The antinatriuretic action of γ -L-glutamyl-5-hydroxy-L-tryptophan is dependent on its decarboxylation to 5-hydroxytryptamine in normal man

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- 1 The effects of inhibition of peripheral aromatic L-amino acid decarboxylase during infusion of the relatively renally selective 5-hydroxytryptamine (5-HT) prodrug, γ -L-glutamyl-5-hydroxy-L-tryptophan (glu-5-HTP), were examined in eight healthy male subjects in a randomised, placebo-controlled, cross-over study.
- 2 Each subject received oral carbidopa (100 mg) or placebo followed, 1 h later, by a 60 min intravenous infusion of glu-5-HTP ($16.6 \mu\text{g kg}^{-1} \text{min}^{-1}$) or placebo.
- 3 After administration of glu-5-HTP, cumulative urinary excretion of 5-HT was 430-fold greater than that after placebo, and was associated with a period of sodium retention.
- 4 Pretreatment with carbidopa substantially attenuated the increase in 5-HT excretion after glu-5-HTP and abolished its antinatriuretic effect.
- 5 These results are in keeping with the proposition that the antinatriuretic action of glu-5-HTP is dependent on its decarboxylation to 5-HT.

Keywords 5-hydroxytryptamine γ -L-glutamyl-5-hydroxy-L-tryptophan carbidopa kidney sodium excretion aldosterone

Introduction

The first step in the biosynthesis of 5-hydroxytryptamine (5-HT; serotonin) involves the hydroxylation of the essential amino acid L-tryptophan to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan-5-hydroxylase. 5-HTP is decarboxylated by aromatic L-amino acid decarboxylase (LAAD) to 5-HT. The latter is degraded primarily by monoamine oxidase to produce 5-hydroxyindoleacetic acid (5-HIAA) which is the major catabolic and excretory product of 5-HT metabolism. All these enzymes are present in renal tissue, suggesting that the kidney might have the capacity to synthesise and degrade 5-HT locally [1, 2]. The enzyme γ -glutamyl transferase (γ GT) is also present in high concentrations and the kidney is highly active in the uptake and metabolism of γ -glutamyl derivatives of amino acids [3, 4]. We previously demonstrated marked increases in urinary 5-HT excretion after infusion of the 5-HT prodrug, γ -L-glutamyl-5-hydroxy-L-tryptophan (glu-5-HTP), in

keeping with intrarenal synthesis of 5-HT following the conversion of glu-5-HTP to 5-HTP by γ GT and its subsequent decarboxylation by renal LAAD to 5-HT [5, 6]. Glu-5-HTP was relatively more selective for the kidney than 5-HTP. It reduced urinary sodium excretion without significant alterations in renal haemodynamics and this was due, presumably, to intrarenally generated 5-HT.

The present study was designed to investigate whether carbidopa, a peripheral inhibitor of LAAD [7], blocks the formation of 5-HT from glu-5-HTP and interferes with the actions of glu-5-HTP in normal volunteers.

Methods

Eight male volunteers, age range 18–39 years (mean 28 years) and weighing 59.9–80.9 kg (mean 69.0 kg),

gave informed written consent to take part in this randomised, single-blind, placebo-controlled, within-subject cross-over study which was approved by the Healthy Volunteer Studies Ethics of Medical Research Sub-Committee, Lothian Health Board. They were all healthy as judged by medical history and physical examination, ECG, urinalysis, full blood count and biochemical blood analyses. They abstained from medications from at least 2 weeks before the start of the study and until its completion.

Each subject attended on four separate occasions, at least 1 week apart. They refrained from alcohol for 24 h, abstained from caffeine-containing beverages from 18.00 h, and fasted from 22.00 h the evening before each of the study days. They arrived at the clinical investigation unit at about 08.00 h on each study day, having drunk 500 ml tap water 1 h previously. A cannula was inserted into a vein in each antecubital fossa for blood sampling and administration of infusions. The subjects received an intravenous loading dose of 0.5 g *p*-aminohippurate sodium (PAH; Merck Sharp & Dohme, PA, USA) and 3.5 g polyfructosan (Inutest; Laevosan-Gesellschaft, Linz, Austria) at the start of the study (time 0 h) followed by a maintenance infusion of PAH (3.75 g l⁻¹) and polyfructosan (4.5 g l⁻¹) in 0.9% sodium chloride (saline; 150 mmol l⁻¹) at a rate of 5 ml min⁻¹ for the next 6 h. They emptied their bladders at 1.5 h and accurately timed consecutive urine collections of about 30 min duration were made thereafter until the end of the study. Two hours after the start of the study, the subjects took either 100 mg carbidopa (Merck Sharp & Dohme Ltd, Hoddesdon, UK) or placebo orally. This was followed 1 h later by a 60 min infusion of glu-5-HTP (Aalto Bio Reagents Ltd, Dublin, Eire), made up to 30 ml with 0.9% saline, at a rate of 16.6 µg kg⁻¹ min⁻¹ or placebo (saline alone at 0.5 ml min⁻¹). Each subject therefore received the following four combinations in a randomised sequence: placebo + placebo; placebo + glu-5-HTP; carbidopa + placebo; and carbidopa + glu-5-HTP. The subjects remained supine throughout the experiment except when standing to pass urine and drank 150 ml water every 30 min to promote an adequate diuresis. Blood pressure and pulse rate were measured in duplicate by a semi-automated recorder (Dinamap; Critikon Inc., Tampa, FL, USA) every 0.5 h during the study.

Venous blood samples were collected into lithium heparin tubes at 30 min intervals for measurement of plasma PAH and polyfructosan. Blood for determination of plasma renin activity (PRA) and aldosterone were collected at 0.5, 2, 3, 3.5, 4, 5 and 6 h into pre-cooled glass tubes containing sodium ethylenediamine tetra-acetate and kept on ice. Plasma was separated after centrifugation at 4° C and stored at -40° C until analysis. The volume of each urine collection was measured and aliquots removed and stored at -40° C for analysis of sodium, PAH, polyfructosan, 5-HTP, 5-HT, 5-HIAA and dopamine. Urine samples for 5-HT, 5-HIAA and dopamine were acidified (pH < 3.0) with 5M hydrochloric acid to prevent their oxidation.

Blood and urine analyses

Sodium was measured by an ion-selective electrode analyser (Radiometer KNA1). Plasma aldosterone concentrations were measured by radioimmunoassay with a commercially available kit ('Coat-a-count', Diagnostic Products Ltd, Caernarfon, Gwynedd, UK) with intra- and inter-assay coefficients of variation of 5% and 7% respectively. PRA was measured by radioimmunoassay of angiotensin I generated under standard conditions [8]. The intra- and inter-assay coefficients of variation were 5% and 9% respectively. PAH, 5-HTP, 5-HT and dopamine were measured by h.p.l.c. and polyfructosan and 5-HIAA by spectrophotometry as described previously [5, 6]. The values of the lower limit of detection of the assays for 5-HTP, 5-HT, 5-HIAA and dopamine were 45 nmol l⁻¹, 45 nmol l⁻¹, 5.2 µmol l⁻¹, and 26 nmol l⁻¹ respectively.

Data analysis

Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were estimated from the renal clearances of PAH (C_{PAH}) and polyfructosan (C_{ln}) respectively using the standard formula UV/P, where U is the urine concentration, V is the urine flow rate and P is the mean of the plasma concentrations at the beginning and end of each clearance period.

Results are expressed as means ± s.d. except for the figures where the mean ± s.e. mean values are shown. Statistical comparisons of the parameters measured serially on the four experimental days were made by repeated measures analysis of variance for overall statistical significance with the pretreatment values included as covariates. Two-way ANOVA was employed to identify any differences between the 3–6 h cumulative data on the 4 days. When significant differences were found, relevant pairs of data were compared by Student's *t*-test for paired observations with Bonferroni correction for multiple comparisons. A value of zero was assumed when the measured variable was below the limit of detection of the assay technique used to allow statistical comparisons to be made. Differences were considered to be statistically significant when the *P* value was less than 0.05. SPSS/PC + 4.0 statistical software package (SPSS Inc, Chicago, Illinois, USA) was employed for all statistical analyses.

Results

The urinary excretion rates of 5-HTP, 5-HT, 5-HIAA and dopamine for each 30 min period on the 4 study days are shown in Table 1. The urinary excretion of 5-HT was about 0.4 nmol min⁻¹ at baseline. This rose to a peak value of 278 ± 44 nmol min⁻¹ after glu-5-HTP infusion but did not change significantly after placebo infusion. The cumulative urinary 5-HT excretion over the 3 h period after the start of glu-5-HTP infusion was 430-fold higher than that after placebo

Table 1 Mean (s.d.) urinary 5-HTP, 5-HT, 5-HIAA and dopamine excretion rates for each 0.5 h period on the 4 study days ($n = 8$). Carbidopa or placebo was given at 2 h and time 3–4 h represents the infusion (glu-5-HTP or placebo) period. * = dopamine was not detectable in seven subjects

	1.5–2.0	2.0–2.5	2.5–3.0	3.0–3.5	Time (h) 3.5–4.0	4.0–4.5	4.5–5.0	5.0–5.5	5.5–6.0
<i>5-HTP excretion (nmol min⁻¹)</i>									
Placebo + placebo	0	0	0	0	0	0	0	0	0
Placebo + glu-5-HTP	0	0	0	232.6 (69.0)	436.5 (132.3)	368.9 (134.9)	246.9 (55.8)	149.8 (48.0)	84.3 (23.6)
Carbidopa + placebo	0	0	0	0	0	0	0	0	0
Carbidopa + glu-5-HTP	0	0	0	264.6 (71.1)	682.5 (133.5)	593.3 (173.7)	410.1 (79.6)	260.9 (85.4)	209.9 (42.8)
<i>5-HT excretion (nmol min⁻¹)</i>									
Placebo + placebo	0.3 (0.2)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.0)	0.4 (0.2)	0.5 (0.1)
Placebo + glu-5-HTP	0.5 (0.1)	0.4 (0.1)	0.4 (0.0)	113.1 (42.6)	274.3 (51.5)	278.4 (43.9)	198.8 (41.7)	126.1 (21.9)	88.4 (11.2)
Carbidopa + placebo	0.5 (0.2)	0.4 (0.1)	0.1 (0.1)	0	0	0	0	0	0
Carbidopa + glu-5-HTP	0.4 (0.2)	0.3 (0.2)	0.2 (0.1)	0.6 (0.4)	2.0 (1.1)	2.6 (1.6)	2.2 (1.0)	1.7 (0.7)	1.5 (0.9)
<i>5-HIAA excretion (nmol min⁻¹)</i>									
Placebo + placebo	0	0	0	0	0	0	0	0	0
Placebo + glu-5-HTP	0	0	0	73.7 (27.8)	199.2 (100.9)	222.8 (46.7)	213.2 (46.2)	171.4 (32.7)	144.9 (27.2)
Carbidopa + placebo	0	0	0	0	0	0	0	0	0
Carbidopa + glu-5-HTP	0	0	0	32.1 (35.3)	29.1 (15.2)	49.2 (21.1)	60.4 (35.7)	49.4 (21.3)	43.5 (16.9)
<i>Dopamine excretion (nmol min⁻¹)</i>									
Placebo + placebo	1.3 (0.2)	1.3 (0.3)	1.3 (0.2)	1.3 (0.2)	1.3 (0.3)	1.4 (0.3)	1.4 (0.3)	1.4 (0.3)	1.4 (0.2)
Placebo + glu-5-HTP	1.4 (0.2)	1.3 (0.3)	1.2 (0.2)	1.3 (0.1)	1.2 (0.2)	1.2 (0.2)	1.2 (0.4)	1.2 (0.1)	1.2 (0.1)
Carbidopa + placebo	1.6 (0.2)	1.6 (0.4)	0.7 (0.6)	0.2 (0.3)	0.1 (0.2)	0	0	0	0
Carbidopa + glu-5-HTP	1.6 (0.5)	1.6 (0.3)	0.9 (0.6)	0.2 (0.5)	0.3 (0.8)	0.3* (0.7)	0.2* (0.6)	0.2* (0.6)	0.2* (0.5)

infusion ($32.4 \pm 3.6 \mu\text{mol}$ vs $75.6 \pm 12.7 \text{ nmol}$; $P < 0.001$). Similarly, 5-HTP and 5-HIAA which were undetectable in urine during placebo infusion, increased markedly after administration of glu-5-HTP. Carbidopa suppressed urinary 5-HT and dopamine excretion to undetectable levels in all subjects during placebo infusion. It markedly attenuated the increase in 5-HT excretion after glu-5-HTP. Compared with placebo pretreatment, carbidopa reduced the 3 h cumulative 5-HT excretion by 99% from $32.4 \pm 3.6 \mu\text{mol}$ to $0.3 \pm 0.1 \mu\text{mol}$ ($P < 0.001$). It increased the 3 h cumulative 5-HTP excretion by 60% from $45.6 \pm 10.9 \mu\text{mol}$ to $72.8 \pm 14.8 \mu\text{mol}$ ($P < 0.001$) and reduced 5-HIAA excretion by 74% from $30.8 \pm 6.3 \mu\text{mol}$ to $7.9 \pm 3.2 \mu\text{mol}$ ($P < 0.001$).

There was a steady increase in urinary sodium excretion from $278 \pm 77 \mu\text{mol min}^{-1}$ (1.5–2.0 h period) to $379 \pm 144 \mu\text{mol min}^{-1}$ (5.5–6.0 h period) during the placebo day in response to infusion of saline (Figure 1). Glu-5-HTP produced a significant attenuation of sodium excretion when compared with

placebo infusion ($P < 0.01$). The 3 h cumulative sodium excretion values after placebo and glu-5-HTP administration were 63.3 ± 19.1 and $45.9 \pm 9.2 \text{ mmol}$ respectively ($P < 0.05$). Pretreatment with carbidopa abolished the antinatriuretic effect of glu-5-HTP. The 3 h cumulative sodium excretion when the subjects received carbidopa and glu-5-HTP infusion was $63.5 \pm 15.6 \text{ mmol}$ which is similar to that during placebo infusion only. Carbidopa had no significant effect on cumulative sodium excretion during placebo infusion ($63.3 \pm 5.2 \text{ mmol}$).

Glu-5-HTP significantly increased plasma aldosterone when compared with placebo infusion ($P < 0.001$) (Figure 2). Pretreatment with carbidopa attenuated the increase in plasma aldosterone concentrations produced by glu-5-HTP ($P < 0.005$). Carbidopa had no effect on the steady fall in plasma aldosterone concentration during placebo infusion. PRA declined progressively in response to saline loading during all experimental days (Figure 2).

GFR, ERPF, blood pressure and pulse rate were not

Figure 1 Sodium output (U_{NaV}) on the 4 study days: placebo + placebo (●); placebo + glu-5-HTP (▲); carbidopa + placebo (■); and carbidopa + glu-5-HTP (▼). Values shown are means $\pm$ s.e. mean ($n = 8$).

Figure 2 Plasma aldosterone concentrations and PRA on the 4 study days: placebo + placebo (●); placebo + glu-5-HTP (▲); carbidopa + placebo (■); and carbidopa + glu-5-HTP (▼). Values shown are means $\pm$ s.e. mean ($n = 8$).

significantly different between the 4 study days (data available from authors on request). No side effects were reported or recorded.

Discussion

This study confirms our previous observations that glu-5-HTP markedly increases urinary 5-HT excretion and causes retention of sodium without significant alterations in renal haemodynamics in nor-

mal man [5, 6]. The prodrug also increases aldosterone production as previously reported but the time course of the changes in sodium excretion and the lack of effect on urinary potassium excretion suggest that the antinatriuresis occurs independently of the known actions of aldosterone [6, 9]. Both the enzymes required for 5-HT synthesis from glu-5-HTP, γ GT and LAAD, are highly concentrated in the proximal tubular cells of the kidney [3, 10] and the high urinary levels of 5-HT would indicate that 5-HT is probably formed in the renal tubules and then excreted. That 5-HT is produced intrarenally is further supported by our recently reported findings that the marked increases in urinary excretion of 5-HT occur without significant changes in circulating 5-HT [11].

Urinary dopamine results mainly from dopamine synthesis in proximal tubular cells by the renal decarboxylation of L-dopa [12]. Similarly, it has been suggested that urinary 5-HT reflects intrarenal synthesis of 5-HT [2]. In the present study, a single 100 mg dose of carbidopa, an extracerebral LAAD inhibitor, suppressed dopamine and 5-HT excretion to below the levels of detection of the assays indicating effective inhibition of renal LAAD. We did not observe a significant effect of carbidopa on urinary sodium excretion during saline infusion in agreement with most previous studies [13–15]. Carbidopa substantially reduced the increment in urinary 5-HT excretion that followed administration of glu-5-HTP. There was an increase in urinary 5-HTP excretion and a reduction in 5-HIAA excretion. These results are consistent with significant inhibition of renal LAAD. The lesser reduction in 5-HIAA excretion suggests that there are body compartments, for example the brain, where LAAD may still remain active and be capable of 5-HT synthesis, despite carbidopa administration. Carbidopa abolishes the antinatriuresis induced by glu-5-HTP coincident with the near suppression of 5-HT synthesis, in keeping with the hypothesis that the sodium retention results from intrarenal generation of 5-HT.

Glu-5-HTP increased aldosterone production, without a concomitant increase in PRA, indicating that the release of aldosterone does not depend on the activation of the renin angiotensin system in man [5, 6]. Similar observations have been reported after 5-HTP administration although the rise in aldosterone occurs earlier and is of a greater magnitude [5, 6, 16, 17]. It is possible that the adrenal gland may possess γ GT and LAAD activity [18]. 5-HT could then be produced locally and stimulate aldosterone release since 5-HT has been shown to release aldosterone from the adrenal gland [19, 20]. Alternatively, 5-HTP and 5-HT formed from glu-5-HTP in the kidney may recirculate and act on the adrenal gland. 5-HTP, unlike 5-HT, can cross the blood-brain barrier and the release of aldosterone could, therefore, also be mediated by central 5-HT pathways [16, 17]. Carbidopa does not penetrate the central nervous system to any appreciable extent and would not be expected to inhibit conversion of 5-HTP to 5-HT in the brain. It has been reported to increase plasma 5-HTP follow-

ing administration of 5-HTP and to increase the stimulatory effect of 5-HTP on aldosterone suggesting that central 5-HT pathways are involved in the stimulation of aldosterone induced by administration of 5-HTP [17, 21]. In the present study, however, carbidopa attenuated the increase in aldosterone secretion following glu-5-HTP. This finding suggests that release of aldosterone by glu-5-HTP is predominantly a peripheral effect.

We conclude that the results of this work support the proposition that the effect of glu-5-HTP on

urinary excretion of sodium is caused by a direct tubular action of 5-HT formed within renal tubular cells by LAAD and is independent of any effect on renal haemodynamics. 5-HT may act as a counter-regulatory paracrine substance to dopamine in the local control of sodium handling in the kidney. It remains to be determined how the balance between 5-HT and dopamine is regulated [22] and whether this is important in the pathogenesis of conditions in which alteration in renal sodium handling may be important such as essential hypertension.

References

- 1 Adler S. Serotonin and the kidney. In *Serotonin in health and disease*, vol 4, ed. Essman WB. New York: Spectrum Publications Inc., 1977: 99–137.
- 2 Sole MJ, Madapallimattam A, Baines AD. An active pathway for serotonin synthesis by renal proximal tubules. *Kidney Int* 1986; **29**: 689–694.
- 3 Orlowski M, Szweczek A. Colorimetric determination of γ -glutamyl transpeptidase activity in human serum and tissues with synthetic substrates. *Acta biochim pol* 1961; **8**: 189–200.
- 4 Orlowski M, Wilk S. Metabolism of γ -glutamyl amino acids and peptides in mouse liver and kidney *in vivo*. *Eur J Biochem* 1976; **71**: 549–555.
- 5 Li Kam Wa TC, Freestone S, Samson RR, Johnston NR, Lee MR. A comparison of the effects of two putative 5-hydroxytryptamine renal prodrugs in normal man. *Br J clin Pharmac* 1993; **36**: 19–23.
- 6 Li Kam Wa TC, Freestone S, Samson RR, Johnston NR, Lee MR. A comparison of the renal and neuroendocrine effects of two 5-hydroxytryptamine renal prodrugs in normal man. *Clin Sci* 1993; **85**: 607–614.
- 7 Porter CC. Aromatic amino acid decarboxylase inhibitors. *Fed Proc* 1971; **30**: 871–876.
- 8 Haber E, Koerner T, Page LB, Kliman B, Purnode A. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J clin Endocrinol Metab* 1969; **29**: 1349–1355.
- 9 Sonnenblick EH, Cannon PJ, Laragh JH. The nature of the action of intravenous aldosterone: evidence for a role of the hormone in urinary dilution. *J clin Invest* 1961; **40**: 903–913.
- 10 Goldstein M, Fuxe K, Hökfelt T. Characterization and tissue localisation of catecholamine synthesizing enzymes. *Pharmac Rev* 1972; **24**: 293–308.
- 11 Li Kam Wa TC, Burns N, Williams BC, Freestone S, Lee MR. Blood and urine 5-hydroxytryptophan and 5-HT levels after intravenous administration of two 5-HT prodrugs in normal man. *Br J clin Pharmac* 1994; **37**: 515P–516P.
- 12 Lee MR. Dopamine and the kidney. *Clin Sci* 1982; **62**: 439–448.
- 13 Castellano M, Beschi M, Agabiti-Rosei E, *et al*. Renal and hemodynamic effects of atrial natriuretic peptide infusion are not mediated by peripheral dopaminergic mechanisms. *Am J Hypertension* 1991; **4**: 385–388.
- 14 Eadington DW, Swainson CP, Lee MR. Oral carbidopa has no effect on the renal response to angiotensin II in normal man. *Clin Sci* 1991; **80**: 149–154.
- 15 Jeffrey RF, MacDonald TM, Freestone S, Lee MR. The effect of carbidopa and lithium on the systemic and renal response to acute intravenous saline loading in normal man. *Nephrol Dial Transplant* 1989; **4**: 271–277.
- 16 Maestri E, Camellini L, Rossi G, *et al*. Serotonin regulation of aldosterone secretion. *Horm metabol Res* 1988; **20**: 457–459.
- 17 Shenker Y, Gross MD, Grekin RJ. Central serotonergic stimulation of aldosterone secretion. *J clin Invest* 1985; **76**: 1485–1490.
- 18 Rahman MK, Nagatsu T, Kato T. Aromatic L-amino acid decarboxylase activity in central and peripheral tissues and serum of rats with L-dopa and L-5-hydroxytryptophan as substrates. *Biochem Pharmac* 1981; **30**: 645–649.
- 19 Müller J, Ziegler WH. Stimulation of aldosterone biosynthesis *in vitro* by serotonin. *Acta Endocrinol* 1968; **59**: 23–35.
- 20 Rosenkrantz H. A direct influence of 5-hydroxytryptamine on the adrenal cortex. *Endocrinology* 1959; **64**: 355–362.
- 21 Magnussen I, Engbaek F. The effects of aromatic amino acid decarboxylase inhibitors on plasma concentrations of 5-hydroxytryptophan in man. *Acta pharmacol* 1978; **43**: 36–42.
- 22 Lee MR. Dopamine and the kidney: ten years on. *Clin Sci* 1993; **84**: 357–375.

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