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SPECIFIC ALDOSTERONE-BINDING PROTEINS IN HUMAN PLASMA: PARTIAL CHARACTERISATION*

KEITH S. C. RICHARDSON, WOJCIECH NOWACZYNSKI and JACQUES GENEST

Steroid Research Department, The Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal H2W 1R7, Quebec, Canada

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SUMMARY

In vitro evidence of two new aldosterone-binding proteins in human plasma is presented. These were resolved by gel chromatography (Sephadex G-75 and G-200) and one, a specific, high affinity aldosterone-binding globulin (ABG) has been partially characterised. Both proteins were precipitated from plasma by the addition of ammonium sulfate at 0.57 M–1.40 M concentration, pH 7.4. Polyacrylamide gradient gel (PAA 4/30) electrophoresis (pH 7.80) and electrofocusing (Sephadex G-75 S) showed a single homogeneous band for each one. They showed no interaction with bromophenol blue or Sudan black, but gave a positive periodic acid–Schiff (PAS) stain after electrophoresis. There was no binding of cortisol to either of the precipitated proteins at 23° or 37° but an appreciable binding to both was seen at 4°. Under similar conditions, progesterone showed no binding affinity. Reversible binding of aldosterone for ABG was demonstrated, confirming the specificity of the binding properties. ABG was inactivated by heating at 60° for 35 min but the other aldosterone-binding protein was not. The estimated molecular weights (Sephadex gel filtration) were 26,500–27,500 daltons for ABG and 35,000–37,000 for the other. Two apparent association constants of aldosterone binding affinity for ABG were determined ($2.33 \times 10^7\ M^{-1}$ and $2.50 \times 10^6\ M^{-1}$), indicating a dual binding system. Other physico-chemical properties differentiated ABG from the corticosteroid binding globulin (CBG) and the α_1-acid glycoprotein (AAG).

INTRODUCTION

There is an interaction between some circulating plasma proteins and a variety of small molecules and ions to form protein–ligand complexes [1, 2] and this interaction has been shown to affect the hormonal activity of corticosteroids [3, 4]. Although it is generally accepted that cortisol, the major glucocorticoid in man, is bound to a specific circulating plasma protein, the corticosteroid-binding globulin (CBG) or transcortin, and the gonadal steroids to the sex hormone-binding protein, similar plasma binding of aldosterone, the most potent mineralocorticoid, remained unresolved [2–10]. Albumin was first recognised as the major aldosterone-binding plasma protein [3, 5] but later, the high affinity binding of aldosterone to a plasma globulin thought to be transcortin [2–5, 9] and perhaps a weak affinity for the α_1-acid glycoprotein (AAG or orosmucoid) [2] were also demonstrated. Meanwhile, three separate investigations [6–8] showed that aldosterone was similarly bound to another unidentified plasma protein. Nowaczynski *et al.* [10], studying the dynamic aspects of aldosterone metabolism in man, concluded that part of the total steroid was indeed bound to a 'transcortin-like plasma fraction' (TLPF) postulated to play an important role in essential hypertension (EH).

Gel chromatographic resolution and partial characterisation of a specific, high affinity aldosterone-binding globulin (ABG) and another, heat-stable aldosterone-binding protein in human plasma is reported here. A preliminary account of this work has appeared as a letter to the editor [11].

EXPERIMENTAL METHODS

Reagents. All reagent grade chemicals were used without further purification. Chromatographic experiments were done in distilled, deionized water (Milli-RO: Milli-Q System, Millipore Corp., MA), adjusted to pH 6.4 and with sodium azide (0.02–0.05%) added to inhibit bacterial growth. Plasma obtained from the Canadian Red Cross and also pooled sera from carefully selected EH patients were stored at −20° until used. The results were consistent using either plasma or serum. Radioactivity measurements were recorded on a Packard Liquid Scintillating Counter (Model 3375) at about 60% efficiency. [^{3}H]-aldosterone (100 Ci/mMol), [^{3}H]-cortisol (47 Ci/mMol) and [^{3}H]-progesterone (60 Ci/

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Requests for reprints should be addressed to: Dr. W. Nowaczynski, The Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal H2W 1R7, Quebec, Canada.

mMol) were purchased from Amersham Searle. Sephadex gels from Pharmacia, dialysis membrane from Spectrum Medical Industries Inc., L. A., CA 90054, and human serum albumin (HSA) and bovine serum albumin (BSA) from Sigma Chemical Company.

Experimental techniques. Plasma, diluted 1:1 with isotonic saline, was chromatographed separately on Sephadex G-75 and G-200 columns (50 × 1 cm) at 23° using a modification of the method of Morris *et al.* [12], before or after incubation for 3 h at 37° [10] with either [^{3}H]-aldosterone, [^{3}H]-cortisol, or [^{3}H]-progesterone. Similar gel chromatography was done on unheated and preheated (60° for 35 min) protein fractions precipitated from plasma with ammonium sulfate at 1.14 M concentration, pH 7.4. The columns were eluted with water (pH 6.4), 1 or 2 ml vols collected and the elution profile monitored automatically at 280 nm. In the [^{3}H]-steroid treated samples, aliquots of appropriate column eluates were simultaneously counted for radioactivity in 10 ml of Aquasol 2 (New England Nuclear) and also analysed for protein-steroid binding by the dextran-coated charcoal assay [10]. The elution vol. of the unbound radioactive steroid was determined separately.

The method of Heyns *et al.*[13] for the removal of endogenous steroid from plasma binding proteins was adapted to study the reversible binding of labelled steroid. To aliquots of precipitated plasma protein solutions, previously incubated with [^{3}H]-aldosterone, an equal vol. of dextran-coated charcoal suspension (0.4% dextran, 4% charcoal) was added and the mixtures shaken continuously throughout the experiment. Duplicate samples (0.5 ml) were assayed for radioactivity at 2, 6, 24 and 48 h intervals after the mixtures had been centrifuged at 3,500 rev./min for 10 min. The binding was verified at 4°, 23° and 37°. Similar experiments were done with [^{3}H]-cortisol and [^{3}H]-progesterone.

The Scatchard plot technique [1] was used to analyse binding properties as follows: a mixture of 0.3 ml of the appropriate column eluate (T-16, see Results), 0.1 ml of 0.05 M phosphate buffer pH 8.0, and 0.1 ml of increasing quantities of [^{3}H]-aldosterone (2,500–15,000 c.p.m.) in buffer was incubated at 37° for 3 h. In parallel experiments, 0.1 ml of buffer containing 1000 pg of cold aldosterone was replaced by plain buffer of comparative tubes to maintain a constant vol. of 0.5 ml. The tubes were then chilled to 4° and 0.5 ml of dextran-coated charcoal [14] suspended in deionized water added, the combined mixture briefly vortexed, kept at 4° for 10 min and finally centrifuged as above. The supernatant (0.5 ml) was counted for radioactivity. The amounts of 'bound' and 'free' steroid were calculated as described [15]. The Lowry technique [16] with bovine serum albumin as standard and the spectrophotometric absorption at 280 nm, established the protein content of the eluate.

Equilibrium dialysis [9, 17] was employed to study the displacement of [^{3}H]-aldosterone from plasma proteins other than albumin by unlabelled aldosterone and cortisol. In a second set of displacement reactions, plasma aliquots were incubated [10] with [^{3}H]-aldosterone alone as well as with increasing amounts of unlabelled aldosterone or cortisol and the binding determined by the dextran-coated charcoal assay. At each increment of unlabelled steroid, the TLPF [10] was also determined. The dissociation rate of labelled ABG-bound steroid was measured as follows: in parallel experiments, test tubes containing 0.1 ml of the T-16 eluate were incubated at 37° with 5000 c.p.m. of [^{3}H]-aldosterone in 0.1 ml of 0.05 M phosphate buffer, pH 7.4 and at 2 and 3 h intervals, 1000 pg of unlabelled steroid in 0.3 ml of buffer were added to appropriate tubes and the dissociation rate of [^{3}H]-aldosterone determined at the times indicated.

Electrophoresis was performed on polyacrylamide gradient gel (Pharmacia, PAA 4/30) in a Tris–borate (pH 7.8) continuous buffer system and the proteins localized by staining with Ponceau S (Beckman) or coomassie blue (Canalco). When sections of the gel were analyzed for steroid protein complexes, two identical slabs were run in parallel and one was stained. The matching area of the unstained gel was minced with a pair of scissors in a counting vial containing 15 ml of Aquasol 2, the vials were shaken continuously until the gel dissolved (3 h) and then counted. Periodic acid Schiff (PAS-) staining for the location of glycoproteins [18] and electrofocusing [19] on Sephadex G-75 Superfine containing 3% Ampholine (LKB) at pH 3 10 and 4 6 were performed as described. Molecular weight was estimated by gel filtration [20, 21] on Sephadex G-75 and G-200 (50 × 1 cm) columns calibrated with standard proteins of known molecular weight (Pharmacia Calibration Kit No. 73N394) and BSA.

Aldosterone concentrations were measured by radioimmunoassay [22] on the intact plasma, the ammonium sulfate (1.40 M) precipitate and the resultant supernatant medium and the cortisol concentrations by a chromatographic ligand assay [23]. The percentage of [^{3}H]-aldosterone bound to TLPF was estimated [10] for comparison.

RESULTS

Sephadex column chromatography. Figure 1(b) shows a typical spectrophotometric elution profile of dilute preincubated plasma chromatographed on Sephadex G-75 (23°) and monitored at 280 nm. The radioactivities of [^{3}H]-aldosterone and [^{3}H]-cortisol associated with their respective protein–steroid complexes are shown in Figure 1(a). A single peak of radioactivity associated with cortisol–protein complexes extended from tubes 7–12 (12–24 ml) with an absorption maximum in tube 10. Radioactivity for aldosterone–protein complexes extended from tubes 7–17 (12–34 ml) but was divided between two peaks,

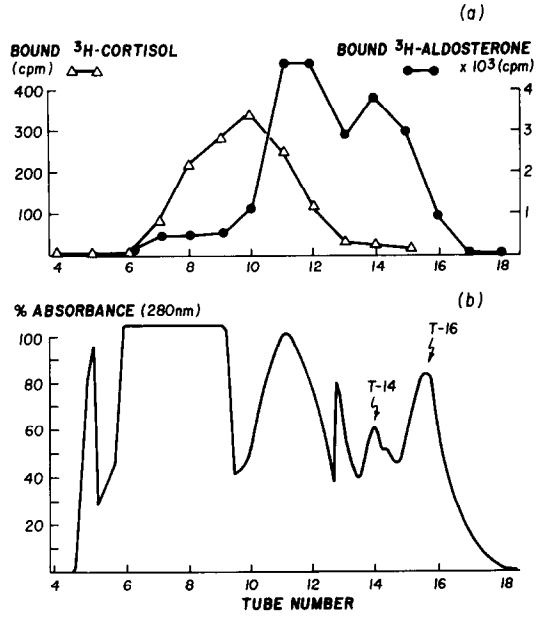

Fig. 1. Sephadex G-75 chromatography of labelled whole plasma diluted 1:1 with 0.9% saline. (a) Each tube (0.5 ml) was assayed for the protein binding of [^{3}H]-cortisol (△——△) and [^{3}H]-aldosterone (●——●). (b) The elution profile monitored at 280 nm. C.p.m./0.25 ml of plasma is plotted against tube number.

the first overlapping the single cortisol peak in tubes 11–12 (20–24 ml) while the second emerged separately in tubes 14–16 (26–32 ml). The second peak of [^{3}H]-aldosterone was distributed among two major protein absorption bands (280 nm) coinciding with tubes 14 and 16 (Fig. 1(b)) and designated T-14 and T-16 respectively. A third peak composed of unbound hormones (not shown in this figure) was eluted in tubes 23–28 (46–56 ml). The U.V. elution profile and the distribution of radioactivity on Sephadex G-200 were virtually the same on G 75 chromatogram in the effluent vol. 20–36 ml. Figure 2 shows the distribution of radioactivity (2(a)) and the spectrophotometric elution profile (2(b)) from a Sephadex G-75 column for a solution of the intact protein-precipitates and for a similar solution heated [10]. The spectrophotometric absorption pattern showed that T-14 and T-16 protein bands eluted in similar respective effluent vols (Fig. 2(b)) for both the heated and control samples, but the distribution of radioactivity for the heated moiety was associated with the T-14 band only. Interestingly, near the void vol. (11–15 ml) in the heated sample, a new peak of radioactivity (T-7) appeared. This was later found to result from partial polymerisation of the T-14 macromolecule after a similar peak had appeared following concentrating *in vacuo* a partially purified solution of this protein.

Ammonium sulfate fractionation. The above aldosterone binding plasma proteins began to precipitate at an ammonium sulfate concentration of 0.57 N, the major portion precipitating at 1.14 M (pH 7.4) and no more appearing above 1.40 M. Contaminating proteins, representing non-specific binding of unknown significance, shown in Fig. 2 (tubes 10–13), were omitted from the spectral pattern by heating a solution of the precipitate at 45° for 20 min. Precipitation of the specific aldosterone-binding plasma proteins resulted in partial loss of binding activity.

Binding and the effect of temperature. The binding activity of T-16 for [^{3}H]-aldosterone decreased sharply but not completely when either the intact plasma or ammonium sulfate-precipitated fractions were heated at 60° for 20 min before incubation, while the spectrophotometric pattern remained unchanged. Increasing the heating period to 35 min resulted in complete disappearance of binding activity for T-16 (Fig. 2(a)) but not for T-14 and its subsequently polymerised product, T-7.

Under our experimental conditions, ammonium sulfate-precipitated aldosterone-binding proteins showed no binding activity for [^{3}H]-cortisol at either 37° or 23° but there was appreciable binding of the hormone at 4° which remained constant throughout the 48-h experiment [13]. Under identical conditions, no binding activity was shown for [^{3}H]-progesterone. In contrast, an appreciable decrease in [^{3}H]-aldosterone binding occurred after 2 h at 37° with no further changes seen thereafter. Binding activity for aldosterone at 4° and 23° remained unchanged but the amount of initially ABG-bound steroid increased with decreasing temperature.

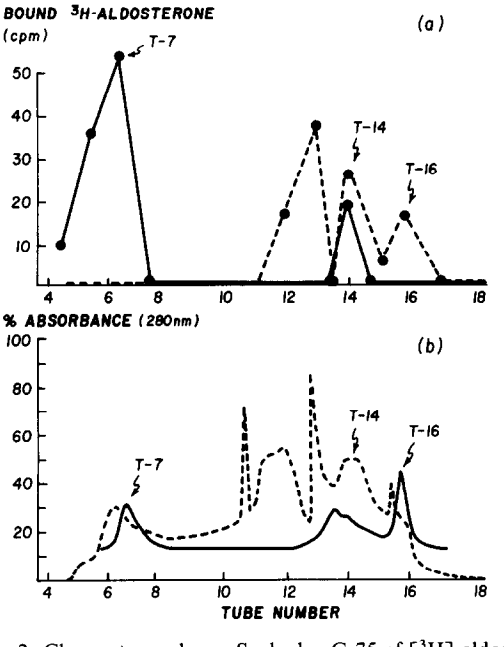

Fig. 2. Chromatography on Sephadex G-75 of [^{3}H]-aldosterone-labelled plasma proteins precipitated by addition of ammonium sulfate (1.14 M). Isotonic saline solutions of the precipitated proteins (2 ml) previously incubated with the steroid (10^5 c.p.m./ml) were applied to the column and the protein-bound radioactivity determined for each tube (c.p.m./0.25 ml). (a) (solid line) solution heated (60° for 35 min) prior to incubation: (b) (dotted line) control solution. Absorbance of the heated (solid line) and unheated (dotted line) solutions monitored at 280 nm.

Table 1. The displacement of [^{3}H]-aldosterone with cortisol and aldosterone from the binding sites of the TLPF of human plasma by the dextran-coated charcoal assay. The TLPF binding* was determined at each increment of unlabelled steroid

Added cortisol pg/0.1 ml	% TLPF	Added aldosterone pg/0.1 ml	% TLPF
0	8.2	0	8.2
25	7.6	25	8.0
50	7.2	50	7.1
100	6.2	100	5.5
200	6.0	200	4.6
250	6.0	250	3.3

* Binding experiments were performed as described elsewhere [10] except that parallel experiments were carried out with unlabelled steroid added as shown above in both the heated (60° for 35 min) and unheated plasma aliquots.

Table 2. Equilibrium dialysis at 37° and the effect of added aldosterone and cortisol on the binding of [^{3}H]-aldosterone to serum proteins and albumin*

Cold steroid μg	Labelled steroid (100,000 c.p.m.)	[^{3}H]-Aldosterone bound and free in serum dialysate: Albumin %	Other protein %	Free %
Aldosterone	[^{3}H]-Aldosterone			
0		30	27	43
1		33	25	42
3		33	22	45
5		33	21	46
8		35	20	45
10		34	17	49
11		37	16	47
12		37	15	48
Cortisol				
1		35	14	51
2		37	11	52
2.5		38	10	52
4		39	8	53
5		40	8	52

* Dialysis was performed for 24 h on 2 ml serum essentially as described [9], and triplicate aliquots were counted for radioactivity. Values are the mean of three determinations.

Displacement reactions. Using both dextran-coated charcoal (Table 1) and equilibrium dialysis (Table 2) at 37°, the addition of unlabelled steroid gave significant displacement of tritiated aldosterone from the binding sites of the plasma proteins believed to be TLPF. Similar displacement of [^{3}H]-aldosterone by unlabelled cortisol was minimal in the charcoal assay but not in the equilibrium dialysis method.

Figure 3 shows the rate of dissociation of [^{3}H]-aldosterone from ABG. Two apparent binding systems were evident: the first showed fairly rapid exchanges which remained relatively constant. According to Lefkowitz *et al.* [24] this suggests a thermodynamic equilibrium point at which binding and dissociation occur simultaneously at rates too rapid to be recorded by the method used. The other binding system showed only slight dissociation.

The Scatchard plot [1] analysis (Fig. 4) of the binding data for the T-16 protein eluate by the dextran-coated charcoal technique [15, 16] gave two apparent

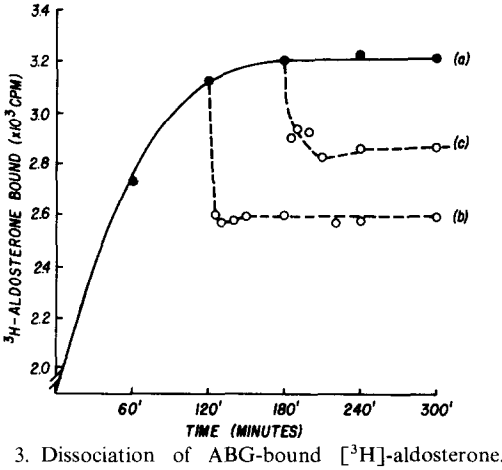

Fig. 3. Dissociation of ABG-bound [^{3}H]-aldosterone. Effect of time on the incubation at 37° (a). Effect of added unlabelled aldosterone after 2 h (b) and 3 h incubation (c). The rate of dissociation was followed by the dextran-coated charcoal technique at times indicated. Values were means of duplicates.

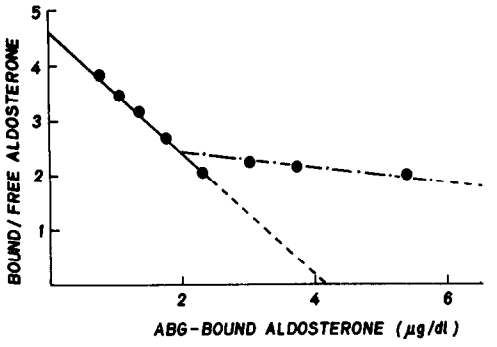

Fig. 4. Scatchard plot analysis of specific binding of [^{3}H]-aldosterone to ABG at 37°. The straight lines were calculated by the method of Baxter and Tomkins[16]. The apparent association constant $K_1 = 2.33 \times 10^7\ M^{-1}$ was derived from the slope of the line (●——●) of best fit for the first five points and $K_2 = 10^6\ M^{-1}$ from the slope of the line (●–·–●) for the remaining three points. Extrapolation of each line gave intercepts on the abscissa equivalent to binding capacities of (N_1) 4.3 μg/dl and (N_2) 18.6 μg/dl of plasma, respectively. The binding data was obtained by the dextran-coated charcoal assay. Each value represents the mean of three separate observations*.

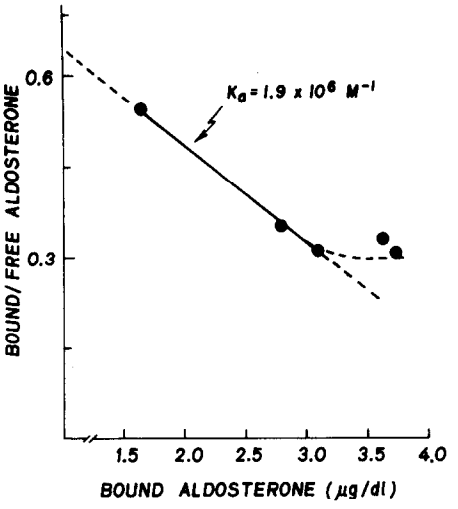

Fig. 5. Scatchard analysis of the specific binding of aldosterone to the TLPF [10]. Points calculated from the data of equilibrium dialysis at 37° (Table 2). Each bag containing 2 ml of serum was dialysed against 20 ml of saline–Tris buffer [9]. K_a, the apparent association constant, was determined from the slope of the straight line of best fit joining the first three points. Values are the mean of three separate experiments. Bound aldosterone is expressed in μg/dl of plasma.

association constants for aldosterone, which indicated a dual binding system; $K_1 = 2.33 \times 10^7\ M^{-1}$ and $K_2 = 2.5 \times 10^6\ M^{-1}$ with binding capacity of 4.3 μg/dl and 18.6 μg/dl of plasma, respectively. Similar values were obtained with the intact plasma. The association constant of aldosterone binding for albumin, not shown here, was $3.2 \times 10^3\ M^{-1}$.

In contrast, Scatchard plot analysis of binding data (Table 2) derived from equilibrium dialysis [9, 17] of whole plasma gave an apparent association constant $K_a = 1.9 \times 10^6\ M^{-1}$ (Fig. 5) for aldosterone bound to plasma proteins other than albumin, which is in close agreement with the above K_2 and with other reported values [9] for aldosterone bound to a plasma protein thought to be transcortin.

Electrophoresis and electrofocusing. The two aldosterone-binding proteins in human plasma migrated with the mobility of albumin and some α_1-globulins [25] but unlike albumin, they did not interact with bromophenol blue. Similarly, the unchromatographed precipitates or appropriate eluates incubated with Sudan black and then elecrophorised showed no stained protein band. Both ABG and the heat stable aldosterone-binding protein demonstrated a positive PAS- stain after electrophoresis while albumin, as expected, did not. Electrophoresis on polyacrylamide gradient gel showed a single homogeneous band in the α_1-globulin region for both proteins run as a mixture or appropriate eluates. Protein fractions, incubated separately with [^{3}H]-cortisol and [^{3}H]-aldosterone and simultaneously electrophorised, showed no radioactivity for [^{3}H]-cortisol in regions of the gel similar to where [^{3}H]-aldosterone was found.

In repeated experiments, albumin demonstrated an isoelectric point ranging from pI 4.55–4.60 whereas both aldosterone-binding proteins ranged between pI 4.75 and 4.80. Analytical isoelectrofocusing on granular gel also showed a single homogeneous band for both aldosterone-binding proteins.

Molecular weight determinations. Figure 6 shows a typical calibration curve using standard proteins

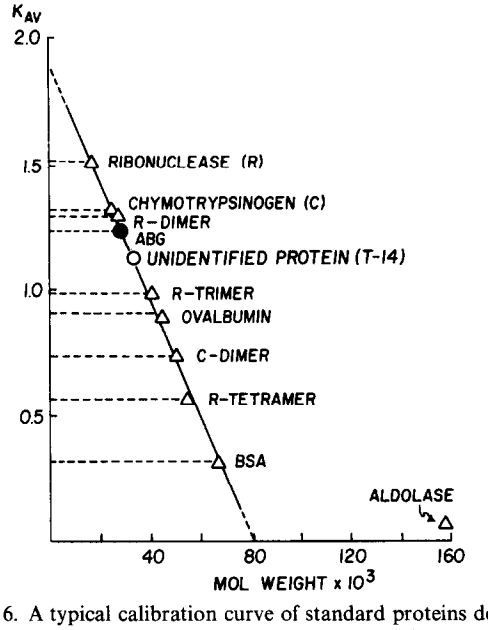

Fig. 6. A typical calibration curve of standard proteins derived from gel filtration on a Sephadex G-75 column (△). The diffusion coefficient (K_{av}) was calculated from a standard equation (Pharmacia Bulletin. Sephadex: Gel Filtration in theory and practice (1974), p. 56) and K_{av} plotted against the molecular weights. The mean K_{av} for ABG (●) and the unidentified heat-stable aldosterone-binding protein, T-14 (○) were determined as described.

* These values were uncorrected for endogenous steroid.

Table 3. Radioimmunoassay for total aldosterone [22] and the determination of cortisol concentration [23] performed on the ammonium sulfate precipitate (1.40 M), the corresponding supernatant and the intact serum from EH patients

Sample	Aldosterone ng/dl	Cortisol μg/dl
Precipitate	3.2	0
Supernatant	19.7	22.6
Intact Serum	22.4	23.8

Values are means of duplicates at two different concentrations.

eluted from Sephadex G-75 column. The diffusion constant (K_{av}) [26] for T-16 ranged from 1.30–1.26 and for T-14 from 1.08–1.03 corresponding to a molecular weight of 26,500–27,500 for the former and 35,000–37,000 for the latter. Elution vols obtained on G-200 were almost identical, whereas on G-25 and G-50, also tested, no distinct isolation pattern was seen, probably because of elution rates too rapid for effective separation.

Plasma corticosteroid determinations. Table 3 shows total plasma aldosterone and corresponding plasma cortisol in the intact plasma, the ammonium sulfate-precipitated proteins and the remaining supernatant. In repeated determinations, aldosterone concentration in the precipitate corresponded very closely to the TLPF [10] determined in the intact plasma indicating the specificity of this method for ABG. In a typical experiment, the fraction of the total endogenous aldosterone in the precipitate was very similar (14.3%) to the percentage of the total bound steroid measured in the intact plasma as TLPF (14.7%). The same salt-precipitated ABG plasma fraction, which should not contain CBG, showed neither cortisol nor its binding. In contrast, the resultant supernatant medium gave a value for endogenous cortisol concentration of 22.6 μg/dl, very similar to that in the intact plasma, 23.8 μg/dl.

DISCUSSION

This investigation was stimulated by observations in EH patients of decreased aldosterone metabolic clearance rate (MCR) apparently due in part to increased plasma protein binding [10].

Sephadex G-75 and G-200 gel chromatography of human plasma presented a simple method for the resolution and partial separation of the two aldosterone-binding plasma proteins and G-75 gel was preferred for the separation of globular proteins and estimating molecular weights between 3000 and 37,000 daltons [26]. The use of dextran-coated charcoal to investigate steroid–protein interaction has been critically evaluated by some researchers [27, 28] and extensively employed by many others to study the kinetics of the separation of 'free' from 'bound' ligands [10, 13–16]. We found that this technique gave remarkable reproducibility of results and also saved time, simplified the methodology and provided milder assay conditions.

Precipitation of CBG, AAG, and albumin requires a greater concentration of ammonium sulfate [29] than that needed to precipitate ABG and the other aldosterone binding protein. Like CBG, the ABG demonstrated a considerable loss of binding activity on precipitation. Ammonium sulfate is not expected to precipitate endogenous steroids unless bound to plasma components. Hence, the similarity of cortisol concentrations in the supernatant and the intact plasma indicated that CBG was indeed not precipitated under these conditions. Moreover, the absence of cortisol in the corresponding precipitate and the close agreement between the aldosterone level in this plasma fraction and that determined by the TLPF method [10] in the intact plasma clearly indicated two distinct new conticosteroid binding globulins in human plasma. This conclusion is further supported by the finding that ABG showed no binding affinity for progesterone while this hormone is bound more strongly to CBG than cortisol at 37° [30].

CBG was irreversibly inactivated at 60° for 20 min [4, 31], while ABG required a slightly longer time for complete and irreversible inactivation. In contrast, orosmucoid (AAG) has been reported to withstand temperatures of 100° for 5–10 min [4]. Moreover, of the two aldosterone binding proteins studied, ABG showed no tendency to polymerize under routine procedures while human CBG was reported to undergo self-aggregation [32].

The apparent association constants of aldosterone for ABG at 37° and for albumin found in our study are in good agreement with other reports [3–5, 9]. In addition, Scatchard plot analysis of our data from equilibrium dialysis on intact plasma gave an apparent association constant consistent with K_2 from this study. Since the Scatchard plots for the column eluates, precipitate and intact plasma all gave similar affinity constants by the dextran-coated charcoal technique, the slightly lower value obtained by equilibrium dialysis [9, 12] was perhaps a result of different experimental conditions. Analysis of dissociation rates of [^{3}H]-aldosterone from ABG supports the possibility of two binding systems [24], the first of which may be related to the specific high affinity binding constant K_1. It is not certain whether the lower affinity binding (K_2) is characteristic of a different binding site on the same plasma component or is rather related to another chromatographically unresolved protein.

The routine use of Sephadex gel filtration to estimate molecular weights of proteins [20, 21, 33] and the excellent reproducibility of the elution of both aldosterone-binding proteins allowed a reliable estimation of their molecular weights which were much lower than either CBG (52,000) or AAG (41,000) [2]. From electrophoretic and electrofocusing data, the α_1-acid nature of these proteins was established and

evidence given that they were not lipoproteins or albumin artifacts.

The finding of the specific aldosterone-binding to ABG supports previous observations that subtle modifications in aldosterone metabolism in EH patients were due partly to an appreciable increase in this binding [10]. Firmer evidence of the possible regulatory role of this binding has been provided by our group [34, 35] in which acute ACTH infusion, besides raising plasma aldosterone, also accelerated the hepatic catabolism by reducing the plasma ABG binding of this hormone [34, 35]. The decrease in this binding by only a few per cent was paralleled by a significant increase in the MCR. Moreover, ACTH infusion increased the MCR and inversed the pattern of aldosterone urinary metabolites with no change in hepatic blood flow [34–36]. In addition, angiotensin II infusions increased plasma aldosterone, decreased its MCR and, in contrast to ACTH, lowered the hepatic blood flow with no change in aldosterone bound to ABG [37]. Other possible physiological significance of this new plasma protein has been suggested [38].

Our study does not explain why the percentage of aldosterone bound to ABG is much less than that reported for cortisol bound to CBG [4]. The difference may, however, be related to very different plasma concentrations of these two hormones and different plasma concentrations of CBG and ABG. An interaction with the albumin binding system of a lower affinity but much greater plasma concentration, and consequently its total binding capacity, could be an important factor also. Further research is necessary to explain this particular point.

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