A Study on the Origins of Urinary Serotonin and Tryptamine

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A sensitive gas chromatography/negative ion chemical ionization mass spectrometric (GC/NICIMS) method was devised and by using deuterated L-tryptophan-3,3-d2(Trp-d2) as a tracer, the detailed in vivo metabolism of serotonin (5-HT) and tryptamine (TA) was investigated. A human was administered orally with 10 mg/kg Trp-d2 and rats were injected intra-peritoneally with 50 mg/kg Trp-d2. The ratios of the level of 5-HT-d2 derived from Trp-d2 to that of endogenous 5-HT-d0 and that of TA-d2 to that of TA-d0 were measured in urine and some organs of rats at times up to 4 hr after administration. It is concluded that urinary excretion of 5-HT might reflect its turnover in the central nervous system and that of TA might reflect its peripheral turnover.

(Key Words: Serotonin, Tryptamine, GC/NICIMS, Deuterated-Tryptophan, CNS)

INTRODUCTION

Since the discovery of Serotonin (5-HT) in mammalian brain (20), its in vivo metabolism and biological role have been widely studied. As a result, it is presently considered that 5-HT is an established central neurotransmitter, and that abnormal metabolism in the central nervous system might be related to several psychiatric disorders, such as depression, autism, schizophrenia and so on. Many methods for the determination of 5-HT and related compounds have been developed (10, 12, 13, 19) and the relationships between their levels in physiological fluids and in psychiatric diseases were studied by many investigators (4, 8, 11, 16, 17, 22). However, these investigators did not reach any conclusive results.

Recently, many investigators showed that gas chromatography/mass spectrometry using stable isotope labelled compounds as a tracer was a powerful tool for the in vivo metabolic investigations of phenylalanine, tyrosine, etc (6, 15, 18, 21). We also developed a sensitive gas chromatography/negative ion chemical ionization mass spectrometric (GC/NICIMS) method using L-tryptophan-3,3-d2 and as tracer for the investigation of detail in vivo metabolism of Tryptophan (Trp), 5-HT, Tryptamine (TA) (9). It was also reported that comparable amounts of 5-HT-d2 and TA-d2 to those of endogenous ones were excreted into urine several hours after the administration of Trp-d2 (10 mg/kg), though measurable amounts of these compounds were not found in blood during the same period. Whether these indole amines excreted into urine were metabolized from Trp-d2 was investigated in this work.

MATERIALS AND METHODS

Apparatus

A Finnigan 4000 GC/MS equipped with a pulsed positive ion negative ion chemical ionization accessory (Finnigan Co., C.A.) was used. Sample injection were carried out with a solvent-cut injector (Gaskuro Kogyo Co., Tokyo).
Conditons of GC/NICIMS for the determination of Trp, TA and 5-HT

The separations were made on an OV-101 coated fused silica capillary column (25 m × 0.25 mm i.d.). The capillary column was directly connected to the mass spectrometer. Methane was used as the GC carrier gas and the chemical ionization reagent gas. The flow rate of methane carrier gas was 1 ml/min and the ion-source pressure was kept at 0.15 Torr. The ionizing potential and emission current were 90 eV and 300 μA respectively. The temperature of the injector, the ion-source and the capillary column was kept at 280°C, 250°C and 200°C respectively.

Reagents
Serotonin-α, α, β, β-d4 creatinine sulphate and tryptamine-α, α, β, β-d4 hydrochloride were purchased from Merck Frost Canada, Inc. L-tryptophan-3,3-d2 and D, L-tryptophan-2', 3, 3', 4', 5', 6', 7'-d7 synthesized according to the methods reported in the previous paper were used (Hayashi et al., in press). Cation exchanged resin AG 50 W × 2 (200-400 mesh) obtained from Bio-Rad Lab. was used after treating with 10 fold volume on 2N HCl at 80°C for 4 hrs and washing with 50 fold volume of double distilled water. The other reagents and solvents were all reagent grade.

Experimental Animals
Male Wistar rats (260–377 g) were housed at constant temperature (24°C) and humidity (50%) using light/dark schedule of for at least 2 weeks before tracer experiments.

Administration of Trp-d2
In the case of the administratin to a healthy human, Trp-d2 (10 mg/kg) was administered orally at 11:00 a.m. and heparinized blood was obtained from the forearm vein at certain intervals. An aliquot of the blood sample was centrifuged at 10,000 g for 15 min in a refrigerated centrifuge. Plasma obtained was stored at −20°C until analyzed. The remaining blood sample was diluted with an equal volume of water and stored at −20°C. Urine was also collected at certain intervals. Also, an aliquot of the urine sample was stored in the same conditions.

In the case of rat, Trp-d2 (50 mg/kg) was administered intra-peritoneally as a saline solution (10 mg/ml). The rats were killed by decapitation at certain intervals and the organs were immediately take out. Plasma was obtained by centrifuging the heparinized blood at 10,000 g for 15 min in a refrigerated centrifuge. The organs and plasma obtained were stored at −20°C until analyzed.

For the collection of urine samples, 10 cm of silicon tube (0.025 inches i.d., 0.047 inches o.d.) was connected into the bladder of the rat which was fixed on a board. Urine samples were collected at certain intervals (30 min).

Determination of deuterated and non-deuterated Trp in plasma
Twenty microliters of the plasma were deproteinized with 0.5 ml of 80% aqueous ethanol containing 200 mg of D, L-tryptophan-2', 3, 3', 4', 5', 6', 7'-d7 and centrifuged in a microcentrifuge (Sakuma, Model M-15-3). The supernatant was evaporated to dryness on a rotary evaporator and the residue was reconstituted with 1 ml of 25 mM pyridine-formate buffer (pH 2.5). The solution was applied to a 150 mm × 5 mm i.d. glass column containing 0.3 ml of AG 50 W × 2. After washing with 5 ml of 25 mM pyridine-formate buffer (pH 3.0), the amino acids were eluted from the column with 6 ml of 25 mM pyridine-formate buffer (pH 4.5). The elute was evaporated to dryness and the residue was redissolved with 300 μl of ethanol. The solution was transferred into a siliconized glass ampoule and the ethanol was evaporated in a nitrogen stream.

One hundred micro-liters of the mixture of trifluoroacetic anhydride (TFAA) and acetonitrile (1:1) were added to the residue. After 5 min reaction at room temperature, the excess TFAA and acetonitrile were evaporated in a nitrogen stream. Three hundred microliters of diazomethane solution in ether (about 1%) were added to the residue and allowed to stand 10 min at room temperature. The excess diazomethane solution was removed in a nitrogen stream. Then, the residue was redissolved with 50 μl of the mixture of TFAA and acetonitrile. One microliter of the resulting solution was applied into GC/NICIMS. The GC/NICIMS conditions were described above.
Determination of deuterated and non-deuterated TA and 5-HT

To whole urine collected from a rat during 30 min, redistilled water was added to 1 ml. One hundred microliters of analytical internal standard solution which was prepared by diluting (1/200) stock solution (tryptamine-α, α, β, β-δ4, serotonin-α, α, β, β-δ4 400 μg/ml 0.01N HCl) with redistilled water were added to the diluted sample. Then, the amines were extracted with 5 ml of 20% n-butanol solution in ether by shaking vigorously for 10 min after addition of 1 ml of 0.75M sodium phosphate buffer (pH 10.0) which contained 260 mg of NaCl and 1 mg of ethyldiamine tetraacetic acid disodium salt and four milliliters of n-hexane from the organic layer with 300 μl of 0.1N HCl by shaking for 5 min. After the aqueous phase was frozen in a dry-ice acetone bath, the upper organic layer was discarded by decantation. The aqueous phase was washed with 4 ml of n-hexane and the n-hexane layer was removed in the same way. The aqueous layer was transferred into a glass ampule and evaporated to dryness in a nitrogen stream. Then, 50 μl of the mixture of TFAA and acetonitrile (1:1) was added to the residue. The ampule was sealed and allowed to react at 60°C for 30 min. One microliter of the reaction mixture solution was placed into GC/NICIMS. The GC/NICIMS conditions were described above. To 1 ml of plasma, 0.5 g of homogenized tissue sample from a rat every 30 min, and 100 μl of urine, 1 ml of plasma sample from a normal adult every 60 min, the same procedure was used.

RESULTS AND DISCUSSION

Tracer experiments in healthy persons

Fig. 1 shows the concentrations of Trp-d2 and endogenous Trp-d0 in plasma. The plasma Trp-d2 level rapidly increased. The maximum level were found in both subjects around 45 min after the administration. Trp-d0 levels ranged from 49 to 74 nmol/ml. It is considered that the differences in the levels of Trp-d2 in plasma between the two subjects were caused by the difference in absorption. On the other hand, measurable amounts of 5-HT-d2 and TA-d2 derived from Trp-d2 were not found in whole blood samples.

The concentrations of deuterated and non-deuterated 5-HT and TA in urine were shown in Fig. 2. The maximum was found in both subjects between 1 and 2 hr after administration and decreased gradually. The maximum ratio of the level of TA-d2 to that of TA-d0 was also found in the same period as shown in Fig. 3. These differences of the ratios (5-HT-d2/5-HT-d0) in urine between two subjects might be caused by different levels of Trp-d2 in plasma as shown in Fig. 1. But the differences of ratios (TA-d2/TA-d0) between the two subjects is unknown.

Tracer experiments in rats

Trp-d2 (50 mg/kg) was administered intraperitoneally. And urine was collected continuously at 30 min intervals through a silicone tube connected into the bladder. Fig. 4 shows the ratios of the levels of 5-HT-d2 and TA-d2 to those of their non-deuterated ones in urine. The maximum ratios were urine. The maximum ratios were found in both amines around 60 min after the administration and decreased gradually. The maximum ratios were found earlier than those in humans. These differences might be due to different methods of administration of Trp-d2.

Next, the levels of deuterated 5-HT, TA and those of non-deuterated ones were measured in some organs (Table 1). The ratios of the level of 5-HT-d2 to that of 5-HT-d0 were significantly higher than in other organs for brain (Fig. 5) and the curve was similar to that for urine. But, for the other organs, the ratios were low and changes of the ratios as observed for urine were not found. On the other hand, remarkable changes in the ratios of the level of TA-d2 to that of TA-d0 were observed for the heart, the lungs, the liver and the small intestine, but not for the brain.

These data might suggest that urinary excretion of 5-HT-d2 reflects its turnover in the central nervous system (CNS) and that of TA-d2 reflects its peripheral turnover (heart, liver, lung, kidney, etc.). For the ratio of the level of 5-HT-d2 to that of 5-HT-d0, there was a difference of the maximum between that in the brain and that in the urine. The maximum ratio was lower in the brain than in the urine, probably because the ratio in the whole brain was measured and there were many regions in the brain which might show high or low ratios.

It is believed that 5-HT is metabolized to
Fig. 1  Time course of plasma levels of Trp-d₀ and Trp-d₂ (10 mg/kg) to normal adults

- ○ - : Trp-d₀  - ● - : Trp-d₂ (Sample 1)
- △ - : - ▲ - : Trp-d₂ (Sample 2)

Fig. 2  Ratios of 5-HT-d₂ to 5-HT-d₀ in urine from normal adults after oral administration of Trp-d₂ (10 mg/kg)

- ● - : Sample 1  - ○ - : Sample 2
A Study on the Origins of Urinary Serotonin and Tryptamine

Fig. 3 Ratios of TA-d$_2$ to TA-d$_0$ in urine from normal adults after oral administration of Trp-d$_2$ (10 mg/kg)

Fig. 4 Ratios of 5-HT-d$_2$ to 5-HT-d$_0$ (A) and those of TA-d$_2$ to TA-d$_0$ (B) in urine from rats after intra-peritoneal administration of Trp-d$_2$ (50 mg/kg)
Table 1 Total amount of 5-HT-d0, 5-HT-d2, TA-d0 and TA-d2 in various organs of rats after intraperitoneal administration of Trp-d2 (50 mg/kg)

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Fig. 5 Ratios of 5-HT-d2 to 5-HT-d0 (A) and TA-d2 to TA-d0 (B) in various organs of rats after intra-peritoneal administration of Trp-d2 (50 mg/kg)

- : brain  - : lung
- : heart  - : liver
- : stomach - : small intestine
- : kidney  - : plasma
A Study on the Origins of Urinary Serotonin and Tryptamine—251

5-hydroxyindoleacetic acid (5-HIAA) in the CNS and 5-HIAA is excreted into urine. However, it has been recently reported that urinary 5-HIAA does not reflect quantitatively central 5-HT metabolism and CSF level of 5-HIAA is of value in studies of 5-HT metabolism in the CNS (1, 2, 3, 7, 14). On the other hand, it is well known that 5-HT is excreted into urine. But less attention has been paid to 5-HT in urine. It is considered that urinary 5-HT reflects mostly peripheral metabolism (5), though this has not been proved. Our data showed that peripheral organs such as gastrointestinal tract, liver, heart, and so on contained large amounts of 5-HT (Table 1). However, a large pool size of 5-HT does not mean a high turnover rate. In fact, in our data, the turnover rate of 5-HT in these peripheral organs which contain large amounts of 5-HT was remarkably lower than that in brain. And time course of the ratio of 5-HT-d$_2$/5-HT-d$_0$ in urine was related to only that in the brain.

In this study, each sample of some organs was obtained in only one rat. It is because that Trp-d$_2$ was too expensive and this tracer experiment using stable-isotope-labelled compounds in a complicated but sensitive method. However, further detailed experiments are necessary for the clear conclusion of the main origin of urinary 5-HT.

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