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

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Six healthy male subjects received equimolar amounts of two 5-hydroxytryptamine (5-HT) precursors, 5-hydroxy-L-tryptophan (5-HTP) and γ-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 γ-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, γ-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

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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.

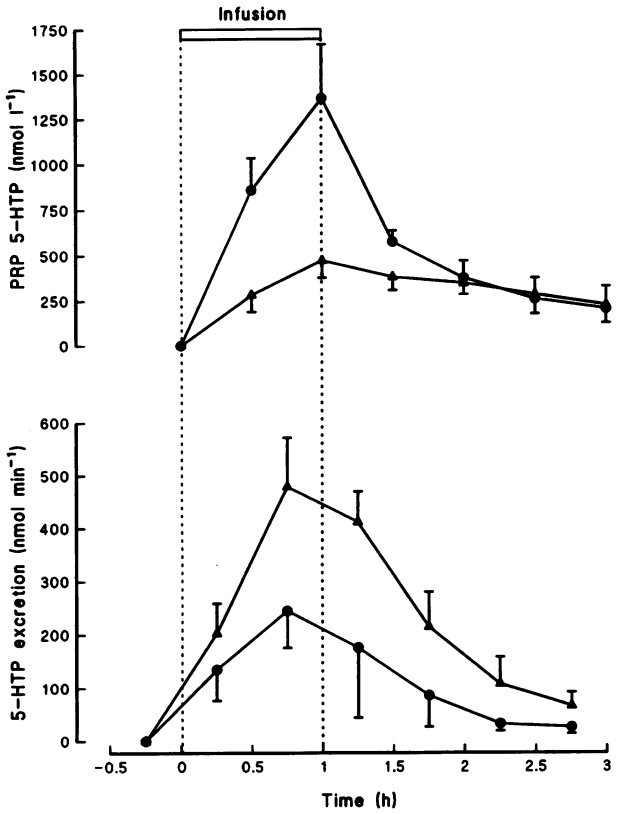

Figure 1 5-HTP concentrations in PRP and urinary 5-HTP excretion rates before, during and after infusion of 5-HTP (●) and glu-5-HTP (▲). Values shown are means ± s.d. (n = 6).

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

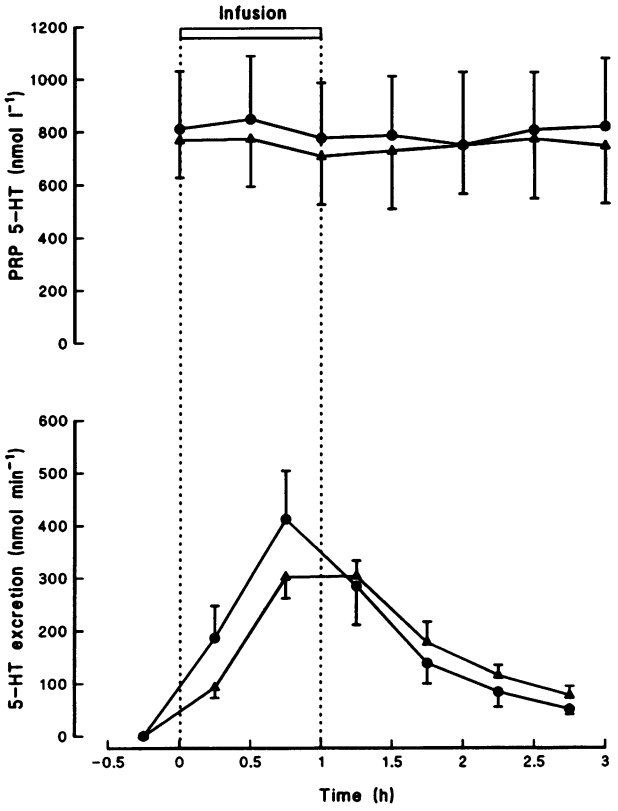

Figure 2 5-HT concentrations in PRP and urinary 5-HT excretion rates before, during and after infusion of 5-HTP (●) and glu-5-HTP (▲). Values shown are means ± s.d. (n = 6).

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 transformation of glu-5-HTP to 5-HTP since the enzyme γ-glutamyl transferase required for the conversion of glu-5-HTP to 5-HTP is widely distributed in the body although its concentration in renal tissue is considerably greater than elsewhere [10]. Gastrointestinal side effects after administration of 5-HTP appear to be related to the plasma 5-HTP concentrations [11, 12]. In the present study, two subjects developed nausea at the end of 5-HTP infusion at the time when peak circulating levels occurred. In contrast, peak circulating 5-HTP levels were much lower after infusion of the glutamyl compound and no adverse effects were observed. 5-HTP is decarboxylated to 5-HT by aromatic L-amino acid decarboxylase. This enzyme has a ubiquitous distribution with high activity in the kidney and liver [13]. The absence of an increase in circulating 5-HT, particularly after administration of 5-HTP, suggests that 5-HT, if produced extrarenally, is rapidly metabolised and cleared from the circulation.

This study therefore provides further evidence that 5-HT is synthesised intrarenally after administration of both 5-HTP and glu-5-HTP in man. Definitive proof of this will require estimations of 5-HTP and 5-HT in the renal artery and vein, in addition to urinary measurements. The glutamyl compound exhibits greater renal selectivity and is better tolerated [6]. It could be used as a valuable pharmacological research compound for the investigation of the renal formation and effects of 5-HT in man.

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