Solubilization of angiotensin II receptors in rat glomeruli

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^{125}I-labelled angiotensin II (A II) specifically binds to solubilized receptors extracted from rat isolated glomeruli using CHAPS (3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate). The yield of solubilization of the binding sites was 3.3%. Equilibrium was reached after 15–20 min and specific binding represented 75% of total binding. Dissociation of the hormone–receptor complex after addition of an excess of A II was very slow in the presence of Ca^{2+} and Mg^{2+}. [Sar^1 Ala^8] A II and [Sar^1 Ile^8] A II were more potent as competitive inhibitors of ^{125}I-labelled A II than A II itself and its heptapeptide. These basic features of ^{125}I-labelled A II binding to the extracted material were similar to those observed previously with untreated glomeruli.

Angiotensin II *Glomerulus* *Solubilized receptor* *Detergent* *Binding study* *Angiotensin II heptapeptide*

1. INTRODUCTION

Murine and human glomeruli possess specific receptors for angiotensin II (A II) which are linked to glomerular vasoreactivity [1,2]. Solubilization and characterization of high-affinity A II receptors derived from rabbit aorta membranes, bovine adrenal cortex or dog adrenal cortex and uterus have been performed by extraction with various detergents, deoxycholate [3], digitonin [4] or Triton X-100 [5]. A major problem in solubilizing A II receptors is the rapid loss of binding activity when particulate receptors are treated with ionic or nonionic detergents [5,6]. This problem has been overcome using photoaffinity labelling [5] or chemical crosslinking [7] which both provide a stable hormone–receptor complex. These techniques allow the analysis of the receptor species itself but prevent us from studying the characteristics of binding to the soluble material. Here, we report the solubilization of the previously studied A II-binding sites of rat glomeruli with a more recently described Zwitterionic detergent, CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) which has been demonstrated as both more effective at breaking protein–protein interactions and less capable of denaturing activity than the other solubilizing agents [8,9].

2. MATERIALS AND METHODS

[Asn^1, Val^5] A II was donated by Ciba-Geigy (Basel) and labelled with ^{125}I as in [10]. Labelled and unlabelled molecules were separated with polyacrylamide gel electrophoresis as in [11]. The specific activity of the labelled hormone was 2000 Ci/mmol which corresponds to one atom iodine per molecule. [Asp^1, Ile^5] A II, [Des Asp^1, Ile^5] A II, [Sar^1, Ala^8] A II and [Sar^1, Ile^8] A II were purchased from Beckman (Geneva, Switzerland) and ACTH-(1–24) was a gift of Ciba-Geigy. CHAPS was obtained from Sigma (St. Louis, MO).

Glomeruli were isolated from the renal cortex of male Sprague-Dawley rats weighing 150–200 g as in [12]. The final pellet was checked for purity under light microscopy. Tubular fragments were always below 2% of the total number of glomeruli.

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Glomeruli were suspended in 20 mM Tris–HCl buffer, pH 7.5, containing 135 mM NaCl, 10 mM KCL, 10 mM $NaCH_3COO$ and 5 mM glucose (buffer A). The preparation was kept frozen and used within the next 48 h. On the day of the experiment, glomeruli were resuspended in buffer A plus 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Both cations have been demonstrated as enhancing the binding of A II to its glomerular receptors [13]. The suspension was sonicated for 2 min (Branson Sonic Power, Danbury, CO), adjusted to a protein concentration of 15 mg/ml and incubated at room temperature for 1 h under continuous agitation with 2 mM CHAPS. At the end of the incubation, the preparation was centrifuged at 100000 × *g* for 60 min at 5°C. The clear supernatant fraction containing approximately 4 mg protein/ml was carefully removed and used for the binding studies.

^{125}I-labelled A II receptor binding assays were carried out at room temperature for 30 min under continuous gentle shaking in 800 μl buffer A containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ with 200–400 μg of solubilized receptor protein, 1 nM ^{125}I-labelled A II, 0.5 mM CHAPS and 125 μg/ml 1–24 ACTH. The latter product inhibited degradation of the tracer without affecting binding kinetics [1,14]. Non-specific binding was estimated in parallel with tubes containing an additional amount of unlabelled A II (1 μM). In order to separate free and bound radioactivities, the reaction mixture was cooled at 0°C and applied to a Sephadex G-200 (Pharmacia, Uppsala) column (0.9 × 15 cm) equilibrated and eluted (100 μl/min) with 20 mM Tris–HCl (pH 7.5). Aliquots (600-μl) were collected and ^{125}I radioactivity was counted with a crystal-type scintillation detector giving 63% efficiency. Bound radioactivity was eluted in the initial 6 ml. Elution of proteins was monitored by absorbance lecture at 280 nm on a model 25 Beckman spectrophotometer. This technique was long and not ideal for handling great series but we found it was the only one providing correct separation of bound and free radioactivities in the system studied.

Specific binding to intact isolated glomeruli suspended in the same incubation medium was studied in parallel in some experiments using similar time of incubation and concentration of ^{125}I-labelled A II. Free and bound radioactivities were separated by filtration through a Millipore filter as in [1].

Specifically bound radioactivity (total binding minus binding in the presence of 1 μM A II) was expressed as fmol A II per mg receptor protein, both with soluble material and isolated glomeruli.

3. RESULTS

^{125}I-labelled A II binding was measured as a function of time at varying hormonal concentrations between 0.1 and 2 nM. Equilibrium was reached after 15–20 min. Specific binding represented approximately 75% of total binding (fig.1a). Dissociation after addition of an excess of

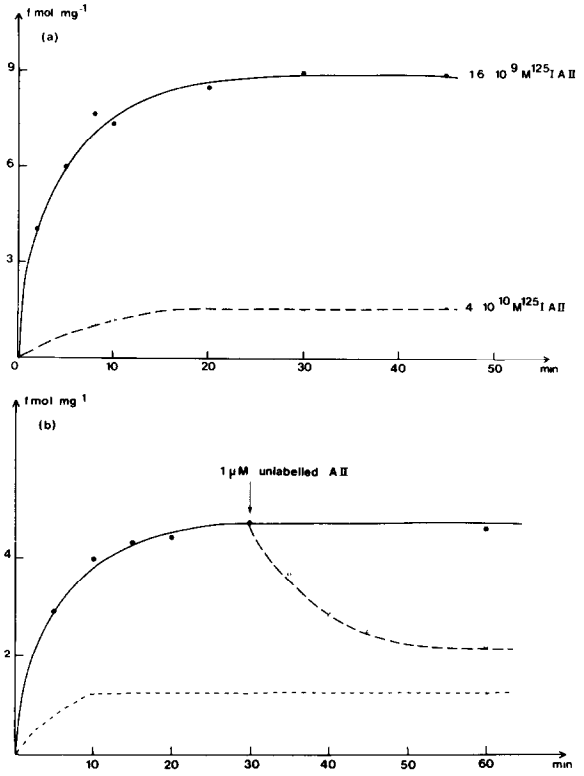

Fig.1. (a) Time course of specific binding of ^{125}I-labelled A II to glomerular solubilized receptors at two concentrations of ^{125}I-labelled A II (0.4 and 1.6 nM) in the incubation medium. (b) Time-course of total (●—●) and non-specific (○—○) binding of ^{125}I-labelled A II to glomerular solubilized receptors. Concentration of ^{125}I-labelled A II was 1.2 nM. $CaCl_2$ and $MgCl_2$ were omitted in the incubation medium. Dissociation of the hormone–receptor complex (Δ—Δ) was obtained after addition of 1 μM unlabelled A II after equilibrium had been reached.

unlabelled A II was difficult to obtain in the presence of Ca^{2+} and Mg^{2+} in accordance with the results that we reported for binding studies with untreated glomeruli [13]. When these two cations were omitted in the incubation medium, the height of the equilibrium plateau was smaller. Dissociation in the presence of an excess of unlabelled A II was obtained but the rate of dissociation was slow and the level of non-specific binding was not reached 30 min after addition of the unlabelled hormone (fig.1b). Specific binding was found to vary linearly with increasing glomerular protein concentration up to 500 μg per assay tube. The equation of the regression line obtained in the presence of 1.8 nM ^{125}I-labelled A II was:

$$y \text{ (fmol} \cdot \text{mg}^{-1} \text{ } ^{125}\text{I-labelled A II)} =$$
$$0.007\ x\ (\mu\text{g glomerular protein}) +$$
$$0.073\ (r = 0.98,\ n = 8)$$

Dilution of ^{125}I-labelled A II (0.6 nM) with increasing concentrations of unlabelled A II from 10 pM to 1 μM decreased the percentage of labelled bound hormone. Residual binding at 1 μM was less than 20%. The concentration corresponding to 50% of specific binding at zero dose was 0.76 nM (fig.2). Table 1 illustrates the relative potency of various analogs and antagonists for competition with ^{125}I-labelled A II binding at two concentrations (1 nM and 1 μM). Both [Sar^1 Ala^8] A II and [Sar^1 Ile^8] A II were more potent than

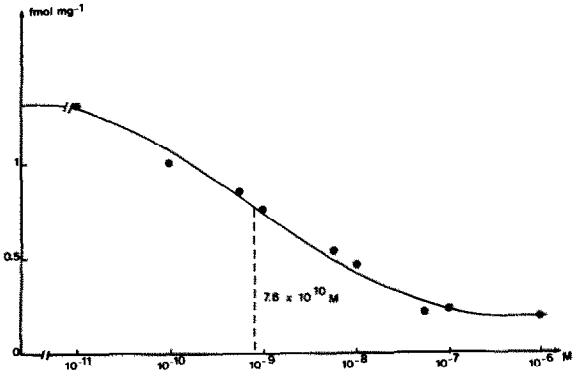

Fig.2. Competitive inhibition of ^{125}I-labelled A II binding to glomerular solubilized receptors in the presence of increasing concentrations of unlabelled A II. Concentration of ^{125}I-labelled A II was 0.57 nM. The dotted line indicates the concentration corresponding to 50% inhibition of maximum binding.

Table 1

Potency order for displacement of ^{125}I-labelled A II from glomerular solubilized receptors

Peptide tested	Concentrations	
	1 nM	2 μM
[Asn^1 Val^5] A II	61.9 ± 1.4	19.8 ± 4.3
[Asp^1 Ile^5] A II	65.1 ± 5.2	22.1 ± 2.9
[Des Asp^1 Ile^5] A II	70.8 ± 2.1	25.7 ± 1.8
[Sar^1 Ala^8] A II	33.0 + 2.4	20.2 ± 2.0
[Sar^1 Ile^8] A II	42.3 ± 3.9	18.7 ± 1.6

Means ± SE of 4 individual values are given. Residual binding expressed as percentage of control binding

A II itself. [Asp^1 Ile^5] A II, [Asn^1 Val^5] A II and [Des Asp^1 Ile^5] A II had similar inhibitory activities. At 30 min of incubation, less than 20% degradation of free ligand was observed as determined by rebinding to fresh glomeruli when 1–24 ACTH was added to the incubation medium.

The solubilization yield of the ^{125}I-labelled A II receptors was calculated by comparison of ^{125}I-labelled A II binding to isolated glomeruli and to the CHAPS-extracted material under the same conditions. After 30 min incubation and in the presence of 0.6 nM ^{125}I-labelled A II, isolated glomeruli and their solubilized fraction bound 106.3 ± 6.5 and 3.5 ± 0.3 fmol ^{125}I-labelled A II/mg protein, respectively. This corresponded to a yield of 3.3%.

4. DISCUSSION

These findings indicate that the components extracted from rat isolated glomeruli with CHAPS contain A II receptors. The basic features of ^{125}I-labelled A II binding to the CHAPS-extracted material are similar to those observed with untreated glomeruli [1,14–16]. Both the particulate and the soluble system bind ^{125}I-labelled A II rapidly at concentrations of hormone between 0.1 and 2 nM reaching maximum levels in 15–20 min and this binding is a linear function of protein concentration. Binding is enhanced in presence of Ca^{2+} and Mg^{2+}. The relative potency of A II analogs for competition with ^{125}I-labelled A II binding is similar to that observed in [16] with particulate glomerular receptors. Finally, ^{125}I-labelled

A II is rapidly inactivated by the preparation in the absence of 1–24 ACTH. Thus, taken together, these observations suggest that the soluble ^{125}I-labelled A II binding components are the same as those formerly identified in untreated isolated glomeruli and that their hormone-binding function is preserved during the extraction procedure. Under the experimental conditions used ^{125}I-labelled A II binding was very slowly reversible even when Ca^{2+} and Mg^{2+} had been omitted in the incubation medium. The calculation of association and dissociation rate constants was therefore considered as incorrect as well as the precise determination of a K_d-value from the Scatchard's transformation of the data obtained in competitive inhibition studies.

Several marked differences between the particulate and the extracted receptors are also evident. For the extracted receptors, the apparent K_d value estimated from the concentration corresponding to 50% inhibition of binding in competitive inhibition studies was greater than the corresponding value observed with the particulate receptors for the higher affinity group of sites [15]. However, other authors using isolated glomeruli found a single population of specific receptors with an equilibrium dissociation constant between 0.1 and 1 nM, thus closer to that of the soluble receptors [14,16]. The concentration of specific receptors per mg soluble protein was markedly lower than that observed with intact glomeruli despite the fact that almost all binding activity had disappeared from the residual pellet of detergent-treated glomeruli. Low recovery of solubilized receptors has been already reported in [17] with vasopressin receptors in pig kidney plasma membranes and in [18] in the case of rat testis gonadotropin receptor.

In conclusion, the availability of glomerular solubilized receptors for A II in non-membranous form can be considered as the first step for their further purification and characterization.

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