Aldosterone Receptors and the Evaluation of Plasma Mineralocorticoid Activity in Normal and Hypertensive States

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ABSTRACT Aldosterone receptors from rat kidney slices were utilized in a competitive binding technique to analyze the contribution of various steroids to plasma "mineralocorticoid" activity and to assess their possible role in hypertension. To consider simultaneously the plasma binding, steroids were incubated with slices in undiluted plasma; competitor activities for [^{3}H]aldosterone binding were aldosterone, 100%; deoxycorticosterone, 16.2%; cortisol, 0.4%; and 18-hydroxy-deoxycorticosterone and 18-hydroxy-corticosterone, 0.1%. These steroids were more active in buffer than plasma, suggesting that they bind to plasma and that this reduces their receptor binding. Analysis of the competition data suggests that at normal plasma concentrations, aldosterone occupies the receptors to a major extent, cortisol occupies some of the receptors, and deoxycorticosterone and 18-hydroxydeoxycorticosterone contribute little to receptor occupancy. Two steroids implicated in low-renin essential hypertension, 16β-hydroxy-dehydroepiandrosterone and 16-oxoandrostenediol, did not have significant competitor activity.

Competitor activity in plasmas from normal subjects taken at 12 noon (upright) was greater than that in those taken at 8 a.m. (supine). Since the 12 noon samples had higher aldosterone and lower cortisol levels than the 8 a.m. samples, the competitor activity under these physiological circumstances reflects aldosterone more than cortisol. The competitor activities of plasmas from patients relative to normal subjects (100±12.1%; mean±SEM) were: normal renin "essential" hypertension, 117±14.3%; low-renin essential hypertension, 101±6.6%; and primary aldosteronism, 176±14.3%. Thus, a significant increase in activity of steroids that interact with mineralocorticoid receptors was detected in primary aldosteronism ($P < 0.01$) but was not detected in low-renin or normal-renin essential hypertension.

INTRODUCTION

Excessive secretion of adrenal steroids with sodium-retaining activity can result in hypertension in man (1, 2). The characteristic clinical findings in patients with primary aldosteronism (2) result from hypersecretion of aldosterone; those in patients with 11- and 17-hydroxylase defects (3, 4) presumably result from hypersecretion of deoxycorticosterone. Both involve hypertension, but the mechanism is not known. Expansion of extracellular fluid volume (5, 6) may account for the suppressed plasma renin activity characteristic of these syndromes (7).

The observation that 15–30% of patients with "essential" [1] hypertension also have subnormal plasma

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[1] The term "essential" applied to hypertension is used herein to describe high blood pressure without known factors to which it could be secondary.

renin activity has led to the suggestion that steroids with sodium-retaining activity may contribute to the hypertension in these patients as well (8–12). This hypothesis has received further support from reports that in low-renin essential hypertension, the elevated blood pressure is "volume-dependent" and responds to diuretic therapy more readily than other forms of essential hypertension (8, 10, 13, 14). That adrenal factors may be involved in this syndrome is further suggested by the findings that blockade of aldosterone biosynthesis by aminogluthetimide (15) or of the action of sodium-retaining steroids by spironolactone (8, 9–11, 13–15) results in greater amelioration of the hypertension in patients with low renin as compared to those with normal renin levels.

These considerations have led investigators to look for steroids with sodium-retaining activity in plasma or urine of patients with low-renin essential hypertension. The aldosterone secretory rate is ordinarily normal in such patients (16). With further investigation, a number of other steroids have been implicated, including deoxycorticosterone (17), 18-hydroxy-deoxycorticosterone (18–20) or a metabolite of it (20), 16α,-18β-dihydroxy-deoxycorticosterone (21), and more recently (22) 16β-hydroxy-dehydroepiandrosterone (16β-OH-DHEA)[2] and 16-oxo-androstenediol. In all cases, a major problem has been to determine whether the amount of steroid is sufficient to account for an excess of sodium-retaining activity.

To determine the quantitative contributions of steroids to the plasma sodium-retaining activity and whether a single steroid or combination of steroids are present in amounts sufficient to produce an elevation of total sodium-retaining activity in low-renin essential hypertension, we have utilized the "mineralocorticoid" receptor in rat kidney. These proteins have a high affinity for aldosterone and have been called mineralocorticoid[3] receptors since the available evidence suggests that they mediate the steroidal sodium-retaining response. Thus, there is an excellent correlation between the degree of binding of agonist steroids with the receptors and their sodium-retaining activity (23–26). With rat kidney slices exposed to low concentrations of aldosterone (e.g., less than 1 nM), most of the aldosterone bound in cytosol is associated with these mineralocorticoid receptors; some (around 15–35%) of the binding is by other sites[4] that have a lower affinity for aldosterone and that most likely are predominantly glucocorticoid receptors (24–26).

The present approach is based on the notion that plasma steroids with sodium-retaining activity can bind to the aldosterone receptors and therefore inhibit the binding of [^{3}H]aldosterone. Thus, if there are high concentrations of such steroids in plasma in low-renin essential hypertension or other conditions, plasma from these patients should exhibit an increased inhibition of [^{3}H]aldosterone binding. Since free and not plasma-bound steroids probably determine receptor occupancy (27), we incubated receptors with whole plasma. By this method, bound steroids, unavailable for receptor binding, are not detected. This technique should reflect the competition of all of the free mineralocorticoids in the plasma sample.

METHODS

Materials. Radioactive aldosterone (91 Ci/mmol, New England Nuclear, Boston, Mass.) was used without further purification. Nonradioactive aldosterone, 11-deoxycorticosterone and cortisol were obtained from Sigma Chemical Co. (St. Louis, Mo.). 18-Hydroxy-corticosterone was obtained from Ikapharm Ltd. (Ramat-Gan, Israel). Spironolactone (SC14266), 18-hydroxy-deoxycorticosterone, 16β-OH-DHEA, and 16-oxo-androstenediol were acquired from G. D. Searle & Co. (Columbus, Ohio).

Steroid-free plasma was prepared by a charcoal treatment procedure modified from that described previously (27). Activated charcoal (Norit A, American Norit Co., Inc., Jacksonville, Fla.), treated as described previously, was incubated with plasma (50 mg charcoal/ml plasma) at 37°C for 30 min in a gyrotory shaker bath (New Brunswick Scientific Co., New Brunswick, N. J.) (100 rpm). The mixture was then centrifuged at 25,000 *g* for 10 min. The supernatant plasma was removed and treated with charcoal two more times in a similar way. Then the plasma was filtered through cheesecloth (Hermitage Cotton Mills, Camden, S. C.), and the filtrate was centrifuged twice at 48,000 *g* for 20 min and filtered through glass filter paper (Whatman, GF/C). This procedure effectively removes steroids (27); aldosterone, 11-deoxycorticosterone,[5] and cortisol were undetectable in the treated samples.

Binding studies. The procedure for preparation of rat kidney slices and measurement of [^{3}H]aldosterone binding are slight modifications of those previously described (24, 28). Sprague-Dawley rats (200–220 g) were adrenalectomized and maintained on 1% normal saline for 3 days to 2 wk before use. Rats were sacrificed by cervical dislocation and perfused with buffer A (133 mM NaCl, 0.5 mM $MgCl_2$, 6 mM KH_2PO_4, 1 mM $CaCl_2$, 5 mM Tris-HCl, and 5 mM

[2] *Abbreviations used in this paper:* 16β-OH-DHEA or 16β-OH-dehydroepiandrosterone, androst-5-ene-3β,16β-diol-17-one; 16-oxo-androstenediol, androst-5-ene-3β,17β-diol-16-one; K_d, equilibrium dissociation constant; 11-deoxycorticosterone, deoxycorticosterone; spironolactone SC14266, potassium 3-(3-oxo-17β-hydroxy-4,6-androstadein-17α-Y1)propionate.

[3] Whereas the term "mineralocorticoid" has a precise meaning in this context, it has sometimes been used more loosely in other contexts with respect to the physiological effects of steroids on electrolyte balance, particularly potassium.

[4] The range of 15–30% at 1 nM is obtained by calculation with affinity and site concentration data obtained in previous (24, 25) and the present studies.

[5] Measured by a recently developed radioimmunoassay. B. Chang, M. Schambelan, and E. Biglieri, unpublished observations.

glucose, pH 7.4) through a cardiac injection. The kidneys were removed, decapsulated, and cut into 275-μm-thick slices with a McIlwain mechanical tissue chopper (Brinkman Instruments, Westbury, N. Y.). Slices (from about 0.1 kidney) were incubated in 20-ml vials in a gyrotory shaker bath (New Brunswick Scientific Co.) at 120 rpm for 30 min at 37°C in 2 ml whole plasma or buffer A previously equilibrated with [^{3}H]aldosterone (1 nM final concentration) with or without competitor steroids for 30 min at 37°C. After incubation, samples were placed in an ice bath and centrifuged at 0°C. All further procedures were done at 0–4°C. Under these conditions, the total uptake of radioactivity was less than 5% of that added, as determined by measurement of radioactivity in the supernatant medium. The pellet was washed three times with ice-cold solution B (25 mM KH_2PO_4 and 0.1 M NaCl, pH 7.6). After washing, the samples were resuspended in 0.2 ml of solution C (2 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM *N*-Tris (hydroxymethyl) methyl glycine (tricine), 0.01 mM dithiothreitol (Sigma) in 5% glycerol, pH 7.4), and homogenized with a Teflon pestle (six strokes at 2,000 rpm). The homogenate was centrifuged at 8,000 *g* for 5 min, and 0.2 ml of the supernatant medium was placed on a Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) column (0.4 cm × 9.5 cm). The fractions excluded from the gel were assayed for radioactivity at 45% efficiency in 5 ml of 4 g/liter Omnifluor (Beckman Instruments, Inc., Fullerton Calif.) in toluene with 25% Triton X-100 (Beckman), and for protein as previously described (27). "Background" (27) was assessed from parallel experiments in tubes containing, in addition to the radioactive aldosterone, a 1,000-fold excess of nonradioactive aldosterone. Background determinations were less than 10% of the total binding. Binding was calculated as counts per minute per milligram protein and all measurements are reported as specific binding (i.e. the background subtracted from the experimental binding [29]).

Other assays. Plasma renin activity (29) and aldosterone (30) were measured by radioimmunoassay. Plasma cortisol was measured by the competitive protein binding assay (31).

The testing of plasma for competitive activity. Plasma specimens were tested for their ability to inhibit the binding of [^{3}H]aldosterone, and the relative competitive activity of the plasma samples were compared to those of other samples run in the same experiment. However, to obtain some indication of the magnitude of the differences within the experiment, all the values were related to the inhibition of binding obtained in an experiment in which competition by nonradioactive aldosterone for [^{3}H]aldosterone binding in charcoal-treated plasma was measured. The latter was plotted in a linear form as discussed elsewhere (27), and the degree of competitive inhibition was related to the curve. In principle, the linear plot could be used to provide a precise measure of plasma "mineralocorticoid equivalents"; however, this cannot be done until it can be demonstrated that charcoal-treated plasma reflects a true "zero" point. Thus, for the experiment in which postural changes are studied, the mean of the competitor values determined from the samples taken in the recumbent position at 8 a.m. was arbitrarily assigned a value of 100%, and all of the determinations were expressed as a percent of this mean value. For the experiment in which samples from normals and from patients with various forms of hypertension were studied, the mean of the values from normals was assigned a value of 100% and the other determinations were expressed as a percent of this value. Thus, the linearized competition curve was used to give a better indication of the relative differences than would be reflected by a simple reporting of differences in specific binding that are not linearly related to differences in competitor. The main emphasis in these two experiments was on whether differences in the various situations can be detailed. The "control" in both cases was internal, and the comparisons presented were valid and independent of the establishment of a zero point.

Statistical comparisons of the data were performed by Student's *t* test (32). The intra-assay coefficient of variation ranged from 2% to 26%, with a mean of 13%.

Clinical studies. For the study relating competitor activity in normals and various hypertensive states, 23 hypertensive patients and 9 normal control subjects were studied. 5 patients had primary aldosteronism, as characterized by the classic findings of hypertension, hypokalemia, subnormal plasma renin activity, elevated urinary and/or plasma aldosterone concentration, and subsequent demonstration of an aldosterone-secreting adenoma at operation. 18 patients were considered to have essential hypertension after a complete evaluation had failed to disclose any form of secondary hypertension. These patients were further divided into normal-renin (7 patients) and low-renin (11 patients) subgroups by analysis of their plasma renin concentration, in the supine and standing posture on both normal and low sodium intakes. Further details are provided in the Results and legends. The normal control subjects were nonhypertensive volunteers or patients being evaluated for unrelated problems.

For the latter study and for the investigation of changes due to posture and time of day, all subjects were studied under metabolic ward conditions either at the Clinical Study Center, San Francisco General Hospital, or the Hypertension-Endocrine Branch of the National Heart and Lung Institute, NIH. They received a constant diet containing 110–120 meq of sodium daily. After their body weights and urinary sodium had stabilized, plasma samples were obtained at 8 a.m. after overnight recumbency, and again at 12 noon, after 4 h of upright activity. For anticoagulant, either disodium EDTA (final concentration, 30 mM), citrate phosphate dextrose (final concentration, 11 mM trisodium citrate, 2 mM citric acid, 17 mM dextrose, 2 mM monobasic phosphate) or heparin (0.15 mg/ml) was used; plasma was rapidly separated in a refrigerated centrifuge and stored at −20°C before analysis. In control plasma, the effect of freezing was found to be negligible.

RESULTS

Characteristics of receptor binding by aldosterone. When [^{3}H]aldosterone was incubated with kidney slices from adrenalectomized rats, binding was maximal before 30 min, as reported previously (28). The data in Fig. 1 indicate that this binding reaction was also reversible. Slices were incubated with [^{3}H]aldosterone for 30 min. After that, an excess ("chase") of nonradioactive aldosterone was added at various times to inhibit subsequent [^{3}H]aldosterone binding. Whereas there was no decrease in the binding of [^{3}H]aldosterone in the "control" incubations that did not receive the chase, the specific binding in the experimental sample decreased rapidly ($t_{\frac{1}{2}}$ about 3 min).

Fig. 2 shows the results of an experiment in which increasing concentrations of [^{3}H]aldosterone were in-

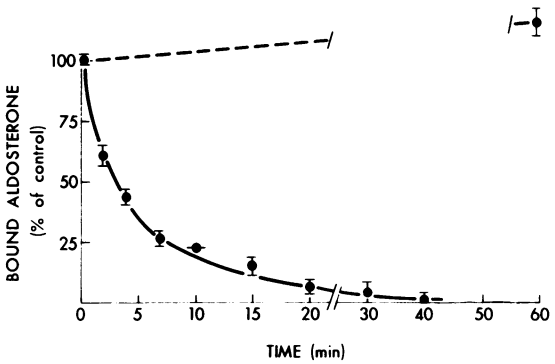

FIGURE 1 Kinetics of dissociation of [^{3}H]aldosterone from the cytosol binding sites. Tubes containing slices were first incubated with [^{3}H]aldosterone for 30 min in buffer A and samples were assayed for [^{3}H]aldosterone binding. Then, at various times thereafter, an excess chase (2.5 μM final concentration) of nonradioactive aldosterone was added to reaction mixtures; specific binding of all the samples was measured after 60 min more. Shown (solid line) is the specific binding as a function of the time the samples were exposed to the chase. For example, when the chase was added 20 min after the initail 30-min incubation, the sample was exposed to the chase for 40 min as shown on the abscissa; the control binding after the initial 30-min incubation (bars indicate the range of two determinations) is shown at 60 min and the control at the termination of the experiment (1,967 cpm/mg protein; bars show the SEM of 11 determinations) is shown at 0 time. As indicated, the two controls differed by less than 15%. For the various time points, brackets show the range of duplicate determinations.

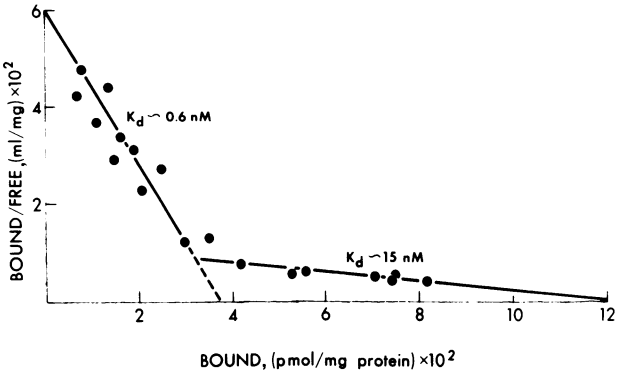

FIGURE 2 Scatchard analysis of the binding of various concentrations of [^{3}H]aldosterone by rat kidney slices incubated with buffer A (27). The free aldosterone concentration was determined by measuring the [^{3}H]aldosterone in the supernatant medium after removal of the slices at the end of the incubation. For determination of the affinity and site concentrations reported in the text, the curves are resolved into two straight lines, as described elsewhere (33).

cubated in buffer with the slices. The data were analyzed according to the Scatchard technique and, consistent with previous analyses (24, 26) suggest the presence of predominantly two classes of binding sites.[6] Aldosterone binds to the higher-affinity component with an estimated equilibrium dissociation constant (K_d) of 0.6 nM. (The mean and range of three experiments were 0.59 and 0.54–0.64 nM, respectively). The affinity of aldosterone for the lower-affinity component sites, more difficult to determine precisely, is between 10 and 30 nM. The mean concentration of aldosterone-binding sites in the higher- and lower-affinity components, respectively, in three experiments was calculated to be 26 (range, 23–30) and 69 (range, 60–85) fmol/mg cytosol protein. When the binding by kidney slices incubated with [^{3}H]aldosterone in plasma was studied, similar equilibrium constants and concentrations of sites are obtained, if one utilizes the "free"[7] rather than total aldosterone in plasma for the affinity calculations.

Steroidal specificity in competition for [^{3}H]aldosterone binding. The activity of different steroids for inhibition of [^{3}H]aldosterone binding is shown in Fig. 3 and in Table I. As indicated, of the steroids tested, aldosterone has the highest activity. Deoxycorticosterone and corti-

[6] The possibility that the two components of the Scatchard plot reflect negative cooperativity has not been excluded. This possibility seems less likely since the binding properties of the low-affinity component (25, 26) are similar to those of glucocorticoid receptors studied in other tissues in which the high affinity component is absent (25, 34, 35). In any event, the experimental approach of measuring the relative competitive properties of steroids in plasma presented in this communication does not depend on answering this question.

[7] Under our conditions, about 55% of the aldosterone in plasma is bound, as measured by a steady-state gel filtration procedure or by equilibrium dialysis (D. T. Matulich, and J. D. Baxter. In preparation).

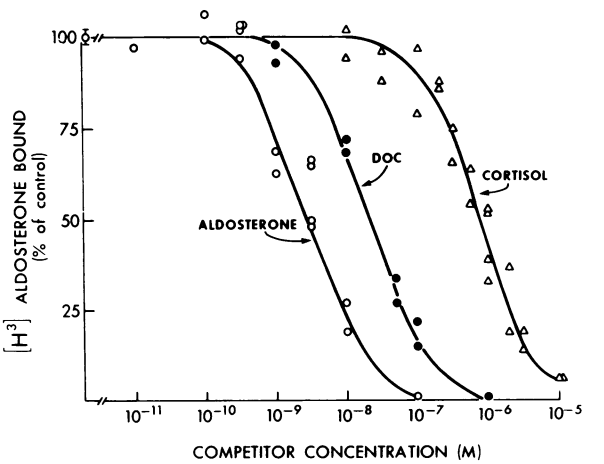

FIGURE 3 Competition by nonradioactive aldosterone (○), deoxycorticosterone (DOC [●]), and cortisol (△) for [^{3}H]aldosterone (1 nM) binding by rat kidney slices incubated in steroid-free plasma (in citrate-phosphate-dextrose). The control (100%) represents specific binding in the absence of added competitor and was 604 cpm/mg protein. For aldosterone, a similar competition curve was obtained with five different plasma samples.

TABLE I

*Inhibition of [^3H]Aldosterone Binding by Nonradioactive Steroids**

Steroid	Activity			
	In plasma		In buffer	
	C_{50}	%	C_{50}	%
	nM		*nM*	
Aldosterone	2.7±0.6	100	2.0±0.3	100
Deoxycorticosterone	16.5±4.5	16.2	4.7±0.6	41.5
Cortisol	690±60	0.4	88±14	2.2
18-Hydroxy-deoxycorticosterone	10^3	0.1	—	1.4‡
18-Hydroxy-corticosterone	10^3	0.1	—	0.5‡
Spironolactone (SC14266)	5×10^4	0.05	—	0.4§
16β-OH-DHEA	No inhibition detected‖		No inhibition detected‖	
16-Oxo-androstenediol	No inhibition detected‖		No inhibition detected‖	

* Various concentrations of nonradioactive steroids or of spironolactone were equilibrated for 30 min with plasma or buffer A and 1 nM [^{3}H]aldosterone, before addition of kidney slices and measurement of binding. The specific binding of [^{3}H]aldosterone in the absence of competitor ranged from 810 to 1,350 cpm/mg protein in buffer A and from 380 to 670 com/mg protein in plasma. The concentration required for 50% inhibition of specific binding (C_{50}) was determined, and the mean±SEM of three experiments (performed in duplicate) is reported. The percentage values were obtained from the C_{50} for aldosterone divided by the C_{50} for the steroid in question times 100.

‡ Taken from Feldman and Funder (45).

§ Taken from Funder, et al. (40).

‖ Tested at 0.5 and 1.0 μM.

sol have 16.2% and 0.4%, respectively, of the activity of aldosterone. 18-Hydroxy-deoxycorticosterone and 18-hydroxycorticosterone have definite but weak activity as competitors. The capacity of these steroids as competitors for [^{3}H]aldosterone binding generally correlates with their known sodium-retaining activity. The spironolactone SC14266 also inhibited [^{3}H]aldosterone binding.

These data suggest that the pattern of observed competitive inhibition of [^{3}H]aldosterone binding is predominately specific for the mineralocorticoid receptors. This is true although some of the [^{3}H]aldosterone is bound by the larger population of lower-affinity sites, probably mostly glucocorticoid receptors. In fact, using the determined values for affinity and site concentrations for both classes of sites, we calculate that at 1 nM, around 65–85% of the [^{3}H]aldosterone binding is by the higher-affinity sites, and the competitive inhibition by nonradioactive aldosterone (at levels of binding inhibition to 75%) is almost exclusively inhibition of [^{3}H]aldosterone binding by the higher-affinity sites.[8] The competition by nonradioactive aldosterone for [^{3}H]aldosterone binding is also plotted (Fig. 4) as the ratio of binding in the absence of competitor divided by that in the presence of the competitor, as described in the Methods and elsewhere (27). The linear relationship obtained provides additional evidence that the competition in this case is predominantly by the mineralocorticoid sites.

The two C_{19} steroids, 16β-OH-DHEA and 16-oxo-androstenediol, were tested in both plasma and buffer at concentrations up to 1 μM. No inhibition of [^{3}H]-aldosterone binding by either steroid was observed (Table I).

Table I shows also the binding avidity of several of these steroids examined in the absence of plasma. This gives a much better estimate of the relative intrinsic affinity of the steroids for the receptor. Of note is that on a concentration basis, cortisol and deoxycorticosterone are less potent competitors for aldosterone sites

[8] Given high enough concentrations, potent glucocorticoids such as dexamethasone can competitively inhibit [^{3}H]aldosterone binding by the mineralocorticoid receptors (and can inhibit the smaller amount of [^{3}H]aldosterone bound by the glucocorticoid receptors). For example, Funder et al. (25) reported that dexamethasone has 2% the affinity of aldosterone for binding to the mineralocorticoid receptors, and our own estimates (5%, data not shown) are close to this. It is possible, therefore, that potent glucocorticoids such as dexamethasone may exert some of their effects on electrolyte balance through the mineralocorticoid receptors, although the influences of these steroids in physiologic studies are complex (36, 37). Whereas the presence of such steroids could result in detection of competitor activity, this did not appear to be a problem in the current studies.

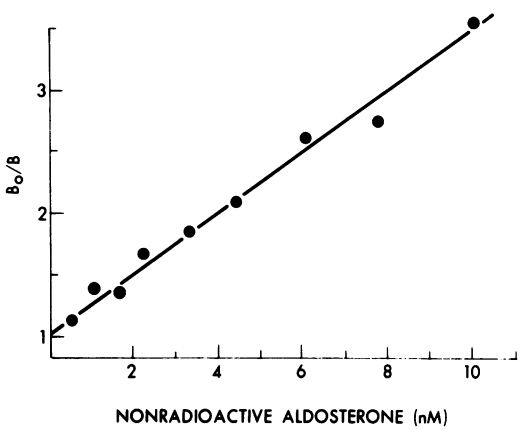

FIGURE 4 Plot of the specific binding in steroid-free plasma of [^{3}H]aldosterone (1 nM) in the absence of competitor (B_o) (359 cpm/mg protein) divided by that determined in the presence of the added competitor (B) as a function of the competitor concentration. (See Methods for other details.) Points represent the means of duplicate determinations.

when incubations are performed in plasma instead of buffer. This gives further support for the idea that the known extensive plasma binding of these steroids (38, 39)[9] does reduce their ability to bind to the receptor. The value for 50% inhibition of binding by the spironolactone analogue in plasma is roughly 10% of that determined in buffer; this suggests that this antagonist may also circulate mostly bound in plasma. Also, 18-hydroxydeoxycorticosterone and 18-hydroxy-corticosterone are weaker competitors in plasma, as compared with previous estimates (35) in which buffer was used for incubation; this suggests that these steroids also bind significantly to plasma.

Competitive activity of plasma in response to posture and time of day. To determine whether the inhibition of binding of [^{3}H]aldosterone changes when there are physiological variations in endogenously produced steroids, we compared the activity of plasma from normal individuals taken at 8 a.m., after they had been recumbent overnight, with samples taken at 12 noon, after subjects had been erect for 4 h. As discussed in the Methods, the competitor activity is compared with the mean recumbency value, standardized as 100%. As shown in Fig. 5, there was an increase in competitor activity in the noon samples from all four subjects, and this corresponded to an increase in plasma aldosterone concentration. By contrast, the plasma cortisol concentration was lower in the samples taken at 12 noon than in those taken at 8 a.m., reflecting the known circadian rhythm of this steroid. These findings demonstrated that the receptor technique can detect an elevation of competitor under physiological circumstances when plasma cortisol is decreasing and plasma aldosterone is increasing.

Comparison of the plasma mineralocorticoid activity of patients with hypertension. The competitive activity of plasma and other clinical data from 5 normal subjects and 23 patients with hypertension is shown in Table II. The mean and range for normals and for patients with low-renin essential hypertension are almost identical. Further, the mean for patients with normal-renin essential hypertension is also similar to that of the former two groups, although an elevated activity was found in two samples. In one of these patients, plasma aldosterone concentration was high normal (12.2 ng/dl; normal, 2–14 ng/dl [30]); a similar value was not available for the other individual. In striking contrast, the total mineralocorticoid activity in four of the five plasma samples from patients with primary aldosteronism was markedly elevated. Further, the mean value for the primary aldosteronism group was significantly different from that of either of the normal controls ($P < 0.01$) or of the low-renin essential hypertension group ($P < 0.01$). As shown in Table II, the mean age of the low-renin and normal-renin hypertension groups was not significantly different.

DISCUSSION

The present studies suggest that mineralocorticoid receptors can be used to evaluate quantitatively the steroids that interact with them. As suggested by these and

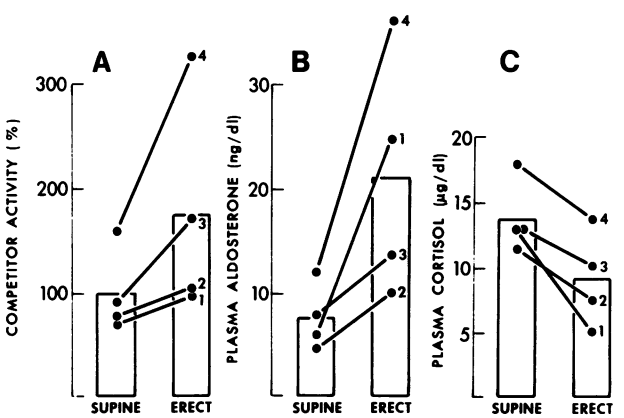

FIGURE 5 Changes in plasma competitor activity (A), aldosterone (B), and cortisol (C) in samples taken (in heparin) from normal individuals at 8 a.m. after overnight recumbency, as compared with those taken in the erect posture at 12 noon. For comparison the numbers placed to the right of the erect value denote the individual sample. Competitor activity is expressed as the percent of the mean activity in plasmas from normals taken at 8 a.m. as described in the Methods. Bars indicate the mean values.

[9] We found using a steady-state gel filtration technique that in normal or steroid-free plasma at 37°C about 96% of deoxycorticosterone is plasma-bound. (D. T. Matulich, and J. D. Baxter. In preparation.)

TABLE II

*Competitor Activity and Other Data on Hypertensive Patients and Normal Controls**

Patient	Age	Aldosterone		Plasma cortisol	Plasma renin activity	Competitor activity
		Urine	Plasma			
		μg/24 h	*ng/dl*	*μg/dl*	*ng/ml/3 h*	%
I. Normals						
1	65	10.8	—	—	3.0	103
2	25	9.4	5.4	14.6	7.7	112
3	21	9.2	7.7	—	3.6	66
4	24	18.7	—	—	7.4	137
5	24	18.8	—	—	2.4	82
Mean	31.8±8.3‡	13.4±2.2	—	—	4.8±1.1	100±12.3
II. Low-renin hypertension						
1	53	5.5	—	—	0.7	96
2	44	12.1	—	—	0.5	90
3	31	1.1	13.2	14.4	<0.5	82
4	38	13.3	—	—	0.6	82
5	17	3.1	9.4	—	<0.5	74
6	43	10.3	—	—	1.3	116
7	47	15.4	—	—	<0.5	73
8	41	10.5	8.3	—	<0.5	132
9	43	12.6	7.4	—	<0.5	111
10	44	9.3	7.9	—	<0.5	127
11	47	10.8	—	—	<0.5	129
Mean	40.7±2.9§	9.5±1.3	9.2	—	<0.5	101±6.8‖
III. Primary aldosteronism						
1	45	24.2	24.5	—	<0.5	196
2	41	48.6	31.7	14.3	<0.5	196
3	43	48.6	31.6	16.3	<0.5	202
4	52	23.1	—	—	<0.5	80
5	55	53.6	33.9¶	14.9	0.8	209
Mean	47.2±2.7	36.5±6.3	30.4	15.2	<0.5	177±24.3**
IV. Normal renin hypertension						
1	27	11.9	12.2	—	6.1	165
2	50	9.6	—	—	3.6	95
3	37	14.4	—	—	9.9	89
4	44	8.8	—	—	1.3‡‡	93
5	30	9.3	—	—	4.3	93
6	47	8.6	—	—	3.3	106
7	37	16.2	—	—	3.0	178
Mean	38.9±3.2§	11.3±1.1	—	—	4.5±1.1	117±14.3
Normal range	—	4–20	2–14	5–20	2–12	—

* Measurements were made as described in Methods. Comparative determinations for each patient in samples (obtained in EDTA) were made on the same day for urine and plasma comparisons or on plasma drawn at the same time.

‡ The ± signs indicate the SEM.

§ The mean ages in low-renin (39.1 yr) and normal-renin (40.7 yr) hypertension groups did not differ significantly (t = 0.356).

‖ This value is not significantly different from the mean of the normals or of the normal-renin hypertension group, but differs significantly ($P < 0.01$) from the primary aldosteronism group.

¶ This determination was made on a different sample taken under comparable conditions.

** This value differs significantly ($P < 0.01$) from the normal or low-renin hypertension group.

‡‡ Subsequent measurements during upright posture and during sodium restriction demonstrated normal renin responsiveness in this patient.

previous investigations (24), at low concentrations binding of [^{3}H]aldosterone by rat kidney slices is mostly by high-affinity sites, termed mineralocorticoid receptors. More importantly, competition by nonradioactive aldosterone for [^{3}H]aldosterone binding by the slices results predominantly from inhibition of binding by these high-affinity sites. The ability of deoxycorticosterone and cortisol to inhibit binding also generally correlates with their potency as sodium-retaining steroids, and the binding can be inhibited by spironolactone (24, 40).

For studying the competitive activity of plasmas, particular use is made of the technique of incubating whole plasma with the slices. If the total tissue uptake of steroid is small in relation to the total steroid present in plasma (as was true for the present studies), the normal plasma binding of steroids should be preserved. Thus, only the free and not the protein-bound steroid should be available for competition. In support of this hypothesis, we found that the ability of steroids in plasma to inhibit binding of [^{3}H]aldosterone was less than the activity of the same steroids incubated with slices in buffer. Further, the extent of these differences correlated well with known properties of plasma binding of the steroids. Thus, aldosterone, relatively weakly bound in plasma (39),[7] is only slightly less effective as a competitor in plasma than in buffer, whereas deoxycorticosterone and cortisol, tightly bound in plasma (38),[9] are much less effective as competitors in plasma than in buffer.

The results of the present investigations may provide an indication as to the relative importance of various sodium-retaining steroids in vivo. Of particular interest in this regard is deoxycorticosterone. In normals, the plasma deoxycorticosterone concentration is around 7–10 ng/dl or 0.2–0.3 nM (17, 41, 42). Concentrations of this steroid in the 20–100 ng/dl range have been reported in low-renin essential hypertension (17). The data shown in Fig. 3 suggest that at a concentration of deoxycorticosterone of 0.2 nM, there is no detectable inhibition of the binding of [^{3}H]aldosterone. In fact, only at plasma deoxycorticosterone concentrations 10-fold higher (2 nM, or around 70 ng/dl) is significant inhibition observed; even higher concentrations are required for marked inhibition. If binding to receptors is necessary for sodium-retaining activity, these findings imply that at normal concentrations, deoxycorticosterone contributes little to the sodium-retaining activity of plasma. Of course, if the plasma binding of deoxycorticosterone were for some reason decreased, its competitive activity for receptors would be greater. One might expect modest sodium-retaining activity from deoxycorticosterone at plasma concentrations in the 50–70 ng/dl range. However, at concentrations of 100 ng/dl or greater, such as might be anticipated in patients with 17-hydroxylase deficiency (2, 4) or certain forms of Cushing's syndrome (43, 44), deoxycorticosterone should produce marked sodium-retaining activity. Consistent with this, plasma deoxycorticosterone concentrations in the 100–500 ng/dl range are occasionally observed in patients with adrenal carcinoma who show hypertension and hypokalemia (41, 43). A further indication that our procedure for measuring competitive activity can detect deoxycorticosterone when it is present in sufficient concentration to exhibit marked clinical effects is our finding (unpublished) that marked elevations of competitor activity are found in plasma after administration of large dosages of deoxycorticosterone (20 mg/day) to patients.

Using a buffer system, Feldman and Funder (45) found that aldosterone was 70 and 210 times more effective as a competitor than 18-hydroxy-deoxycorticosterone and 18-OH-corticosterone, respectively. In the present studies, performed with plasma, aldosterone was found to be 1,000 times more potent than either of these steroids as a competitor (Table I). The lower activity of 18-hydroxy-deoxycorticosterone and 18-hydroxy-corticosterone as competitors in plasma as compared with buffer suggests that these steroids are bound to a significant extent by plasma proteins. Accordingly, it appears that a marked elevation of the plasma concentrations of either of these steroids would be required to produce significant receptor occupancy and thus mineralocorticoid activity. Thus, it is unlikely that plasma 18-hydroxy-deoxycorticosterone concentrations in the 20–60 ng/dl range, as reported for normals (46), have important sodium-retaining effects.

Relative to aldosterone, the concentrations of the spironolactone, SC14266, required to inhibit [^{3}H]aldosterone binding in plasma were also greater than those reported for inhibition in buffer (40). Thus, it is likely that this compound binds significantly to plasma proteins as well.

It has been recently reported that most of the mineralocorticoid activity in urine of patients with low-renin essential hypertension is caused by substances other than aldosterone (22). This activity was identified as 16β-OH-DHEA, found in greater quantities in patients with low-renin essential hypertension than in those with normal-renin essential hypertension. This steroid was found by bioassay in the rat to have one fortieth the sodium-retaining activity of aldosterone. The 16-oxo isomer of 16β-OH-DHEA, 16-oxo-androstenediol, was also found to have sodium-retaining activity (22). Since it is readily formed from 16β-OH-DHEA, it was questioned whether 16-oxo-androstenediol could also be present in low-renin essential hypertension (22). In the present studies, 16β-OH-DHEA and the 16-oxo isomer, tested in

plasma and in buffer, did not inhibit [^{3}H]aldosterone binding, even at concentrations 500- to 1,000-fold higher than those of aldosterone. Funder et al. also reported in abstract form (47) that 16β-OH-DHEA and the 16-oxo isomer do not inhibit [^{3}H]aldosterone binding as measured in a buffer system. Furthermore, these workers verified the authenticity of 16β-OH-DHEA before and after the incubations. Thus our findings, and those of Funder and co-workers, suggest that 16β-OH-DHEA and its 16-oxo isomer are not potent sodium-retaining steroids that can act through the rat mineralocorticoid receptor. They do not exclude the possibility that the steroids exhibit aldosterone-like actions through another receptor system. Although a question may be raised as to the appropriateness of the rat receptor for these studies, it should be noted that the sodium-retaining activity of 16β-OH-DHEA and the 16-oxo isomer was demonstrated in a rat bioassay system (22).

In the present studies, we used the competition procedure to obtain estimates of the general pattern of relative competitor activity in plasma. We have not standardized or refined the procedure in sufficient detail to be usable as a clinically applicable radioreceptor assay. Thus, we have emphasized relative differences in the activity of plasma samples assayed within an experiment.

Using the competition procedure, we compared the activity of plasma obtained at 8 a.m. after the subjects had been recumbent, with those obtained at 12 noon, after they had been in the erect posture for 4 h. We found that the competitor activity of plasma increases under these physiological circumstances in which plasma aldosterone increases and plasma cortisol decreases. These findings suggest that the competition procedure is sensitive to physiological variations in plasma steroids. Thus, although cortisol is present in plasma at concentrations much higher than those of aldosterone, it does not ordinarily prevent detection of physiological changes in plasma aldosterone. It should be emphasized, however, that at physiological concentrations, cortisol can inhibit the binding of [^{3}H]aldosterone by the receptors (Fig. 3). This inhibition may result partly from the blockage by cortisol of aldosterone binding by the glucocorticoid receptors. However, it is unlikely that all of the inhibition is due to this; some or in fact most must be due to occupancy by cortisol of mineralocorticoid receptors. Indeed, at physiological concentrations in plasma, cortisol doubtless exhibits some sodium-retaining activity (48). These data further suggest that if plasma aldosterone is increasing and plasma cortisol is decreasing, the competition assay would reflect a composite influence of these changes. This appears to be the case. The difference between the competitor activity of samples taken with the subjects erect at 12 noon and that of samples taken with them supine at 8 a.m. appears to be less than that attributable to aldosterone alone (Fig. 5). However, confirmation of this point will have to await refinement of the assay so that changes in competitor activity can be more precisely related to mineralocorticoid equivalents.

Competitor activity was markedly elevated in plasma of patients with primary aldosteronism, a condition known to be associated with sodium retention and suppressed plasma renin. Thus, these techniques can detect changes in competition for mineralocorticoid receptor binding under physiological stimuli such as change of posture, in pathological states, and after pharmacological administration of deoxycorticosterone.

Of particular note is that the mean and range of competitor activity in plasma from patients with low-renin essential hypertension did not differ from that of normals and was not greater than the activity in plasma from patients with normal-renin essential hypertension. Thus, these studies do not support the hypothesis that in low-renin essential hypertension there are major elevations of plasma steroids with mineralocorticoid activity that can interact with the aldosterone receptor. It should be realized that the assay procedure should detect such elevations if present, even if they represented a combination of steroids or reflected a decrease in plasma binding of steroids.

From the presently available data, we cannot exclude the possibility that in low-renin essential hypertension there are minor elevations of plasma steroids with sodium-retaining activity that interact with the mineralocorticoid receptor. However, it should be stressed that changes in competitor activity can be detected in response to the physiological influence of a change in posture. Thus, if there are minor elevations of steroids with sodium-retaining activity in the plasma of patients with low-renin essential hypertension, which are not detectable by this method, the significance of these elevations might be questioned.

The present studies do not exclude the possibility that in low-renin essential hypertension, elevations of plasma steroids with sodium-retaining activity may utilize another receptor system. The question might also be raised as to whether certain important metabolites could be formed intracellularly and that these might not be detected by the assay. However, since the competition experiments were performed with an intact cell preparation at 37°C, one would have to assume that these metabolites could not accumulate under the conditions employed or would only accumulate in the kidneys of humans, but not rats. Also, there could be elevations of steroids that act through human but not rat mineralocorticoid receptors. Finally, since the present studies are retrospective, done on frozen specimens, it seems warranted to examine the question of an elevated plasma

mineralocorticoid activity in low-renin essential hypertension in a prospective way, to exclude the possibility that an unstable and presently unidentified steroid is present in fresh plasma. In such studies, plasma can be examined from many more patients and can be taken under a variety of conditions and at different times of the day.

In addition to the doubt raised by these binding studies, the idea that elevated plasma concentrations of sodium-retaining steroids are a major factor in low-renin hypertension might also be questioned for other reasons. This condition appears to differ from syndromes known to involve such steroids (2–6), in that hypokalemia, although infrequently present (49), has not been a prominent feature of the disease (3, 8–11, 14, 49). Further, whereas an elevation in total body sodium has been reported for primary aldosteronism (5, 6), the earlier suggestion that this exists in low-renin essential hypertension (15) has not been confirmed (6). Finally, other studies suggest that plasma and extracellular fluid volume are also normal in low-renin essential hypertension (49). Thus, whereas clear evidence against the steroid-excess hypothesis for low-renin hypertension has not been presented, the available data should at least raise some doubt as to their validity, and other potential mechanisms should be considered.

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