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PLASMA URIC ACID AND SPIRONOLACTONE RESPONSE IN HEALTHY SUBJECTS

In healthy subjects treated with fludrocortisone there was a negative relationship between plasma uric acid concentration (PUA) and the urinary log 10 Na/K ratio following single doses of the aldosterone antagonist spironolactone (Aldactone[®], Searle) (Ramsay, Hessian & Tidd, 1975), a finding which has been confirmed in subsequent unpublished studies. Interpretation of these observations has been hampered by the complexity of the studies, involving as they did treatment with both fludrocortisone and spironolactone. In the present study we have examined the relationship between PUA and spironolactone response in a simplified protocol.

Twelve healthy subjects were studied on two occasions separated by an interval of one week. After an overnight fast they received at 09.00 h either spironolactone (100 mg) or identical placebos in a random balanced crossover design. In the 24 h following treatment the subjects consumed a diet which was constant on each occasion and designed to provide mild sodium restriction (calculated sodium content 90 mmol). Urine was collected during the 24 h period for measurement of sodium, potassium and creatinine excretion. Venous blood for measurement of PUA and creatinine was taken immediately before treatment, and at 2, 4, 8 and 24 h after treatment. The subjects were ambulant. Alcohol and all forms of medication were prohibited during the study. Uric acid in plasma and urine was measured by an automated colorimetric method (Nishi, 1967);

sodium and potassium concentration by flame photometry using lithium as the internal standard; creatinine by an automated method and canrenone by a fluorimetric method (Gochman & Gantt, 1962). Statistical methods used were the analysis of variance and product moment correlations. Log transformation of the urine Na/K ratio was used as this provides a sensitive index of response to mineralocorticoid antagonists (Ramsay, Harrison, Shelton & Tidd, 1975).

In the 24 h following spironolactone mean sodium excretion was increased by 35% ($P < 0.001$), urine log Na/K was increased by 15% ($P < 0.01$) and potassium concentration was reduced by 23% ($P < 0.05$). Potassium excretion, urine volume and creatinine clearance did not differ significantly from placebo values ($P > 0.1$) (Table 1). PUA concentration after spironolactone did not differ from placebo values at any sampling time. Two values of PUA were calculated for each subject: the pretreatment PUA (means of two estimates per subject) and the overall PUA (means of all estimates before and after treatment, i.e. 10 estimates per subject). True spironolactone responses (Δ) were calculated as the difference between placebo and spironolactone results for each subject. PUA concentration correlated significantly with Δ sodium excretion and Δ potassium concentration using either the pretreatment or the overall PUA values (Table 2). Correlations between PUA and Δ log 10 Na/K were negative but not significant. The direction of these correlations was

such that subjects with high levels of PUA excreted less sodium, and had smaller reductions in potassium concentration, than those with low PUA. In other words a higher PUA concentration was associated with reduced responses to spironolactone.

We examined data from two studies of the bioavailability of single doses of spironolactone tablets to determine whether there was a relationship between PUA and spironolactone metabolism. PUA was measured immediately before each treatment and bioavailability was assessed from plasma and urinary levels of canrenone, the principal unconjugated metabolite of spironolactone. All observations in each individual were averaged to give a single value for PUA and for the parameters of bioavailability. The design of the studies was such that the between subject correlations were not biased by different tablet formulations or by different study phases. There were no consistent or significant relationships between PUA and the AUC canrenone from 0-24 h, the urinary excretion of canrenone from 0-96 h, or the renal clearance of canrenone following spironolactone treatment (Table 2). The range of PUA concentrations observed in these studies was similar to that in the study demonstrating the correlations with pharmacological responses.

The correlation between PUA and spironolactone response is similar to that reported

previously (Ramsay *et al.*, 1975) although in prior studies correlations with the urine log 10 Na/K have generally been higher than those with either sodium or potassium separately. From the present study it may be concluded that the relationship between PUA and spironolactone response is not dependent on pretreatment with fludrocortisone. In separate studies there was no significant relationship between PUA and the plasma or urinary levels of canrenone, the principal unconjugated metabolite of spironolactone, and there was therefore no evidence that the pharmacological relationship reflects a link between PUA and spironolactone metabolism. It has been shown that PUA concentration also relates to the urinary electrolyte excretion following single doses of frusemide (Ramsay, Tidd, Auty, Levine & Branch, 1975; Branch, Levine, Read, Vander Elst, Rupp, Shelton & Ramsay, 1976). The evidence available at present would favour a relationship not specifically between PUA and spironolactone response, but some more general relationship between PUA and the response to diuretic agents, the nature of which remains to be elucidated. It would seem of interest to examine this relationship for diuretic agents other than spironolactone and frusemide, and also to seek a relationship between PUA and the therapeutic response to these drugs in conditions such as oedema or essential hypertension.

Table 1 Mean (and s.e. mean) results for urinary variables over 24 h after spironolactone (100 mg) and placebo in twelve healthy subjects

	Spironolactone (100 mg)	Placebo	P
Sodium excretion (mmol)	144.8(13.6)	106.9(13.1)	<0.001
Potassium excretion (mmol)	48.7(3.2)	53.6(2.6)	>0.1
Potassium concentration (mmol/l)	48.0(6.0)	62.0(8.0)	<0.05
Log 10 Na/K	1.46(0.05)	1.27(0.06)	<0.01
Volume (ml)	1112(91)	981(98)	>0.1
Creatinine clearance (ml/min)	90.9(6.8)	97.7(5.3)	>0.1

Table 2 Correlations of (a) pretreatment and overall PUA values with spironolactone responses in twelve healthy subjects and (b) pretreatment PUA with canrenone levels in two bioavailability studies ($n = 10$ and $n = 11$)

	Pretreatment PUA	Overall PUA
(a) Sodium excretion	-0.58*	-0.65**
Potassium concentration	+0.63*	+0.68**
Log 10 Na/K	-0.40	-0.46
(b) Canrenone AUC 0-24 h	+0.33, +0.41	
Canrenone excretion 0-96 h	-0.09, +0.50	
Canrenone clearance	-0.50, -0.03	

* $P < 0.05$, ** $P < 0.02$

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A SINGLE COMPREHENSIVE GAS CHROMATOGRAPHIC ASSAY FOR TRICYCLIC AND TETRACYCLIC ANTIDEPRESSANT DRUGS AND THEIR MAJOR METABOLITES IN PLASMA

The relationship between plasma levels of tricyclic antidepressive drugs and their therapeutic action remains controversial, but evidence suggests that there is a therapeutic range for optimal effect. It is well documented that for a standard oral dose a wide range of steady-state blood levels is achieved. Therefore, in the routine management of patients on tricyclic antidepressive therapy there is a need for an accurate, rapid and relatively easy method of analysis. The methods at present available for such plasma estimations are only suitable for research purposes as often they are long, complicated and require highly specialised equipment.

The basis of a simple system was introduced by Gifford (1976) and Gifford, Turner & Pare (1975), who described a single extraction procedure for tricyclic antidepressants and demonstrated the relative retention index and detector response to standard concentrations of each drug in methanol. This system has been further developed and a single assay procedure can now be used for most of the tricyclic and tetracyclic antidepressants, the extraction procedure differing only in the composition of the organic solvent from that used by Gifford *et al.* (1975).

To plasma or serum (4 ml) containing the drug to be estimated, in a silanized ground glass 30 ml centrifuge tube, was added 4 ml of distilled water and 1 ml (200 ng) of the chosen internal standard. 10N NaOH (1 ml) was then added to make the solution alkaline ensuring that both drugs were in unionized form. The drugs were extracted into 10 ml of an organic solvent (*n*-heptane: 0.5% isoamyl alcohol) by gentle shaking on a horizontal shaker for 20 min. The mixture was then centrifuged to separate the two phases and to break any emulsions formed during the shaking. A maximal amount of the organic layer was aspirated off and placed in a 15 ml centrifuge tube. To this was added 100 μ l of glacial acetic acid and 100 μ l of isoamyl alcohol to reduce the volatility of the drugs and to minimise the amount adhering to the glass. The organic extract was then reduced to 50 μ l by evaporation in a water bath at 60°C under a gentle stream of nitrogen. Aliquots (2 μ l) of the final extract were injected into the chromatograph for analysis.

All analyses were performed using an isothermal chromatograph with an electrically heated nitrogen-phosphorus specific detector (Perkin