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STRUCTURE–ACTIVITY RELATIONSHIP OF NEW STEROIDAL ALDOSTERONE ANTAGONISTS

COMPARISON OF THE AFFINITY FOR MINERALOCORTICOID RECEPTORS *IN VITRO* AND THE ANTIALDOSTERONE ACTIVITY *IN VIVO*

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Abstract—The ability of 18 steroids with structures similar to spironolactone, progesterone or aldosterone to compete with [^{3}H]aldosterone for binding at rat renal cytosol receptors *in vitro* and the antialdosterone activity *in vivo* were tested in comparison with spironolactone. The affinity of these compounds for mineralocorticoid receptors was then compared with their pharmacological action in rats. Replacement of the 17-spirolactone ring by a 17α-hydroxypropyl group and a 17β-hydroxyl group resulted in a loss of affinity for the [^{3}H]aldosterone binding sites but not in a reduction in antialdosterone activity *in vivo*. Compared to spironolactone, C_6/C_7 unsaturated compounds showed a reduced activity both *in vitro* and *in vivo*. Substitution of the 7α-thioacetyl group of spironolactone by a 6,7-methylene group in the β position (prorenone) increased the affinity to the receptor as well as the biological activity by 52 and 41%, respectively. Introduction of a methyl-group at the D-ring of spironolactone resulted in similar significant drops in activity both *in vitro* and *in vivo*. The progesterone like steroids were weak competitors for aldosterone *in vitro* and *in vivo*. Two of three aldosterone like steroids (18-deoxy-aldosterones) still exhibit mineralocorticoid activity and one analogue could be classified as a weak aldosterone antagonist. The studies show in general that the comparison of the affinity for mineralocorticoid receptor sites with the antimineralocorticoid activity *in vivo* is a valuable procedure in the search for new antimineralocorticoid substances.

The biological action of potential antimineralocorticoid substances is usually tested in expts on adrenalectomized animals *in vivo*. Under these conditions the antimineralocorticoid effect of the substances to be studied is determined on the basis of various factors: (a) bioavailability, (b) affinity for the receptors and ratio of agonistic to antagonistic action at the target cell, and (c) metabolization rate and activity of the metabolites. The decisive factor for the natriuretic and potassium-retaining action of antimineralocorticoid drugs is the blocking of the mineralocorticoid receptors at the distal part of the renal tubules.

The intracellular activity of aldosterone and other mineralocorticoids in the tubular cells is initiated by the binding of these steroids to specific cytoplasmic receptor proteins. Previous studies have shown that the antimineralocorticoid effect of spironolactone is based on a competitive antagonism at the intracellular aldosterone-binding receptors [1–3]. *In vitro* investigations with renal cytoplasmic mineralocorticoid receptors can thus be used to test how changes in the molecular structure of spironolactone and other known antimineralocorticoid steroids influence the affinity for the receptor proteins in the cytoplasm.

These results could facilitate the design and synthesis of new specific and potent aldosterone antagonists. Furthermore, the comparison with the aldosterone-antagonistic action of these derivatives *in vivo* should show to what extent the affinity for the receptor proteins *in vitro* correlates with the mineralocorticoid-antagonistic activity *in vivo* and whether determination of the receptor affinity *in vitro* can be incorporated in the screening of new potential antimineralocorticoids.

METHODS

In vitro *experiments: binding to renal cytoplasmic mineralocorticoid receptors (MCR).* Male Sprague–Dawley rats (body weight 180–200 g) were adrenalectomized under phenobarbitone anaesthesia 4 days prior to the actual mineralocorticoid receptor test, and were given 1% sodium chloride solution to drink and fed Purina rat diet. On the day of the test, 3–4 rats were anaesthetized with phenobarbitone (5 mg/100 g body weight, given i.p.) and exsanguinated by the carotid artery. The kidneys were perfused with ice-cold buffer (0.17 M NaCl, 0.25 M K_2HPO_4, at pH 7.4) via vena cava; the kidneys were then removed and homogenised in Tris buffer (0.1 M Tris, 3 mM $CaCl_2$, at pH 7.4). The homogenate was centrifuged for 45 min at 30,000 *g*. The supernatant (cytosol) was taken for further incubation immediately afterwards.

[^{3}H]Aldosterone (2.5 nM) and a number of unlabelled steroids in increasing concentrations were

incubated together with 500 μl of cytosol for 1 hr at 23°. This concentration of [^{3}H]aldosterone represents the range of the Scatchard analysis, in which aldosterone was predominantly bound to Type I binding sites [4].

Protein bound [^{3}H]aldosterone was separated from the free activity by passage through a Biogel P-10 column (0.5 × 10 cm) which had been previously equilibrated with Tris buffer. The void vols. were collected in scintillation vials. The activity in the protein fraction was measured in the scintillation counter after addition of 10 ml of aquasol (Tricarb Model 3385, Packard Co.).

[^{3}H]Aldosterone which was non-specifically protein bound was determined by incubating [^{3}H]aldosterone (2.5 nM) together with unlabelled aldosterone at a 1000-times higher concentration. This 'non-specifically bound fraction' of the total protein bound [^{3}H]aldosterone accounted for about 10–20% and was subtracted from the total bound [^{3}H]aldosterone. The inhibitory effect of a non-labelled steroid on the binding of [^{3}H]aldosterone to the receptor sites was calculated from the following relation:

$$\frac{\text{specific bound [}^3\text{H]aldosterone in presence of the substance} \times 100}{\text{specific bound [}^3\text{H]aldosterone in absence of the substance}}.$$

The affinity for aldosterone receptors of the various substances investigated was compared with that of spironolactone in each incubation sample. The relative affinity, referred to that of spironolactone (which was taken to be 100) was established by the following relation:

$$\frac{\text{concentration of spironolactone at 50\% displacement} \times 100}{\text{concentration of reference substance at 50\% displacement}}.$$

For each component and concentration, at least two determinations were performed in duplicate. The method used is similar to that described previously [5, 6].

In vivo *experiments: antialdosterone activity in rats.* The method used for evaluation of the antialdosterone activity in rats was described previously [7]. Adrenalectomized Wistar rats with a body weight of 140–160 g were substituted with 1 mg fluocortolone caproate/kg at the day of surgery and 10 mg fluocortolone/kg s.c. 1 day before the diuresis expt. These glucocorticoid-substituted rats were infused intravenously with a saline–glucose solution (0.05% NaCl; 5% glucose) containing aldosterone (50 μg/l.) at a rate of 3 ml/hr for 10 or 15 hr. The aldosterone antagonist was administered 1 hr before the start of the aldosterone infusion. Urine excretion was measured in fractions of 1 hr. Sodium and potassium concentrations in urine were determined by flame photometry. The antialdosterone activity was assessed by the ability of the compounds to reverse the aldosterone effect on the urinary Na/K ratio.

Substances which showed antialdosterone activity in a preliminary test were examined in the diuresis expt over a period of 10 or 15 hr, to determine their relative potency in comparison to spironolactone after oral administration. The various steroids and spironolactone were administered at oral doses of 6.7, 13.4 and 26.8 mg/kg. The dose–response relationship was tested for each fraction (hr) by regression analysis after logarithmic transformation of the doses. The relative potency of a steroid, compared to spironolactone was established by comparing linear and parallel dose–response curves. The potency of the standard substance, spironolactone, was allocated the value of 100. The maximum relative potency (MRP) achieved by the investigated steroid vs spironolactone during the long-term expt is specified in Tables 1 and 2.

Substances. d-Aldosterone (Aldocorten®) was from Ciba-Geigy. The following compounds were synthesized by Schering Aktiengesellschaft, Berlin/Bergkamen: fluocortolone (caproate): 6α-fluor-21,11β-dihydroxy-16-methyl-1,4-pregnadiene-3,20-dione(21-caproate); spironolactone: 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)-propionic acid γ-lactone; compound I: 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)-*n*-propanol; compound II: 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)-3,3-dimethylpropanol; compound III: 4-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)-butanol; compound IV (canrenone): 3-(3-oxo-17β-hydroxy-4,6-androstadiene-17α-yl)-propionic acid γ-lactone; compound V: 3-(3-oxo-17β-hydroxy-4,6-androstadiene-17α-yl)-*n*-propanol; compound VI: 3-(3-oxo-17β-hydroxy-4,6,15-androstatriene-17α-yl)-propionic acid γ-lactone; compound VII (prorenone): 3-(3-oxo-17β-hydroxy-6β,7β-methylene-4-androstene-17α-yl)-propionic acid γ-lactone; compound VIII: 3-(3-oxo-17β-hydroxy-6β,7β-methylene-4-androstene-17α-yl)-*n*-propanol; compound IX: 3-(3-oxo-17β-hydroxy-6β,7β-methylene-1,4-androstadiene-17α-yl)-*n*-propanol; compound X (potassium prorenoate): potassium 3-(17β-hydroxy-6β,7β-methylene-3-oxo-4-androstene-17α-yl)-propionate; compound XI: 3-(3-oxo-7α-acetylthio-17β-hydroxy-15β-methyl-4-androstene-17α-yl)-propionic acid γ-lactone; compound XII: 3-(3-oxo-7α-acetylthio-17β-hydroxy-16α-methyl-4-androstene-17α-yl)-propionic acid γ-lactone; compound XIII (18-deoxyaldosterone): 21-hydroxy-11β,18-oxido-4-pregnene-3,20-dione; compound XIV (18-deoxyaldosterone acetate): 21-acetoxy-11β,18-oxido-4-pregnene-3,20-dione; compound XV: 11β,21-dihydroxy-18-hydroxymethyl-4-pregnene-3,20-dione (18α,20-hemiketal form); compound XVI: 1,4-pregnadiene-3,15,20-trione; compound XVII: 4,6-pregnadiene-3,15,20-trione; compound XVIII: 7α,14α-dihydroxy-4-pregnene-3,20-dione. [1,2,6,7-^{3}H]Aldosterone (85 Ci/mmol) was purchased from New England Nuclear.

RESULTS

Standard compounds

Compared to unlabelled aldosterone, spironolactone had a distinctly weaker affinity for [^{3}H]aldosterone-binding receptor sites in rat kidney cytosol (Fig. 1). A reduction by 50% in specific bound [^{3}H]aldosterone was attained at a spironolac-

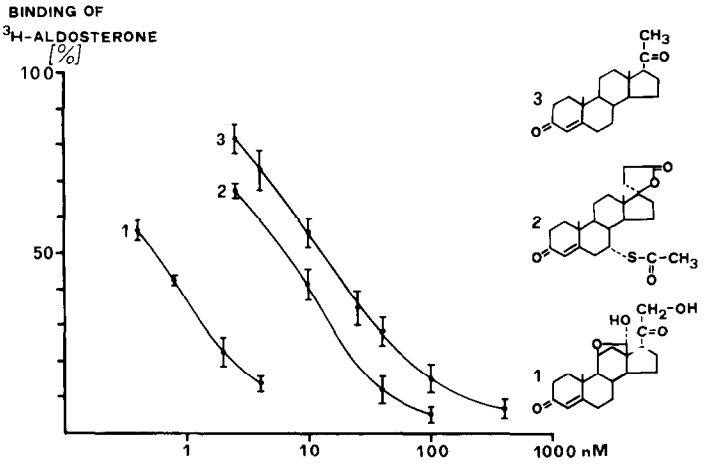

Fig. 1. Affinity for renal mineralocorticoid receptors of rats *in vitro* of aldosterone (1), spironolactone (2) and progesterone (3). Dose–response curves: the response is expressed as binding of [^{3}H]aldosterone. The points are mean values (n = 4) and the vertical bars represent S.E.M.

tone concentration 10 times higher than that of aldosterone. The relative affinity of spironolactone was thus 10% compared to aldosterone.

The displacement of [^{3}H]aldosterone by progesterone is also shown, for purposes of comparison. Progesterone had approximately 50% the affinity of spironolactone for the receptor sites.

In the following it will be shown how changes in the molecular structure of spironolactone, progesterone and aldosterone affect the affinity for MCR and the antimineralocorticoid action of these compounds.

Spironolactone derivatives (Fig. 2 and Table 1)

17α-Hydroxypropyl derivates. The replacement of the 17-spirolactone group of spironolactone by a 17α-hydroxypropyl and a 17β-hydroxyl group (compound I) resulted in almost complete loss of affinity for the cytoplasmic receptor sites. Derivatives of this prototype with differences in chain branching (compound II) or in chain length (compound III) showed also a negligible receptor affinity. The relative affinity of these three steroids was less than 1% of that of spironolactone. In contrast, compound I was even 1.84 times more potent than spironolactone *in vivo*

Table 1. Affinity for renal mineralocorticoid receptors (MCR) of rats *in vitro* and maximal relative antialdosterone potency (MRP) in rats of spironolactone derivatives in comparison to spironolactone

Compound	Relevant modifications compared to spironolactone	Affinity for MCR vs spironolactone (=100%) (%)	Maximal MRP vs spironolactone (=100%) (95% confidence limits) (%)
17α-Hydroxypropyl compounds			
I	17α-Hydroxypropyl, 17β-OH	<1	184 (108–387)
II	17α-(3-Hydroxy-3,3-dimethylpropyl), 17β-OH	<1	No activity
III	17α-Hydroxybutyl, 17β-OH	<1	No activity
C_6/C_7 unsaturated compounds			
IV	Δ^6 (= canrenone)	24	46 (28–83)
V	Δ^6-17α-Hydroxypropyl, 17β-OH	<1	32 (21–45)
VI	$\Delta^{6,15}$	54.4	34 (19–56)
6β,7β-Methylene compounds			
VII	6β,7β-Methylene (prorenone)	152.0	141 (98–221)
VIII	6β,7β-Methylene; 17α-hydroxypropyl, 17β-OH	4.0	71 (21–157)
IX	Δ^1; 6β,7β-methylene; 17α-hydroxypropyl, 17β-OH	<1	72 (21–157)
X	6β,7β-Methylene-17α-propionate, K^+ (= K^+ prorenoate)	37.1	114 (51–268)
Methylation at the D-ring			
XI	15β-Methyl	7.9	47 (19–153)
XII	16α-Methyl	<1	No activity

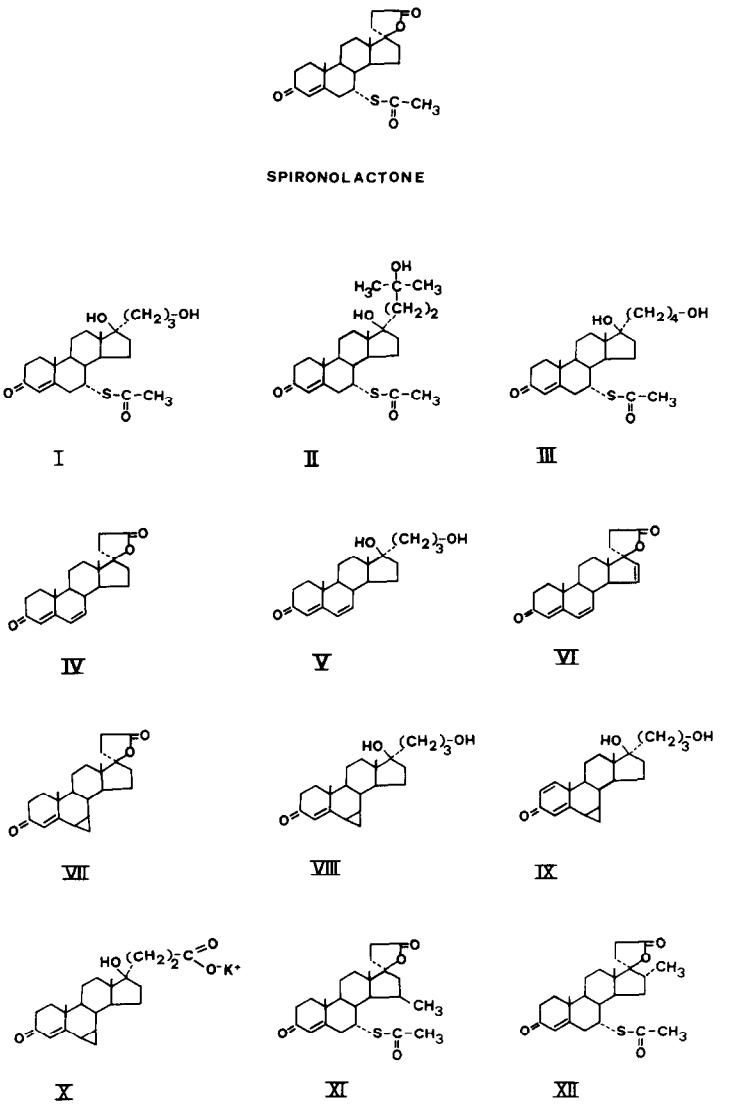

Fig. 2. Structures of spironolactone and its derivatives marked by the compound number (I–XII).

whereas compounds II and III with lengthened or branched 17α-hydroxyalkyl chains did not show antialdosterone activity *in vivo*.

C_6/C_7 *unsaturated compounds.* The substitution of the 7α-thioacetyl group of spironolactone by a 6,7-double bond leads to canrenone (compound IV) which showed 24% of the affinity of spironolactone in the *in vitro* test for mineralocorticoid receptors. A very low affinity was displayed by the 17α-hydroxypropyl derivative of canrenone (compound V). In contrast, the Δ^{15} derivative (compound VI) of canrenone was characterized by a manifest receptor affinity (54.4% in comparison to spironolactone).

The antialdosterone activity *in vivo* of these three Δ^6-spirolactone derivatives was of comparable magnitude but all of them showed a lower potency in comparison to spironolactone (Table 1).

6β,7β-*Methylene compounds.* If the 7α-thioacetyl group of spironolactone was substituted by a 6,7-methylene group in beta position (prorenone, compound VII) the affinity to the mineralocorticoid receptor and the biological activity increases by 52 and 41%, respectively, in comparison with spironolactone. The substitution of the 17-spirolactone ring by the 17α-hydroxypropyl and 17β-hydroxyl groups (compound VIII) resulted in a strong reduction in affinity for mineralocorticoid receptors but only a minor and not significant decrease of the antimineralocorticoid activity *in vivo*. The relative affinity of the 17α-hydroxypropyl derivative of prorenone (compound VIII), however, appeared to be higher than that of the corresponding derivative of spironolactone (compound I).

Introduction of a 1,2-double bond in the 17α-hydroxypropyl derivative of prorenone (compound IX) resulted in a further fall in relative affinity compared to the saturated congener but the biology activity remained unchanged. Finally, the affinity of potassium prorenoate (compound X) was lower than that of prorenone and spironolactone, but higher

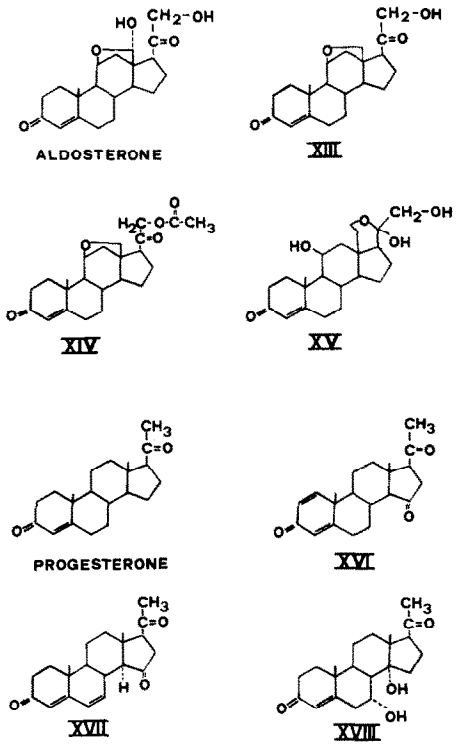

Fig. 3. Structures of aldosterone and progesterone and their respective derivatives marked by the compound number (XIII–XVIII).

than that of compounds VIII and IX. Its antimineralocorticoid activity *in vivo* was similar to that of the other 6β,7β-methylene derivatives.

Methylation at the D-ring. The position of the methyl group at the D-ring with reference to the 17-spirolactone ring was of particular interest. 15β-Methylspironolactone (compound XI) had a relative affinity of 7.9% and 16α-methylspironolactone (compound XII) an affinity of less than 1% compared to spironolactone. Similarly, compound XI showed less antimineralocorticoid activity *in vivo* than spironolactone while compound XII did not show antagonistic activity at all.

Aldosterone derivatives (Fig. 3 and Table 2)

The affinity for mineralocorticoid receptors of the three aldosterone derivatives (compounds XIII–XV) tested was considerably lower than that of spironolactone and negligibly small in comparison to that of aldosterone. Compounds XIII and XIV were weak agonists and only compound XV was a weak antagonist in correlation with its low receptor affinity.

Progesterone derivatives (Fig. 3 and Table 2)

The three progesterone derivatives examined showed an affinity for the mineralocorticoid receptor and an antimineralocorticoid effect *in vivo*. The antialdosterone activity was significantly lower than that of spironolactone. But, on the other side, the three compounds, Δ^1-15-ketoprogesterone (compound XVI), Δ^6-15-ketoprogesterone (compound XVII) and the 7α,14α-dihydroxyprogesterone (compound XVIII) displaced [^{3}H]aldosterone from the receptor sites to only a minor degree.

DISCUSSION

In comparing the *in vivo* aldosterone antagonistic effect of various steroids with their relative affinities for the renal mineralocorticoid receptor, there are a number of aspects to be taken into consideration.

In the receptor assay system, the investigation is confined to the competition between a steroid and [^{3}H]aldosterone for binding to cytoplasmatic receptor sites. However, *in vitro* affinity for the receptor of a given compound could induce an agonistic or antagonistic action [8, 9].

In the bioassay, on the other hand, there are differences in intestinal absorption, distribution, metabolism and excretion which effect the aldosterone-agonistic or -antagonistic action of a substance *in vivo*.

For these reasons, a good correlation between the affinity for aldosterone binding receptor sites and *in vivo* antimineralocorticoid activity of a given compound cannot be expected. However, comparison of the *in vivo* and *in vitro* antimineralocorticoid action of a series of compounds elucidates the importance of certain molecular structures and of

Table 2. Affinity for renal mineralocorticoid receptors (MCR) of rats *in vitro* and maximal relative antialdosterone potency (MRP) in rats of aldosterone analogues (compounds XIII–XV) and progesterone derivatives (compounds XVI–XVIII) in comparison to spironolactone

Compound	Trivial name	Affinity for MCR vs spironolactone (= 100%) (%)	MRP vs spironolactone (= 100%) (95% confidence limits) (%)
Aldosterone derivatives			
XIII	18-Deoxyaldosterone	30.0	Mineralocorticoid activity
XIV	18-Deoxyaldosterone acetate	53.6	Mineralocorticoid activity
XV	18-Hydroxymethyl corticosterone (18α,20-hemiketal form)	<1	Very weak activity
Progesterone derivatives			
XVI	Δ^1-15-Ketoprogesterone	1.3	56 (30–127)
XVII	Δ^6-15-Ketoprogesterone	1.7	20 (8–38)
XVIII	7α,14α-Dihydroxyprogesterone	1.2	61 (29–209)

the metabolism for the antimineralocorticoid action of various compounds.

For the spironolactone derivatives an intact structure of the 17-spirolactone ring seems to be essential for the binding to the mineralocorticoid receptor. Opening of the spirolactone ring, e.g. in spironolactone, canrenone or prorenone, leads either to a partial or an almost complete loss of the binding capacity, depending on the remaining structure at C-17. Similar observations were also made by Funder *et al.* [2] and Peterfalvi *et al.* [10].

Among the compounds without the 17-spirolactone ring, the prototype of the series containing a 17α-hydroxypropyl side chain and a 17β-hydroxy group, compound I, showed a striking result: its pharmacological activity *in vivo* was higher than that of spironolactone while its affinity for the mineralocorticoid receptor appeared to be negligible. Assuming that a blockade of aldosterone binding sites in the cytoplasm is essential for competitive antagonism, this result implies that compound I is converted *in vivo* to one or more active metabolites which then have affinity for the mineralocorticoid receptors.

It is likely that the *in vivo* conversion to active substances takes place by oxidation of the hydroxyl group of the side chain at C-17 to a carboxyl group and further a closure to the spirolactone ring.

Peterfalvi *et al.* [10] already pointed to the importance of the lactonic ring at C-17 for the antialdosterone activity. They found that 17-*O*-methyl-5,6-dihydrocanrenoic acid, a derivative which cannot be lactonized, was inactive *in vivo* as well as in the receptor assay *in vitro*. This assumption for ring closure is supported in the present study by compounds II and III. A branched 17α side chain—the 17α-hydroxy-3,3-dimethylpropyl chain of compound II—or an extended 17α side chain—the 17α-hydroxybutyl chain of compound III—seems to impede a ring formation by oxidation of the hydroxyl group and ring closure. Thus, not only the affinity to the mineralocorticoid receptor is very low but also their activity *in vivo* is abolished. Nevertheless, this mechanism can, however, only be answered on the basis of additional biochemical and pharmacological investigation.

Modifications of the structural features at the B-ring of spironolactone influence the affinity of its derivatives for the aldosterone receptor sites to a variable extent. Canrenone (compound IV) presented a reasonable affinity for the receptors *in vitro* and a corresponding antialdosterone activity *in vivo*. This compound is one of the active metabolites of spironolactone [11]. As in the case of compound I, the 17α-hydroxypropyl derivative did not show affinity for the receptor sites but caused a clear antialdosterone effect *in vivo*.

The double bond between C-15 and C-16 in compound VI increased slightly the action *in vitro* as well as *in vivo* when compared to canrenone (compound IV). The second group of 7α-dethioacetylated compounds is the 6β,7β-methylene series. The 6β,7β-methylene moiety, as shown by prorenone (compound VII), increases both the affinity for the receptor sites *in vitro* and the pharmacological activity *in vivo*, as shown extensively by Claire *et al.* [12]. As in the previous group, the introduction of the 17α-hydroxypropyl and 17β-hydroxyl groups (compounds VIII and IX) practically abolished the affinity for the receptor but reduced only to a minor extent the antialdosterone activity. In the case of potassium prorenoate (compound X), the open form of prorenone, a part of the binding capacity to the receptor was lost but the antialdosterone power *in vivo* was kept in comparison to prorenone.

The affinity for mineralocorticoid receptors was in accordance with the *in vivo* activity for the spironolactone derivatives methylated at the D-ring. The two aldosterone derivatives, compounds XIII and XIV, bound relatively well to the receptor but acted as agonists in the *in vivo* test. Compound XV, the third compound of this series, was inactive in both tests. Finally, the progesterone derivatives (compounds XVI–XVIII) showed similar binding capacity *in vitro* as well as antialdosterone activity *in vivo*.

The results of the present study are in agreement with previous investigations performed with other spironolactone derivatives [13, 14]. In these studies Feldman [14] indicated a poor correlation between the affinity for rat renal cytoplasmic mineralocorticoid receptors *in vitro* and the biological activity of these compounds measured *in vitro* in the toad bladder short-circuit current model, although this technique provides an accurate measurement of the effect of a steroid on active sodium transport in the absence of pharmacokinetic factors.

For the principal reasons discussed above, one cannot expect a complete agreement between affinity for receptors sites and antimineralocorticoid activity *in vivo* of a given compound. However, the present studies demonstrate the usefulness of studying the affinity, for mineralocorticoid receptors *in vitro*, of compounds with expected mineralocorticoid or antimineralocorticoid activity in the search for more specific mineralocorticoid antagonistic steroids, and for a better understanding of the metabolism as well as the mechanism of action of new compounds.

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REFERENCES

1. D. D. Fanestil, *Biochem. Pharmac.* **17**, 2240 (1968).
2. J. W. Funder, D. Feldman, E. Highland and I. S. Edelman, *Biochem. Pharmac.* **23**, 1493 (1974).
3. J. W. Funder, D. Marver, J. Stewart, D. Feldman and I. S. Edelman, *Clin. Exp. Pharmac. Physiol. Suppl.* **2**, 99 (1975).
4. G. Rousseau, J. D. Baxter, J. W. Funder, I. S. Edelman and G. M. Tomkins, *J. Steroid Biochem.* **3**, 219 (1972).
5. G. Wambach and I. R. Higgins, *Endocrinology* **102**, 1686 (1978).
6. G. Wambach and I. R. Higgins, in *Antihormones* (Ed. M. K. Agarwal) p. 167. Elsevier/North Holland, BioMedical Press, Amsterdam (1979).
7. J. Casals-Stenzel, M. Buse and W. Losert, *Eur J. Pharmac.* **80**, 37 (1982).

8. M. R. Sherman, in *Aldosterone Antagonists in Clinical Medicine* (Eds. G. M. Addison, N. Wirefeldt Asmussen, P. Corvol, P. W. C. Kloppenborg, N. Norman, R. Schröder and J. I. S. Robertson) p. 1. Excerpta Medica, Amsterdam (1978).
9. J. P. Raynaud, M. M. Bouton, M. Moguilewsky, T. Ojasoo, D. Philibert, G. Beck, F. Labrie and J. P. Mornun, *J. Steroid Biochem.* **12**, 143 (1980).
10. M. Peterfalvi, V. Torelli, R. Fournex, G. Rousseau, M. Claire, A. Michaud and P. Corvol, *Biochem. Pharmac.* **29**, 353 (1980).
11. A. Karim, J. Zagarella, J. Hribar and M. Dooley, *Clin. Pharmac. Ther.* **19**, 158 (1976).
12. M. Claire, M. E. Rafestin-Oblin, A. Michaud, C. Roth-Meyer and P. Corvol, *Endocrinology* **104**, 1194 (1979).
13. C. Sakauye and D. Feldman, *Am. J. Physiol.* **231**, 93 (1976).
14. D. Feldman, in *Aldosterone Antagonists in Clinical Medicine* (Eds. G. M. Addison, N. Wirefeldt Asmussen, P. Corvol, P. W. C. Kloppenborg, N. Norman, R. Schröder and J. I. S. Robertson) p. 18. Excerpta Medica, Amsterdam (1978).