

Measurement of Serotonin Distribution and 5-Hydroxyindoleacetic Acid Excretion after Oral Administration of Serotonin using HPLC Fluorescence Detection

Islam J¹, Shirakawa H^{1,2*}, Aso H^{3,4} and Komai M¹

¹Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

²Food Evaluation Unit

³Livestock Immunology Unit, International Education and Research Center for Food Agricultural Immunology (CFAI), Graduate School of Agricultural Science, Tohoku University, Sendai 981-5555, Japan

⁴Cellular Biology Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

***Corresponding author:** Hitoshi Shirakawa, Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan, Tel : +81 227178813; E-mail: shirakah@m.tohoku.ac.jp

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Abstract

Serotonin (5-hydroxytryptamine) is a well-known neurotransmitter with both central and peripheral functions and is involved in diverse physiological processes. The aim of this study was to determine bioavailability and overall excretion of orally administered serotonin, as well as its metabolism to 5-hydroxyindole-3-acetic acid. Serotonin was administered to mice by oral gavage (0, 1, 10, and 100 mg/kg body weight). Serum as well as liver and lung tissue samples were collected at 0.5, 1, 3, 6, 12, and 24 h after administration. Urine and fecal samples were collected for the entire 24 h post-administration period to determine overall excretion. Serotonin and 5-hydroxyindole-3-acetic acid levels were measured by a high-performance liquid chromatography-fluorescence detection method. The serum concentration of serotonin increased a dose- and time-dependent manner. Accumulation of serotonin was observed in the various tissues analyzed, primarily in the high dose group. Urinary 5-hydroxyindole-3-acetic acid excretion was higher in treated mice than in untreated mice; however, urinary serotonin levels were unchanged among the treatment groups. In contrast, high levels of serotonin were observed in the feces. The results of the present study provide important information for the use of orally administered serotonin to reduce risk factors associated with life style-related diseases, notably metabolic syndrome.

Keywords: HPLC fluorescence detection; Oral administration; Serotonin

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from the essential amino acid tryptophan in the enterochromaffin cells of the gastrointestinal tract and in serotonergic neurons in the central nervous system [1]. Approximately 2% of dietary tryptophan is converted to 5-HT [2]. Peripheral 5-HT is metabolized primarily in the liver and lung via enzymatic conversion by *monoamine oxidase-A* (MAO-A; EC 1.4.3.4), resulting in 5-hydroxyindole-3-acetic acid (5-HIAA) [2,3]. 5-HIAA is the predominant metabolite of 5-HT and is subsequently excreted in the urine [4,5]. Peripheral 5-HT has been extensively researched because of its activity as a peripheral hormone that affects vasoconstriction and intestinal motility, as well as functioning as an intrinsic cofactor for the T-cell mediated immune system [6]. In recent years, 5-HT has gained attention for its peripheral effects and to prevent metabolic disorders that can be attributed to its wide range of physiological effects. For example, 5-HT has been shown to be involved in glucose and lipid metabolism [7]. In addition, 5-HT has been shown to reduce weight gain, hyperglycemia, insulin resistance, and the expansion of intra-abdominal adipocytes without affecting food intake in mice fed a high-fat diet [8]. However, most research regarding 5-HT has been conducted using intraperitoneal injections [8,9], and there is little information regarding orally administered 5-HT. Several fruits and vegetables, such as the cherry tomato, contain high levels of 5-HT [10,11], suggesting that the use of these foods has potential as a therapeutic strategy for the treatment of metabolic disorders in the future. Thus, the main objective of the present study was to determine the time- and dose-dependent fate of 5-HT and its metabolite 5-HIAA by using a high-performance liquid chromatography (HPLC)-fluorescence detection method. Our results provide important information for determining the dose of 5-HT needed to realize maximum benefits after oral administration.

Materials and Methods

Chemicals and reagents

HPLC-grade acetonitrile and water were obtained from Kanto Kagaku (Tokyo, Japan). 5-HT, ammonium formate, and formic acid were purchased from Wako Pure Chemicals (Osaka, Japan). 5-HIAA and 5-hydroxytryptophan (5-HW) were purchased from Sigma-Aldrich (Tokyo, Japan).

Animals

Seventy-two male ICR mice (6-week-old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were housed in a pathogen-free mouse colony (temperature, $23\pm 3^\circ\text{C}$; relative humidity, $55\pm 10\%$; lighting cycle, 12 h/d) and had free access to diet and drinking water. The Animal Research-Animal Care Committee of Tohoku University approved the experimental plan of the present study. All experiments were conducted under the guidelines issued by this committee in accordance with Japanese governmental legislation (2005).

Experimental design

In this study, a single oral dose of 5-HT was administered to mice at different concentrations by gavage after dividing them into the following treatment groups: (1) control group: vehicle only (0.3 ml of 0.4% cellulose), (2) low dose group: 1 mg/kg body weight of 5-HT (0.3 ml of 0.1 mg/ml), (3) medium dose group: 10 mg/kg body weight of 5-HT (0.3 ml of 1 mg/ml), and 4) high dose group: 100 mg/kg body weight of 5-HT (0.3 ml of 10 mg/ml). The 5-HT doses (0.1, 1 and 10 mg/ml solutions) were freshly diluted by 0.4% cellulose solution from a stock solution of 5-HT (500 mg/ml) that was stored at -30°C . Mice were fasted overnight before administration of 5-HT at 10 AM. Three mice per experimental group were sacrificed at every time point (0.5, 1, 3, 6, 12, and 24 h) after administration of 5-HT, and serum as well as liver and lung tissues were collected. In addition, feces and urine were collected at 24 h to determine overall 5-HT excretion. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C . All specimens were mixed with 4 volumes of 0.2M perchloric acid and a known amount of internal standard, 5-HW, which is an intermediate in the synthesis of 5-HT from tryptophan. 5-HW was not detected in significant concentrations in the serum and organs tested, validating its use as the internal standard. Serum and urine samples were vortexed thoroughly for 1 min. Tissue samples were homogenized with a polytron homogenizer (Kinematica AG, Luzern, Switzerland) for 30 sec. All specimens were then centrifuged at $12,000 \times g$ for 10 min at 4°C and supernatant collected. Prior to HPLC analysis, specimens were diluted 10-fold with 100 mM HCOONH_4 (pH 3.4) and then passed through a $0.45\text{-}\mu\text{m}$ microfilter.

Collection of feces and urine

Mice were individually housed in metabolic cages under controlled environmental conditions of light and temperature as previously described. Chow diet and drinking water were provided in each cage *ad libitum*. Total feces were collected for 24 h after gavage, weighed, and stored at -30°C . The metabolic cages were thoroughly examined to identify any food ingredients attached to the cage wall. In order to determine creatinine excretion in urine for 24 h after gavage, each metabolic cage was thoroughly washed with 10 ml distilled water. All the diluted urine samples were collected and then filtered using filter paper and preserved at -30°C for further analysis. Creatinine levels were determined using a commercial assay (Lab Assay Creatinine, Wako Pure Chemicals). Excretion percentages of 5-HT were determined with the following equation: (excreted amount of 5-HT/oral dose) \times 100. Group values were estimated by subtracting the control group value.

HPLC figure

The HPLC analytical method was conducted as previously described with slight modifications [10]. In brief, linear gradients were applied and separations were performed with 10 mM HCOONH_4 (pH 3.4) from 0–5 min and a linear gradient of 0–25% acetonitrile in 10 mM HCOONH_4 (pH 3.4) from 5–15 min; the run was continued for column washing (90% acetonitrile for 9 min), followed by re-equilibration (10 mM HCOONH_4 (pH 3.4) for 8 min). The total HPLC run time for each sample was 32 min, and the injection volume was 20 μl in an Atlantis C18 column (4.6 \times 50 mm, 5 μm , Waters, Milford, MA, USA) at 30°C with a flow rate of 1.0 ml/min. Fluorescence was detected

at an excitation and emission of 300 nm and 355 nm, respectively.

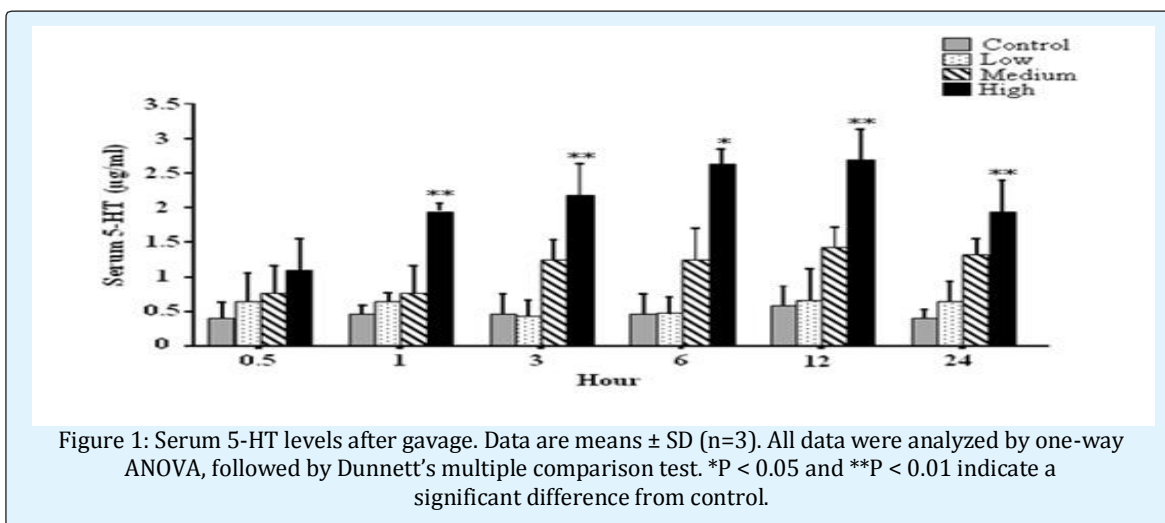
Statistics

Results are presented as means \pm standard deviation (SD). Differences between the time intervals were analyzed for significance with one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using SigmaPlot version 12.5 (San Jose, CA, USA). Significance was set at $P < 0.05$.

Results and Discussions

Serum levels of 5-HT after oral gavage

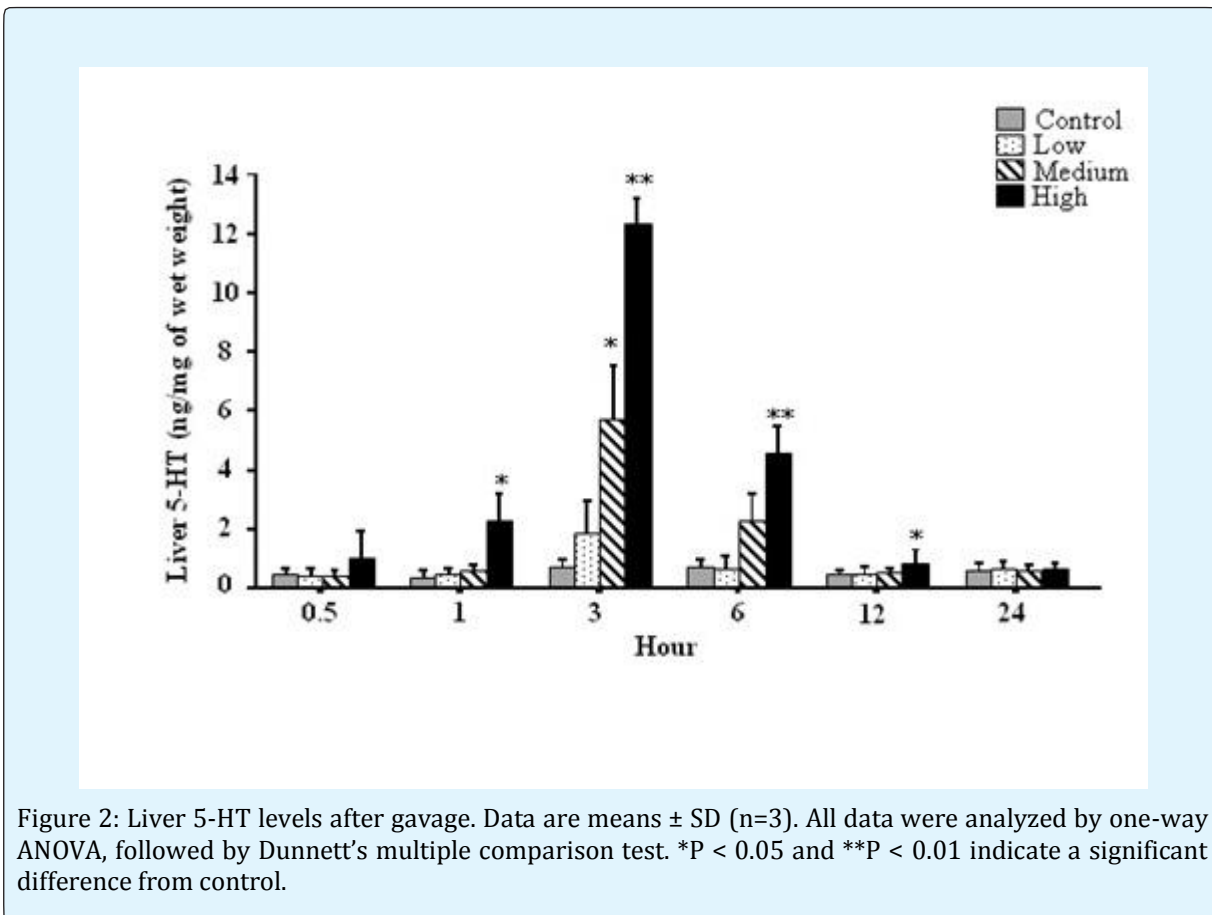
Serum 5-HT levels increased within 30 min after oral gavage (Figure 1). 5-HT levels were consistent in the control group until 12 h after oral gavage, which may be attributable to the effects of circadian rhythms [12]. There were no apparent differences in the measured 5-HT levels between the control and low dose (1 mg/kg) treatment groups. In the group receiving the medium dose of 5-HT (10 mg/kg), the concentration of 5-HT reached its maximum level 12 h after administration and then began to decline. In the group receiving the high dose of 5-HT (100 mg/kg), the concentration of 5-HT also reached its maximum level 12 h after administration as shown in Figure 1. Serum 5-HT levels in the control group were similar to the results reported in another study [5]. Once ingested, 5-HT is taken up by different receptors present in various tissues and blood platelets. Exogenous 5-HT affects the uptake capacity of the serotonin transporter (SERT) [1].

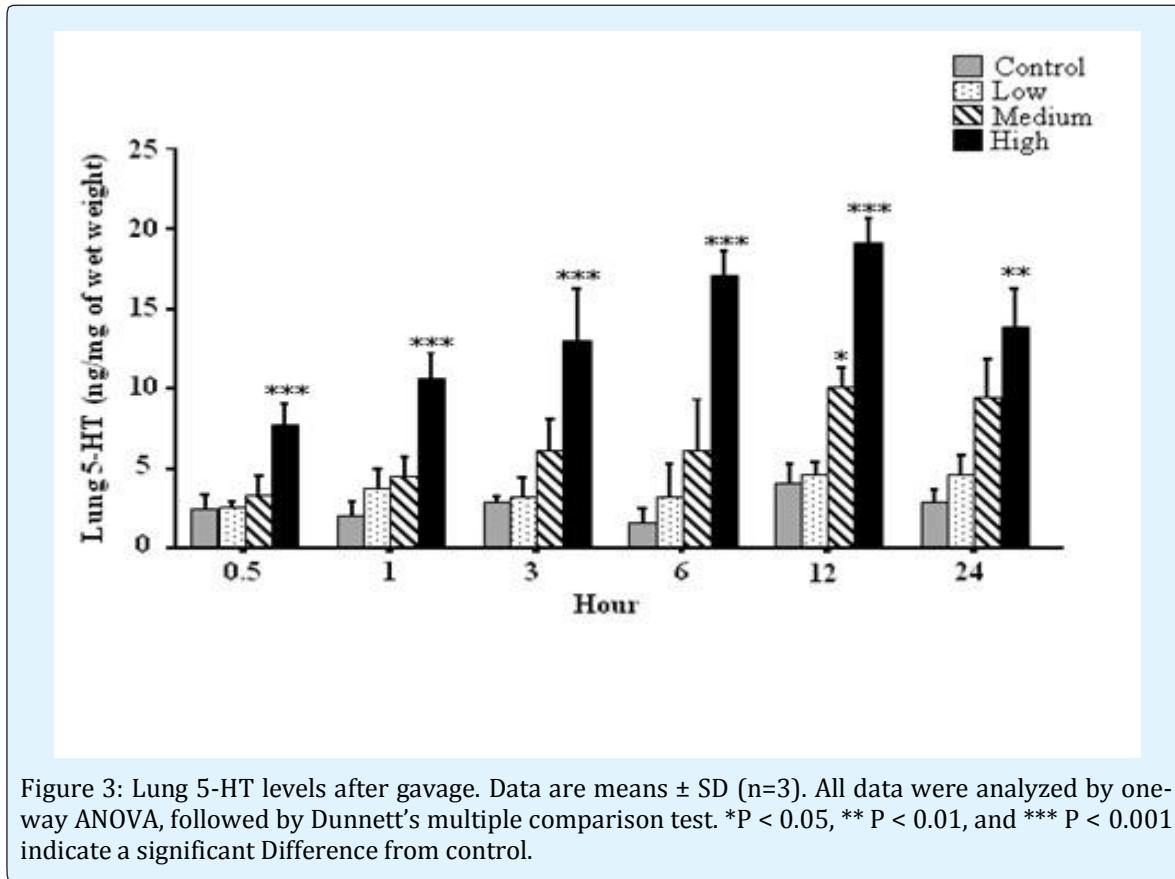


Determination of 5-HT in the liver and lung

5-HT accumulation was analyzed in the liver and lung because they are the predominant organs involved in 5-HT metabolism. In the liver, significant increases in 5-HT levels were observed 1 h after treatment in the high dose group and reached maximum levels 3 h after administration (Figure 2). In addition, the concentration of 5-HT was significantly higher 3 h after gavage in the medium dose group than that of the control group. While 5-HT concentrations in the liver of mice in the low dose group were numerically higher than that of the control group, the differences did not reach significance. All values returned to baseline levels 6 h after gavage in the low dose group, 12 h after gavage in the medium dose group, and 24 h after gavage in the high dose group. In lung, 5-HT concentrations were found to rapidly increase

within 30 min of administration in the high dose group (Figure 3). Overall, 5-HT levels increased in a dose- and time-dependent manner. Maximum 5-HT levels were observed in the high dose groups during the 6- to 12-h time intervals. Lung 5-HT levels declined to baseline levels by 24 h after administration in all treatment groups except the high dose group. In the blood, virtually all of the 5-HT is contained in the platelets and is quickly removed during systemic circulation through the liver and lung. In this study, 5-HT accumulation in the lung was more extensive than that in the liver, which is likely attributable to the higher concentration of platelets found in the lung [13]. Lung 5-HT levels of the control group were found to be similar to that reported in another study [14].





5-HT and 5-HIAA excretion in feces and urine

Administration of 5-HT resulted in increased excretion of 5-HIAA in the urine. Urinary creatinine ranged from 8-10 mg/24 h in the different groups ($P > 0.05$, data not shown). Table 1 illustrates the total 5-HT and 5-HIAA levels measured in the urine. Total excretion of 5-HT and 5-HIAA from feces is described in Table 1. We determined that approximately 8.46, 2.42, and 0.42% of 5-HT was excreted in the urine, whereas 9.18, 1.99, and 11.11% of 5-HT was excreted in the feces for the low, medium, and high dose treatment groups, respectively. The metabolic pathway that converts 5-HT to 5-HIAA has been extensively investigated by other researchers [5,15]. In

our study, we found significant amounts of 5-HIAA excreted in the urine, but not 5-HT (Table 1). It has been shown that dietary 5-HT increases the urinary excretion of 5-HIAA [16]; however, there is a paucity of data regarding excretion of 5-HT and 5-HIAA in the feces. In this study, we included fecal analysis of 5-HT and 5-HIAA levels in order to determine the extent of 5-HT that escapes metabolism. 5-HT and 5-HIAA were eliminated via the urine in a dose-dependent manner after oral administration; these phenomena were similar to that reported in another study wherein high levels of 5-HT were administered by intraperitoneal injection [9].

Group	Urinary excretion		Feces excretion	
	5-HT (μg)	5-HIAA (μg)	5-HT (μg)	5-HIAA (μg)
Control	6.36 \pm 2.81	411.69 \pm 85.34	0.64 \pm 0.49	2.60 \pm 1.37
Low	8.90 \pm 3.98	423.11 \pm 98.04	3.59 \pm 2.55	7.71 \pm 3.39
Medium	13.63 \pm 7.04	604.41 \pm 10.89	6.63 \pm 3.47	21.56 \pm 14.38
High	18.97 \pm 2.06	1739.08 \pm 635.16*	334.12 \pm 54.24*	52.80 \pm 17.32*

Table 1: Twenty-four h urinary 5-HT and 5-HIAA excretion analysis.

Data are means \pm SD (n=3). All data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

*P < 0.05 indicates significant difference from control.

Here, we report that oral administration of 5-HT results in a rapid and prolonged increase in serum 5-HT levels in a dose- and time-dependent manner. These results provide new information on the importance of foods and supplements that contain high levels of 5-HT for the prevention of metabolic syndrome. As it is well known that peripheral 5-HT is involved in metabolic homeostasis and may offer new approaches for developing therapeutic drugs for hyperlipidemia, diabetes, and obesity treatment [8,17] when administered via intraperitoneal injection. Commonly prescribed medications such as selective serotonin reuptake inhibitors (SSRIs) influence 5-HT availability by interfering with its metabolic clearance and uptake capacity and blocking serotonin transporter; however, they have side effects when used long term [18]. Our results indicate that orally administered 5-HT rapidly increases serum 5-HT levels and prolongs bioavailability. Mice in the high dose group excreted 5-HT in high amounts and showed extensive metabolism of 5-HT to 5-HIAA, which may be attributable to the high dose being above the pharmacological range. Overall, these data suggest that orally administered 5-HT or dietary 5-HT may be as efficacious as intraperitoneal injections of 5-HT. The peripheral effects of 5-HT are not yet completely understood. There is a clear need for further investigations regarding elevated levels of 5-HT that can act peripherally. This study also describes a simplified method for 5-HT and 5-HIAA detection using HPLC-fluorescence analysis as a selective, specific, and sensitive assay. Finally, this study reports important findings about the accumulation, metabolism, and excretion of 5-HT after oral administration that have significant implications for future studies of lifestyle-related diseases.

Conclusion

This study describes the bioavailability of orally administered 5-HT and subsequent increases in serum 5-HT levels. Given the increased recognition of a role for peripheral 5-HT in metabolic disorders, these results may offer new strategies for preventing metabolic syndrome.

Acknowledgements

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