Br. J. clin. Pharmac, **18**, 369–375

Metabolic effects of high dose amiloride and spironolactone: a comparative study in normal subjects

J. A. MILLAR*, R. FRASER, P. MASON, B. LECKIE, A. M. M. CUMMING & J. I. S. ROBERTSON
MRC Blood Pressure Research Unit, Western Infirmary, Glasgow G11 6NT

1 Amiloride (75 mg daily) and spironolactone (300 mg daily) were given to five normal subjects for 7 days in order to compare metabolic effects at maximal doses.
2 Blood pressure, body weight, Na^+ and K^+ balance, and plasma concentrations of Na^+, K^+, active and total renin, angiotensin II, aldosterone, 11-deoxycorticosterone (DOC), 18-hydroxydeoxycorticosterone (18-OH DOC), corticosterone (B), 18-hydroxycorticosterone (18-OH B) and cortisol were measured before and on each day of treatment.
3 Natriuresis and K^+ retention were significantly greater with amiloride. Plasma K^+ increased from 4.1 ± 0.2 to 4.9 ± 0.2 mmol/l (mean ± s.d.) on amiloride and from 4.0 ± 0.2 to 4.4 ± 0.2 mmol/l with spironolactone. Stimulation of renin, angiotensin II, aldosterone and 18-OH B occurred with both drugs but was greater with amiloride in each case. A transient decrease in systolic and diastolic blood pressure was observed after 2 days of spironolactone treatment but not with amiloride. The slope of the regression of aldosterone on angiotensin II during spironolactone treatment was less than that with amiloride, consistent with partial blockade of aldosterone synthesis by spironolactone.
4 These data suggest that the maximum metabolic effects of amiloride exceed those of spironolactone.

Keywords aldosterone amiloride metabolic effects renin spironolactone

Introduction

Spironolactone and amiloride are potassium-sparing diuretics which act at the same or neighbouring sites on the nephron at the distal tubule. However, their mechanisms of action differ. Spironolactone antagonises the action of aldosterone on the kidney by specific binding to aldosterone receptors (Sakauye & Feldman, 1976), and partially inhibits aldosterone synthesis in the adrenal zona glomerulosa (Erbler, 1972; Cheng *et al.*, 1976; Abshagen *et al.*, 1978). Aldosterone responses to acute administration of ACTH are diminished (Kim *et al.*, 1979). Impairment of hepatic aldosterone metabolism (Tsai & Morris, 1978) may also contribute. The molecular pharmacology of amiloride is less well defined. One effect is to inhibit epithelial cell transport of sodium and potassium (Davis & Finn, 1982), and hence to decrease tubular cell permeability to sodium (Knauf *et al.*, 1978).

Several studies have attempted to assess the relative potency of amiloride and spironolactone on a weight basis by comparing the dose-response relationships with respect to plasma potassium, sodium, aldosterone and blood pressure (Bull & Laragh, 1968; Ramsay *et al.*, 1980; McInnes, 1982). Reported values from these studies varied from 2.8:1 to 5:1. However, the relative potency suggested by the maximum recommended daily doses (20 and 200 mg for amiloride and spironolactone, respectively) is 10:1. We have studied this apparent inconsistency by measuring the metabolic

* Present address: Department of Pharmacology, University of Otago, P.O. Box 913, Dunedin, New Zealand

and endocrine effects of both drugs at high doses in normal subjects. Since insufficient data are available on dose-response relationships for amiloride when given in repeated doses, we approached the problem by using a daily dose of spironolactone known to produce maximal effects on plasma K^+ (300 mg) and that of amiloride calculated from a relative potency of 4:1 (75 mg). We measured the effects of each drug on sodium and potassium balance and plasma levels of active and inactive renin, angiotensin II, aldosterone and mineralocorticoid steroid precursors, at daily intervals over a period of 7 days.

Methods

Five normal male subjects aged 25–44 years participated in the study, which was approved by the Research and Ethical Committee of the Western Infirmary, Glasgow. Each subject took a fixed defined diet containing 140–160 mM Na^+/24 h and 60 mM K^+/24 h for a period of 11 days, on two occasions separated by at least 4 weeks. Spironolactone (Aldactone, G. D. Searle, Ltd; 100 mg 3 times daily) or amiloride (Midamor, Thomas Morson Pharmaceuticals; 25 mg 3 times daily) was taken by mouth from days 5–11. The order of treatment with each drug was varied. Blood samples were taken each morning without forearm exercise between 08.00 h and 09.00 h after 30 min quiet supine rest and overnight fasting, from day 4 onwards. Urine was collected in 24 h batches from the start of the experiment, and daily urinary sodium, potassium, and creatinine excretion were measured. Sodium balance was calculated as the difference between dietary sodium intake and urinary output. Each subject was weighed daily.

Active plasma renin concentration was measured by radioimmunoassay of angiotensin I generated during incubation of untreated plasma at 37°C in the presence of ox angiotensinogen (Millar *et al.*, 1980). Total renin was measured after acid activation of inactive renin at pH 3.0. Inactive renin concentration was calculated as the difference between total and active renin. Results for renin concentrations are expressed in terms of the Human International Standard Renin Preparation (Bangham *et al.*, 1975).

Angiotensin II was measured by the method of Düsterdieck & McElwee (1971) as described by Morton *et al.* (1976). Plasma corticosteroids (corticosterone (B), 18-hydroxycorticosterone (18-OHB), deoxycorticosterone (DOC), 18-hydroxy deoxycorticosterone (18-OH DOC), cortisol and aldosterone) were measured by gas-liquid chromatography after extraction and separation by paper chromatography as described by Mason & Fraser (1975) and Wilson *et al.* (1976).

Plasma and urine Na^+ and K^+ were measured by flame photometry.

Blood pressure was measured throughout by the same observer.

Statistical methods

Sequential changes in circulating hormones, urinary electrolyte excretion, cumulative electrolyte balance, blood pressure and body weight were assessed by two-way analysis of variance. Alterations in serum creatinine were analysed by paired *t*-test. Linear regressions were calculated using the method of least squares. A P value < 0.05 was taken to indicate statistical significance.

Results

Effects of amiloride and spironolactone on Na^+ and K^+ balance

Twenty-four hour urinary Na^+ excretion fell from 215 ± 34 (s.e. mean) and 166 ± 18 mmol/24 h on the first day of the study to levels of 127 ± 6 and 125 ± 10 mmol/24 h before administration of amiloride and spironolactone respectively. Following treatment there was a natriuresis with both drugs, but during the first 24 h of treatment this was greater with amiloride (Figure 1). Hence the cumulative negative salt balance over 7 days with amiloride (385 ± 22 mmol Na^+) was greater than that for spironolactone (242 ± 33 mmol Na^+; $F = 35.57$, $P < 0.001$). However the difference in total 7-day cumulative salt balance was also due to a positive 24 h Na^+ balance in two subjects on day 10 (mean −3.5 ± 6 mmol/24 h) and all five subjects on day 11 (mean 13.9 ± 6 mmol/24 h) with spironolactone. No corresponding 'escape' was seen during amiloride treatment; values on days 10 and 11 were −37.2 ± 18 and −11.8 ± 6 mmol/24 h, respectively.

Data for potassium balance show similar differences. Twenty-four hour urine K^+ did not change significantly with spironolactone but decreased from 64 ± 5 to 33 ± 5 and 41 ± 5 mmol/24 h during the 2 days following administration of amiloride. Thus potassium retention was a feature of treatment with amiloride (cumulative 7 day K^+ balance +90 ±

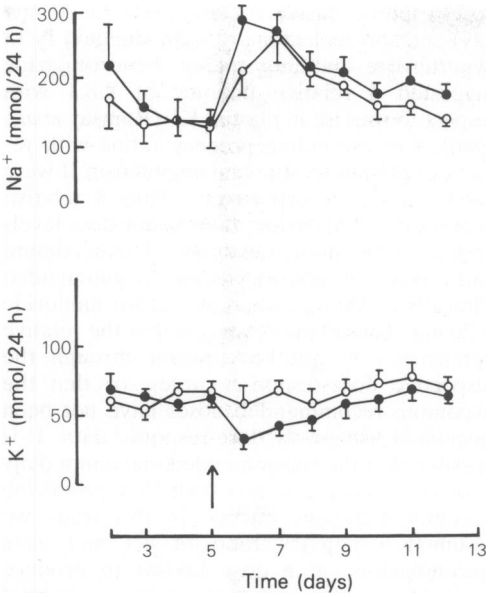

Figure 1 Urine Na^+ and K^+ (mmol/24 h) before and during treatment with either spironolactone (300 mg/day; ○—○) or amiloride (75 mg/day; ●——●) in five normal subjects. Treatments were commenced on day 5 of the study as indicated by the arrow. Values are shown as mean ± s.e. mean.

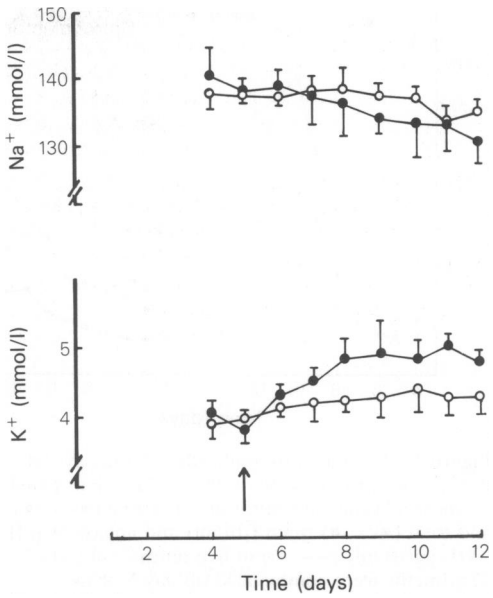

Figure 2 Plasma Na^+ and K^+ (mmol/l) from day 4 of the experiment. For explanation of symbols, see legend to Figure 1.

20 mmol) but not with spironolactone (−8.7 ± 17 mmol; $F = 152.70$, $P < 0.001$).

Plasma Na^+ decreased progressively from 141 ± 1 to 131 ± 2 mmol/l during amiloride treatment. With spironolactone there was a corresponding decrease during the first 5 days, from 138 ± 1 to 134 ± 1 mmol/l, but on the sixth day of treatment, when sodium balance had become positive, plasma Na^+ increased to 136 ± 1 mmol/l. Plasma K^+ increased progressively with both drugs, but the increase was greater with amiloride (amiloride, 4.0 ± 0.2 to 4.9 ± 0.2; spironolactone, 4.0 ± 0.2 to 4.4 ± 0.2 mmol/l; $F = 66.84$, $P < 0.001$). Hyperkalaemia (plasma $K^+ > 5.0$ mmol/l) was observed on 7 days in three subjects on amiloride, with a maximum individual value of 5.7 mmol/l on day 5 of treatment, but not with spironolactone (Figure 2). Body weight decreased with both drug treatments (amiloride 69.9 ± 9 to 66.4 ± 5; spironolactone, 70.6 ± 4 to 67.6 ± 4 kg) but the changes were not significant ($F = 0.16$). Serum creatinine increased significantly with spironolactone, from 89.4 ± 11.2 to 120.0 ± 8.4 μmol/l ($t = 5.25$; $P < 0.001$; but not with amiloride (105.6 ± 12.9 to 111.6 ± 14.1 μmol/l ($t = 1.09$, NS). Blood pressure showed no significant changes with amiloride but after 2 days of treatment with spironolactone systolic blood pressure had declined from 111 ± 5 to 100 ± 7 ($t = 2.19$, $P \sim 0.05$) and diastolic pressure from 65 ± 4 to 54 ± 5 ($t = 2.01$, $P \sim 0.05$) mm Hg.

Effect of amiloride and spironolactone on active renin, total renin, and angiotensin II (Figure 3)

Plasma active and total renin were significantly increased by both diuretics, with maximal levels achieved by day 5 of treatment (active: $F = 6.26$, $P < 0.01$; total: $F = 9.25$, $P < 0.001$). The increase in active renin with amiloride (13.6-fold) was greater than with spironolactone (9.5-fold) $F = 11.43$, $P < 0.001$). Corresponding changes in plasma angiotensin II were also observed. The increase in total renin was accounted for by the changes in active renin, and therefore no consistent changes occurred in circulating inactive renin.

Effect of amiloride and spironolactone on mineralocorticoids and mineralocorticoid precursors

Table 1 gives values for plasma aldosterone, 18-OH B, B, DOC, 18-OH DOC and cortisol during treatment with amiloride and spirono-

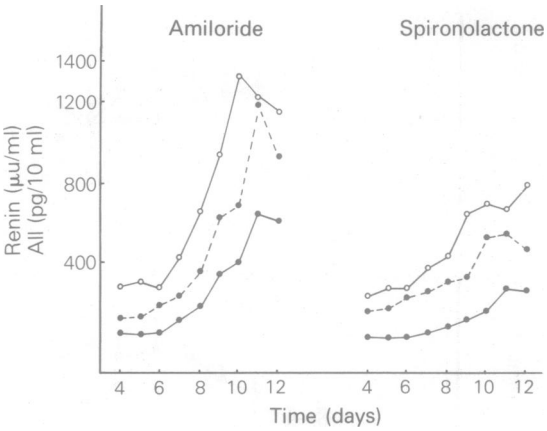

Figure 3 The effect of amiloride (75 mg/day; left panel) and spironolactone (300 mg/day; right panel) on mean plasma concentrations of active (●——●) and total (●- - -●) renin (μu/ml) and angiotensin II (AII, pg/10 ml; ○——○) in five normal subjects. Treatments were commenced on day 5 of the experiment.

lactone. Data for DOC, 18-OH B and B were available in only four subjects. Both aldosterone and 18-OH B increased progressively during treatment with both drugs, but the increase was greater with amiloride by factors of 3.3 and 2.5 respectively (aldosterone: $F = 41.75$, $P < 0.001$; 18-OH B: $F = 61.05$, $P < 0.001$). Deoxycorticosterone levels increased with spironolactone but not with amiloride ($F = 8.39$, $P < 0.01$). No changes were seen in plasma cortisol, 18-OH DOC, or B. Sequential increments in aldosterone were greater with amiloride treatment ($F = 41.75$, $P < 0.001$), and a significant correlation between concurrent individual plasma angiotensin II and aldosterone concentrations was found for each diuretic (amiloride: $r = 0.90$, $n = 35$, $P < 0.001$; spironolactone: $r = 0.54$, $n = 43$, $P < 0.001$). The slopes of the regression lines were significantly different ($P < 0.01$) indicating that aldosterone levels relative to angiotensin II were significantly less with spironolactone (Figure 4).

Discussion

The relative potency of amiloride and spironolactone has been the subject of some controversy. It has been stated from single dose studies that the potency ratio of amiloride and spironolactone on a weight basis is 5:1 (Bull & Laragh, 1968). However, examination of the dose-response curves for amiloride (5–20 mg/day) and spironolactone (25–200 mg/day) in 15 hypertensive patients taking bendrofluazide suggested a relative potency of 2.8:1 with respect to the rise in plasma K^+ (Ramsay *et al.*, 1980). Corresponding potency ratios with respect to plasma sodium and angiotensin II were 3.9:1 and 3.2:1 respectively. Thus a relative potency of 5:1 at current therapeutic dose levels appears to be an over-estimate. The maximum daily dose of spironolactone recommended clinically is 200 mg, whereas that for amiloride is 20 mg. These figures suggest that the relative potencies may not be constant through the respective dose-response curves, or that the maximum recommended doses have not been calculated using only dose-response data. It is possible that the recommended maximum daily dose of amiloride is less than that producing maximal metabolic effects. In this study we assumed a potency ratio of 4:1 and gave spironolactone at a dose known to produce maximal or near maximal effects in normal subjects, in order to test the effectiveness and tolerability of a higher dose of amiloride than used previously, and to compare the metabolic and renal effects of these doses.

Amiloride at a daily dose of 75 mg was well tolerated and free of side effects. There were greater effects on urinary sodium excretion, renal potassium retention and plasma Na^+ and K^+ than with spironolactone (300 mg/day) due to more pronounced effects during the first day of treatment and absence of 'escape' from the diuretic action of the drug within the period of study. These findings indicate that the relative potency of amiloride and spironolactone at high dose is substantially greater than 4:1. This could arise if the log-dose response relationship was steeper for amiloride, or if the maximal response was greater, or both. Interestingly, the dose-response curve for spironolactone is reportedly not 'monotonic' and exhibits a lower plateau at doses between 100 and 200 mg (McInnes *et al.*, 1982). This characteristic would tend to increase the potency ratio at higher doses. Previous studies indicate that the relative potency of the two drugs is constant for 'normal' therapeutic doses, and hence that the dose-response curves at these doses are parallel. However, no additional increase in plasma potassium occurs with spironolactone when the daily dose is increased from 100 to 200 mg (Ramsay *et al.*, 1980), and no increase in urinary potassium occurs when the dose is increased above 200 mg (Casals-Stenzel *et al.*, 1978). Thus maximum effects of spironolactone in normal subjects are achieved at doses of approximately 200 mg, less than that used in the

Table 1 Values of aldosterone (aldo; ng/100 ml), 11-deoxycorticosterone (DOC: ng/100 ml), 18-hydroxydeoxycorticosterone (18-OH DOC; ng/100 ml), corticosterone (B; μg/l), 18-hydroxycorticosterone (18-OH B; ng/100 ml) and cortisol (μg/100 ml) in five normal subjects given either spironolactone (300 mg/day; S) or amiloride (75 mg/day; A) for 7 days. Both drugs were given from the morning of day 5 of the study (see text); blood samples for the above compounds were taken from day 4 of the study. Values are shown as mean ± s.e. mean. For Aldo, 18-OH DOC, 18-OH B, and cortisol, $n = 5$; for DOC and B, $n = 4$.

		Day								
		4	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Aldo	S	7 ± 2	7 ± 2	7 ± 4	13 ± 6	20 ± 10	26 ± 10	33 ± 16	38 ± 19	39 ± 12
	A	10 ± 5	10 ± 6	23 ± 5	45 ± 22	54 ± 32	81 ± 38	109 ± 56	107 ± 44	104 ± 47
DOC	S	12 ± 4	12 ± 3	14 ± 6	14 ± 7	21 ± 6	23 ± 7	20 ± 1	18 ± 5	12 ± 3
	A	11 ± 9	10 ± 6	10 ± 6	11 ± 5	14 ± 5	14 ± 5	5 ± 7	14 ± 10	15 ± 4
18-OH DOC	S	14 ± 8	15 ± 8	16 ± 12	13 ± 7	13 ± 2	14 ± 5	22 ± 14	21 ± 10	12 ± 2
	A	11 ± 1	13 ± 4	13 ± 4	13 ± 3	12 ± 4	14 ± 6	12 ± 4	12 ± 6	13 ± 6
B	S	2.2 ± 1	2.6 ± 1	2.2 ± 2	2.5 ± 2	3.4 ± 3	2.7 ± 2	3.4 ± 2	4.1 ± 4	4 ± 3
	A	2.9 ± 2	3.7 ± 2	2.8 ± 2	4.5 ± 3	4.1 ± 3	3 ± 1	4.3 ± 3	5.5 ± 5	6 ± 6
18-OH B	S	14 ± 7	12 ± 6	10 ± 4	21 ± 10	44 ± 23	61 ± 31	74 ± 42	65 ± 45	67 ± 40
	A	17 ± 11	22 ± 16	42 ± 21	42 ± 21	96 ± 9.2	155 ± 29	137 ± 37	126 ± 27	149 ± 54
Cortisol	S	4 ± 1	3 ± 2	3 ± 1	4 ± 2	4 ± 1	4 ± 1	4 ± 1	5 ± 1	3 ± 1
	A	4 ± 1	4 ± 1	4 ± 1	3 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 2	5 ± 1

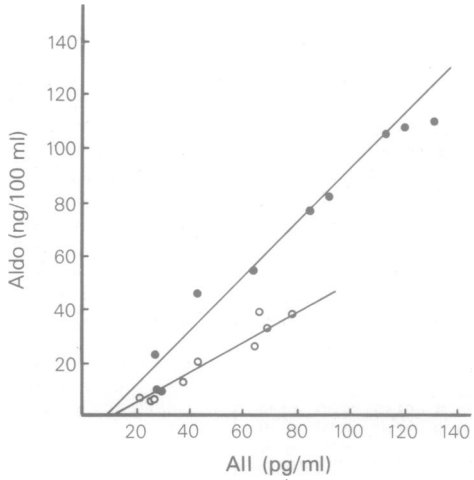

Figure 4 Regression lines for plasma aldosterone (Aldo, ng/100 ml) on angiotensin II (AII, pg/ml) in five normal subjects given amiloride (●) or spironolactone (○) for 7 days, calculated using mean daily values. For spironolactone treatment, $y = 8.16 + 0.61x$, $r = 0.975$, $n = 9$, $P < 0.001$. For amiloride, $y = 8.08 + 0.943x$, $r = 0.984$, $n = 9$, $P < 0.001$. The regression coefficients are significantly different ($t = 7.37$, d.f. = 14, $P < 0.01$).

present study. Our findings therefore suggest that maximum effects of amiloride on sodium excretion and potassium retention are greater than those of spironolactone. This suggestion is in agreement with *in vitro* studies (Knauf *et al.*, 1978).

The greater stimulation of active renin by amiloride was in keeping with the larger cumulative sodium loss, as were the higher values achieved for plasma angiotensin II and aldosterone. The rise in aldosterone with amiloride was probably also due in part to the greater increase in plasma potassium. The angiotensin II and aldosterone-stimulating effect of amiloride may be expected to limit both the effectiveness and possible toxicity due to hyperkalaemia of this agent in a manner similar to that previously documented for thiazide diuretics (Darracott-Vaughan *et al.*, 1978). Indeed, these endocrine responses may explain the absence of a fall in blood pressure in this study during the period of maximum natriuresis with amiloride.

Interpretation of the aldosterone levels after spironolactone is complicated by the additional inhibitory action of the parent drug or its metabolites on aldosterone biosynthesis (Erbler, 1972; Cheng *et al.*, 1976). This effect may explain the early decrease in aldosterone observed by some authors during treatment with spironolactone (Abshagen *et al.*, 1978) and the temporary appearance of 'spironolactone bodies' in the zona glomerulosa of treated animals and man (Conn & Hinerman, 1977). In this study we did not observe an early fall in aldosterone although a slight decrease in 18-hydroxycorticosterone levels after 2 days treatment was observed. In addition, the lower slope of the aldosterone on angiotensin II regression line with spironolactone is consistent with impaired mineralocorticoid synthesis. Conn & Hinerman (1977) inferred from the absence of spironolactone bodies in the zona fasciculata that the action of spironolactone was restricted to the zona glomerulosa. More direct evidence of such an effect has been provided in recent *in vitro* studies on duck and sheep adrenal (Aupetit *et al.*, 1978). Assuming that plasma levels reflect the rates of synthesis, our results may indicate a zone-specific inhibitory effect on 11β-hydroxylation, leading to the temporary increase in DOC which was observed during spironolactone treatment. This result is consistent with the possibility that the 11β-hydroxylases from the two zones are genetically distinct (Cheng *et al.*, 1976).

The effect of both drugs on inactive renin is in keeping with previous studies which suggest that circulating inactive renin is not stimulated in man by dietary salt depletion, treatment with thiazide diuretics or converting enzyme inhibitors, and infusion of angiotensin II. In this study active and total renin concentration changed in proportion at two levels of salt loss, supporting our previous conclusion that inactive renin is not released into the blood or consumed by activation during stimulation of active renin release by salt depletion, and is not an important precursor pool for plasma active renin (Millar *et al.*, 1980; Millar, 1982).

In summary, the maximal effects of amiloride on electrolyte balance and the renin-angiotensin-aldosterone system are greater than those of spironolactone. Previous dose-response studies using single doses of amiloride suggested that maximum effects on urine volume and sodium, chloride, and potassium excretion occurs with a dose of 40 mg (Lant *et al.*, 1969). In the light of these data and our previous long-term use of amiloride at a dose of 40 mg (Kremer *et al.*, 1977), we suggest that the maximum recommended daily dose of amiloride should be doubled. Hyperkalaemia was associated in this study with amiloride treatment, and higher doses of this drug may be associated with increased risk of toxicity due to this side effect. Due caution is therefore required when using higher doses of amiloride.

We thank Mr K. McIlroy for measurements of urine Na^+ and K^+, and Mrs R. Watt for preparing the special diets. Amiloride and spironolactone were provided by Thomas Morson Pharmaceuticals and G. D. Searle Ltd respectively.

References

Abshagen, U., Sporl, S., Schöneshöfer, M., L'age, M. & Oelkers, W. (1978). Interference of spironolactone therapy with adrenal steroid metabolism in secondary hyperaldosteronism. *Klin. Wochenschr.*, **56**, 341–349.

Aupetit, B., Duchier, J. & Legrand, J. C. (1978). Action des spironolactones sur la synthesise de l'aldosterone et sur le metabolisme surrénalien. *Ann. d'Endrocrinologie (Paris)*, **39**, 355–372.

Bangham, D. R., Robertson, I., Robertson, J. I. S., Robinson, C. J. & Tree, M. (1975). An international collaborative study of renin assay: establishment of the international reference preparation of human renin. *Clin. Sci. mol. Med.*, 48 (Suppl 2), 135s–159s.

Bull, M. B. & Laragh, J. H. (1968). Amiloride, a potassium-sparing natriuretic agent. *Circulation*, **37**, 45–53.

Casals-Stenzel, J., Schmalbach, J. & Losert, W. (1980). Acute effects of aldosterone antagonists in volunteers. In *Aldosterone antagonists in clinical medicine*, eds Addision, G. M. *et al*, pp. 207–216. Amsterdam: Excerpta Medica.

Cheng, S. C., Suzuki, K., Sadéc, W. & Harding, B. W. (1976). Effects of spironolactone, canrenone, and canrenoate-K^+ on cytochrome P450 and 11β and 18-hydroxylation in bovine and human adrenocortical mitochondria. *Endocrinology*, **99**, 1097–1106.

Conn, J. W. & Hinerman, D. L. (1977). Spironolactone-induced inhibition of aldosterone biosynthesis in primary aldosteronism: morphological and functional studies. *Metabolism*, **26**, 1293–1307.

Darracott-Vaughan, E., Carey, R. M., Peach, M. J., Ackerly, J. A. & Ayers, G. R. (1978). The renin response to diuretic therapy: limitation of antihypertensive potential. *Circ. Res.*, **42**, 376–380.

Davis, C. W. & Finn, A. L. (1982). Sodium transport inhibition by amiloride reduces basolateral membrane potassium conductance in tight epithelia. *Science*, **216**, 525–526.

Dusterdieck, G. & McElwee, G. (1971). Estimation of angiotensin II concentration in human plasma by radio-immunoassay. Some application to physiological and clinical states. *Eur. J. clin. Invest.*, **2**, 32–38.

Erbler, H. C. (1972). Stimulation of aldosterone production *in vitro* and its inhibition by spironolactone. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **273**, 366–375.

Kim, K. S., Morimoto, S., Uchida, K., Miyamori, I., Miyamoto, M. & Takeda, R. (1979). Decreased response of plasma aldosterone to acute ACTH stimulation during long-term treatment with spironolactone in essential hypertension. *Hormone Res.*, **11**, 4–11.

Knauf, H., Lübeke, R. & Wais, U. (1978). Potassium-retaining diuretics. A comparative study on their mechanisms of action. In *Aldosterone antagonists in clinical medicine*, eds Addison, G. M. *et al.*, pp. 70–76. Amsterdam: Excerpta Medica.

Kremer, D., Boddy, K., Brown, J. J., Davies, D. L., Fraser, R., Lever, A. F., Morton, J. J. & Robertson, J. I. S. (1977). Amiloride in the treatment of primary hyperaldosteronism and essential hypertension. *Clin. Endocrinol.*, **7**, 151–157.

Lant, A. F., Smith, A. J. & Wilson, G. M. (1969). Clinical evaluation of amiloride, a potassium-sparing diuretic. *Clin. Pharmac. Ther.*, **10**, 50–63.

McInnes, G. T. (1982). Relative potency of amiloride and spironolactone in healthy man. *Clin. Pharmac. Ther.*, **31**, 472–477.

McInnes, G. T., Perkins, R. M., Shelton, J. R. & Harrison, I. R. (1982). Spironolactone dose-response relationships in healthy subjects. *Br. J. clin. Pharmac.*, **13**, 513–518.

Mason, P. & Fraser, R. (1975). Estimation of 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone, corticosterone, cortisol, and 11-deoxycortisol in human plasma by gas-liquid chromatography with electron capture detection. *J. Endocrinol.*, **64**, 277–288.

Millar, J. A. (1982). Plasma active and inactive renin in man during infusion of angiotensin II with and without prior administration of nifedipine. *Clin. exp. Hypertension*, **4**, 2415–2424.

Millar, J. A., Hammat, M. & Johnston, C. I. (1980). Effect of converting enzyme inhibition on circulating inactive renin in salt replete and deplete normal subjects. *J. Endocrinol.*, **86**, 329–335.

Millar, J. A., Leckie, B. J., Morton, J. J. & Tree, M. (1980). A microassay for plasma renin concentration in human plasma based on antibody trapping. *Clin. Chim. Acta*, **101**, 5–15.

Morton, J. J., Semple, P. F., Waite, M. A., Brown, J. J., Lever, A. F. & Robertson, J. I. S. (1976). Estimation of angiotensin I and II in the human circulation by radioimmunoassay. In *Hormones in human blood*, ed. Antoniades, H. N., pp. 607–612. Cambridge, Mass: Harvard University Press.

Ramsay, L. E., Hettiarachchi, J., Fraser, R. & Morton, J. J. (1980). Amiloride, spironolactone, and potassium chloride in thiazide-treated hypertensive patients. *Clin. Pharmac. Ther.*, **27**, 533–543.

Sakauye, C. & Feldman, D. (1976). Agonist and antimineralocorticoid activities of spironolactone. *Am. J. Physiol.*, **231**, 93–97.

Tsai, R. & Morris, D. J. (1978). Effect of spironolactone on hepatic metabolism of aldosterone in male rats. *Endocrinology*, **103**, 1239–1244.

Wilson, A., Mason, P. A. & Fraser, R. (1976). Estimation of 18-hydroxycorticosterone concentration in human plasma by gas-liquid chromatography with electron captive detection. *J. steroid Biochem.*, **7**, 611–613.

(Received September 19, 1983, accepted April 27, 1984,)