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RELATIVE POTENCY AND STRUCTURE ACTIVITY RELATIONSHIPS OF ALDOSTERONE ANTAGONISTS IN HEALTHY MAN: CORRELATION WITH ANIMAL EXPERIENCE

G.T. McINNES, J.R. SHELTON, L.E. RAMSAY, I.R. HARRISON, M.J. ASBURY, J.M. CLARKE, R.M. PERKINS & G.R. VENNING

Division of Clinical Research, G.D. Searle & Co. Ltd, Lane End Road, High Wycome, Bucks HP12 4HL

1 The renal antimineralocorticoid potency of single doses of thirteen compounds with properties in animals compatible with competitive aldosterone antagonism was compared to that of spironolactone in healthy men.

2 Twelve compounds showed significant activity when compared to placebo but only one, prorenoate potassium, was significantly more potent than spironolactone on a weight basis.

3 The results allowed ranking of the compounds in order of potency relative to spironolactone and general observations on structure activity relationships in man.

4 Animal bioassays and *in vitro* aldosterone binding studies are unreliable predictors of the human activity of competitive mineralocorticoid antagonists.

Introduction

Spironolactone is a competitive aldosterone antagonist (Kagawa, 1960a; Drill, 1962) which is used in the treatment of hypertension and in conditions where there is salt and water retention (Ochs *et al.*, 1978). Since its introduction numerous related structures have been synthesised and their renal antimineralocorticoid activity has been studied in animals. However, few compounds have been assessed in man. Thus the hypothesis that the potency of a competitive mineralocorticoid antagonist in animal laboratory screening reflects its potency in human subjects is largely untested.

For two compounds, canrenoate potassium and prorenoate potassium, studies in rats and dogs did not predict reliably the potency observed in healthy man (Ramsay *et al.*, 1975a, 1976). We have now examined by standardised methods in healthy men, the pharmacological activity of 13 structures related to spironolactone and showing antimineralocorticoid activity in animals. We use these data to explore structure activity relationships in man, and to determine the predictive value of screening methods using laboratory animals, both *in vivo* and *in vitro*.

Methods

The methods used in these studies closely followed those described by Ramsay *et al.* (1975a, 1976) and McInnes *et al.* (1980) which in turn evolved from earlier work by Ross (1962), who showed that single doses of the orally active synthetic mineralocorticoid, fludrocortisone, given in the evening, consistently depressed the ratio of sodium to potassium in the overnight urine of normal men with a dose-response which exhibited parallelism with that of aldosterone. The ratio returned towards normal in the presence of competitive mineralocorticoid antagonists by virtue of an increase in sodium excretion and a decrease in potassium excretion. Use of these parameters and functions of them allows comparison of the activity of mineralocorticoid antagonists and an estimate of their potency relative to a standard, spironolactone.

Study designs

The experiments were carried out according to double-blind, randomised, balanced crossover designs. Eleven drugs were examined in three-phase studies, the treatments compared being new aldosterone antagonist, spironolactone and placebo. Four were examined in six-phase studies, the treatments being the new aldosterone antagonist and spironolactone, each at three dose levels, the intention being to yield two drug, three dose parallel line bioassays in each instance. Two drugs were studied in both methods. The phases of the studies were separated by 1 week intervals.

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Subjects

A total of 114 male subjects considered to be healthy after medical history, physical examination and laboratory screening, were studied, six or twelve volunteers taking part in each experiment. All forms of medication were prohibited from 1 week before the studies until their completion. Salicylates were specifically forbidden since they are known to interfere with the urinary electrolyte response to spironolactone (Tweeddale & Ogilvie, 1973). Alcohol was not allowed from 24 h before each medication until the urine collections were completed.

Procedure

Until 17.30 h on study days, the subjects continued normal activities and diet. At 19.00 h fludrocortisone was taken and in the next 30 min, a prescribed light meal, identical on all occasions, was consumed. At 21.00 h, the test medication was administered with a volume of water constant within a given study and a further constant volume of water was taken at 23.00 h. All urine passed between 23.00 h and 07.00 h the next day was collected. In the three-phase studies, a further 0.5 mg fludrocortisone was taken immediately thereafter and the subjects ate a prescribed light breakfast. Urine was again collected from 09.00 h until 13.00 h. This modification of the basic procedure allows examination of the later phases of activity of the antagonists (McInnes *et al.*, 1980; Ramsay *et al.*, 1975a). Urine and venous blood for laboratory screening, including urinalysis, blood urea, electrolytes, liver function tests and haematology, were sampled 12 h before and after each medication and 1 week after completion of each study. All screening was at 09.00 h to avoid possible effects of diurnal variation. General enquiry for possible side effects was made 12 h and 1 week after each treatment.

Drugs

The compounds studied were spirolactones or closely related soluble potassium salts of steroid acids (Figure 1). Full chemical names are given in the Appendix.

Three-phase studies

1. New aldosterone antagonist, 50 mg chemical in an opaque gelatin capsule plus placebo spironolactone.
2. Spironolactone, 50 mg (2 × 25 mg Aldactone tablets) plus placebo capsule.
3. Double placebo
   Fludrocortisone (Florinef, Squibb), 0.5 mg (5 × 0.1 mg tablets) at 19.00 h and 07.00 h the next day.

Six-phase studies

1. New aldosterone antagonist:
   (a) SC23992: 10 mg, 20 mg and 30 mg (10 mg tablets) plus placebo spironolactone
   (b) SC27169: 50 mg, 100 mg and 200 mg (50 or 100 mg chemical in opaque gelatin capsules) plus placebo spironolactone
   (c) SC14266: 100 mg, 150 mg and 200 mg (in 300 ml aqueous solution)
   (d) SC8109: 250 mg, 500 mg and 1000 mg (125 mg chemical in opaque gelatin capsules) plus placebo spironolactone
2. Spironolactone 25 mg, 50 mg and 100 mg* (25 mg tablets)† plus placebo new aldosterone antagonist
   *In study of SC23992: 75 mg
   †In study of SC14266: spironolactone in 300 ml aqueous suspension
   Fludrocortisone 0.5 mg (5 × 0.1 mg tablets)—SC23992 and SC8109 studies or 1 mg (1 × 1 mg tablets)—SC27169 and SC14266 studies.

Laboratory

Urine was collected without preservatives and after measurement of volume, an aliquot was stored at −20°C. Urinary sodium and potassium concentrations were measured by atomic absorption spectrophotometry. Samples were batched by study phase for laboratory analysis. Screening tests were by conventional laboratory procedures.

Statistical analysis

The statistical methods employed are described by Armitage (1973). The antagonist treatment effects were compared by analysis of variance, allowing for subject and phase effects in the linear model. Logarithmic transformation was performed to stabilise the variance of the ratio variable (10 × Na/K) and hence validate the analysis of variance, with the additional advantage of linearising the dose-response relationship.

In the three-phase experiments, where there was an overall difference between the treatments, the Newman-Keuls (Studentized Range) multiple comparison technique was used to test:

1. Activity of new aldosterone antagonist (ν placebo).
2. Activity of spironolactone (ν placebo).
3. New aldosterone antagonist ν spironolactone.

In the 2–10 h collection, the slope of the log spironolactone dose-$\log_{10}$ 10 Na/K response relationship derived from previous studies was used to estimate the potency of each new aldosterone antagonist relative to spironolactone, with 95% confidence limits. This estimation requires the assumptions of a linear

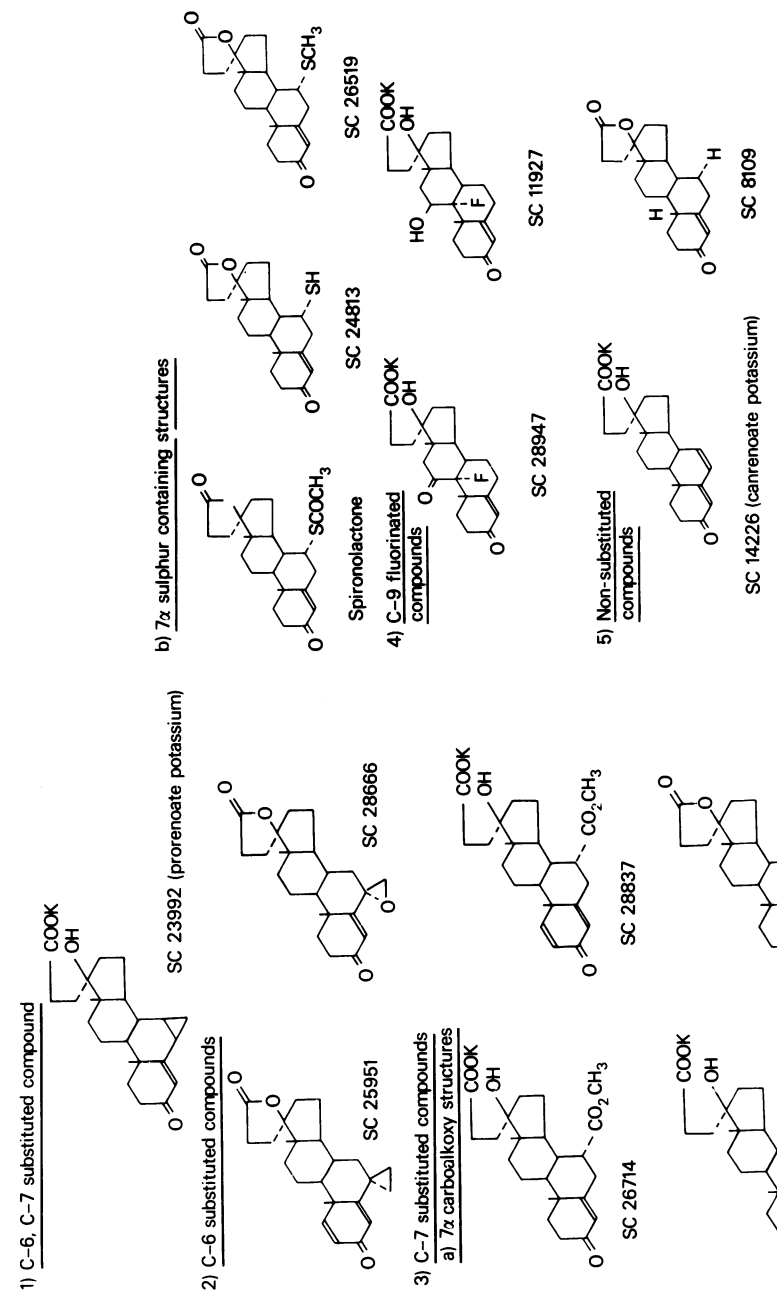

Figure 1 Structures of the steroidal aldosterone antagonists.

log dose-response relationship for the new aldosterone antagonist, which is not unreasonable in the presence of significant activity compared with placebo, and parallelism with the spironolactone relationship. This approach has been described in detail (McInnes *et al.*, 1981a).

In the six-phase studies, the significance of the linear log dose-$\log_{10}$ 10 Na/K response trends for the new aldosterone antagonists and spironolactone were tested and their slopes compared. Provided the slopes were significant and there was not significant contra-indication to either linearity of parallelism, the validity of the parallel line bioassay was accepted and the potency of the new aldosterone antagonists relative to spironolactone were estimated with 95% confidence limits.

Data for four compounds have been published previously (McInnes *et al.*, 1980; Ramsay *et al.*, 1975a, 1976).

Results

Three-phase studies

Urine electrolytes between 2–10 h after treatment When compared to placebo values, following spironolactone and the new aldosterone antagonists, urinary sodium excretion was higher, potassium excretion was lower except after SC28666 and the ratio $\log_{10}$ 10 Na/K was increased. The mean $\log_{10}$ 10 Na/K results are given in Table 1. There were highly significant increases in urinary $\log_{10}$ 10 Na/K after all the new aldosterone antagonists ($P < 0.05$ or $P < 0.01$), with the exception of SC28666, and after spironolactone ($P < 0.05$–$P < 0.01$). The $\log_{10}$ 10 Na/K response to spironolactone was significantly greater than that to SC14266, SC26519, SC24813 (all $P < 0.01$), SC26714, SC28837, SC30822, SC11927 and SC28666 (all $P < 0.05$). There were no significant differences between spironolactone and the other drugs tested.

Urine electrolytes between 12–16 h after treatment The sodium and potassium responses followed the expected pattern. The mean results for $\log_{10}$ 10 Na/K are shown in Table 1. Significant increases in the ratio $\log_{10}$ 10 Na/K were observed after SC25951, SC14266 (both $P < 0.01$), SC27169, SC26519 (both $P < 0.05$) and spironolactone in six experiments (P 0.05–$P <$ 0.01). Significant differences in $\log_{10}$ 10 Na/K responses were noted between spironolactone and SC25951 ($P < 0.05$), favouring SC25951 and between spironolactone, SC24813 ($P < 0.01$) and SC28666 ($P < 0.05$), both favouring spironolactone. No other significant differences between the drugs were noted in this period.

Approximate potency of new aldosterone antagonists relative to spironolactone Using urinary $\log_{10}$ 10 Na/K in the 2–10 h collection as response, the potency of the test drugs relative to spironolactone, with 95% confidence limits, were calculated from the data presented in Table 1. The estimates are shown in Table 2. The potency estimates ranged from 1.10 to 0.20. Four of the compounds SC25951, SC26714, SC27169 and SC28947 had potency not significantly different from that of spironolactone (confidence limits bracketed unity) but the remaining seven agents were significantly less potent than spironolactone and indeed SC28666 had no demonstrable antimineralocorticoid activity in this model.

Six-phase studies

The criteria necessary for a valid estimate of relative potency in this experimental model are:

1. that the log dose-response trend for each drug does not deviate significantly from linearity;
2. that the slope of the log dose-response trend for each drug (or the average of the two drugs) is significantly different from zero;
3. that the log dose-response trends for the two drugs do not deviate significantly from parallelism.

The criteria were satisfied for all the drugs tested using urinary $\log_{10}$ 10 Na/K, the best single index of response in studies of this type. The mean log dose-urinary $\log_{10}$ 10 Na/K response trends for the new aldosterone antagonists and spironolactone are shown in Figure 2. The estimate of potency of each compound relative to the standard, spironolactone, using this response variable, and the 95% confidence limits for each estimate, are given in Table 2. These potency estimates ranged from 2.69–0.08. Judging from the confidence intervals, SC23992 was significantly more potent and the other three drugs significantly less potent than spironolactone. The confidence intervals were such as to allow clear ranking of the compounds in order of potency: SC23992 > spironolactone > SC27169 > SC14266 > SC8109.

Laboratory screen results

Clinically significant laboratory abnormalities were encountered only in the studies involving SC25951 and SC26714. In the former experiment, each subject showed an increase in serum glutamic pyruvic transaminase (SGPT) level 1 week after treatment with SC25951 and in one case a value (70 iu/ml) above the normal range (5–35 iu/ml) was seen. A similar trend for serum glutamic oxalacetic transaminase (SGOT) was apparent. In the study of SC26714, one volunteer had elevated values of SGPT (38–51 iu/ml) from 7 to 16 days after treatment with that drug. No such changes were seen after spironolactone or placebo.

Table 1 Mean ± s.e. mean for urinary $\log_{10}$ 10 Na/K between 2–10 h and 12–16 h after test medication ($n = 6$)

	Urine $\log_{10}$ 10 Na/K					
	2–10 h			*12–16 h*		
	Placebo	*Spironolactone*	*New drug*	*Placebo*	*Spironolactone*	*New drug*
SC25951	0.84 ± 0.08	1.18 ± 0.04***	1.20 ± 0.06***	0.51 ± 0.10	0.77 ± 0.06*	1.04 ± 0.08***††
SC26714[1]	0.65 ± 0.07	1.37 ± 0.04***	1.16 ± 0.09***††			
SC27169	0.80 ± 0.07	1.39 ± 0.11***	1.28 ± 0.05***	0.40 ± 0.11	0.73 ± 0.08**	0.64 ± 0.04**
SC28947	0.87 ± 0.12	1.34 ± 0.06***	1.18 ± 0.07***	0.68 ± 0.17	0.91 ± 0.12	0.77 ± 0.13
SC28837	1.10 ± 0.11	1.47 ± 0.07***	1.31 ± 0.11**††	0.84 ± 0.14	0.96 ± 0.14[2]	0.85 ± 0.13
SC30822	0.78 ± 0.05	1.28 ± 0.04***	1.13 ± 0.07***††	0.48 ± 0.14	0.78 ± 0.07**	0.67 ± 0.09
SC14266[3]	0.64 ± 0.08	1.42 ± 0.10***	0.99 ± 0.07***†††	0.52 ± 0.08	0.97 ± 0.09***	0.86 ± 0.10***
SC26519[4]	0.78 ± 0.09	1.31 ± 0.07***	1.08 ± 0.06***†††	0.50 ± 0.08	0.79 ± 0.07***	0.68 ± 0.06**
SC24813			1.02 ± 0.08***†††			0.54 ± 0.06†††
SC11927	0.69 ± 0.17	1.24 ± 0.08***	0.96 ± 0.08**††	0.42 ± 0.15	0.79 ± 0.05	0.64 ± 0.09
SC28666	0.93 ± 0.12	1.36 ± 0.13**	1.02 ± 0.13††	0.64 ± 0.03	0.94 ± 0.08***	0.78 ± 0.07††

Significantly different from placebo $*P = 0.05$; $**P < 0.05$; $***P < 0.01$
Significantly different from spironolactone $\dagger P = 0.05$; $\dagger\dagger P < 0.05$; $\dagger\dagger\dagger P < 0.01$
[1]SC26714 50 mg (5 × 10 mg tablets) compared to spironolactone 100 mg (4 × 25 mg tablets)
Fludrocortisone 1 mg (1 × 1 mg tablet) at 19.00 h. No extended urine collection.
[2]One estimated missing value.
[3]SC14266 95 mg in 200 ml aqueous solution compared to spironolactone 100 mg in 200 ml aqueous suspension.
Fludocortisone 1 mg (1 × 1 mg tablet) at 19.00 h ($n = 12$).
[4]SC26519 and SC24813 compared to spironolactone and placebo in one experiment using 12 volunteers.

Table 2 Estimates of the single dose potency of thirteen new aldosterone antagonists relative to spironolactone for renal antimineralocorticoid activity in man (with 95% confidence limits) and animals using urinary $\log_{10}$ 10 Na/K as response and estimates of relative binding affinity for aldosterone receptors *in vitro*

	Human	*Animal*			*Rat kidney aldosterone*
Compound		*Monkey*	*Dog*	*Rat*	*receptor binding*
SC23992	2.69 (2.08–3.52)*	3.09	3.04[1]	4.60–8.09[1]	0.1[5]
SC25951	1.10 (0.54–2.26)			4.25	0.4[6]
SC26714	0.67 (0.12–1.92)	0.65	2.14–2.45	4.17–5.64	
SC27169	0.60 (0.24–1.42)	1.58	1.18	3.86–11	0.2[6]
	0.61 (0.48–0.79)*				
SC28947	0.47 (0.18–1.14)	4.10			
SC28837	0.47 (0.21–0.99)			7.06	
SC30822	0.47 (0.22–0.95)			5.8	
SC26519	0.33 (0.15–0.62)	0.12		1.06[2]	2[6]
SC11927	0.26 (0.07–0.86)	1.87			
SC24813	0.26 (0.12–0.49)	0.58	0.70[2]	0.06	2[6]
SC28666	0.20 (0.05–0.69)	1.05		0.88	0.52 –0.86
SC14266	0.11 (0.04–0.22)	0.66	1.26[1]	0.90[3]	0.025–0.04[6, 7]
	0.31 (0.23–0.40)*				
SC8109	0.08 (0.04–0.13)*			0.2[4]	1.19 –1.33[6, 7]

*Six-phase studies
[1]Hofmann *et al.* (1975)
[2]Hofmann (1974)
[3]Kagawa *et al.* (1964)
[4]Kagawa (1960a)
[5]Funder *et al.* (1976)
[6]Funder *et al.* (1974)
[7]Sakauye & Feldman (1976)
Unreferenced animal results—unpublished data, G.D. Searle & Co.

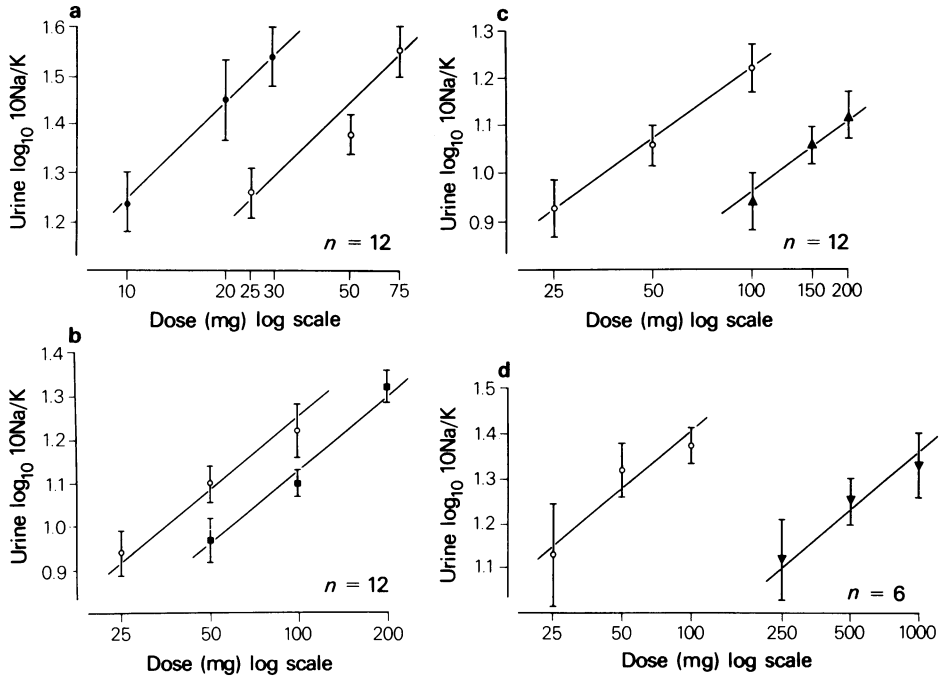

Figure 2 Mean log dose-$\log_{10}$ 10 Na/K response curves for spironolactone (○) and (a) prorenoate potassium (●). (b) SC27169 (■), (c) canrenoate potassium (▲), (d) SC8109 (▼). Mean (vertical bars s.e. mean) results.

Discussion

In the three-phase studies, eleven compounds including spironolactone, showed significant antimineralocorticoid activity when compared to placebo and five agents, SC25951, SC27169, SC14266, SC26519 and spironolactone, exhibited activity for up to 16 h after administration of single doses. Only SC28666 had no significant renal antimineralocorticoid activity. Although each drug was administered at only one dose level, it was possible to estimate their potencies relative to spironolactone using an approach previously described (McInnes *et al.*, 1980, 1981a). The variable used in these estimations, urinary $\log_{10}$ Na/K, is generally considered to be the best single index of mineralocorticoid (Johnson, 1954) and antimineralocorticoid activity (Gantt & Dyniewicz, 1963; Kagawa, 1964).

The potency of four compounds relative to spironolactone was more precisely defined using multiple dose level comparisons (Ramsay *et al.*, 1975a, 1976). The convincing parallelism of the dose-response curves of spironolactone and these compounds (Figure 2) indicated that the drugs have qualitatively similar pharmacological activity and suggested that these agents also behaved as competitive mineralocorticoid antagonists at renal level. Results for the two drugs studied in both methods suggest that the assumptions implicit in the first approach were valid. Relative potencies in the three phase and six phase studies respectively were 0.11, 0.31 (canrenoate potassium: spironolactone) and 0.60, 0.61 (SC27169: spironolactone).

These estimates allowed us to rank the compounds according to their potency relative to spironolactone in man (Table 2). Where compounds were examined by both experimental methods, the rank order of potency relative to spironolactone was maintained offering some support for the overall rank order. Only prorenoate potassium, was significantly more potent than spironolactone; SC25951, SC26714 and SC28947 had potency not significantly different from that of spironolactone and all the other compounds tested were significantly less potent than spironolactone.

The efficacy of this group of compounds in antagonising the urinary effects of mineralocorticoids has been examined in animals using similar methodology (Kagawa, 1960a; Kagawa *et al.*, 1964; Hofmann, 1974; Hofmann *et al.*, 1975; Weier & Hofmann, 1976; Hofmann *et al.*, 1977). The aldosterone antagonists were administered orally or intragastrically to adrenalectomised rats, intact dogs and monkeys. The agonist, aldosterone, or deoxy-

corticosterone acetate in most rat experiments, was given by intramuscular injection and the response variable was urinary $\log_{10}$ 10 Na/K. The results of quantitative comparisons with spironolactone are summarised in Table 2. In some animal models, the results show poor reproducibility and where confidence limits for potency estimates have been quoted, it is clear that the sensitivity or precision of the assay is often inadequate. It is encouraging in this respect, that the human assay performs relatively well, allowing the estimates to be treated with some confidence.

Investigation of the relationship between the results of different screening models, with only a small number of compounds tested in the screening systems, should be approached with caution. However, at best, only a very weak correlation between animal and human experience can be observed. A likely reason for this is that the pharmacokinetic characteristics of these compounds vary from species to species (Karim *et al.*, 1976). Experience to date indicates that the single dose rank order in man is confirmed in chronic dosing stuides in healthy men (Ramsay *et al.*, 1977; McInnes *et al.*, 1981b). The limitations of studies of this nature have been discussed elsewhere (Ramsay *et al.*, 1975a, 1975b; 1977) and it remains to be seen how well clinical performance is predicted.

The results of *in vitro* studies of the affinity of these compounds for rat renal aldosterone receptors which have binding activities similar to the corresponding human receptors (Matulich *et al.*, 1976) show weak correlation with human experience *in vivo* (Table 2). Three compounds exhibited greater affinity than spironolactone but had weak antimineralocorticoid activity in man. One of these agents SC8109 has properties in animals consistent with partial mineralocorticoid agonism (Kagawa, 1960b) and receptor binding studies cannot differentiate between antagonist and agonist activity (Funder *et al.*, 1974; Sakauye & Feldman, 1976). Compounds with poor affinity are weak agonists but are likely antagonists (Raynaud *et al.*, 1975) further complicating the interruption of these studies. *In vitro* studies take no account of drug absorption, disposition and metabolism, and steroidal aldosterone antagonists might be expected to undergo extensive biotransformation to active metabolites. Indeed, the corresponding spirolactones, which are predicted major metabolites of potassium salts studied here have better binding characteristics than the open ring structures (Funder *et al.*, 1974; Funder *et al.*, 1976; Sakauye & Feldman, 1976; Claire *et al.*, 1979). Thus the results of these *in vitro* studies should be considered only as screening tests for structures with possible properties of competitive aldosterone antagonism and cannot be expected to predict accurately anti-aldosterone potency.

Although we evaluated the largest group of steroidal aldosterone antagonists to be studied in man, it represents a small sample for the examination of structure activity relationships. This difficulty was exaggerated since most of the agents had similar potencies with confidence limits showing considerable overlap and the structures were not always readily comparable. The influence on potency of opening the lactone ring could not be assessed but since conversion of potassium salts to the corresponding spirolactones is expected to be a major pathway of biotransformation, relationships were considered regardless of the integrity of the lactone ring. This allowed some general observations on structure activity relationships in man:

1. A cyclopropyl linkage between C-6 and 7 in the C-1 unsaturated structure (SC23992) endowed greatest activity.
2. Of the C-1 unsaturated structures, 6-spirocyclopropyl substitution (SC25951) seemed to endow greater activity than the 7α carboalkoxy ester (SC28837) although the differences were insignificant, statistically.
3. The influence of ring saturation on activity was seen in the comparison of the corresponding 7α carboalkoxy esters, SC26714 (4-ene) and SC28837 (1-4 diene). The 4-ene structure was more potent but again the confidence limits showed considerable overlap. Animal work had suggested that insertion of a double bond at C-1 increased oral potency (Cella & Tweit, 1959).
4. 7α carboalkoxy substitution endowed moderate activity but the similarity in the potency estimates of the four such compounds studied does not allow us to comment on the influence of individual side chains. 6β hydroxylation (SC30822) of the lactone corresponding to a 7α carboalkoxy ester (SC27169) did not significantly affect potency.
5. Structures with sulphur containing moeities at the 7α position, spironolactone and its presumed intermediate metabolites (SC24813 and SC26519), showed moderate activity but 7α thio- and 7α thiomethyl-spirolactone were significantly less potent than the parent drug.
6. 11 dioxo-substutition (SC28947) endowed greater activity than 11β hydroxylation (SC11927) but the difference was statistically insignificant.
7. SC14266, the potassium salt of the open lactone corresponding to canrenone, quantitatively the major metabolite of spironolactone, was significantly less potent than spironolactone itself.
8. SC8109. the 19-nor derivative of the C-6 saturated ring structure corresponding to canrenone showed significant activity but was much less potent than spironolactone or SC14266.
9. 6β cyclopropyl structures exhibited variable activity and 9α fluorination had no clear influence on activity suggesting that these substituents may be of limited importance in determining activity. 6-spiroepoxide structures have little if any activity.

The low potency of SC11927 contrasts with earlier experience of its oral activity in reversing the urinary electrolyte responses to fludrocortisone in healthy men (Noel & Leahy, 1962; Ross, 1962). However, an important difference between these studies and ours was that antagonists were administered repeatedly over several days before challenge with the agonist. The relative potency relationship found after single doses of anti-mineralocorticoids may not necessarily hold after chronic dosing (Ramsay *et al.*, 1977; McInnes *et al.*, 1981b), possibly as a consequence of the pharmacokinetic properties of the antagonists. At present there is insufficient information on these aspects of the pharmacology of SC11927 in man to ascertain whether the disparities alluded to above can be explained in such terms. SC8109 was among the first steroidal aldosterone antagonists used clinically (Liddle, 1957), and the available data suggested a potency relative to the present formulation of spironolactone of about 1 : 10 in favour of the latter drug (Drill, 1962). Thus the estimate of relative potency (SC8109: spironolactone, 0.08 with 95% confidence limits of true potency of 0.04–0.13) derived from the experiment reported here shows excellent agreement with clinical experience, lending support to the validity of the human volunteer model employed by us to study aldosterone antagonists.

The apparent hepatoxicity induced by SC25951 and SC26714 may have been structurally related, the potassium salt of the open lactone corresponding to SC25951 having been implicated in the development of similar enzyme changes (McInnes *et al.*, unpublished data). It is disappointing that two of the most potent compounds tested should produce these effects.

In summary, we have studied the renal antimineralocorticoid activity in man of single oral doses of 13 compounds with properties compatible with competitive aldosterone antagonism in animals. Our results allowed us to rank the agents in order of potency relative to spironolactone and to gain information on structure activity relationships in man. There was poor correlation with the results of animal experiments using similar methodology and *in vitro* aldosterone receptor binding studies. In particular animal bioassays completely failed to reflect the rank order or potency in man leading us to conclude that this study has shown beyond reasonable doubt that estimates of renal anti-aldosterone potency in rats, dogs and monkeys have unreliable predictive value for man.

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Appendix

1. *C-6, C-7 substituted compound*
   6β, 7β cyclopropyl structure, prorenoate potassium (SC23992): 17-hydroxy-3-oxo-6β, 7β-methylene-17α-pregn-4-ene-21-carboxylic acid, monopotassium salt.
2. *C-6 substituted compounds*
   (i) C-1 unsaturated 6-spirocyclopropyl (SC25951): 17-hydroxy-3-oxo-6, 6-spirocylcopropyl-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
   (ii) 6-spiroepoxide (SC28666): (6R)-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone-6-spiro-2-oxiran.
3. *C-7 substituted compounds*

(a) *7α carboalkoxy structures*
   (i) Methyl ester (SC26714): 17-hydroxy-3-oxo-17α-pregn-4-ene-7α, 21-dicarboxylic acid, 7-methyl ester, monopotassium salt.
   (ii) C-1 unsaturated methyl ester (SC28837): 17-hydroxy-3-oxo-17α-pregn-1, 4-diene-7α, 21-dicarboxylic acid, 7-methyl ester, monopotassium salt.
   (iii) Isopropyl ester (SC27169): 17-hydroxy-3-oxo-17α-pregn-4-ene-7α, 21-dicarboxylic acid, 7-(1-methylethyl) ester, monopotassium salt.
   (iv) 6β hydroxylated isopropyl ester (SC30822): 17, 6β-dihydroxy-3-oxo-17α-pregn-4-ene-7α, 21-dicarboxylic acid, 7-(1-methylethyl) ester, monopotassium salt.

(b) *7α sulphur containing structures*
   (i) Spironolactone: 7α-acetylthio-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
   (ii) 7α thio-spirolactone (SC24813): 17-hydroxy-3-oxo-7α-thiol-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
   (iii) 7α thiomethyl-spirolactone (SC26519): 17-hydroxy-3-oxo-7α-thiomethyl-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.

4. *C-9 fluorinated compounds*
   (i) SC28947: 9α-fluoro-17-hydroxy-3, 11-dioxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
   (ii) SC11927: 9α-fluoro-11β, 17-dihydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
5. *Non-substituted compounds*
   (i) Canrenoate potassium (SC14266): 17-hydroxy-3-oxo-17α-pregn-4, 6-diene,21-carboxylic acid, monopotassium salt.
   (ii) C-10 demethylated (19-nor) structure (SC8109): 17-hydroxy-3-oxo-19-nor-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.

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