Blood and urine 5-hydroxytryptophan and 5-hydroxytryptamine levels after administration of two 5-hydroxytryptamine precursors in normal man

T. C. LI KAM WA, N. J. T. BURNS1, B. C. WILLIAMS1, S. FREESTONE & M. R. LEE
Clinical Pharmacology Unit, Department of Medicine, Royal Infirmary, Edinburgh EH3 9YW and 1Department of Medicine, Western General Hospital, Edinburgh EH4 2XU

Six healthy male subjects received equimolar amounts of two 5-hydroxytryptamine (5-HT) precursors, 5-hydroxy-L-tryptophan (5-HTP) and y-L-glutamyl-5-hydroxy-L-tryptophan (glu-5-HTP), on two occasions in a randomised cross-over study. There were marked increases in urinary 5-HTP and 5-HT excretion after infusion of both compounds. Mean urinary excretion rate of 5-HT, which was < 0.7 nmol min⁻¹ before dosing, rose to a peak value of 412 ± 92 nmol min⁻¹ at the end of 5-HTP infusion and 303 ± 29 nmol min⁻¹ after administration of glu-5-HTP. This occurred without significant changes in blood 5-HT levels measured in platelet-rich plasma. These findings provide further evidence that the increase in urine 5-HT after administration of both 5-HT precursors is largely due to 5-HT synthesised within the kidney.

Keywords 5-hydroxytryptamine 5-hydroxytryptophan y-L-glutamyl-5-hydroxy-L-tryptophan kidney

Introduction
The mammalian kidney contains all the major enzymes required for the synthesis and degradation of 5-hydroxytryptamine (5-HT) [1, 2]. Intrarenal synthesis of 5-HT occurs in rats given its immediate precursor, 5-hydroxytryptophan (5-HTP), and it has been suggested that 5-HT may act as a counterregulatory paracrine substance to dopamine in the local regulation of sodium excretion [2–4]. We previously administered 5-HTP and its glutamyl derivative, y-L-glutamyl-5-hydroxy-L-tryptophan (glu-5-HTP), in healthy men and demonstrated a marked increase in urinary 5-HT excretion after both compounds [5, 6]. We argued that this large increment in 5-HT excretion cannot be explained by extrarenal production of 5-HT and that it is principally due to intrarenally generated 5-HT. In the present study, we have estimated 5-HTP and 5-HT concentrations in platelet-rich plasma (PRP), in addition to urinary 5-HTP and 5-HT excretion, after infusion of equimolar amounts of both 5-HT precursors to investigate our hypothesis further. We chose to measure 5-HT in PRP rather than whole blood since processing of whole blood for 5-HT assay inevitably leads to disruption of red blood cells with release of oxyhaemoglobin resulting in oxidation of 5-HT [7].

Methods
Six healthy male volunteers (age range 22–35 years) gave informed written consent to be studied on two separate days, at least 1 week apart, in this randomised cross-over study which was approved by the Lothian Ethics of Medical Research Committee. They abstained from alcohol for 24 h and fasted from 22.00 h the evening before each study day. They attended the clinical investigation unit at 08.00 h after drinking 500 ml of water 1 h previously. The subjects received intravenous 0.9% saline at 5 ml min⁻¹ and drank 150 ml of water half-hourly over the next 6 h. They emptied their bladders at 2 h and serial urine collections of 30 min duration were made thereafter. One hour later, an equimolar dose of 5-HTP (10 µg kg⁻¹ min⁻¹) or glu-5-HTP (16.6 µg kg⁻¹ min⁻¹) was infused intravenously for 60 min. Venous blood samples were collected via a 16 G cannula before and every 30 min for 3 h after the start of the infusion. The blood sample (9 ml) was dispensed into an acid-citrate-dextrose anticoagulant (1 ml) consisting of citric acid (8 g l⁻¹), trisodium citrate (22 g l⁻¹) and glucose (20 g l⁻¹) [8]. The citrated whole blood was centrifuged at 120 g for 20 min at room temperature and the upper two-thirds of the supernatant (PRP) were harvested and stored at −40° C in a

Correspondence: Dr T. C. Li Kam Wa, Clinical Pharmacology Unit, Department of Medicine, Royal Infirmary, Edinburgh EH3 9YW

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sealed polystyrene tube until analysis. The volume of each urine collection was measured and aliquots stored at −40 °C for analysis of 5-HTP and 5-HT. The urine samples were acidified with 5 M hydrochloric acid to prevent their oxidation. PRP 5-HTP and 5-HT were assayed by h.p.l.c. (Waters Associates, Millford, UK) after deproteinisation with perchloric acid (15%) containing cysteine (2 mM) using N-methylserotonin as the internal standard [8]. The electrochemical detector operated at a potential of 0.6 V and a sensitivity of 10 nA. The mobile phase (flow rate 2 ml min −1) consisted of phosphate buffer (0.1 M) containing EDTA (1 M), octane sulphonic acid (25 mg l −1) and methanol (5%). Urine 5-HTP and 5-HT were measured by h.p.l.c. as described previously [5]. Glu-5-HTP was supplied by Aalto Bio Reagents Ltd, Dublin, Eire, and 5-HTP was obtained from Sigma Chemical Co. Ltd, Poole, UK.

Results are expressed as means ± s.d. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. The apparent renal clearance of 5-HTP was estimated by dividing the amount of 5-HTP excreted in urine by the corresponding area under the concentration-time curve. The data on the 2 experimental days were compared by Student’s paired t-test and 95% confidence intervals (CI) of the differences between means quoted where appropriate. Differences were considered statistically significant when the P value was less than 0.05.

Results

The 5-HTP concentrations in PRP and urinary excretion rates of 5-HTP on the two study days are shown in Figure 1. 5-HTP was undetectable in baseline PRP and urine samples. C max for 5-HTP in PRP was 1365 ± 302 nmol l −1 and AUC(0–3 h) was 1763 ± 250 nmol l −1 h after administration of 5-HTP. The corresponding values after glu-5-HTP infusion were lower at 471 ± 95 nmol l −1 (95% CI of the difference: 539 to 1249, P < 0.005) and 934 ± 185 nmol l −1 h (95% CI of the difference: 464 to 1193, P < 0.005). The 3 h cumulative 5-HTP excretion was 2.5 times greater after glu-5-HTP (44.0 ± 8.6 µmol) than after 5-HTP infusion (17.6 ± 2.1 µmol; 95% CI of the difference: 18.5 to 34.4, P < 0.001). The apparent renal clearance of 5-HTP over the first hour was higher after glu-5-HTP (1357 ± 348 ml min −1) than after 5-HTP (246 ± 56 ml min −1; 95% CI of the difference: 734 to 1487, P < 0.001).

PRP 5-HT concentration was 812 ± 218 nmol l −1 before administration of 5-HTP and 769 ± 140 nmol l −1 before glu-5-HTP and did not change significantly after administration of either compound (Figure 2). There were, however, huge increases in urinary 5-HT excretion. Mean urinary excretion rate of 5-HT, which was < 0.7 nmol min −1 before dosing, rose to a peak value of 412 ± 92 nmol min −1 at the end of 5-HTP infusion and 303 ± 29 nmol min −1 after administration of glu-5-HTP. The 3 h cumulative 5-HT excretion values after 5-HTP and glu-5-HTP were, however, huge
were not significantly different at 37.4 ± 4.6 μmol and 32.0 ± 4.5 μmol respectively (95% CI of the difference: −0.6 to 11.5).

Two subjects complained of nausea, and one of these two vomited, at the end of 5-HTP infusion. There were no ill-effects following glu-5-HTP infusion.

Discussion

The present study confirms our previous observations that both 5-HTP and glu-5-HTP markedly increase urinary 5-HT excretion [5, 6]. In addition, we have now shown that this occurs without concomitant changes in circulating 5-HT levels. These findings support our hypothesis that urine 5-HT, after infusion of both 5-HT precursors, is largely derived from intrarenal synthesis of 5-HT. Although a placebo day was not included in this study, we previously showed that the saline infusion and water loading employed in the protocol do not affect urinary 5-HTP or 5-HT excretion [5, 6, 9].

The high renal clearance value of 5-HTP observed after administration of glu-5-HTP suggests that urine 5-HTP after glu-5-HTP is also predominantly produced intrarenally. The rise in circulating 5-HTP is probably caused both by its formation within the kidney (followed by recirculation) and extrarenal transmembrane assay for serotonin in platelet-rich plasma. Clin Chim Acta 1987; 162: 175–188.


References


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