive patients, the levels of 5-HIAA/TRP were not lower in depressive patients. This result indicates that 5-HT is degraded to 5-HIAA in depressive patients almost to the same extent to healthy old and young women (**Figures 11** and **12**).

We measured the levels of KYN in healthy old men and young women and depressive patients. TRP seems to be degraded to KYN pathways to the same extent in these three groups.

These results suggest that in depressive patients 5-HT was quickly degraded to 5-HIAA, and this seems to be a reason of low 5-HT levels in depressive patients.

As to a relationship between serotonin pathway and KYN pathway, Lapin IP suggested that in depression tryptophan 2,3-dioxygenase in the liver shunted metabolism of serotonin away from 5-HT production to KYN production, resulting in serotonin deficiency [35]. KYN,

QN, and 3-HKYN were shown to be anxiogenic and KYNA were anxiolytic [36]. From these results, he tried to explain the effects of antidepressive drugs.

Our results do not support this hypothesis. Metabolites of KYN pathways were not high in depressive patients.

There have not been enough studies as to 5-HT levels in the brain of patients of bipolar depression. Serotonin levels in cerebrospinal fluids of patients of bipolar depression were shown to be high [37] or normal [38]. So it seems to be very important to discriminate monopolar and bipolar depression to study roles of serotonin in the pathogenesis of disease.

Our results show that plasma 5-HT levels were low and metabolites of KYN pathway were not changed in patients of monopolar depression.

3. Statistics

Standard ANOVA methodology was used and $p < 0.05$ was considered as significant difference. Results are expressed as mean ± SD. Bars of figures represent standard deviations.

4. Ethics

This work has been approved by the Ethical committees of Showa Women's University, Showa University School of Medicine, and NPO "International projects on food and health" and has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments.

Acknowledgements

Experiments were designed and performed by all of the authors. AT wrote the manuscript. Statistical analyses were done by FS. All authors read the manuscript and approve the final manuscript. All the authors had responsibilities for a final content. A part of the work was reported in Japanese (http://www5c.biglobe.ne.jp/~takada-a/protein%20and%20brain.pdf).

Author details

Akikazu Takada[1]*, Fumiko Shimizu[2] and Junichi Masuda[3]

*Address all correspondence to: takadaa@mwd.biglobe.ne.jp

1 International Projects on Food and Health (NPO), Tokyo, Japan

2 Faculty of Life and Environmental Sciences, Showa Women's University, Tokyo, Japan

3 Global Application Development Center, Shimadzu Corporation, Kanagawa-ken, Japan

References

[1] Canli T, Lesch KP. Long story short: The serotonin transporter in emotion regulation and social cognition. Nature Neuroscience. 2007;**10**:1103-1109. DOI: 10.1038/nn1964pmid: 17726476

[2] Yates CA, Herbert J. Differential circadian rhythms in pineal and hypothalamic 5-HT induced by artificial photoperiods or melatonin. Nature. 1976;**262**:219-220. DOI: 10.1038/262219a0pmid:934338

[3] Stone TW, Stoy N. Darlington LG an expanding range of targets for kynurenine metabolites of tryptophan. Trends in Pharmacological Sciences. 2013;**34**:136-143. DOI: 10.1016/j.tips.2012.09.006pmid:23123095

[4] Matsuoka K, Kato K, Takao T, Ogawa M, Ishii Y, Shimizu F, Masuda J, Takada A. Concentrations of various tryptophan metabolites increase in patients of diabetes mellitus compared to healthy aged male adults. Diabetology International. 2016;**8**:69-72. DOI: 10.1007/s13340-016-0282-y

[5] Hervè E, Beyne E, Jamault H, Delacoux E. Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection. Journal of Chromatography B. 1996;**675**:157-161

[6] Marklová E, Makovičková H, Krákorova I. Screening for defects in tryptophan metabolism. Journal of Chromatography. A. 2000;**870**:289-293

[7] Zhu W, Stevens AP, Dettmer K, Gottfried E, Hoves S, Kreutz M, Holler E, Canelas AB, Kema I, Oefner PJ. Quantitative profiling of tryptophan metabolites in serum, urine, and cell culture supernatants by liquid chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry. 2011;**401**:3249-3261

[8] Zhang A, Rijal K, Kah S, Ravid K, Chitalia V. A mass spectrometric method for quantification of tryptophan-derived uremic solutes in human serum. The Journal of Biological Methods. 2017;**4**:e75

[9] Malyszko J, Urano T, Takada Y, Takada A. Amino acids, serotonin, and 5-hydroxyindole acetic acid following foot shock to rats. Brain Research. 1995;**36**:137-140

[10] Malyszko J, Urano T, Yan D, Serizawa Y, Koima Y, Takada Y, Takada A. Foot shock induced changes blood and brain serotonin and related substances in rats. The Japanese Journal of Physiology. 1994;**44**:35-47

[11] Takahashi H, Takada Y, Nagai N, Urano T, Takada A. Effects of nicotine and foot shock stress on dopamine release in the striatum and nucleus accumbens. Brain Research Bulletin. 1998;**145**:157-162

[12] Takahashi H, Takada Y, Nagai N, Urano T, Takada A. Nicotine increase stress-induced serotonin release by stimulating nicotinic acetylcholine receptors in rat striatum. Synapse. 1998;**28**:212-219

[13] Pawlak R, Takada y, Takahashi H, Urano T, Ihara H, Nagai N, Takada A. Differential effects of nicotine against stress induced changes in dopaminergic system in rat striatum and hippocampus. European Journal of Pharmacology. 2000;**387**:171-177

[14] Takahahi H, Takada Y, Nagai N, Urano T, Takada A. Previous exposure to foot shock stress attenuates nicotine-induced serotonin release in rat striatum during the subsequent stress. Brain Research Bulletin. 2000;**52**:285-290

[15] Pawlak D, Takada Y, Urano T, Takada A. Serotonergic and kynurenic pathways in rats exposed to foot shock. 2000;**53**:197-205

[16] Adel A, Trullas R, Gelpi E. Time course of changes in serotonin and noradrenaline in rat brain after predictable and unpredictable shock. Brain Research. 1988;**459**:54-59

[17] Dunn AJ. Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxy indole acetic acid after foot shock stress. Life Science. 1988;**42**:1847-1853

[18] Harris C, Miranda AF, Tanguay JL, Beninge RJ, Jhamands K. Modulation of atrial quioline neurotoxicity by elevation of endogenous brain kynurenic acid. British Journal of Pharmacology. 1998;**124**:391-399

[19] Hodkins MP, Schwarcz R. Metabolic control of kynurenic acid formation in the rat brain. Developmental Neuroscience. 1998;**20**:408-416

[20] Zung WW. A self-rating depression scale. Archives of General Psychiatry. 1965;**12**:63-70

[21] Freese A, Swartz KJ, During MJ, Martin JB. Kynurenine metabolites of tryptophan:Implication for neurologic diseases. Neurology. 1990;**40**:691-695

[22] Stone TW. Neuropharmacology of quinolinic acid and kynurenic acid. Pharmacological Reviews. 1993;**45**:309-379

[23] Saito K, Heyes MP. Kynurenine pathway enzymes in brain. In Fillipini GA, editor. Recent Advances in Tryptophan Research. New York: Prenum Press. 1996;485-492

[24] Moroni F, Russi P, Lombardi G, Beni M, Carla V. Presence of kynurenic acid in the mammalian brain. Journal of Neurochemistry. 1988;**51**:177-180.45

[25] TW S, Addae JI. The pharmacological manipulation of glutamate receptors and neuroprotection. European Journal of Pharmacology. 2002;**447**:285-296

[26] Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: Physiopathological implications. Journal of Neurosurgical Sciences. 2001;**21**:7463-7473

[27] Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nature Medicine. 2004;**10**:1216-1221

[28] Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy States. International Journal of Tryptophan Research. 2009;**2**:1-19

[29] Okuno E, Nishikawa T, Nakamura M. Kynurenine aminotransferases in the rat. Localization and characterization. Advances in Experimental Medicine and Biology. 1996;**398**:455-464

[30] Okuda S, Nishiyama N, Saito H, Katsuki H. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. Proceedings of the National Academy of Sciences. 1996;**93**:12533-12558

[31] Schwarcz R, Du F. Quinolinic acid and kynurenic acid in mammalian brain. Advances in Experimental Medicine and Biology. 1991;**294**:185-199

[32] Connick JH, Heywood JC, Sills GJ, Thompson GG, Brodie MJ, Stone TW. Nicotinylalanine increases central kynurenic acid content and anticonvulsant activity. General Pharmacology. 1992;**23**:235-239

[33] Mann JJ, Arango V, Marzuk PM, Theccanat S, Reis DJ. Evidence for the 5-HT hypothesis of suicide. A review of postmortem studies. The British Journal of Psychiatry. 1989;**155**(Suppl 8):7-14

[34] Oquendo MA, Sullivan GM, Sudol K, Baca-Garcia E, Stanley BH, et al. Toward a biosignature for suicide. The American Journal of Psychiatry. 2014;**171**:1259-1277

[35] Lapin IP. Kynurenines as probable participants of depression. Pharmakopsychiatr-Neuropsychopharmakol. 1973;**6**:273-279

[36] Lapin IP. Antagonism of kynurenic acid to anxiogens in mice. Life Sciences. 1998;**63**: 231-236

[37] Pålsson E, Sellgren C, Rydén E, Kizza R, Pelanis A, Zetterberg H, Blennow K, Landén M. Cerebrospinal fluid monoamine metabolite profiles in bipolar disorder, ADHD, and controls. Journal of Neural Transmission (Vienna). Sep 2017;**124**(9):1135-1143. Epub 2017 Jun 27. PMID: 28656371. DOI: 10.1007/s00702-017-1746-3

[38] Berrettini WH, Nurnberger JI Jr, Scheinin M, Seppala T, Linnoila M, Narrow W, Simmons-Alling S, Gershon ES. Cerebrospinal fluid and plasma monoamines and their metabolites in euthymic bipolar patients. Biological Psychiatry. 1985;**20**:257-269

IntechOpen