SPIRONOLACTONE DOSE-RESPONSE RELATIONSHIPS IN HEALTHY SUBJECTS

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1 The effect of single oral doses of spironolactone 25 mg, 50 mg, 100 mg, 200 mg, 400 mg, and placebo in reversing the urinary electrolyte changes induced by fludrocortisone between 2-10 h and 12-16 h after treatment was examined in healthy subjects.

2 In the two collection periods, there were statistically significant log linear dose-response relationships for sodium excretion ($P < 0.001$), potassium excretion ($P < 0.001$ and $P < 0.025$ respectively) and $\log_{10} 10 \text{ Na/K}$ ($P < 0.001$).

3 However, there was evidence that the log spironolactone dose-urinary sodium responses did not increase monotonically, while the relationship for urinary potassium appeared to enter the lower 'plateau' at doses between 100 mg and 200 mg, and when compared to placebo values, potassium excretion was not significantly depressed 12-16 h after treatment ($P > 0.1$). Thus, sodium and potassium responses were dissociated in dose producing maximal effect and in duration of activity, reinforcing the view that functions of the urinary sodium/potassium ratio alone cannot be considered an adequate description of renal antimineralocorticoid activity.

4 Dose-response relationships for all urinary electrolyte variables seem consistently steep and linear between 25 mg and 100 mg of spironolactone, suggesting that, in studies employing this model, the doses of spironolactone should be restricted to this range.

Introduction

Spironolactone is a synthetic steroid lactone (spiro-lactone) displaying properties compatible with specific, competitive mineralocorticoid antagonism (Drill, 1962; Kagawa, 1960). It is used clinically to treat primary hyperaldosteronism, essential hypertension and oedematous states (Ochs *et al.*, 1978), where daily doses of 400 mg may be necessary (Ferguson *et al.*, 1977; Ogilvie *et al.*, 1977; Papadoyanakis *et al.*, 1972). In view of the prolonged elimination kinetics of canrenone (Karim *et al.*, 1976a, b), quantitatively its major active metabolite (Gochman & Gannt, 1962), single daily dosing seems logical.

Healthy subjects have been used to evaluate the renal antimineralocorticoid activity of spironolactone because it is inconvenient and difficult to establish dose-response relationships in patients (Sadée *et al.*, 1974). Since competitive mineralocorticoid antagonists have no demonstrable activity under basal conditions (Liddle, 1957; Ross & Winternitz, 1961), a state of mineralocorticoid excess is necessary and this can be achieved conveniently by administering single oral doses of the synthetic mineralocorticoid, fludrocortisone (Ross, 1962). Under these circumstances, significant linear log spironolactone dose-urinary electrolyte responses can be demonstrated

after single doses in the range 25-100 mg (Ramsay *et al.*, 1975, 1976a, b). However, evidence from single (Ramsay *et al.*, 1975, 1976a) and repeated dosing (McInnes *et al.*, 1981; Ramsay *et al.*, 1977) studies have suggested that natriuretic and antidiuretic responses may be dissociated with respect to the steeply graded portion of the log dose-response relationship, urinary potassium excretion appearing to reach the lower plateau at doses of approximately 100 mg (McInnes *et al.*, 1981; Ramsay *et al.*, 1977). There is no published evidence on the electrolyte responses to greater single doses of spironolactone in the fludrocortisone model. In this study, we undertook to define spironolactone dose-urinary electrolyte response relationships in healthy volunteers over a wide range of single doses, similar to that which might be employed in patients.

Methods

Rationale

We used the method of Ramsay *et al.* (1975, 1976a) which was based on the original observations of Ross (1962). Single oral doses of fludrocortisone given in

the evening produce highly reproducible changes in the electrolyte composition of overnight urine in healthy men. These changes, reduced sodium excretion, increased potassium excretion and reduction of the Na/K ratio, are reversed by single doses of aldosterone antagonists and the dose-response curves for fludrocortisone and aldosterone exhibit parallelism. In this study, urine was collected for 16 h after aldosterone antagonist treatment (Ramsay *et al.*, 1976a).

Study design

The study was of double-blind (placebo controlled), crossover, randomised, balanced (Latin square) design. Each of the six treatments were taken on one occasion by each of the subjects with intervals of at least one week between treatments.

Subjects

Six males, aged 24 to 39 years, who were judged healthy after medical history, physical examination and laboratory screening participated in the study. The subjects were ambulant, took no medication other than that prescribed throughout the full study period and abstained from alcohol from 24 h before each treatment until urine collection was completed. The volunteers gave written informed consent for the study which was approved by a peer review committee.

Medication

The treatments consisted of single oral doses of placebo, 25 mg, 50 mg, 100 mg, 200 mg and 400 mg spironolactone administered double-blind as 25 mg tablets with matching placebo tablets. Fludrocortisone was dispensed as one 1 mg tablet (1 mg dose) and five 0.1 mg tablets (0.5 mg dose).

Procedure

On study days, the subjects took fludrocortisone 1 mg with 150 ml water at 19.00 h and a single dose of spironolactone or placebo with 300 ml water at 21.00 h. An additional 300 ml water was ingested at 23.00 h, the bladder emptied and all urine passed from then until 07.00 h the next morning collected. At 07.00 h the subjects took a further oral dose of fludrocortisone (0.5 mg) with 150 ml water and urine was collected from 09.00 h until 13.00 h. From 16.00 h on study days until the urine collection was completed, the subjects consumed nothing orally other than light meals, identical on all occasions, medication and water prescribed. The drug responses were assessed from electrolyte excretion in the two urine collections, i.e. between 2–10 and 12–16 h after treatment with spironolactone or placebo.

Table 1 Mean (s.e. mean) results in six subjects for urinary responses to placebo, spironolactone 25 mg, 50 mg, 100 mg, 200 mg and 400 mg

Response	Spironolactone dose (mg)					Statistical significance*			
	Placebo	25	50	100	200	400	Difference between six treatments	Log dose-response	Non-linearity
2–10 h									
Sodium excretion (mmol)	14.8 (5.1)	18.4 (3.9)	26.3 (5.1)	34.5 (4.1)	30.2 (7.2)	38.8 (4.2)	$P < 0.001$	$P < 0.001$	$P < 0.1$
Log ₁₀ 100 sodium/creatinine	1.40 (0.13)	1.60 (0.09)	1.73 (0.07)	1.86 (0.05)	1.78 (0.07)	1.92 (0.06)	$P < 0.001$	$P < 0.001$	NS
Potassium excretion (mmol)	23.1 (3.3)	21.4 (3.6)	18.7 (2.5)	17.4 (2.7)	14.1 (2.8)	14.7 (2.3)	$P < 0.001$	$P < 0.001$	NS
Log ₁₀ 10 Na/K	0.73 (0.12)	0.93 (0.08)	1.13 (0.07)	1.30 (0.07)	1.32 (0.05)	1.44 (0.09)	$P < 0.001$	$P < 0.001$	NS
Volume (ml)	628 (97)	481 (68)	527 (93)	552 (76)	418 (42)	623 (97)	$P < 0.1$	NS	$P < 0.1$
12–16 h									
Sodium excretion (mmol)	4.9 (1.8)	5.9 (1.5)	7.7 (1.5)	10.4 (1.7)	10.9 (1.6)	17.1 (2.9)	$P < 0.001$	$P < 0.001$	NS
Log ₁₀ 100 sodium/creatinine	1.18 (0.14)	1.31 (0.15)	1.45 (0.10)	1.61 (0.06)	1.64 (0.07)	1.82 (0.10)	$P < 0.001$	$P < 0.001$	NS
Potassium excretion (mmol)	18.0 (1.1)	21.3 (2.7)	19.6 (2.1)	15.6 (2.3)	16.6 (1.8)	15.6 (2.1)	NS	$P < 0.025$	NS
Log ₁₀ 10 Na/K	0.30 (0.16)	0.38 (0.13)	0.56 (0.13)	0.82 (0.09)	0.81 (0.10)	1.03 (0.11)	$P < 0.001$	$P < 0.001$	NS
Volume (ml)	145 (9)	152 (10)	157 (12)	153 (23)	144 (12)	180 (29)	NS	NS	NS

*NS, $P < 0.1$

Laboratory analysis

Urine was collected without preservative. After measurement of volume, an aliquot was stored at -20°C until immediately prior to assay. Urine sodium concentration was measured by atomic absorption spectrophotometry, potassium concentration by flame photometry with lithium as internal standard and creatinine concentration by an automated colorimetric method (Chasson *et al.*, 1961). Samples from each phase of the study were analysed as a single batch.

Statistical analysis

The responses were compared by analysis of variance appropriate to the Latin square design, allowing for subject, phase and treatment effects in the fitted linear model. The between treatments variations were partitioned to investigate the log dose-response relationships using regression analysis. The statistical methods employed are described by Armitage (1971).

Figure 1 Log spironolactone dose-urinary sodium response curves in the periods 2–10 h (●) and 12–16 h (○) after treatment. Mean (vertical bars, s.e. mean) results in six subjects.

Results

The mean results and between subjects standard errors (s.e. mean) for urinary responses in the 2–10 h and 12–16 h collection periods are presented in Table 1.

Urinary sodium excretion

In both periods, the differences between the six treatments and the slopes of the log dose-response relationships achieved a high level of statistical significance ($P < 0.001$ in each case) with no important contraindication to linearity. However, in the 2–10 h collection, mean sodium excretion was lower after 200 mg than after 100 mg spironolactone and, between 12 and 16 h, the sodium response to 200 mg was less than that to 100 mg in three of the six subjects. In each collection, 400 mg produced the greatest response (Figure 1). The log dose-response profiles were similar when the results were taken relative to creatinine excretion.

Urinary potassium excretion

The overall difference between placebo and the five dose levels of spironolactone was significant in the 2–10 h collection ($P < 0.001$) but not in the 12–16 h collection ($P > 0.1$). However, the slopes of the fitted log dose-response relationships were significantly negative in both cases ($P < 0.001$ and $P < 0.025$, respectively). There was no significant departure from linearity but there was evidence of the relationship entering the lower 'plateau' at around 200 mg in the first collection and around 100 mg in the second (Figure 2a, b).

Urinary log₁₀ Na/K

The difference between treatments and the slope of the log dose-response relationship were highly significant in each collection period ($P < 0.001$) with no significant departure from linearity.

Urine volume

In neither collection period was there a statistically significant difference between placebo and the five dose levels of spironolactone, nor was there evidence of a log dose-response relationship.

Discussion

In this study, log spironolactone dose-urinary electrolyte response relationships have been defined precisely in healthy men. On the face of it, the results suggest that, between single doses of 25 mg and 400 mg in the period up to 16 h after treatment, significant

Figure 2 Log spironolactone dose-urinary potassium response curves (a) in the period 2–10 h after treatment and (b) in the period 12–16 h after treatment. Mean (vertical bars, s.e. mean) results in six subjects.

linear relationships exist for sodium excretion, potassium excretion and urinary $\log_{10} 10$ Na/K, usually considered the best single index of renal mineralocorticoid (Johnson, 1954) and antimineralocorticoid activity (Kagawa, 1964; Ramsay *et al.*, 1975).

However, this view may be an oversimplification. In each collection period, mean sodium excretion following 200 mg spironolactone was not significantly different from that after 100 mg. Indeed, in the overnight collection, the sodium response to 200 mg was less than that to 100 mg. These observations may indicate that the sodium response does not increase monotonically with dose in this region. There was no obvious explanation for this from within the study. The similarity of the $\log_{10} 100$ sodium/creatinine results provided no evidence of incomplete urine collections and covariate analysis for urine volume could not eliminate this paradoxical response. Taken alone, this does not preclude a log linear dose-response relationship. However, the phenomenon has been demonstrated repeatedly in unpublished studies utilising single doses of spironolactone greater than 100 mg (Table 2). The striking similarity of these findings makes it unlikely that the paradoxical response is merely a chance finding, an artefact of study design or due to an atypical study population.

The upper 'plateau' of the dose-response relationship cannot explain this effect since the response to 400 mg is consistently greater than those to lower

doses. Similar reasoning makes it unlikely that mineralocorticoid agonist properties have been uncovered at very high doses as have been noted in animal and *in vitro* studies of spiro lactones (Gantt, 1961; Sakayue & Feldman, 1976). However, at least two metabolites contribute to the activity of spironolactone (McInnes *et al.*, 1980; Ramsay *et al.*, 1976a, b) and it remains possible that one or more of these exhibit agonist activity in concentrations present after single doses of 100–200 mg spironolactone, while the overall anti-mineralocorticoid activity of other metabolites might predominate at very high doses (e.g. 400 mg) of spironolactone.

Although there was no statistical contraindication to linearity of the log spironolactone dose-urinary potassium excretion relationship in either of the urine collection periods, the curves seemed to enter the lower 'plateau' at doses of about 200 mg in the 2–10 h collection and about 100 mg in the later period. In contrast to sodium excretion, potassium excretion was not significantly depressed in the second urine collection, indicating that spironolactone's influence on potassium handling was relatively short lived. A valid comparison of the spironolactone dose-response relationships for sodium excretion and potassium excretion is difficult due to the paradoxical behaviour of the sodium response but they seem to be dissociated both in duration of activity and in dose producing maximal response. Furthermore, the slopes of

Table 2 Examples from other studies of mean urinary sodium excretion (mmol) 2–10 h after single doses of spironolactone in healthy subjects

Study	Number of subjects	Spironolactone dose (mg)						
		25	50	75	100	150	200	400
1	12		26.8		48.5	41.6		
2	8				39.3	52.7	51.1	65.3
3 ^a	12	27.8	31.9	43.1				
4 ^b	12	22.8	27.6		41.1			
5 ^c	12	25.9	33.8		38.2			
6 ^d	6	25.1	32.7		38.5			
7 ^e	12	19.1	23.7		30.8			

^aRamsay *et al.* (1975)^bRamsay *et al.* (1976a)^cRamsay *et al.* (1976b)^dMcInnes *et al.* (1981a)^eMcInnes *et al.* (1981c)

the dose-response curves for natriuresis were steeper than those for antikaliuresis, which is in keeping with previous experience with this model (Ramsay *et al.*, 1975, 1976a).

The results of this study reinforce the view that the urinary sodium/potassium ratio alone cannot be considered an adequate description of renal mineralocorticoid and antimineralocorticoid activity (McInnes *et al.*, 1980; Ramsay *et al.*, 1975). In bioassay procedures, functions of this ratio have been accepted as the best index of activity (Johnson, 1954; Kagawa, 1964; Ramsay *et al.*, 1975), and not infrequently have been used exclusively. The $\log_{10} 10 \text{ Na/K}$ ratio may provide a useful index of overall response to aldosterone antagonists but the dose-response tends to follow that for sodium excretion in duration and in dose range, and fails to reflect the urinary potassium response.

Dose-response relationships for all urinary electrolyte variables seem consistently steep and linear between 25 to 100 mg of spironolactone as evidenced by the results of this study and previous observations

(Table 2). Thus, in acute studies it may be wise to limit the doses of spironolactone to this range, although extrapolation of information so gained to all doses may not be strictly valid. Likewise, relating the findings from single dose studies to the clinical situation where the drug is prescribed continuously and maximal activity is observed only after some days medication, should be cautious. However, repeated dosing studies in healthy subjects have confirmed dissociation of urinary sodium and potassium handling (McInnes *et al.*, 1981a; Ramsay *et al.*, 1977). The relatively narrow dose range over which the steep portion of the potassium excretion relationship is encountered may explain the difficulties in discriminating between the urinary potassium responses to different doses of spironolactone in those studies.

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