Excretion of the Uraemic Metabolite 3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoic Acid in Human Urine

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Dear Sir,

The furanoid acid 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (5-propyl FPA) occurs in human urine and blood [1] and concentrations approaching 400 μM have been observed in uraemic plasma [2–4]. Such levels compare with those of well-known uraemic metabolites such as hippurate and indoxyl-3-sulphate [5]. 5-Propyl FPA is a potent inhibitor of drug plasma protein binding [6] and it is highly bound to albumin [7, 8]. Since 5-propyl FPA accumulates chronically in renal failure the question arises of how is it normally eliminated. Two mechanisms are likely for a highly bound dicarboxylic acid: further metabolism particularly by conjugation (phase II) pathways or tubular secretion. The urinary excretion of 5-propyl FPA by normal males was reported to be about 2 mg/day [3] which is relatively low and so we sought to determine if this represents the total amount of 5-propyl FPA in urine or whether conjugates are also excreted. The concentration of 5-propyl FPA in human urine was measured by high-pressure liquid chromatography (HPLC) both before and after hydrolysis by one of three methods: (a) enzymatic (choloylglycine hydrolase), (b) acid hydrolysis of glycine conjugates and (c) hydrolysis of glucuronides with β -glucuronidase. (a) Choloylglycine hydrolase solution (120 units in 0.2 ml distilled water) was mixed with equal aliquots (0.2 ml) of sodium acetate buffer (0.025 M, pH 5.6), 2-mercaptoethanol (0.75% in water) freshly prepared, EDTA disodium (0.055 M) and normal human urine as substrate (adjusted to pH 5.6). The mixture was bubbled with oxygen-free nitrogen and incubated overnight at 37 °C. Ice-cold trichloroacetic acid (20% w/v; 1 ml) was added to terminate the reaction and the supernatant obtained after centrifugation at 2,000 g for 10 min was analysed by HPLC. (b) Samples (1 ml) of human urine were boiled at 100 °C in screw-topped glass tubes for 16 h with 10 MHO (1 ml), neutralised with 10 M NaOH, centrifuged and the supernatant analysed by HPLC. (c) Human urine was adjusted to pH 5 and β -glucuronidase enzyme (type 3, bovine liver) added to produce a concentration of 20,000 units/ml. Aliquots (0.5 ml) were mixed with 2.5 ml 0.2 M sodium acetate buffer (pH 5) and incubated at 37 °C for 16 h in a shaking water bath. The samples were then centrifuged and the supernatant analysed. In each procedure blank samples contained water instead of either the enzyme or acid.

Samples were prepared for HPLC analysis with Sep-Pak C₁₈ cartridges which were first washed with acetoni-trile (5 ml) then with distilled water (5 ml). Samples (1–3 ml) were loaded onto the cartridge and eluted with distilled water (5 ml) followed by 2 ml of water: acetoni-trile (80/20 by

vol.) and finally with acetonitrile (5 ml). This final wash was collected, evaporated under nitrogen and reconstituted in 300 μ l of the HPLC solvent and 20 μ l injected onto the column. Mean recovery of authentic 5-propyl FPA was 40% and the detection limit of the system with a 20- μ l injection loop was 1 μ M and was linear to 1,000 μ M. Within-day variation of a 25 μ M standard was 6.5% with a between-day variation of 5.9%.

The concentration of 5-propyl FPA in 24-hour urine samples from 4 normal healthy males (aged 23–26) ranged from 0.93 to 4.71 mg/day (3.9–19.6 μ mol/day) with a mean $\pm$ SD value of 2.52 $\pm$ 1.62 mg/day. This value agrees with that of 1.74 mg/day reported by Mabuchi and Nakahashi [3]. Hydrolysis of glycine conjugates usually requires strong acid or alkali [9] and in order to circumvent the use of such reagents, which may have degraded 5-propyl FPA and/or its metabolites, the enzyme cho-

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loylglycine hydrolase was used under mild conditions. The use of cholesterylglucuronide hydrolase, strong acid or β -glucuronidase did not, however, reveal any evidence for either glycine or glucuronide conjugates of 5-propyl FPA. It is possible that a conjugate exists in urine which is not susceptible to hydrolysis by either of the enzymes used, but which is degraded to something other than 5-propyl FPA by strong acid hydrolysis.

The concentrations of sulphate and glycine conjugates are increased in uraemic plasma and so the apparent lack of conjugation of 5-propyl FPA in normal individuals does not preclude its conjugation when high concentrations are present. The effect of uraemia per se on the production of 5-propyl FPA has not yet been determined but its accumulation correlates with the duration of uraemia [3], and 5-propyl FPA disappears from plasma slowly even after a successful kidney transplantation [10]. If 5-propyl FPA is a final excretion product [1], active renal excretion is likely to be an important mechanism for its removal and so 5-propyl FPA would qualify as another of the solutes for which residual tubular secretory function is important [11].

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