Hydrodynamic properties of the angiotensin II receptor from bovine adrenal zona glomerulosa

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The bovine adrenal angiotensin II receptor was solubilized with the non-ionic detergent octyl β -D-glucoside following its binding with the high-affinity antagonist ^{125}I -labelled $[\text{Sar}^1, \text{Ile}^8]$ angiotensin II. The complex was sufficiently stable to allow the determination of its hydrodynamic properties. The solubilized receptor migrated on a Superose 6 column as a single peak with a Stokes radius of 5.29 nm. Comparison of sedimentation behaviour through a sucrose density gradient in H_2O and $^2\text{H}_2\text{O}$ lead to a partial specific volume of 0.751 ml/g and a sedimentation coefficient ($s_{20,w}$) of 5.17 S. Combination of gel filtration and sedimentation data indicated that the labelled protein–detergent complex has an M_r of 124 000 and a frictional ratio of 1.42. The M_r of the angiotensin II receptor was estimated as 104 000 kDa after correction for the bound detergent. Photoaffinity cross-linking of ^{125}I - $[\text{Sar}^1, (4-\text{N}_3)\text{Phe}^8]$ angiotensin II with bovine adrenal membrane receptor followed by SDS/PAGE under reducing and non-reducing conditions yielded a broad band of M_r 54 000. This suggests that the angiotensin II receptor is a non-covalent dimer in which the two subunits have a similar M_r .

INTRODUCTION

Angiotensin II (AII) is an octapeptide that binds to specific receptors to exert its biological actions. These receptors have been found in a number of tissues, including adrenal cortex (Lin & Goodfriend, 1970; Glossman *et al.*, 1974), uterus (Lin & Goodfriend, 1970; Rouzaire-Dubois *et al.*, 1975; Capponi & Catt, 1979), vascular smooth muscle (Gunther *et al.*, 1980), liver (Campanile *et al.*, 1982), brain (Bennett & Snyder, 1976) and heart (Mukherjee *et al.*, 1982; Rogers, 1984), and their binding properties have been well characterized (reviewed in Capponi *et al.*, 1981). The most important effects resulting from the activation of AII receptors are a stimulation of steroidogenesis (Douglas *et al.*, 1976) in the adrenal cortex and a potent vasoconstriction of the vascular smooth muscle (Gunther *et al.*, 1980) leading to an increase in blood pressure. Thus the AII receptor represents a potential site of intervention in the development of new drugs for the therapy of hypertension (Regoli *et al.*, 1974; Peach, 1977). An essential step towards comprehension of the mechanism of action of AII is the elucidation of its receptor structure. The major drawback to the physical characterization and purification of the AII receptor has been the difficulty in solubilizing the protein in a sufficiently stable and active form (Brecher *et al.*, 1974; Devynck *et al.*, 1974; Forget & Heisler, 1979; Capponi & Catt, 1980; Sen *et al.*, 1984). Some groups of workers have been able to recover binding activity after solubilization, but their methods have not led to further purification or characterization of the receptor (Chang & Lotti, 1981; Capponi *et al.*, 1983; Sen *et al.*, 1983; Akiyama *et al.*, 1984). For this reason, the techniques of photoaffinity labelling and chemical cross-linking have been used to obtain a stable complex which permits the study of the properties of the solubilized AII receptor (Capponi & Catt, 1980; Paglin & Jamieson, 1982; Guillemette & Escher, 1983; Guillemette *et al.*, 1984, 1986; Rogers, 1984; Carson *et al.*, 1987).

We have previously validated the application of the antagonist $[\text{Sar}^1, \text{Ile}^8]\text{AII}$ (SARILE) as a high-affinity radioligand which enabled us to document the existence of an agonist-specific high-affinity conformation of the angiotensin receptor (De Léan *et al.*, 1984). The antagonist–receptor complex formed with ^{125}I -labelled SARILE is only slowly dissociable and is quantitatively recovered following detergent extraction with octyl β -D-glucoside (OG). In contrast, the corresponding agonist–receptor complex was readily dissociable and, following detergent extraction, behaved as a larger molecular complex which appeared to include a guanine-nucleotide-binding protein. Thus the solubilized antagonist–receptor complex obtained with ^{125}I -SARILE appeared to represent a simpler form of the receptor protein and seemed more appropriate for studying its properties.

In this paper, we report the first full hydrodynamic characterization of the AII receptor solubilized in OG. These results, combined with direct photoaffinity labelling of the receptor protein, document the molecular composition of the detergent-isolated antagonist–receptor complex.

EXPERIMENTAL

Chemicals

SARILE was from Institut Armand Frappier (Laval, Canada). $[\text{Sar}^1, (4-\text{N}_3)\text{Phe}^8]\text{AII}$ (azido-SARANG) was kindly provided by the laboratory of E. Escher (Sherbrooke, Quebec, Canada). Carrier-free Na^{125}I was purchased from Amersham; Iodo-beads were from Pierce Chemical Co. and OG was from Calbiochem. BSA, aprotinin, leupeptin, pepstatin A and $^2\text{H}_2\text{O}$ (99.8% pure) were from Sigma. Thyroglobulin, catalase, aldolase, BSA, chymotrypsinogen A and ribonuclease A were from Pharmacia Fine Chemicals. Electrophoresis standards and reagents were from Bio-Rad.

Abbreviations used: AII, angiotensin II; SARILE, $[\text{Sar}^1, \text{Ile}^8]$ angiotensin II; SARANG, $[\text{Sar}^1]$ angiotensin II; azido-SARANG, $[\text{Sar}^1, (4-\text{N}_3)\text{Phe}^8]$ angiotensin II; OG, octyl β -D-glucoside; TAE, 10 mM-Tris acetate/0.1 mM-EDTA/100 mM-sodium acetate, pH 8.0; PABA, *p*-aminobenzoic acid; PMSF, phenylmethanesulphonyl fluoride.

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Iodination of peptides

SARILE or azido-SARANG were radioiodinated with Iodo-beads. Typically, 10 µg of peptide was dissolved in 100 µl of 0.5 M-KH₂PO₄, pH 7.0. The reaction was started by the addition of 1 mCi of Na¹²⁵I and two Iodo-beads, and the mixture was incubated at 4 °C for 15 min. The radiolabelled peptide was then separated from free iodine on a PRP-1 cartridge (Hamilton Co., Reno, NV, U.S.A.) and purified by h.p.l.c. on a reverse-phase RP-18 Spheri-5 column (Brownlee Labs, Santa Clara, CA, U.S.A.) using a 30 min linear 0–40 % CH₃CN gradient in 20 mM-triethylamine/acetate, pH 4.0.

Preparation of membranes

Zona glomerulosa layers from bovine adrenal glands were dissected and homogenized in 20 vol. of homogenization buffer [20 mM-NaHCO₃, 2 mM-EDTA, 1 µM-aprotinin, 1 µM-leupeptin, 1 µM-phenylmethanesulphonyl fluoride (PMSF) and 0.1 µM-pepstatin A]. The homogenate was filtered through cheesecloth and centrifuged at 1000 g for 10 min. The supernatant was then centrifuged at 30000 g for 15 min. The resulting pellet was washed twice with homogenization buffer and finally resuspended in 50 mM-Tris/HCl (pH 7.4)/250 mM-sucrose/0.1 mM-EDTA/1 mM-MgCl₂ (2 ml/g of tissue) and stored at –70 °C until use.

Prelabelling of membranes and solubilization

Radioligand binding was carried out by incubating zona glomerulosa membranes (0.5 mg/ml) with 600 pM-¹²⁵I-SARILE in 50 mM-Tris/HCl (pH 7.4)/1 mM-MgCl₂/0.1 mM-EDTA/150 mM-NaCl/0.2 % (w/v) BSA at 25 °C for 30 min. Non-specific binding was estimated in the presence of 1 µM-SARILE. All subsequent operations were carried out at 4 °C. The membranes were centrifuged at 30000 g for 20 min and the pellet was resuspended in TAE buffer (10 mM-Tris acetate/0.1 mM-EDTA/100 mM-sodium acetate, pH 8.0) containing 40 mM-OG at a protein concentration of 1 mg/ml. The suspension was then gently agitated with a magnetic bar for 30 min on ice. The solubilized fraction was obtained by centrifugation at 30000 g for 20 min followed by filtration through a 0.22 µm pore size Millipore filter. This ultrafiltrate was subsequently used for chromatography and sucrose density gradient centrifugation experiments. The receptor-bound radioligand in the soluble receptor preparation was estimated by chromatography on Sephadex G-50 and found to represent 50 % of the total radioligand initially bound to the membrane receptor.

Steric exclusion h.p.l.c.

The solubilized preparation (250 µl) was injected on to a Superose 6 (Pharmacia) column (10 mm × 300 mm) and eluted at 4 °C in TAE buffer containing 250 mM-sucrose and 40 mM-OG at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected and counted for radioactivity in a gamma counter (LKB 1272). The void volume (*V*₀) and the total volume (*V*_T) were evaluated respectively with Blue Dextran and acetone. The elution volume (*V*_e) of the protein standards was determined by monitoring the absorbance at 280 nm. The distribution coefficient, *K*_{av}, was given by the equation (Laurent & Killander, 1964):

$$K_{av} = (V_e - V_0)/(V_T - V_0) \quad (1)$$

The Stokes radius of the hormone–receptor complex was evaluated from a calibration curve relating $(-\log K_{av})^{1/2}$ and the Stokes radius of the reference proteins.

Sucrose density gradient centrifugation

Linear gradients (4.9 ml) of 5–20 % (w/v) sucrose were prepared using a Beckman density gradient former. The low- and high-density solutions, respectively 0 % and 28 % (w/v) sucrose,

were prepared in TAE buffer containing 40 mM-OG, in either H₂O or ²H₂O. Gradients were stored at 4 °C for at least 4 h prior to use. Portions (250 µl) of the solubilized fractions were layered on to gradients and centrifuged for 18 h at 33000 rev./min (*r*_{av}, 84.6 mm) in an SW 55 Ti rotor of a Beckman model L8-55M ultracentrifuge. Samples of reference proteins of known sedimentation coefficient were run simultaneously. The radioligand–receptor complex was detected by counting the radioactivity in each fraction (0.13 ml). Reference proteins were assayed in each fraction by the BCA method (Pierce) using BSA as standard.

Analysis of hydrodynamic data

The partial specific volume of the labelled receptor–detergent complex (*v*_A) was calculated from the sedimentation data obtained in H₂O and ²H₂O using the relation (O'Brien *et al.*, 1978):

$$\bar{v}_A = \frac{(1 - \bar{v}_B \rho_D^B) - \alpha(1 - \bar{v}_B \rho_H^B)}{\rho_H^A(1 - \bar{v}_B \rho_D^B) - \alpha \rho_D^A(1 - \bar{v}_B \rho_H^B)} \quad (2)$$

where *v*_A and *v*_B are the partial specific volumes of the unknown (A) and reference protein (B) respectively; $\alpha = y_D^B y_H^A / y_H^B y_D^A$ where *y* is the migration distance in H₂O (H) and ²H₂O (D); ρ represents the average density taken at 0.5 *y*. BSA was used as the reference protein (*v*_B = 0.736).

The sedimentation coefficient of the receptor complex (*s*_{20,w}^A) was given by the equation (O'Brien *et al.*, 1978):

$$s_{20,w}^A = \frac{y_A(1 - \bar{v}_A \rho_{20,w})(1 - \bar{v}_B \rho_{t,m}^B)}{y_B(1 - \bar{v}_B \rho_{20,w})(1 - \bar{v}_A \rho_{t,m}^A)} \cdot s_{20,w}^B \quad (3)$$

where $\rho_{20,w}$ is the density of H₂O at 20 °C and $\rho_{t,m}$ is the density at the average position of the molecule in the gradient. BSA was again used as the standard protein (*s*_{20,w} = 4.41 S).

The *M*_r of the labelled receptor–detergent complex (*M*_c) was calculated according to the following equation (Siegel & Monty, 1966):

$$M_c = \frac{6\pi N \eta_{20,w} \alpha s_{20,w}}{1 - \bar{v} \rho_{20,w}} \quad (4)$$

where α is the Stokes radius obtained by gel filtration, $\eta_{20,w}$ is the viscosity of water at 20 °C, *N* is Avogadro's number and $\rho_{20,w}$ is the density of water at 20 °C.

To estimate the molecular asymmetry of the complex in solution, the frictional ratio (*f*/*f*₀) was estimated according to the following equation (Siegel & Monty, 1966):

$$f/f_0 = \alpha \left(\frac{4\pi N}{3M[\bar{v} + (\delta/\rho)]} \right)^{1/3} \quad (5)$$

where δ is the degree of hydration of the protein, estimated at 0.3 g/g (Kuntz & Kauzmann, 1974).

The relative contributions of the protein and detergent in the complex were estimated using partial specific volume values of 0.73 ml/g for the protein (Smith, 1963) and 0.85 ml/g for the detergent (Werner & Reithmeier, 1985) and assuming that the partial specific volumes of the protein and detergent components are additive. The *M*_r of the protein component of the complex was obtained after correction for the bound detergent by using the equation (Tanford *et al.*, 1974):

$$M_r = \frac{M_c}{1 + \delta_D} \quad (6)$$

Photoaffinity labelling of the receptor

Photolabelling of AII receptor was performed using the high-affinity agonist ¹²⁵I-azido-SARANG. Non-covalent binding was first carried out in the dark in 1 ml of buffer containing

50 mM-Tris/HCl, pH 7.4, 150 mM-NaCl, 1 mM-MgCl₂, 0.1 mM-EDTA, 50–100 pM-¹²⁵I-azido-SARANG and 60 µg of protein. After 1 h of incubation, the volume was brought to 10 ml with 150 mM-NaCl containing 100 µM-PABA (*p*-aminobenzoic acid), and photolysis was performed on ice for 5 min with a 100 W u.v. lamp (maximum emission at 365 nm) at a distance of 8 cm. Non-specific binding was determined in the presence of 1 µM-SARILE. The membranes were then centrifuged at 30 000 *g* for 30 min and the pellet was resuspended in SDS/PAGE sample buffer [62 mM-Tris/HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.0005% (w/v) Bromophenol Blue and 5% 2-mercaptoethanol] and heated for 3 min at 100 °C. Binding of ¹²⁵I-azido-SARANG to membranes without subsequent u.v. exposure was assessed by filtration on GF/B filters followed by washing with 4 × 5 ml of ice-cold 50 mM-KH₂PO₄, pH 7.4.

SDS/polyacrylamide-gel electrophoresis

Electrophoresis was carried out on a 7.5%-acrylamide separating gel using the discontinuous buffer system of Laemmli (1970). Equivalent amounts of membrane proteins (200 µg) were loaded in each lane of a 1.5 mm-thick slab gel. Following electrophoresis, the gels were stained with Coomassie Blue R-250, destained, and dried prior to autoradiography on Kodak X-Omat RP film with Du Pont Lightning Plus intensifying screens at -70 °C.

RESULTS

Hydrodynamic properties of the AII receptor

SARILE has been shown to interact with AII receptors with high affinity and a slow dissociation rate (De Léan *et al.*, 1984), thus allowing the study of the hydrodynamic properties of the hormone-receptor complex.

The elution profile of the solubilized membranes prelabelled with ¹²⁵I-SARILE on a Superose 6 column is shown in Fig. 1. The column was calibrated by determining the distribution coefficient (*K*_{av}) of globular proteins of known Stokes radii and a linear relationship was found between (-log *K*_{av})^{1/2} and Stokes radius. The chromatogram showed a single peak of radioactivity

Fig. 1. Steric exclusion h.p.l.c. of AII receptor solubilized in 40 mM-OG
Adrenal zona glomerulosa membranes were incubated with ¹²⁵I-SARILE in the absence (—) or presence (---) of 1 µM unlabelled SARILE and solubilized for 30 min on ice. A sample of the solubilized preparation (250 µl) was injected on to a Superose 6 column and eluted at a flow rate of 0.5 ml/min at 4 °C. Fractions of 0.5 ml were collected and the radioactivity was counted. The arrows show the positions of the protein standards: 1, thyroglobulin (8.5 nm); 2, catalase (5.22 nm); 3, aldolase (4.81 nm); 4, chymotrypsinogen A (2.09 nm); 5, ribonuclease A (1.64 nm).

Fig. 2. Sucrose density gradient centrifugation of the AII receptor solubilized in 40 mM-OG

The solubilized preparation was prepared as described in the legend to Fig. 1. Samples (250 µl) were layered on 5–20% (w/v) sucrose density gradients made up in H₂O (a) or ²H₂O (b) and centrifuged for 18 h at 33 000 rev./min in a Beckman SW 55 Ti rotor. Fractions (10 drops) were collected from the bottom of the tubes and the radioactivity corresponding to total (—) and non-specific (---) binding was counted. Arrows indicate the sedimentation positions of reference proteins: 1, aldolase (*s*_{20,w} = 7.35 S); 2, BSA (*s*_{20,w} = 4.41 S).

eluting at a *K*_{av} of 0.345, corresponding to a Stokes radius of 5.29 ± 0.14 nm. No peak was detected when membranes were prelabelled in the presence of 1 µM unlabelled SARILE.

The sedimentation behaviour of the hormone-receptor complex was studied in 5–20% sucrose gradients containing 40 mM-OG (Fig. 2). Calibration of the gradients with globular proteins yielded a linear relationship between *s*_{20,w} and the fractional migration distance. The labelled receptor sedimented as a single peak in H₂O at a position corresponding to a mean apparent sedimentation coefficient (*s*_H) of 5.11 S. In the presence of 1 µM unlabelled SARILE, no peak of radioactivity was observed. The sedimentation profile of the receptor complex, relative to the reference proteins, was different when the gradients were performed in ²H₂O. The labelled receptor migrated more slowly at a position corresponding to a mean apparent sedimentation coefficient (*s*_D) of 4.89 S.

The partial specific volume of the labelled receptor-detergent complex (*v*) was calculated by comparing each of the six sedimentation values obtained in H₂O gradients with each of the

Table 1. Molecular parameters of the solubilized AII receptor

Values are means ± S.E.M. of numbers of experiments in parentheses.

Parameter	Value
Stokes radius, <i>α</i> (nm)	5.29 ± 0.14 (4)
Partial specific volume, <i>v</i> (ml/g)	0.751 ± 0.003 (36)
Sedimentation coefficient, <i>s</i> _{20,w}	5.17 ± 0.11 (6)
<i>M</i> _r of complex, <i>M</i> _c	124 000
Frictional ratio, <i>f</i> / <i>f</i> ₀	1.42
Detergent bound (g/g of protein)	0.19 [0.38–0.01]*
<i>M</i> _r of protein	104 000 [90 000–123 000]*

* Values in square brackets are those obtained when the partial specific volume of the protein is assumed to be 0.710 and 0.750 ml/g respectively.

Fig. 3. Competition curve of SARILE for the binding of ^{125}I -azido-SARANG to bovine adrenal zona glomerulosa membranes

Increasing amounts of SARILE were incubated with 50 pM- ^{125}I -azido-SARANG and adrenal membranes (40 $\mu\text{g}/\text{ml}$) at 25 $^{\circ}\text{C}$ for 60 min. Bound radioligand was assessed by rapid filtration on GF/B filters. The curves were analysed by non-linear least-squares regression analysis (De Léan *et al.*, 1982). The solid line represents the computerized fit of the data.

six values obtained in $^2\text{H}_2\text{O}$ gradients, thus yielding 36 separate estimates of $\bar{v}$. The mean partial specific volume obtained was $0.751 \pm 0.003 \text{ ml/g}$. This value was used to calculate a corrected $s_{20,w}$ of $5.17 \pm 0.11 \text{ S}$, which yielded an M_r of 124000 for the labelled receptor-detergent complex. The frictional ratio of the complex, calculated from gel filtration and centrifugation data, was 1.42, which corresponds to a prolate ellipsoid having an axial ratio of 8. The M_r of the protein component was estimated to be 104000 after correction for the contribution of the detergent to the mass of the complex. The observed binding of OG was 0.19 g/g of protein. A summary of the hydrodynamic properties of the AII receptor is shown in Table 1.

Photoaffinity labelling of the AII receptor

The subunit structure of the AII receptor protein was studied by SDS/PAGE after photolabelling with the derivative ^{125}I -azido-SARANG. Fig. 3 shows a competition curve of the antagonist SARILE for the binding of ^{125}I -azido-SARANG to bovine adrenal membranes. Computer analysis of the curve indicates that SARILE binds to a single population of sites with a K_d of 540 pM. An M_r -54000 band was labelled when membranes were incubated with ^{125}I -azido-SARANG, irradiated and subjected to SDS/PAGE under reducing conditions (Fig. 4a). A high- M_r band was also observed at the top of the gel which probably represents an aggregate of the receptor proteins. When the gel electrophoresis in SDS was performed under non-reducing conditions, a major M_r -55000 band was still apparent and a minor one appeared at M_r -106000, accounting for less than 10% of the signal as estimated by densitometry (Fig. 5). The specificity of the labelling was demonstrated by the inhibition of the covalent incorporation of the radioligand by increasing amounts of the antagonist SARILE. This inhibition was essentially superimposable, after normalization, to a competition binding curve performed in membranes using the same ligands but without subsequent u.v. exposure (Fig. 4b).

DISCUSSION

Solubilization of the AII receptor was achieved by treatment of bovine zona glomerulosa membranes with OG. This non-ionic

Fig. 4. Concentration-dependent inhibition of ^{125}I -azido-SARANG labelling of bovine adrenal membranes by SARILE

(a) Adrenal membranes (60 $\mu\text{g}/\text{ml}$) were incubated with 50 pM- ^{125}I -azido-SARANG and increasing concentrations of unlabelled SARILE for 1 h at 25 $^{\circ}\text{C}$, and photolabelled as described in the Experimental section. The membranes were centrifuged and solubilized in sample buffer, and the proteins were analysed by SDS/PAGE and autoradiography. The M_r values of standard proteins are: myosin, 200000; β -galactosidase, 116250; phosphorylase b, 97400; BSA, 66200; ovalbumin, 42699. (b) After being revealed by autoradiography, the labelled bands were excised and counted for radioactivity. The dose-response curve of SARILE for the inhibition of ^{125}I -azido-SARANG labelling of the M_r -54000 band (■) was compared with its competition curve for the binding of the radioligand to bovine adrenal membranes (●).

detergent was chosen because it is less denaturant, for its ability to preserve protein-protein interactions and because it forms small micelles. Earlier work from our laboratory has shown that OG allows for solubilization of the hormone-receptor complex with the high-affinity antagonist ^{125}I -SARILE. The complex was sufficiently stable to sustain steric exclusion and ion-exchange chromatography (De Léan *et al.*, 1984).

Assuming that the protein and the detergent have partial specific volumes of 0.73 and 0.85 ml/g respectively, eqns. (5) and (6) yield an M_r of 104000 for the receptor protein and a level of OG binding of 0.19 g/g of protein. Theoretical limitations on the estimation of the M_r for detergent-solubilized proteins through gel filtration and sucrose density gradient centrifugation have been discussed previously (Tanford & Reynolds, 1976). But this

Fig. 5. Photolabelling of the AII receptor with ^{125}I -azido-SARANG

Zona glomerulosa membranes were incubated with ^{125}I -azido-SARANG in the presence (lanes 2, 4, 6 and 8) or absence (lanes 1, 3, 5 and 7) of $1\ \mu\text{M}$ unlabelled SARILE as described in the legend to Fig. 4. The photolabelled membranes were solubilized in the sample buffer containing 8 M-urea in the presence (lanes 1, 2, 3 and 4) or absence (lanes 5, 6, 7 and 8) of 5% 2-mercaptoethanol, incubated at room temperature for 24 h and heated for 3 min at 100°C . Lanes 3, 4, 7 and 8 contain membranes not exposed to u.v. light after binding.

method is still very useful for physicochemical characterization of hydrophobic proteins hardly obtainable in pure form and allows useful comparison. The hydrodynamic estimate of the AII receptor M_r (104000) compared with the M_r 54000 band obtained by gel electrophoresis under reducing conditions suggests that the receptor is a homodimer or a heterodimer in which the M_r -54000 polypeptide chain probably contains the binding site. Moreover, this estimate is in close agreement with the apparent M_r of the minor protein band present when electrophoresis is performed under non-reducing conditions. The fact that the majority of the radioactivity was still found in the M_r 55000 band in these conditions demonstrates that the subunits of the AII receptor are not linked together by disulphide bridges. An incomplete dissociation of these subunits in SDS can account for the presence of the faint band at M_r 106000 on the gel. The M_r -104000 band is comparable with the M_r -116000 specifically labelled protein found in rat adrenal cortex by Paglin & Jamieson (1982), using disuccinimidyl suberate. Rogers (1984) also labelled an M_r -116000 protein in bovine heart with the cross-linking agent dithiobis(succinimidyl propionate), and Capponi & Catt (1980) have reported the presence of two binding species, of M_r 126000 and 65000 or 68000, in canine adrenal gland and uterus. This also tends to confirm that the high- M_r band obtained by chemical cross-linking (Paglin & Jamieson, 1982; Rogers, 1984) is the receptor itself, in which the two subunits are cross-linked together. Carson *et al.* (1987) labelled with azido-SARANG an M_r -58000 protein in the bovine adrenal cortex but they also observed, under non-reducing conditions, an M_r -105000 band on SDS/PAGE which accounted for less than 5% of the signal. Several groups have noted the presence of two peaks of radioactivity by steric exclusion chromatography, suggesting again that the receptor protein could be a dimer (Capponi & Catt, 1980; Guillemette & Escher, 1983; Carson *et al.*, 1987), but as the detergent binding is unknown, the M_r of the protein component of the peaks could not be estimated without using SDS/PAGE, which dissociates protein interactions.

The broad bands observed by SDS/PAGE are consistent with the glycoprotein nature of the AII receptor, which has been extensively demonstrated (Carson *et al.*, 1987). It is also compatible with a heterodimeric protein in which the M_r values of both subunits are similar.

Our results, obtained by applying for the first time the centrifugation through density gradient to the AII receptor, support the hypothesis that this protein is a non-covalent dimer of approx. M_r 104000 with an M_r -54000 hormone-binding subunit. Further studies are required for the isolation and the characterization of the M_r -54000 subunit as well as the identification of the additional membrane component involved in agonist-promoted receptor complex.

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