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Ca^{2+} AND Mg^{2+} DEPENDENCE OF ANGIOTENSIN II BINDING TO ISOLATED RAT RENAL GLOMERULI

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Abstract—[^{125}I]angiotensin II binding to isolated rat renal glomeruli was clearly increased in the presence of either Ca^{2+} or Mg^{2+} (10 μM to 5 mM for both). This effect was more marked at higher pH. In the absence of both Ca^{2+} and Mg^{2+}, [^{125}I]angiotensin II binding decreased with pH whereas at 2 mM of these two cations, the opposite was observed. There was a clear-cut interaction between the effects of pH and those of Ca^{2+} or Mg^{2+}. The increase in [^{125}I] angiotensin II binding was similar for the same molar concentrations of Ca^{2+} or Mg^{2+}. Effects of these two cations were additive at pH 8.5, but negatively interacted at pH 7.5. Mn^{2+} had the same effects as Ca^{2+} and Mg^{2+} and interacted with them. Addition of ethyleneglycol bis (β aminoethylether)-*N,N'*tetraacetic acid or of ethylenediamine tetraacetic acid suppressed the stimulatory effects of Ca^{2+} and Mg^{2+} and decreased [^{125}I]angiotensin II binding even when these two cations were not introduced in the incubation milieu. The extent of increase in [^{125}I]angiotensin II binding at increasing concentrations of Ca^{2+} or Mg^{2+} was greater in the presence than in the absence of these chelating substances. Kinetics of [^{125}I]angiotensin II binding were modified in the presence of Ca^{2+} and Mg^{2+}. The slopes of the association curves were smaller and the heights of the equilibrium plateaus greater. Moreover the dissociation after addition of an excess of angiotensin II was slower and irregular and did not correspond to an exponential function. The constant of affinity estimated from association curves at two different hormonal concentrations or from binding experiments at equilibrium was unchanged. Addition of EDTA at equilibrium also produced dissociation of bound [^{125}I]angiotensin II whether or not Ca^{2+} and Mg^{2+} were present. Enhancement of [^{125}I]angiotensin II binding by Ca^{2+} and Mg^{2+} and interaction of these two cations with pH seem particular to the glomerular binding sites and suggest a role in the binding reaction for the electric charges of both angiotensin II and the glomerular structures.

Specific receptors for angiotensin II (AII) have been demonstrated in glomeruli isolated from rat kidney cortex [1, 2] and related to the glomerular vasoreactivity [1]. The number of these receptors and their affinity for AII are modulated by the sodium balance since binding was increased when glomeruli were prepared from sodium-loaded rats whereas it was diminished with glomeruli from sodium-depleted animals [2]. Receptors for AII within the glomerulus represent several groups of sites which are located on different structures, the mesangial cells [3] and the glomerular basement membranes [4]. AII has multiple effects on the glomerular function. Infusion of this hormone in the rat produces a clear decrease in glomerular filtration rate [5] and an increased permeability of the glomerular capillary walls to proteins [6]. The role of the electric charges of the filtered molecules [7] and of the glomerular structures [8, 9] in the filtration process has been recently emphasized. This has prompted us to try to evaluate the effects of varying the electric charges of both AII and the glomerular structures on the binding reaction. In that purpose we have studied the influence on [^{125}I] AII binding of modifications in pH and divalent cation concentration of the incubation milieu.

MATERIALS AND METHODS

Materials. (Asn^1, Val^5) AII donated by Ciba–Geigy (Basel, Switzerland) was labelled with [^{125}I] by the method of Hunter and Greenwood [10]. Labelled and unlabelled molecules were separated using polyacrylamide gel electrophoresis according to Corvol *et al.* [11]. The specific activity of the labelled hormone assessed by radioimmunoassay as previously described [2] was 2000 Ci/m-mole. This is close to the theoretical value corresponding to one atom iodine per molecule. Biological activity of the tracer was tested *in vitro* from the reduction of the surface of isolated glomeruli [1] and found close to 80 per cent of that of the unlabelled hormone. In order to estimate the electric charge of [^{125}I] AII as a function of pH, isoelectric point of this tracer was determined using isolectric focussing on polyacrylamide gels in two different pH gradients, 3–10 and 5–8 (Ampholines LKB). Conditions of isoelectric focusing and gel elution were described by Finlayson and Chrambach [12]. From four determinations, the apparent isoelectric point (pI) of [^{125}I] AII was 7.3 $\pm$ 0.1 (Fig. 1). Chemicals were analytical grade reagents.

Preparation of isolated glomeruli. Renal glomeruli were isolated from Sprague–Dawley rats of 150–220 g body weight according to the technique of Fong and Drummond [13] with minor modifications. In short, cortex from four kidneys was dissected and minced to a paste-like consistency. The homogenate was gently pushed through a 106 mesh sieve and suspended in 10 ml of 135 mM NaCl, 10 mM Na CH_3 COO, 10 mM KCl, 5 mM glucose, 20 mM Tris-HCl, pH 7.5 (buffer A). The osmolality of this solution ap-

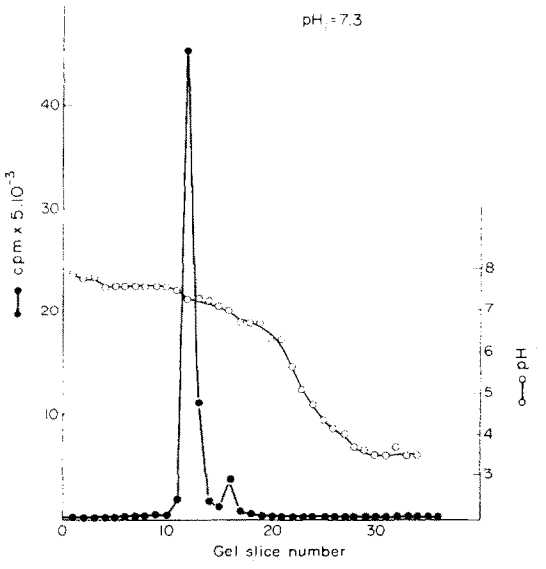

Fig. 1. Isoelectric focusing on polyacrylamide gel of [^{125}I] AII. A constant potential of 200 V was applied to two gels (5% T, 0.2% C) containing 1% carrier ampholytes (Ampholines pH 3–10). The focusing time was 7 hr. The position of [^{125}I] AII was determined by counting the radioactivity content of each gel slice. Elution of the gel slices was performed in 0.5 ml of 0.05 M NaCl, and pH was measured in each eluted solution.

proximates 320 mos/l. The suspension was passed through a 25 gauge needle and then centrifuged at 120 *g* during 90 sec. The supernatant was discarded and the pellet resuspended in the same buffer solution, passed again through the needle and centrifuged. This operation was repeated five times and the final pellet of glomeruli kept at +4° for less than 2 hr. Immediately before the binding experiments, this pellet was washed in buffer A and centrifuged (120 *g* for 90 sec) three times in order to remove extra-cellular cations.

Angiotensin II *receptor binding studies.* The incubation milieu currently consisted of 160 μl of buffer A 40 μl of distilled water. Bovine serum albumin and 1–24 ACTH fragment (Ciba–Geigy, Basel, Switzerland) were added at final concentrations of 1.2% and 125 μg/ml respectively. The latter product as shown in a previous study [1] partly prevented degradation of the tracer without affecting binding kinetics. Incubation was performed at 20–22° in the presence of 70 pM [^{125}I] AII and of 300–500 μg/ml of glomerular protein. Addition to the incubation milieu of mineral salts absent in buffer A or of other reagents was made in a constant volume of 40 μl of distilled water in replacement of the same volume of water present in the control tubes. When the effects of pH were studied, pH of buffer A was modified without changing the Tris molar concentration.

At the end of the incubation, bound radioactivity was separated using filtration through a Millipore filter (HAWP 02500). [^{125}I] was counted with a crystal type scintillation detector giving 55 per cent efficiency. Specific binding was calculated by subtracting the binding in the presence of 50 μM unlabelled AII from total binding and expressed as fmole of bound hormone per mg of glomerular protein. It has been shown in a previous study [1] that the amount of AII bound to glomerular receptors was linearly related to the amount of glomerular protein over the range of protein concentrations studied. Protein concentration was determined according to Lowry *et al.* [14].

RESULTS

Effects of Ca^{2+}, Mg^{2+} *and pH on* [^{125}I] *AII binding.* When the amount of [^{125}I] AII bound at equilibrium (45 min) was plotted against the log doses of Ca^{2+} or Mg^{2+} at different pH, sigmoidal curves were obtained (Fig. 2). Maximum bindings were observed at 5 mM and concentrations corresponding to 50 per cent of maximum binding were in the range of 0.5–2 mM. In the absence of Ca^{2+} and Mg^{2+}, [^{125}I] AII binding decreased progressively with pH. The value obtained at pH 7.1 was approximately three times that measured at pH 8.9. On the contrary, at 5 and 10 mM Ca^{2+} or Mg^{2+}, [^{125}I] AII binding progressively increased with pH. For intermediary concentrations (0.1–1 mM) effects of pH were irregular. This explains that the degree of increase in [^{125}I] AII binding at increasing concentrations of Ca^{2+} or Mg^{2+} was greater at higher pH. Two factor (pH, Ca^{2+} or Mg^{2+} concentration) analysis of variance confirmed there was a significant interaction between

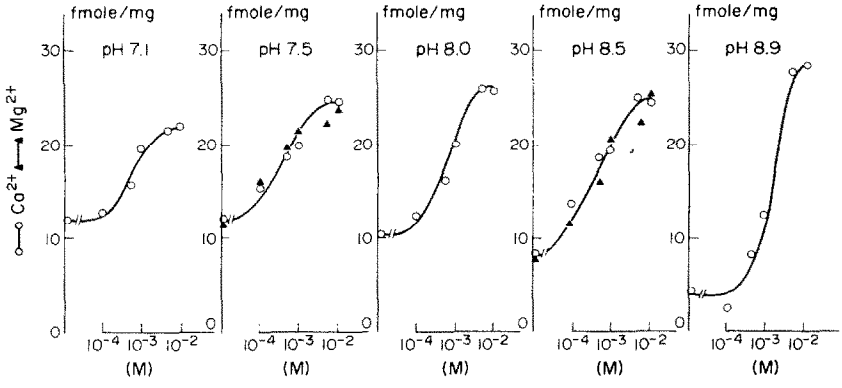

Fig. 2. [^{125}I]AII bound to isolated glomeruli plotted against Ca^{2+} concentrations at five different pH and against Mg^{2+} concentrations at two different pH. Each point represents the mean of duplicates. Binding was measured after 45 min incubation in the presence of 70 pM [^{125}I] AII.

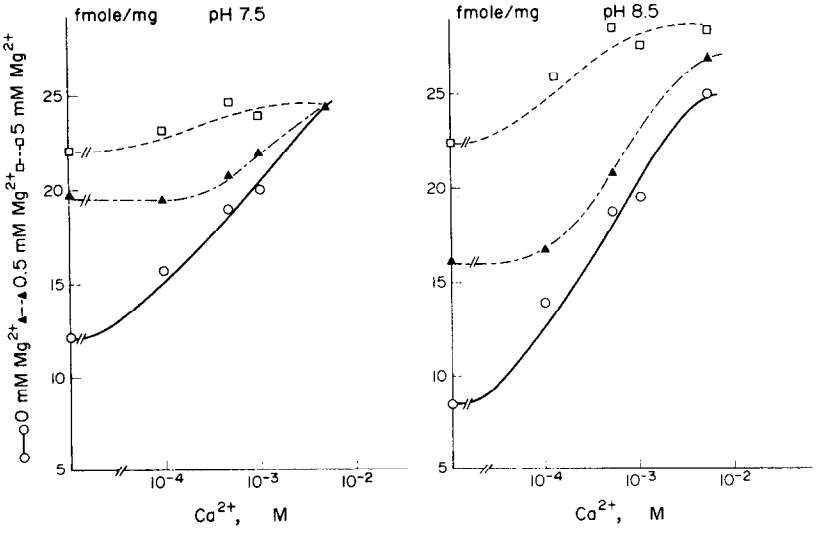

Fig. 3. [^{125}I] AII bound to isolated glomeruli plotted against Ca^{2+} concentration at three different Mg^{2+} concentrations and at two different pH, 7.5 (left) and 8.5 (right). Each point represents the mean of duplicates. Binding was measured after 45 min incubation in the presence of 70 pM [^{125}I] AII.

pH and Ca^{2+} ($P < 0.01$) and between pH and Mg^{2+} ($P < 0.05$). Ca^{2+} and Mg^{2+} had similar effects on [^{125}I] AII binding at the same molar concentrations for the two pH tested (Fig. 2). [^{125}I] AII binding was also measured at increasing concentrations of Ca^{2+} in the presence of three different concentrations of Mg^{2+} (Fig. 3). The effect of Ca^{2+} was much slighter with 5 mM Mg^{2+}. There was a clear-cut interaction between these two cations as well at pH 7.5 as at pH 8.5 as shown by two factor analysis of variance ($P < 0.01$ and $P < 0.05$ respectively). However this interaction was more evident at pH 7.5. As a matter of fact, additivity of 5 mM Ca^{2+} and 5 mM Mg^{2+} effects was completely lost at pH 7.5 but partly persisted at pH 8.5.

Effects of ethylenediaminetetraacetic acid (EDTA) and of ethyleneglycol bis (β aminoethylether)-N,N' tetraacetic acid (EGTA) on [^{125}I] *AII binding.* In the absence of added Ca^{2+} and Mg^{2+}, increasing concentrations of EDTA or EGTA decreased the amount of [^{125}I] AII bound at equilibrium (Fig. 4). Some degree of binding persisted at 5 mM EGTA whereas binding was almost completely abolished at a similar dose of EDTA. EGTA inhibited completely the enhancement of binding due to Ca^{2+} since the amount of [^{125}I] AII bound in the presence of 1 mM Ca^{2+} and 1 mM EGTA was similar to that bound in the absence of these two reagents (Fig. 4, left). EDTA inhibited both Ca^{2+} and Mg^{2+}-stimulated bindings. At 1 mM EDTA, the binding obtained in the presence of 1 mM Ca^{2+} was clearly smaller than the control binding (with no addition of Ca^{2+} and Mg^{2+}) whereas the binding obtained in the presence of 1 mM Mg^{2+} was greater. However at 2 mM EDTA, the

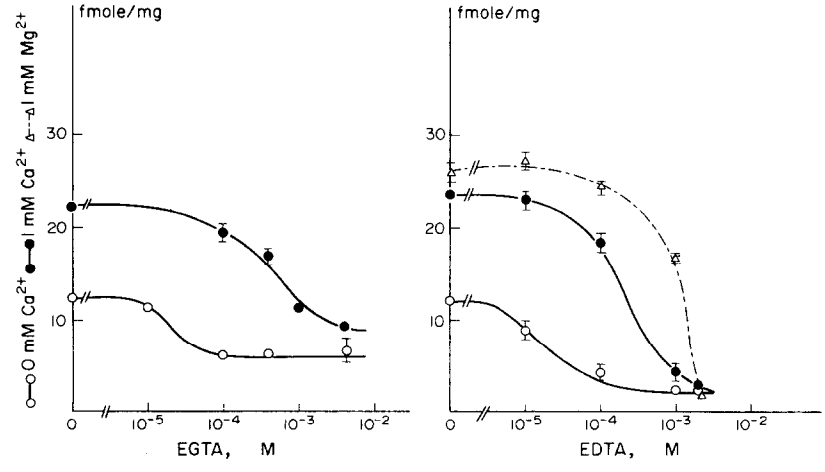

Fig. 4. [^{125}I] AII bound to isolated glomeruli plotted against EGTA concentration at two different Ca^{2+} concentrations (left). [^{125}I] AII bound to isolated glomeruli plotted against EDTA concentration in the presence of 1 mM Mg^{2+} or 1 mM Ca^{2+} or with no addition of these two cations (right). Each point represents the mean of triplicates and each vertical bar twice the standard error or the mean. Binding was measured after 45 min incubation in the presence of 70 pM [^{125}I] AII.

Table 1. [^{125}I] AII binding in the presence of either Ca^{2+} or Mg^{2+} or Mn^{2+} at four different concentrations (mM). Parallel experiments were performed with or without 1 mM EGTA. Values are the means of duplicates and are expressed in each of these two conditions, both in absolute value (fmole/mg) and as percentage of controls obtained without addition of cations. (figures between brackets)

		No EGTA	1 mM EGTA
Ca^{2+}	0	10.3 (100)	3.4 (100)
	0.1	13.4 (130)	4.2 (124)
	1	22.3 (216)	5.0 (149)
	10	27.5 (266)	28.7 (853)
Mg^{2+}	0	15.5 (100)	7.2 (100)
	0.1	20.1 (129)	17.7 (245)
	1	27.5 (177)	27.6 (382)
	10	30.9 (199)	48.9 (676)
Mn^{2+}	0	15.5 (100)	7.2 (100)
	0.1	20.6 (133)	7.7 (106)
	1	26.7 (172)	20.2 (280)
	10	26.4 (170)	26.1 (361)

effects of 1 mM Ca^{2+} or 1 mM Mg^{2+} were completely abolished (Fig. 4, right). The effects of increasing doses of Ca^{2+} or Mg^{2+} on [^{125}I] AII binding with or without EGTA were also compared (Table 1). The degree of increase in hormonal binding was greater in the presence than in the absence of this chelating substance.

Effects of Mn^{2+} *on* [^{125}I] *AII binding.* Mn^{2+} also produced an increase in [^{125}I] AII binding. However the maximum effect was obtained for a smaller concentration (1 mM) than with Ca^{2+} or Mg^{2+}. The degree of increase was also slighter but became more marked in the presence of 1 mM EGTA (Table 1). Mn^{2+} interacted with Ca^{2+} since in the presence of 2.5 mM Ca^{2+}, stimulating effects of Mn^{2+} were completely lost (Table 2).

Table 2. Interaction of Ca^{2+} and Mn^{2+} on [^{125}I] AII binding. Values given are the means of duplicates and are expressed as fmole/mg

Mn^{2+} (mM)	Ca^{2+} (mM)	
	0	2.5
0	15.5	30.7
0.1	20.6	30.2
1	26.7	30.6

Time-course studies, binding at equilibrium and competitive binding experiments of [^{125}I] *AII in the presence of* Ca^{2+} *and* Mg^{2+}. The time-course of [^{125}I] AII binding in the absence of added Ca^{2+} and Mg^{2+} showed that a plateau was reached following 45 min equilibrium. Addition of an excess of unlabeled AII at equilibrium produced a rapid dissociation of [^{125}I] AII from its binding sites (Fig. 5, left). Addition of 1 mM EDTA produced also dissociation (Fig. 5, right). Dissociation constants calculated from first order kinetics (-0.0344 and $-0.0459\ min^{-1}$ after addition of unlabelled AII and EDTA respectively) were not significantly different. When Ca^{2+} and Mg^{2+} were introduced in the milieu at the beginning of the incubation, the plateau was also reached after approximately 45 min but its level was clearly higher than in the absence of these two cations. Both addition of an excess of unlabelled AII or of EDTA produced release of the tracer. However the dissociation curves were irregular and there was no significant correlation between log of binding and time. Kinetic parameters of binding were calculated from time-course studies performed in the absence of Ca^{2+} and Mg^{2+}. In these experiments, association rate (k_{+1}), dissociation rate (k_{-1}) and the affinity constant K_D calculated as the ratio k_{-1}/k_{+1} could be easily determined. Table 3 shows that the correlation coefficients of the association and dissociation curves (binding versus

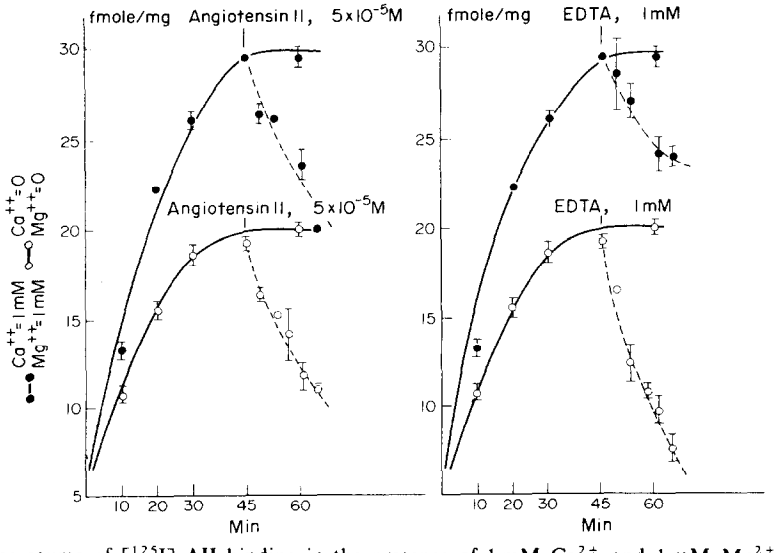

Fig. 5. Time-course of [^{125}I] AII binding in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+} or with no addition of these two cations. At equilibrium (45 min) 50 μM AII (left) or 1 mM EDTA (right) was added in order to obtain dissociation of the tracer from its binding sites (dotted curves). Each point represents the mean of triplicates and each vertical bar twice the standard error of the mean. Binding was measured in the presence of 70 pM [^{125}I] AII.

Table 3. Time-course studies

Association and dissociation curves (expt number)	No Ca^{2+} and Mg^{2+} added				1 mM Ca^{2+} and 1 mM Mg^{2+}			
	Slope of the association curve (min^{-1})	k_{+1} ($min^{-1} M^{-1} \times 10^{11}$)	Slope of the dissociation curve or k_{-1} (min^{-1})	K_D (10^{-11} M)	Slope of the association curve (min^{-1})	Slope of the dissociation curve (min^{-1})	K_D (10^{-11} M)	
1	0.102 (+0.99)	0.0085	−0.0341 (−0.93)	4.01	0.0585 (+0.98)	−0.0100 (−0.54)	Impossible to be calculated	
2	0.121 (+0.90)	0.0135	−0.0282 (−0.87)	2.08	0.0860 (+0.99)	−0.0019 (−0.09)	Impossible to be calculated	
3	0.096 (+0.99)	0.0087	−0.0469 (−0.97)	5.42	0.0936 (+0.98)	−0.0013 (−0.16)	Impossible to be calculated	
4	0.102 (+0.86)	0.0134	−0.0312 (−0.78)	2.32	0.0912 (+0.95)	−0.0016 (−0.07)	Impossible to be calculated	
5	0.116 (+0.97)	0.0149	−0.0235 (−0.78)	1.58	0.0548 (+0.99)	0.0015 (+0.15)	Impossible to be calculated	
6	0.121 (+0.97)	0.0139	−0.0372 (−0.85)	2.68	0.0970 (+0.79)	0.0063 (+0.22)	Impossible to be calculated	
mean ± S.E.M.	0.110 ±0.004	0.0121 ±0.001	−0.0335 ±0.0033	3.02 ±0.59	0.0802 ±0.0076	−0.0012 ±0.0022	Impossible to be calculated	
Rank test					$P < 0.01$	$P < 0.01$		

2 association curves at different [^{125}I] AII concn.		Slope of the association curve (min^{-1})	k_{+1} ($min^{-1} M^{-1} \times 10^{11}$)	k_{-1} (min^{-1})	K_D (10^{-11} M)	Slope of the association curve (min^{-1})	k_{+1} ($min^{-1} M^{-1} \times 10^{11}$)	k_{-1} (min^{-1})	K_D 10^{-11} M
7	(a)	0.112 (+0.91)	0.0119	−0.0123	1.03	0.0594 (+0.92)	0.0047	−0.0199	4.24
	(b)	0.213 (+0.81)				0.0923 (+0.91)			

Numbers between brackets indicate the correlation coefficients. As explained in the text, k_{-1}, k_{+1} and K_D could not be calculated with the data from one association and one subsequent dissociation curve when Ca^{2+} and Mg^{2+} were added, since dissociation did not obey first order kinetics in that case. Calculation was only possible with the data from two simultaneous association curves performed at two different [^{125}I] AII concentrations (h_1 and h_2 respectively). If b_1 and b_2 are the slopes of the two corresponding association curves the two following equations can be written: $b_1 = k_{+1}h_1 + k_{-1}$ and $b_2 = k_{+1}h_2 + k_{-1}$, from which k_{+1} and k_{-1} can be easily calculated.

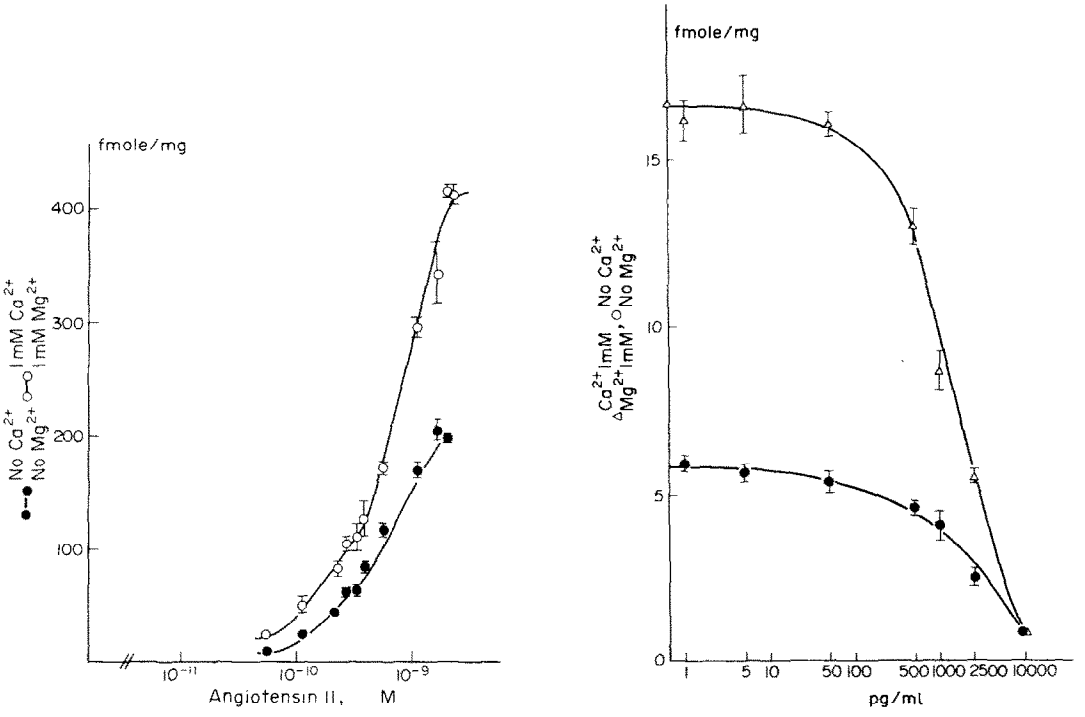

Fig. 6. [^{125}I] AII bound to isolated glomeruli as a function of increasing concentrations of labeled (left) or unlabeled (right) AII in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+} or with no addition of these two cations. Each point represents the mean of triplicates and each vertical bar twice the standard error of the mean. Binding was measured after 45 min incubation.

time) obtained after logarithmic transformation of the data were close to unity in that case*. When the data from the experiments performed in the presence of Ca^{2+} and Mg^{2+} were analyzed, the correlation coefficients obtained were close to unity only for the association curves. Thus we could not calculate in that case the binding parameters from the slopes of the association and dissociation curves. We could only statistically compare the slopes observed in both experimental conditions using the rank test of Mann and Whitney. Both for the association and the dissociation process, the slopes expressed as absolute values were lower when Ca^{2+} and Mg^{2+} were added. In order to get round this difficulty we have calculated k_{+1}, k_{-1} and K_D from association curves performed at two different hormonal concentrations. Table 3 shows that the values obtained are not different from those observed in the absence of cations. Binding at equilibrium as a function of increasing concentrations of [^{125}I] AII in the incubation milieu and competitive binding experiments were also performed both with and without 1 mM Ca^{2+} and 1 mM Mg^{2+} (Fig. 6). These experiments confirmed greater binding of [^{125}I] AII in the presence of Ca^{2+} and Mg^{2+}. The shape of the curves of binding at equilibrium (Fig. 6, left) was similar to that observed in previous studies [2, 4] and suggested either multiplicity of binding sites or a cooperative model. This was reflected in non linear Scatchard's diagrams from which indexes of binding could not be easily calculated. It was however possible to estimate roughly K_D from the concentrations corresponding to 50 per cent of the first plateaus obtained (higher affinity—smaller capacity group of sites) in the curves shown on the left of Fig. 6. These values of K_D were 0.13 and 0.16 nM in the presence and in the absence of Ca^{2+} and Mg^{2+} respectively. They were thus clearly greater than those derived from kinetic studies. This discrepancy can be explained by the imprecise determination of the levels of the binding plateaus observed in the equilibrium binding experiments.

DISCUSSION

The results obtained in the present study clearly show that Ca^{2+} and Mg^{2+} concentrations in the incubation milieu markedly influence the amount of [^{125}I] AII bound to the glomerular receptors. The clear-cut difference in the heights of the equilibrium plateaus gives evidence for a greater number of receptor sites. The precise calculation of this number was made difficult by the non linearity of the Scatchard's plots derived from the data obtained when binding

* The two following equations were used for the association and the dissociation process respectively:

$$\log_e \mathrm{RHeq}/(\mathrm{RHeq} - \mathrm{RH}) = (k_1\mathrm{H} + k_{-1})t \quad (1)$$

$$\log_e \mathrm{RH}/\mathrm{RHeq} = -k_{-1}t \quad (2)$$

RH = bound hormone at any time.
RHeq = bound hormone at equilibrium.
H = hormonal concentration in the incubation milieu.

Equation (1) is valid if H is constant which implicates a negligible degradation and a small fraction of bound hormone related to total hormone. This has been discussed in [1].

at equilibrium was plotted against [^{125}I] AII concentration in the milieu. This can be interpreted as resulting from a cooperative model or from the multiplicity of the groups of receptor sites. The affinity of [^{125}I] AII for its receptors seems unchanged as judged from the hormonal concentrations corresponding to 50 per cent of the binding plateaus and from the values calculated with two association curves.

The effects of Ca^{2+} and Mg^{2+} were maximum for concentrations slightly greater than the physiological levels of these two cations in the plasma. The range of concentrations corresponding to the effect versus log dose curves included the usual physiological or pathological variations around 1.5 mM for ionized Ca^{2+} and 1 mM for ionized Mg^{2+} (Fig. 2). The effects of these two cations were not strictly additive and there was a clear-cut interaction between them. Similarly Mn^{2+} also enhanced [^{125}I] AII binding and interacted with Ca^{2+} but the degree of stimulation obtained with Mn^{2+} was smaller than with the other divalent cations. EGTA inhibited the effects of added Ca^{2+} and EDTA inhibited the effects of added Ca^{2+} and Mg^{2+}. Ca^{2+} and Mg^{2+} provided by the isolated glomeruli play a role in the binding of [^{125}I] AII since introduction into the incubation milieu of EDTA or EGTA produced a marked decrease in the amount of [^{125}I] AII bound. Moreover addition of EDTA after equilibrium of binding had been reached with or without Ca^{2+} and Mg^{2+}, produced a rapid dissociation of [^{125}I] AII from its receptors demonstrating that these cations are implicated in the maintenance of binding.

This Ca^{2+} and Mg^{2+} dependent behaviour of AII towards its receptors seems to be reserved to the glomerular receptors since published studies on AII binding to other tissues led to different conclusions. Lin and Goodfriend [15] observed an increase in [^{125}I] AII bound to rat uterus segments or bovine adrenal particles when 20 mM EDTA was added at 25 or 36°. Devynck and Meyer [16] using [^{3}H] AII and aorta receptors from the rabbit reported that an increase in Ca^{2+} or Mg^{2+} concentrations strongly inhibited the specific binding of AII whereas with the same tracer but using cortical adrenal cells as receptor, Gurchinoff *et al.* [17] observed no effects of these two cations. Glossmann *et al.* [18] concluded that the amount of [^{125}I] AII bound to particulate receptor preparations from the bovine adrenal cortex diminished at increasing concentrations of Mg^{2+} and was influenced by neither 2.5 and 5 mM $CaCl_2$ nor 5 mM EDTA. All these results although varying according to the target organ studied, are opposed to ours since in no case there was an enhancement of binding after addition of Ca^{2+} or Mg^{2+}. Several explanations may account for these discrepancies. In some of these studies [16, 18] high extra-physiological concentrations of Ca^{2+} and Mg^{2+} were used. Another difference is the utilization in some cases [17], of trypsin-collagenase digestion apt to degrade AII receptors which was avoid in the present work. But the more likely hypothesis is a difference in the effects of Ca^{2+} and Mg^{2+} on hormone–receptor interaction according to the receptor studies. These two cations are well known to be able to modify the binding of several polypeptide hormones in different directions as a function of either the hormone or the receptor. At 10 mM Ca^{2+} and Mg^{2+} increased [^{125}I] insulin binding to turkey erythrocytes [19] and [^{125}I] FSH binding to membranes from rat testes tubules [20]. On the contrary, 2–10 mM Ca^{2+} produced a striking inhibition in the binding of [^{125}I] ACTH to adrenal membranes [21] and of [^{3}H]ADH to renal plasma membranes [22] whereas in both cases EGTA had no effect. As far as AII is concerned, Ca^{2+} concentration can increase its binding in another condition in addition to that observed in the present study. This concerns the binding of [^{125}I] AII to specific antibodies. Favre and Valloton [23] reported that among fourteen rabbits having responded to immunization against AII, seven showed specific antibodies which required Ca^{2+} for maximum binding. In parallel, the binding of Ca^{2+}-dependent antibodies was inhibited by adding EDTA in excess. Whereas Mg^{2+} was ineffective, 1 μM Ca^{2+} already increased the binding of [^{125}I] AII.

The explanation for this particular role of Ca^{2+} and Mg^{2+} on the interaction of AII with its glomerular receptor is still unclear. In the absence of Ca^{2+} and Mg^{2+} binding is greater at low pH whereas at 2 mM Ca^{2+} or Mg^{2+} binding is greater at high pH. Thus the degree of increase in binding when Ca^{2+} or Mg^{2+} concentration progressively increases is more marked at higher pH. pH and Ca^{2+} concentration have been shown to affect tachyphylaxis to angiotensin in smooth muscle [24]. For these authors Ca^{2+} can occupy anionic sites present in the vicinity of the specific receptors and interact with AII binding. It has been shown that glomerular basement membranes and other glomerular structures were rich in anionic sites [9]. These sites could be occupied by Ca^{2+} and Mg^{2+} when these cations are added to the incubation milieu. The isoelectric point of [^{125}I] AII has been measured in the present study and is equal to 7.3. Thus in the range of pH studied (7.1–8.9), [^{125}I] AII behaves as an anion above pH 7.3 and binding to its receptor could be facilited by the presence of Ca^{2+} and Mg^{2+} on the glomerular structures. Conversely, in the absence of these two cations, the amount of [^{125}I] AII bound has been found to diminish with pH. This could be due to the increasing negative charge of this tracer preventing binding to the anionic glomerular structures.

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REFERENCES

1. J. D. Sraer, J. Sraer, R. Ardaillou and O. Mimoune, *Kidney Int.* **6**, 241 (1974).
2. M. Beaufils, J. Sraer, C. Lepreux and R. Ardaillou, *Am. J. Physiol.* **230**, 1187 (1976).
3. M. J. Osborne, B. Droz, P. Meyer and F. Morel, *Kidney Int.* **8**, 245 (1975).
4. J. Sraer, L. Baud, J. P. Cosyns, P. Verroust, M. P. Nivez and R. Ardaillou, *J. clin. Invest.* **59**, 69 (1977).

5. R. C. Blantz, K. J. Konnen and B. J. Tucker, *J. clin. Invest.* **57**, 419 (1976).
6. G. M. Eisenbach, J. B. Van Liew and J. Boylan, *Kidney Int.* **8**, 80 (1975).
7. R. L. S. Chang, W. M. Deen, C. R. Robertson and B. M. Brenner, *Kidney Int.* **8**, 212 (1975).
8. H. G. Rennke, R. S. Cotran and M. A. Venkatachalam, *J. Cell. Biol.* **67**, 638 (1975).
9. J. P. Caulfield and M. G. Farquhar, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1646 (1976).
10. W. M. Hunter and F. C. Greenwood, *Nature, Lond.* **194**, 495 (1962).
11. P. Corvol, D. Rodbard, J. Drouet, K. J. Catt and J. Ménard, *Biochim. biophys. Acta* 322, 392 (1973).
12. G. R. Finlayson and A. Chrambach, *Analyt. Biochem.* **40**, 292 (1971).
13. J. J. C. Fong and K. N. Drummond, *J. Lab. clin. Med.* **71**, 1034 (1968).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. S. Y. Lin and T. L. Goodfriend, *Am. J. Physiol.* **218**, 1319 (1970).
16. M. A. Devynck and P. Meyer, *Am. J. Med.* **61**, 758 (1976).
17. S. Gurchinoff, P. A. Khairallah, M. A. Devynck and P. Meyer, *Biochem. Pharmac.* **25**, 1031 (1976).
18. H. Glossmann, A. J. Baukal and K. J. Catt, *J. biol. Chem.* **249**, 825 (1974).
19. B. H. Ginsberg, C. R. Kahn and J. Roth, *Biochim. biophys. Acta* **443**, 227 (1976).
20. H. Abou-Issa and L. E. Reichert, *J. biol. Chem.* **251**, 3326 (1976).
21. J. M. Saez, A. Dazord, A. M. Morera and P. Bataille, *J. biol. Chem.* **250**, 1683 (1975).
22. B. J. Campbell, G. Woodward and V. Boeberg, *J. biol. Chem.* **247**, 6167 (1972).
23. L. Favre and M. B. Valloton, *Immunochemistry* **10**, 43 (1973).
24. J. M. Stewart and R. J. Freer, in *Chemistry and Biology of Peptides* (Ed. Meinhofer), p. 521. Ann Arbor Sci. Publishers (1972).