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Molecular cloning, expression and tissue distribution of a chicken angiotensin II receptor

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Abstract A cDNA encoding a chicken angiotensin II receptor from adrenal gland was isolated to serve as a molecular tool to study the role of AngII in avian embryonic development. This cDNA, sharing a high homology with another avian receptor (turkey), encodes a protein of 359 amino acids with 75% sequence identity with the mammalian type 1 receptor. Transient expression has revealed pharmacological properties distinct from mammalian receptors and a functional coupling leading to the increase in inositol phosphate production. The AngII receptor mRNA is expressed in classical target organs for AngII (adrenal gland, heart, kidney) and, interestingly, in endothelial cells where it may mediate the peculiar vasorelaxation effect of AngII in the chicken.

Key words: Angiotensin II receptor; Molecular cloning; *Gallus domesticus*

1. Introduction

The renin-angiotensin system (RAS), which plays a major role in cardiovascular function and hydro-mineral balance, is known to be functional in all classes of vertebrates. Angiotensin II (AngII), the main effector of this system, acts on a wide range of target tissues that contribute to cardiovascular homeostasis and AngII also exerts growth factor functions [1]. Its involvement in fetal growth and differentiation has been suggested [2].

In mammals, two distinct types of AngII receptors have been pharmacologically characterized [3]. AT_1 receptors bind DuP753 with high affinity, and CGP42112A or PD123319 with low affinity, whereas AT_2 receptors have opposite affinities. The molecular cloning of both receptor types has shown that they are members of the seven transmembrane domain receptor family [4]. Numerous studies have revealed that the AT_1 type is coupled to G-proteins, especially G_q which induces phopholipase C stimulation and subsequent calcium mobilization [3]. In a recent study, AT_2 has been shown to be linked to some G_i proteins ($G_{i\alpha 2}$ and $G_{i\alpha 3}$) but no clear functional coupling has been demonstrated [5].

In non-mammalian species, there is also a RAS, although far less well characterized as in mammals. The only non-mammalian AngII receptors which have been cloned are in *Xenopus laevis*, where two isoforms were identified [6,7], and in the domestic turkey [8]. In both animal species, the peptidic sequences are closely related to the AT_1 type (65 and 75% identity, respectively). These receptors also mediate an AngII-induced mobilization of calcium or inositol phosphate production. However, these receptors have affinities for AngII synthetic analogues different from mammalian types 1 and 2 since, for instance, they do not bind the non-peptidic mammalian AT_1 antagonist DuP753 or the AT_2 antagonist PD 123319.

In avian species, the domestic chicken constitutes an interesting experimental model because of its non-classical response to both avian ([Val^5]-AngII) and mammalian ([Ile^5]-AngII) AngII. Exogenous administration of AngII in anesthetized chickens induces a biphasic action. First appears a vasodepressor response, then immediately followed by a pressor effect [9]. These antagonistic effects may be mediated by distinct AngII receptors or by a single receptor type coupled to distinct signalling pathways depending on the tissue locations of the receptor. The vasodilatation response could be mediated via the stimulation of NO synthase and the generation of cGMP in endothelial cells, whereas the vasoconstriction effect could be mediated by vascular smooth muscle cells and/or neuronal cells. Recently, several authors have brought increasing evidence to demonstrate that there are more than one pharmacologically and functionally distinct avian receptor [10,11]. The elucidation of the mechanisms by which AngII exerts its peculiar effects in the chicken therefore required the molecular cloning of these receptors.

Here, we report the cloning and sequencing of a cDNA encoding a chicken AngII receptor which displays a specific pharmacology. This cDNA has been expressed in COS-7 cells to study the signalling pathway of the receptor, and used to generate riboprobes for in situ hybridization studies on adult chicken organs to show the tissue distribution of its mRNA expression. The molecular characterization of a chicken AngII receptor constitutes a first step toward understanding the chicken vascular physiology and establishing a functional relationship between the mechanisms of action of AngII on blood pressure regulation in non-mammalian and mammalian vertebrate species. It will also serve as a molecular tool to study the role of AngII in avian embryonic development. In addition, because of its peculiar pharmacological profile, this cloned receptor could help to identify the amino acids implicated in the binding recognition of some peptidic and non-peptidic AngII analogues.

2. Materials and methods

2.1. Cloning of chicken AT receptor

The cAT receptor was cloned by homology-based RT-PCR from an adult chicken adrenal gland using sense and antisense primers designed from the previously published sequence of the turkey AT receptor cDNA: 5′-CAGAAGAGATCAAGATGGTCCCAA-3′ and 5′-TGCTGCTTCGTTCACAACCTTGTT-3′. After reverse transcrip-

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Abbreviations: Ang, angiotensin; Sar, sarcosine; AT receptor, angiotensin receptor; tAT, turkey angiotensin receptor; cAT, chicken angiotensin receptor; RAS, renin-angiotensin system

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tion, the 1145 bp fragment containing the entire coding sequence of the chicken AT receptor was amplified with 32 cycles of denaturation at 95°C, 1 min, annealing at 50°C, 1 min, extension at 72°C, 1 min, followed by a 10 min incubation at 72°C. PCR products were directly TA-subcloned into pCRII (In Vitrogen, San Diego, CA). Each insert was sequenced using a Sequenase 2.0 kit (US Biochemical, Cleveland, OH) on both strands. Since the sense primer used for the PCR overlapped the initiation codon, a 5′ RACE-PCR was performed to verify the sequence of the first nucleotides.

2.2. Cell culture and DNA transfection

COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2 incubator. For transient expression studies, one cDNA was subcloned into the *Bgl*II and *Sal*I sites of the expression vector pECE [12]. Transfections were performed when the monolayer reached 90–95% of confluence with 15 μg of this construct per 75 cm^2 flask by the DEAE-dextran method [13]. Transfected cells were immediately seeded in 24- or 12-well plates for subsequent assays which were carried out 72 h after transfection.

2.3. Radioligand binding assays

Radiolabelled ^{125}I[Val^5]-AngII was prepared by chloramine-T iodination and purified by HPLC. Binding assays were performed on intact transfected COS-7 cells 72 h after transfection in the 24-well plates. Incubations were carried out for 1 h at 22°C with increasing concentrations of the radioligand in 200 μl of binding buffer containing 50 mM Tris-HCl, 6.5 mM $MgCl_2$, 125 mM NaCl, 1 mM EDTA and 1 mg/ml BSA, pH 7.6. Non-specific binding was assessed in the presence of 5 μM [Val^5]-AngII. Competition binding experiments were carried out using 1–1.5 nM radiolabeled [Val^5]-AngII and increasing concentration of the various ligands. Each experiment was performed in duplicate or triplicate. Binding data were analyzed with a non-linear least-squares curve-fitting procedure, Ebda-Ligand (Elsevier-Biosoft, Cambridge, UK) [14].

2.4. Determination of inositol phosphate production

As previously described [15], COS cells, subcultured in 12-well plates, were labelled with 2 μCi/ml [^{3}H]myoinositol for 24 h. Cells were subsequently incubated with increasing concentrations of [Val^5]-AngII at 37°C for 30 min in the presence of 10 mM LiCl. The total radiolabelled IP fraction was eluted on a Dowex anion exchange resin (AG®1-X8 resin, Bio-Rad) and measured in a scintillation counter.

2.5. In situ hybridization

Chicken adult tissues were fixed by 4% paraformaldehyde in PBS, dehydrated and embedded in paraffin. Tissue sections (5 μm thick) were mounted on silanated slides. The sense and antisense RNA probes were obtained by in vitro transcription from the whole chicken AngII receptor cDNA subcloned in pCRII. In vitro transcription and radioactive labelling were performed together after linearization of the template with *Hin*dIII or *Xho*I to obtain the antisense or sense cRNA probes. The protocol was as previously described using microwave heating pre-treatment to enhance the hybridization signal [16]. Tissue sections were incubated with probe ($\approx 4\times10^5$ cpm/section). After hybridization and washes, a first observation on Biomax-MR films (Kodak) showing macroscopically the tissue distribution of the receptor mRNA (1–3 day autoradiography) was completed by a second microscopic observation after dipping the slides in liquid emulsion (NTB2, Kodak) (2–5 weeks of exposure).

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                                                                5'CAGAAGAGATCAAG
   1 ATG GTC CCA AAC TAT TCT ACC GAA GAA ACT GTT AAG AGA ATT CAC
     M  V  P  N* Y  S  T  E  E  T  V  K  R  I  H                 15
  46 GTT GAT TGT CCT GTT TCA GGA AGG CAC AGT TAC ATC TAC ATT ATG
     V  D  C  P  V  S  G  R  H  S  Y  I  Y  I  M                 30
  91 GTT CCA ACT GTT TAC AGT ATC ATC TTC ATC ATA GGC ATA TTT GGG
     V  P  T  V  Y  S  I  I  F  I  I  G  I  F  G                 45
 136 AAC AGC CTG GTC GTT ATT GTT ATT TAC TGC TAC ATG AAA TTA AAA
     N  S  L  V  V  I  V  I  Y  C  Y  M  K  L  K                 60
 181 ACA GTG GCC AGC ATC TTT CTG CTA AAC CTG GCA CTG GCT GAC TTG
     T  V  A  S  I  F  L  L  N  L  A  L  A  D  L                 75
 226 TGT TTT CTA ATA ACT CTG CCA CTC TGG GCA GCC TAC ACG GCC ATG
     C  F  L  I  T  L  P  L  W  A  A  Y  T  A  M                 90
 271 GAG TAC CAG TGG CCT TTT GGC AAC TGT TTA TGC AAG CTA GCA TCA
     E  Y  Q  W  P  F  G  N  C  L  C  K  L  A  S                105
 316 GCA GGA ATA AGT TTC AAT TTG TAC GCC AGT GTG TTC CTA CTC ACA
     A  G  I  S  F  N  L  Y  A  S  V  F  L  L  T                120
 361 TGC CTT AGC ATC GAC CGC TAT CTG GCC ATA GTG CAT CCA GTG AAG
     C  L  S  I  D  R  Y  L  A  I  V  H  P  V  K                135
 406 TCA CGA ATC CGA CGT ACC ATG TTT GTT GCC AGA GTA ACC TGC ATT
     S  R  I  R  R  T  M  F  V  A  R  V  T  C  I                150
 451 GTC ATC TGG CTC CTT GCT GGT GTG GCC AGT TTG CCC GTC ATC ATT
     V  I  W  L  L  A  G  V  A  S  L  P  V  I  I                165
 496 CAT CGT AAT ATA TTT TTT GCA GAG AAC TTG AAC ATG ACG GTC TGT
     H  R  N  I  F  F  A  E  N  L  N* M  T  V  C                180
 541 GGC TTT CGA TAT GAC AAC AAT AAC ACA ACA CTG AGG GTT GGG CTA
     G  F  R  Y  D  N  N* N* T  T  L  R  V  G  L                195
 586 GGT TTA TCC AAG AAT TTA CTG GGA TTT TTA ATC CCT TTT CTC ATC
     G  L  S  K  N  L  L  G  F  L  I  P  F  L  I                210
 631 ATA CTA ACA AGT TAC ACC CTA ATT TGG AAG ACA CTG AAG AAG GCA
     I  L  T  S  Y  T  L  I  W  K  T  L  K  K  A                225
 676 TAT CAA ATT CAA AGA AAT AAG ACC AGA AAT GAT GAC ATT TTT AAG
     Y  Q  I  Q  R  N  K  T  R  N  D  D  I  F  K                240
 721 ATG ATT GTG GCA ATA GTA TTT TTC TTC TTC TTT TCC TGG ATT CCT
     M  I  V  A  I  V  F  F  F  F  F  S  W  I  P                255
 766 CAT CAA GTG TTC ACT TTT CTG GAT GTA TTA ATT CAA TTA CAT GTA
     H  Q  V  F  T  F  L  D  V  L  I  Q  L  H  V                270
 811 ATA ACA GAC TGC AAA ATC ACT GAT ATT GTG GAT ACA GCT ATG CCC
     I  T  D  C  K  I  T  D  I  V  D  T  A  M  P                285
 856 TTC ACT ATC TGC ATT GCT TAC TTC AAC AAT TGC TTG AAT CCT TTT
     F  T  I  C  I  A  Y  F  N  N  C  L  N  P  F                300
 901 TTT TAT GTT TTC TTT GGA AAA AAC TTT AAA AAG TAC TTC CTT CAG
     F  Y  V  F  F  G  K  N  F  K  K  Y  F  L  Q                315
 946 CTA ATA AAA TAC ATT CCA CCA AAT GTC AGC ACA CAT CCA AGT CTC
     L  I  K  Y  I  P  P  N  V  S  T  H  P  S  L                330
 991 ACT ACA AAA ATG AGC TCC CTC TCG TAT CGA CCA CCA GAA AAT ATA
     T  T  K  M  S  S  L  S  Y  R  P  P  E  N  I                345
1036 CGC TTG CCC ACT AAA AAG ACT GCT GGG TCT TTC GAC GCT GAG TGA
     R  L  P  T  K  K  T  A  G  S  F  D  A  E  stop             359
1081 TGCATTACGCTACATACTTTTTATCTGAACAAGGTTGTGAACGAAGCAGCA 3'
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Fig. 1. Nucleotide and deduced amino acid sequences of the chicken cDNA receptor. The seven putative transmembrane domains are underlined. The potential N-glycosylation sites are labelled with *. The 5′ and 3′ untranslated regions represented here belong to the clone expressed in COS-7 cells.

3. Results

To generate the cDNA of the chicken AT receptor by PCR, we used two primers corresponding to the tAT sequence in the most conserved region of mammalian AT receptors. Several

Table 1
Pharmacological characterization of chicken AT receptor

a

K_d (nM)	1.39 ± 0.15
B_{max} (sites/cell)	238 439 ± 20 565

b

Peptidic ligands	IC_{50} (nM)	Non-peptidic ligands	IC_{50} (nM)
[Asp^1,Val^5]-AngII	4.74 ± 0.49	CGP42112A	3400 ± 380
[Asp^1,Ile^5]-AngII	5.12 ± 0.34	Dup753	> 10 000
[Asn^1,Val^5]-AngII	165 ± 5	PD123319	> 10 000
[Sar^1,Ile^5]-AngII	61 ± 26		
[Sar^1,Ile^8]-AngII	185 ± 65		
[Asp^1,Ile^5]-AngI	2200 ± 600		
[Asp^1,Ile^5]-AngIII	1650 ± 330		

[a] Binding parameters of ^{125}I[Val^5]-AngII from five experiments.
[b] Affinities of the cAT receptor for peptidic and non-peptidic ligands. Results are expressed as mean ± SEM of 3–7 independent experiments.

products of the expected size and from distinct PCRs were cloned and sequenced. These clones contained an 1080 bp open reading frame, sharing 97.6% homology with the turkey AngII receptor and encoding a protein of 359 amino acid with 99.7% identity with its turkey counterpart (Fig. 1). The protein sequence deduced from the nucleotide sequence possesses many of the characteristic features of the G protein-coupled receptors: (1) the existence of seven hydrophobic regions that are likely to represent the membrane spanning domain found among the G protein-linked receptor superfamily; (2) the protein contains four potential N-glycosylation sites: Asn^{4} present in the amino terminal region and three others, Asn^{176}, Asn^{187} and Asn^{188}, present in the second extracellular loop; (3) four cysteine residues ($Cys^{18,101,180,274}$) may cross-link extracellular loops by disulfide bonding; (4) several serine and threonine residues, putative substrates for protein kinase C- or G-protein coupled receptor kinase phosphorylation, are present in the third intracellular loop and in the carboxyl-terminal tail of the receptor.

The cDNA insert was subcloned into the mammalian expression vector pECE and transfected into COS-7 cells, hereafter designated COS-cAT. Preliminary kinetic analysis revealed that the binding reached equilibrium in 1 h at 22°C (data not shown). The receptor bound $^{125}I[Val^5]$-AngII in a concentration-dependant and saturable manner. Scatchard

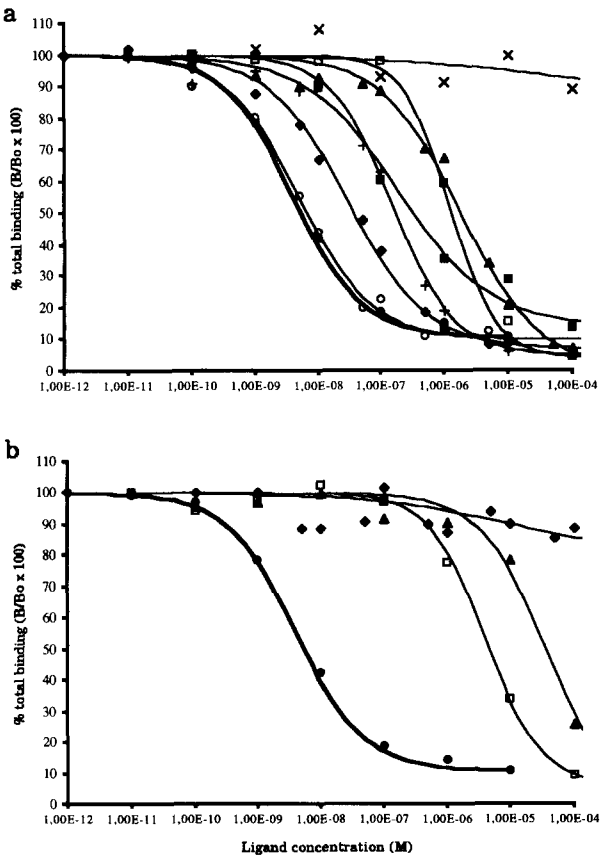

Fig. 2. Competitive inhibition of $^{125}I[Val^5]$-AngII binding to cAT-transfected cells. a: Displacement by unlabelled $[Asp^1,Val^5]$-AngII (●), $[Asp^1,Ile^5]$-AngII (○), $[Asn^1,Val^5]$-AngII (+), AngI (▲), AngIII (□), $[Sar^1,Ile^5]$-AngII (◆), $[Sar^1,Ile^8]$-AngII (■) and the unrelated peptide bradykinin (×). b: Displacement by non-peptidic ligands CGP42112A (□), DuP753 (▲) and PD123319 (◆). Each curve represent the mean of at least three independent experiments with each point performed in triplicate. SEMs were omitted for clarty.

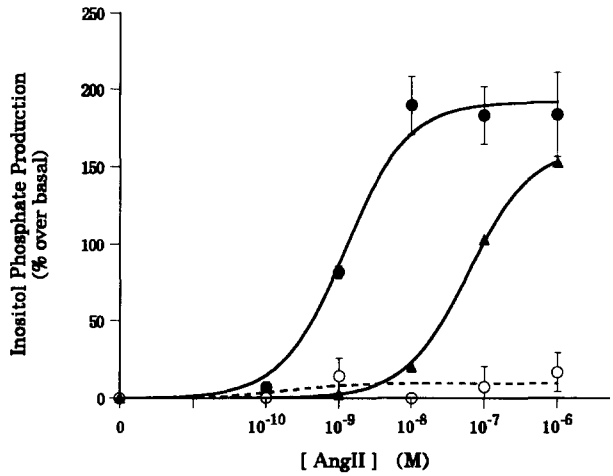

Fig. 3. AngII-induced stimulation of inositol phosphate production. Total inositol phosphates were measured in the absence or presence of increasing concentration of AngII in untransfected (○), and cAT-transfected COS-7 cells with (▲) or without (●) 10^{-6} M of $[Sar^1,Ile^8]$-AngII. Results are expressed as the ratio of the cpm in stimulated versus unstimulated cells and (for the ○ and ● curves) represent the mean ± SEM of three independent experiments with each point performed in triplicate.

transformation of the data revealed a binding site with an estimated K_d of 1.39 nM and an average B_{max} value of approximately 240 000 sites per cell. This binding is inhibited by a variety of angiotensin agonists and antagonists with a pharmacological profile represented in Fig. 2. The inhibitory potency was similar for $[Val^5]$-AngII (the native avian AngII) and $[Ile^5]$-AngII (the native mammalian AngII), whereas the salmon AngII ($[Asn^1,Val^5]$-AngII) — whose first amino acid has lost its acid charge — showed a reduced potency to displace $[Val^5]$-AngII binding (Fig. 2a). The potency of other angiotensins, AngI and AngIII, are in the micromolar range. The mammalian analogue $[Sar^1,Ile^5]$-AngII and the mammalian antagonist $[Sar^1,Ile^8]$-AngII were 10- to 40-fold less potent than the native chicken AngII, respectively (Fig. 2a). The pseudo-peptidic analogue CGP42112A displaced $^{125}I[Val^5]$-AngII binding with an IC_{50} of 3.4 μM, whereas the non-peptidic AT_1 and AT_2 antagonists, DuP753 and PD123319, respectively, were unable to displace it, as the unrelated peptide bradykinin (Fig. 2b). IC_{50} values are listed in Table 1.

In order to verify whether this receptor is functional, experiments were performed to detect the production of IP in response to increasing concentrations of $[Val^5]$-AngII. Activation of the COS-cAT cells reached a plateau at 10^{-8} M AngII. The IP production was then 195% over basal, whereas a very low IP production was detectable in untransfected cells (Fig. 3). The half maximal response (EC_{50}) of the cAT receptor is 1.16 ± 0.17 nM. These results indicate that the cAT cDNA encodes a functional receptor coupled to a G_q family G-protein which activates the phospholipase C signalling pathway. Moreover, to assess whether the mammalian antagonist $[Sar^1,Ile^8]$-AngII was also an antagonist for chicken AT receptor, we incubated transfected COS cells with increasing concentrations of $[Val^5]$-AngII in presence of 10^{-6} M of $[Sar^1,Ile^8]$-AngII. This treatment resulted in a shift of the EC_{50} from 1.16 to 70.71 nM (Fig. 3), demonstrating the antagonistic properties of $[Sar^1,Ile^8]$-AngII in chicken.

The distribution of the cAT mRNA was studied by in situ hybridization. Several organs and tissues expressing the recep-

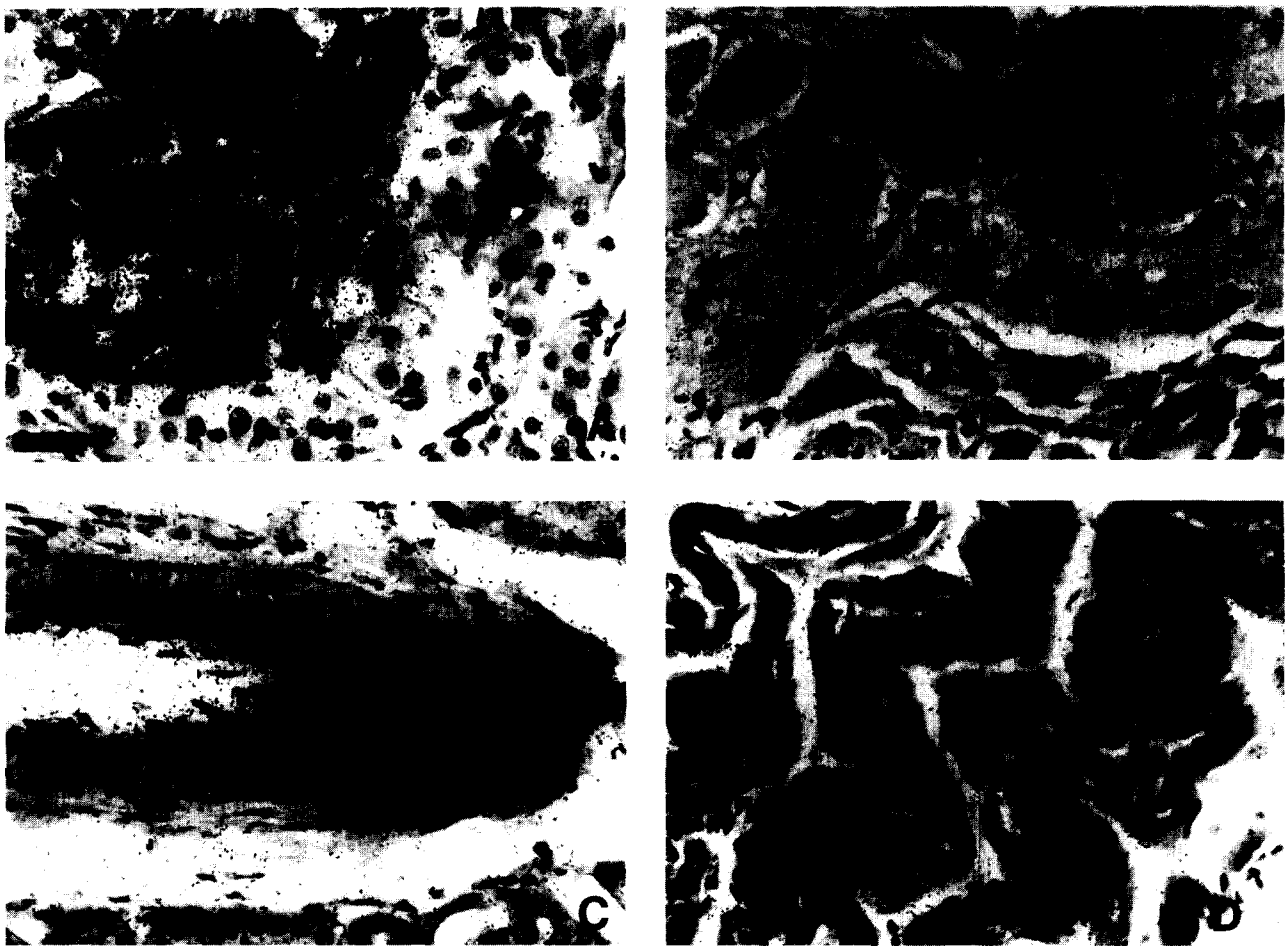

Fig. 4. In situ hybridization. The experiments were performed with a ^{35}S-labelled cAT riboprobe in the adrenal gland (A), sympathetic ganglion (B), renal artery (C) and myocarde (D). Bar: 25 μm.

tor were identified. The adrenal gland (Fig. 4A), from which the cDNA was cloned, showed an intense signal with the antisense probe. In birds, the cortical and medullary zones are not topographically separated but appear as intertwined cord of cells: the cortical cells express the cAT mRNA, but not the cells in the adrenal medulla, histologically recognizable and characterized by tyrosine hydroxylase immunostaining (data not shown). Interestingly, cAT receptor mRNA was detected in the cells of the sympathetic ganglion adjacent to the adrenal gland (Fig. 4B). In the kidney, the hybridization signal is clearly detectable within the glomeruli, and was diffuse and barely distinguishable from background in the proximal tubules (data not shown). Interstingly, in the arteries of large and medium size as shown here in the kidney (Fig. 4C), a strong signal was detected, confined to the endothelial cells. In contrast, the adjacent vascular smooth muscle cells did not show any labelling. In the heart, an homogeneous labelling in the atrium (Fig. 4D), and regional heterogeneities in the wall of the ventricules were observed. The liver, a target organ for AngII in mammals, displayed a low level of mRNA, and in the lung, only the pulmonary parenchyma showed a significant hybridization signal (data not shown).

4. Discussion

In this study, we have cloned and expressed in COS-7 cells a chicken AngII receptor. It differs from the turkey receptor [8] by 27 nucleotide substitutions, of which a single one is not conservative: in the carboxyl-terminal tail, in position 358, the alanine residue is a threonine in the turkey receptor. This change could be important, in particular in desensitization, since it withdraws a potential phosphorylation site present in the tAT. With the other cloned vertebrate receptors (for a review see [4]), the identity is in accordance with what is expected from the phylogeny: 65% and 75% identical to amphibians and mammalians, respectively.

Although the sequence and the signal transduction of the cAT receptor are closely related to the AT_1 type, its pharmacological profile appears different. Like its amphibian and turkey counterparts, it recognizes neither the specific mammalian AT_1 antagonist Dup753, nor the AT_2 synthetic ligand PD123319, and it has a relatively low affinity for the AT_2 non-peptidic ligand CGP42112A. Thus, this receptor is pharmacologically distinct from the two mammalian receptor types. These results are consistent with the physiological and pharmacological studies performed on chicken tissues [10,17,18] or on the cloned receptor in turkey [8]. Also in accordance with these results, Le Noble et al. have demonstrated that the angiogenesis in the chorioallantoic membrane of the chicken embryo is stimulated by AngII and can be inhibited by CGP42112A and not by Dup753 at doses higher than the micromolar [19]. These pharmacological differences suggest that the chicken receptor has a unique structure which contributes to discriminate peptide from non-peptide mamma-

lian AngII receptor antagonists. The replacement of aspartic acid in the first position by asparagine or sarcosine has been shown to reduce in vivo the depressor and pressor effects in chicken [20]. Previous [17] and present data reveal that in vitro binding potencies are also reduced by the first amino acid replacement. Thus, these results confirm the difference between chicken and mammalian receptors, on which sarcosine derivatives show high affinities and potent actions.

In situ hybridization results show that the cloned receptor is present in several organs known to be targets for AngII, including adrenocortical cells where AngII regulates the aldosterone production [11]. AngII binding sites had been shown previously in chicken heart membrane preparations [21] and we can identify as cardiomyocytes the cells in which the receptor is expressed. In mammals, endothelial AngII receptors are detectable by binding studies and have been shown to activate inositol phosphate production and arachidonic acid release [22]. However, because of too low levels of expression, they have not been shown by in situ hybridization. Thus, a particularly interesting observation in the present study is the detection of cAT mRNA in endothelial cells and not in the vascular smooth muscle cells. The presence of AngII receptor mRNA in endothelial cells, which was previously demonstrated by pharmacological studies on the chicken aorta [17,18], support the model proposed by Nishimura et al. to explain the biphasic effect of AngII in the chicken [10]. According to this model, the vasorelaxation observed immediately after AngII administration was caused by the direct interaction of AngII with endothelial receptors, presumably coupled to NO endothelial synthesis and subsequent cGMP production in vascular smooth muscle cells. Then, the vasoconstrictive effect, which can be completely blocked after treatment with α-adrenergic drugs, was attributed to an AngII-mediated catecholamine release triggered by receptors located on adrenergic nerve endings. Our observations are consistent with the presence of cAT receptor in the adrenergic nerve endings, since we detect its mRNA in the catecholaminergic ganglionic cells.

The existence of a second chicken AngII receptor can be postulated to account for some of the results such as the of presence AngII binding sites in the vascular smooth muscle cells in which no in situ hybridization signal was shown in the present study with the cAT riboprobe. Moreover, Kocsis et al. have recently provided evidence for two distinct AngII receptor types or isomorphs in the turkey adrenocortical cells [11]. In amphibian and mammalian species, at least two AngII receptors have been successfully identified. Consequently, it is highly unlikely that the *Aves* class possesses only one such receptor. These data show that further investigation is needed to demonstrate the existence of this putative receptor. The cloning of its cDNA and its pharmacological characterization, together with the cAT receptor cloned in this study, will allow to study the growth-differentiation factor capacities of AngII in the chicken embryo, a developmental model experimentally more simple than the mammalian fetus.

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References

[1] Huckle, W.R. and Earp, H.S. (1994) Prog. Growth Fact. Res. 5, 177–194.
[2] Grady, E.F., Sechi, L.A., Griffin, C.A., Schambelan, M. and Kalinyak, J.E. (1991) J. Clin. Invest. 88, 921–933.
[3] Timmermans, P.B.M.W.M. et al. (1993) Pharmacol. Rev. 45, 206–251.
[4] Sandberg, K. (1994) Trends Endocrinol. Metab. 5, 28–35.
[5] Zhang, J. and Pratt, R.E. (1996) J. Biol. Chem. 271, 15026–15033.
[6] Bergsma, D.J., Ellis, C., Nuthulaganti, P.R., Nambi, P., Scaife, K., Humar, C. and Aiyar, N. (1993) Mol. Pharmacol. 44, 277–284.
[7] Ji, H., Sandberg, K., Zhang, Y. and Catt, K.J. (1993) Biochem. Biophys. Res. Commun. 194, 756–762.
[8] Murphy, T.J., Nakamura, Y., Takeuchi, K. and Alexander, R.W. (1993) Mol. Pharmacol. 44, 1–7.
[9] Nishimura, H., Nakamura, Y., Sumner, R.P. and Khosla, M.C. (1982) Am. J. Physiol. 242, H314–H324.
[10] Nishimura, H., Walker, O.E., Patton, C.M., Madison, A.B., Chiu, A.T. and Keiser, J. (1994) Am. J. Physiol. 267, R1174–R1181.
[11] Kocsis, J.F., Schimmel, R.J., McIlroy, P.J. and Carsia, R.V. (1995) Endocrinology 136, 1626–1634.
[12] Ellis, L., Clauser, E., Morgan, D., Edery, M., Roth, R.A. and Rutter, W.J. (1986) Cell 45, 721–732.
[13] Thibonnier, M., Auzan, C., Madhun, Z., Wilkins, P., Bertimattera, L. and Clauser, E. (1994) J. Biol. Chem. 269, 3304–3310.
[14] Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
[15] Torrens, Y., Daguet de Montety, M.C., El Etr, M., Beaujouan, J.C. and Glowinski, J. (1989) J. Neurochem. 52, 1913–1918.
[16] Sibony, M., Commo, F., Callard, P. and Gasc, J.-M. (1995) Lab. Invest. 73, 586–591.
[17] Stallone, J.N., Nishimura, H. and Khosla, M.C. (1989) J. Pharmacol. Exp. Ther. 251, 1076–1082.
[18] Stallone, J.N., Nishimura, H. and Nasjletti, A. (1990) Am. J. Physiol., E777–E782.
[19] Le Noble, F.A.C., Schreurs, H.H.J.S., Van Straaten, H.W.M., Slaaf, D.W., Smits, J.F.M., Rogg, H. and Struyker Boudier, H.A.J. (1993) Am. J. Physiol. 264, R460–R465.
[20] Nakamura, Y., Nishimura, H. and Khosla, M.C. (1982) Am. J. Physiol. 243, H456–H462.
[21] Baker, K.M. and Aceto, J.A. (1989) J. Mol. Cell Cardiol. 21, 375–382.
[22] Pueyo, M.E., Ndiaye, N. and Michel, J.B. (1996) Br. J. Pharmacol. 118, 79–84.