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Effects of angiotensin II and antagonists on AT_1 receptor expression in mesangial cells

Dominique Chansel [a], Sophie Vandermeersch [a], Catherine Llorens-Cortes [b], Raymond Ardaillou [a,*]

[a] *INSERM U. 489, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France*
[b] *INSERM U. 36, Collège de France, Paris, France*

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Abstract

Rat mesangial cells were exposed to angiotensin II, angiotensin AT_1 receptor antagonists such as losartan, EXP 3174 and candesartan, or dexamethasone for increasing periods (1–24 h). Angiotensin AT_{1A} and AT_{1B} receptor mRNA were measured by reverse transcription-polymerase chain reaction (RT-PCR). Angiotensin II, losartan and EXP 3174 did not modify significantly angiotensin AT_{1A} and AT_{1B} receptor mRNA. Candesartan increased angiotensin AT_{1B} receptor mRNA and, to a lesser extent, angiotensin AT_{1A} receptor mRNA. In contrast, dexamethasone decreased mainly angiotensin AT_{1B} receptor mRNA. As shown by Western blot analysis, exposure of mesangial cells to angiotensin II, losartan or EXP 3174 did not produce any change in angiotensin AT_1 receptor protein, whereas dexamethasone and candesartan exerted inhibitory effects. In conclusion, the angiotensin AT_{1B} receptor subtype, the most abundantly distributed in rat mesangial cells, is inhibited by glucocorticoids. The effect of candesartan is more complex with a slight stimulation of angiotensin AT_{1B} mRNA and a marked inhibition of angiotensin AT_1 receptor protein. In contrast, angiotensin II and the other angiotensin AT_1 receptor antagonists studied are inactive on angiotensin AT_1 mRNA and protein.

Keywords: Angiotensin II receptor; (Rat); Mesangial cell; Angiotensin II receptor antagonist; Dexamethasone

1. Introduction

The biological effects of angiotensin II depend both on the circulating or local levels of the hormone and on the density of the receptors at the surface of the target cells. The latter component is submitted to a complex regulation including transcriptional and post-transcriptional mechanisms. As for many other peptidic hormones, plasma angiotensin II levels play a key-role in these events via interaction of the hormone with its receptors and stimulation of a variety of intracellular pathways. In particular, it has been extensively demonstrated that angiotensin II, after binding to the angiotensin type 1 (AT_1) receptors, induced receptor phosphorylation and internalization with, consequently, a decrease in the surface receptor number (Kai et al., 1994; Richard et al., 1997) occurring within a period of 5–30 min (Conchon et al., 1994). In contrast, binding of angiotensin AT_1 receptor antagonists to angiotensin AT_1 receptors does not result in internalization of the complex (Conchon et al., 1994; Hunyady, 1999). The role of angiotensin II and its antagonists in the control of the expression of angiotensin AT_1 receptor mRNA is a more debated question. This comes in part from the fact that early studies on this problem in the rat did not distinguish the regulation of the two angiotensin AT_1 receptor isoforms, angiotensin AT_{1A} and AT_{1B}, present in this species (Iwai and Inagami, 1992; Makita et al., 1992). Rat mesangial cells represent a unique model because angiotensin AT_{1A} and AT_{1B} receptors are both present in these cells with a slight predominance for angiotensin AT_{1B} receptor. In a previous study, we demonstrated that both isoforms were differently regulated in rat mesangial cells since exposure to dexamethasone induced a down regulation of an-

* Corresponding author. Tel.: +33-1-56-01-67-74; fax: +33-1-56-01-70-03.
E-mail address: raymond.ardaillou@tnn.ap-hop-paris.fr (R. Ardaillou)

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giotensin AT_{1B} receptor expression without affecting angiotensin AT_{1A} receptor (Chansel et al., 1996). By applying a semi-quantitative method of polymerase chain reaction, the regulation of angiotensin AT_{1A} and AT_{1B} receptor mRNA expression in rat mesangial cells was investigated in the present study. We utilized in parallel an anti-angiotensin AT_1 antibody to assess the expression of the angiotensin AT_1 receptor protein. Our aim was to examine the effects of angiotensin II and of several nonpeptidic angiotensin AT_1 receptor antagonists and to reevaluate more precisely the effect of dexamethasone.

2. Materials and methods

2.1. Materials

Reagents for these studies were obtained from the following sources: angiotensin II and dexamethasone from Sigma; RPMI medium and cell culture supplies from Gibco; fetal calf serum and restriction enzymes from Boehringer Mannheim; Taq polymerase from Promega; Moloney murine leukemia virus reverse transcriptase from Life Technologies; dNTP from Pharmacia; anti angiotensin AT_1 antibody (AB 1525) from Chemicon International (Temecula, CA). This rabbit antibody is equally immunoreactive against angiotensin AT_{1A} and AT_{1B} receptors according to the supplier. Losartan and EXP 3174 were gifts from Merck, Sharp and Dohme. Candesartan was a gift from Astra Hässle. All other reagents were purchased from Sigma.

2.2. Cell culture

Primary cultures of mesangial cells were obtained from collagenase-treated glomeruli as previously described (Foidart et al., 1980). Kidneys were removed under pentobarbital anesthesia from 100- to 150-g male Sprague–Dawley rats and glomeruli were isolated by sieving techniques and centrifugation. Collagenase-treated glomeruli were seeded in plastic flasks of 25 cm^2 in the presence of 5 ml of RPMI-1640 medium buffered with 20 mM HEPES (pH 7.4) and supplemented with 10% fetal calf serum, 50 units/ml of penicillin G, 50 μg/ml of streptomycin sulfate and 2 mmol/l glutamine. Culture medium was changed every 2 days. Mesangial cells began to grow from glomeruli after 7–8 days. These cells, stellate or fusiform in shape when observed by phase contrast microscopy, were subcultured at day 21. Confluent cells in the second subculture were studied in all experiments. They exhibited typical morphological and biochemical features of mesangial cells such as tendency to pile up, presence of myosin and α-actin filament bundles and contraction in response to angiotensin II (Foidart et al., 1980).

2.3. RNA isolation

Mesangial cells were preincubated during 24 h in serum-free medium and then incubated under control conditions or in the presence of the agents to be studied for increasing periods (1, 3, 6 and 24 h). At the end of the incubation period, the cells were washed with 0.15 M NaCl. Then total RNA was extracted by the phenol-chloroform method and precipitated with 3 M LiCl (He et al., 1991). RNA concentration was determined from the absorbance reading at 260 nm. Total RNA (15 to 20 μg per lane) was then fractionated by electrophoresis in an agarose gel. The integrity of the purified RNA was determined by visualization of the 28S and 18S ribosomal bands.

2.4. Analysis of angiotensin AT_{1A} and AT_{1B} receptor mRNAs by RT-PCR

Mesangial cell angiotensin AT_{1A} and AT_{1B} receptor mRNAs were quantified as described previously by Llorens-Cortes et al. (1994). The oligonucleotide primers selected corresponded to homologous coding regions of the rat angiotensin AT_{1A} and AT_{1B} receptor genes (positions 295–314 for the sense primer and positions 739–719 for the antisense primer). A synthetic cRNA with a 63-bp deletion removing a unique *Eco*RI site encoding the rat angiotensin AT_{1A} receptor cDNA was used as an internal standard in both the RT and PCR phases of the reaction. cDNAs were synthesized in 20 μl of 50 mmol/l Tris–HCl buffer (pH 8.3) containing 75 mmol/l KCl, 3 mmol/l $MgCl_2$, 20 ng of total mesangial cell RNA, 12×10^5 molecules of the angiotensin AT_1 receptor mutant cRNA, 0.4 μmol/l of the reverse primer, 200 U Moloney murine leukemia virus reverse transcriptase, 2.5 mmol/l dNTP, 10 mmol/l dithiotreitol and 50 U of RNase inhibitor. The reaction was stopped by heating samples for 10 min at 70°C.

Double-stranded cDNAs were synthesized and amplified with 2.5 U of Taq polymerase and 80 nmol/l sense and antisense primers in 0.05 ml of 10 mmol/l Tris–HCl buffer (pH 8.3), 50 mmol/l KCl, 2.0 mmol/l $MgCl_2$, 0.5 mmol/l dNTP, 2 mmol/l dithiothreitol, and 0.01% gelatin for 30 cycles at 92°C, 54°C and 72°C for 60, 60 and 90 s, respectively. Since the coding regions of the rat angiotensin AT_{1A} and AT_{1B} receptor genes are composed of only one exon, contamination of sample RNAs by genomic DNA was excluded by directly subjecting the sample RNAs to PCR amplification without an RT step. Samples in which a DNA PCR product was seen under these conditions were eliminated. A trace amount of [α-^{3}H]dCTP (111 kBq) was included in the PCR reaction for quantification of the different PCR products. After PCR amplification, 20 μl of each PCR reaction product were submitted to *Eco*RI digestion (2000 U/μl) for 90 min at 37°C so

that angiotensin AT_{1A} receptor RNA PCR products could be distinguished from those of angiotensin AT_{1B} receptor. The efficiency of the digestion was verified in each experiment by observation of a complete digestion of a PCR product arising from the amplification of the rat liver angiotensin AT_{1A} receptor cDNA. The different PCR products were separated on a low melting 1.5% agarose gel in the presence of ethidium bromide. The bands were excised, solubilized at 50°C, and the ^3H radioactivity was counted by liquid scintillation spectrometry (Picofluor, Du Pont-New England Nuclear). This technique of PCR can be considered as semi-quantitative. Its main advantage in comparison with the other semi-quantitative methods utilizing an endogenous internal standard (house keeping gene) is that the synthetic internal standard used in the present study exhibits a close similarity with AT_{1A} and AT_{1B} receptor mRNA. For that reason, a unique pair of primers is needed.

Results are currently presented as the ratio of the ^3H radioactivity of the unique angiotensin AT_{1B} receptor cDNA band or of one of the two bands of the angiotensin AT_{1A} receptor cDNA over the ^3H radioactivity of the internal standard band. For precise quantification of the relative distribution of the two subtypes in rat mesangial cells, we took into account the number of C residues present in each fragment (AT_{1A}, 117; AT_{1B}, 113; internal standard, 105).

2.5. Western blot analysis of angiotensin AT_1 receptor

Confluent cells were incubated in the serum-free culture medium with or without dexamethasone, angiotensin II or the angiotensin AT_1 receptor antagonist studied for 48 h. Then they were washed three times in phosphate-buffered saline (PBS), scraped using disposable cell scrapers in 2 ml of PBS and centrifuged at $1800 \times g$ for 10 min. The pellet was stored at −70°C for 150 min, then resuspended in 20 mM Tris buffer containing 10% Triton and centrifuged at $4000 \times g$ for 30 min at 4°C. The final pellet was stored at −20°C.

Western blot analysis was performed with a rabbit anti-angiotensin AT_1 receptor polyclonal antibody according to Sharma et al. (1998) with minor modifications. Sodium dodecylsulphate-polyacrylamide gel electrophoresis was carried out using the Protean II apparatus (Bio-Rad). Separation of proteins was achieved using 10% resolving gels and 4% stacking gels which were placed in a chamber and submerged in electrophoresis running buffer (25 mM Tris, 192 mM glycine and 0.1% sodium dodecylsulphate). A 100 μg of sample protein in 20 μl running buffer and 10 μl sample buffer were mixed, vortexed and spun down for 1 s. A marker lane was added with a commercially produced mixture of molecular weight standards (Amersham, UK). Gels were run for 2 h at 20 mA and transferred to a nitrocellulose membrane using a Transblot (Bio-Rad) at 5 mA/cm^2 for 35 min. The membrane was blocked overnight in 10% nonfat dry milk in PBS at 4°C, followed by incubation with the anti-angiotensin AT_1 antibody at a concentration of 1/1000 in PBS. The membrane was incubated on a rocking platform overnight at 4°C and washed three times with PBS for 10 min before the 2-h incubation with 5/1000 secondary anti-rabbit antibody conjugated with horseradish peroxidase at room temperature. The membrane was washed three times with PBS for 10 min each and visualization was obtained using the enhancement chemiluminescence Western blotting detection kit (Amersham).

2.6. Statistical analysis

All the results are means ± S.E. Statistical differences were assessed using two-way analysis of variance for comparison between the different times of incubation or the different concentrations studied.

3. Results

3.1. Distribution of the angiotensin AT_{1A} and AT_{1B} receptor subtypes in rat mesangial cells

Fig. 1 shows a typical pattern of the distribution of angiotensin AT_{1A} and AT_{1B} receptor mRNA in rat mesangial cells incubated for 24 h in serum-free medium. Angiotensin AT_{1B} receptor appears as a single cDNA species of 444 bp, whereas the angiotensin AT_{1A} receptor cDNA yields after restriction two cDNA fragments of 269 and 175 bp. The internal standard cDNA which lacks *Eco*RI sites remains as a single cDNA species of 384 bp. The percentages of angiotensin AT_{1A} and AT_{1B} receptor mRNA related to total angiotensin AT_1 receptor mRNA were 34 ± 1.7% and 66 ± 1.7%, respectively ($n = 36$) in control rat mesangial cells, thus showing the predominance of the

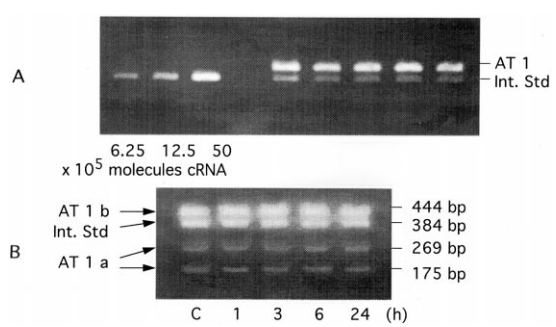

Fig. 1. Analysis of angiotensin II receptor subtypes (angiotensin AT_{1A} and AT_{1B} receptors) in rat mesangial cells incubated for 24 h in serum-free medium. Total RNA from these preparations combined with an internal standard cRNA underwent RT-PCR as described in Materials and methods. PCR products were electrophoresed and visualized under UV light before (top) and after (bottom) *Eco*RI digestion. Int. Std indicates internal standard.

angiotensin AT_{1B} receptor mRNA subtype in accordance with our previous results (Chansel et al., 1996).

3.2. Effects of angiotensin II, angiotensin AT_1 receptor antagonists, and dexamethasone on angiotensin AT_{1A} and AT_{1B} receptor mRNA expression

Rat mesangial cells were exposed to 0.1 μmol/l angiotensin II for increasing periods (1–24 h). Results are presented in Fig. 2. Angiotensin II treatment did not affect significantly angiotensin AT_{1A} and AT_{1B} receptor mRNA expression. Of note, the ratio angiotensin AT_{1A} receptor over internal standard ^3H radioactivity was much lower than the corresponding ratio for angiotensin AT_{1B} receptor. In addition to the real predominance of angiotensin AT_{1B} receptor, this was due to the fact that only one cDNA fragment of angiotensin AT_{1A} receptor was counted. Therefore, the results shown have to be considered for their relative evolution with time. Similarly, losartan (10 μmol/l), an angiotensin AT_1 receptor antagonist, and its active metabolite, EXP 3174 (10 μmol/l), did not modify significantly angiotensin AT_{1A} and AT_{1B} receptor mRNA expressions (Figs. 3 and 4). In contrast, we found a significant increase in total angiotensin AT_1 receptor mRNA as well as in angiotensin AT_{1A} and AT_{1B} receptor mRNAs after treatment with candesartan (10 μmol/l), the active metabolite of candesartan cilexetil (Fig. 5). This increase was maximum after 6 h treatment (+42.8%) for the total angiotensin AT_1 receptor mRNA. It was only significant at 24 h for angiotensin AT_{1A} receptor mRNA (+33.3%). It was the most marked for angiotensin AT_{1B} receptor mRNA with increases of 40.2%, 57.6% and 47.8% at 3, 6 and 24 h, respectively. In contrast, treatment with dexamethasone (1 μmol/l) significantly decreased total angiotensin AT_1 receptor mRNA after 6 and 24 h treat-

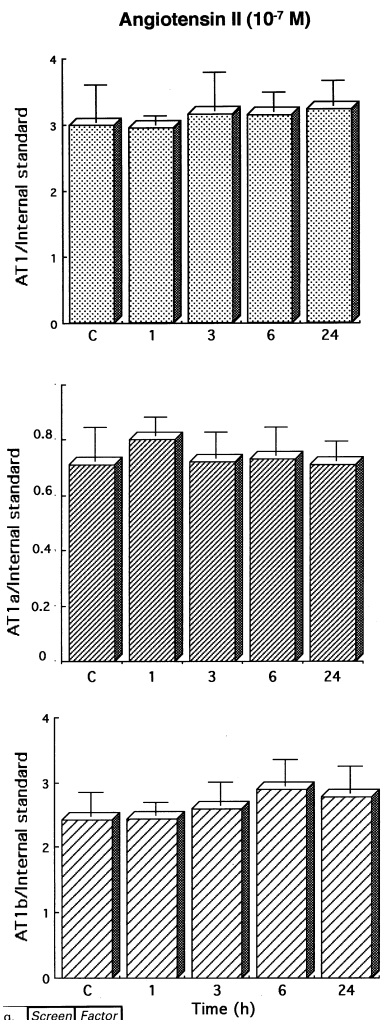

Fig. 2. Effect of angiotensin II (0.1 μM) treatment on angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA levels in rat mesangial cells. Results are presented as the ratio of $[^3H]$ radioactivity for the angiotensin AT_1 receptor cDNA fragments over $[^3H]$ radioactivity for the internal standard. Means ± S.E. of 10 experiments are given. There was no significant change of angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA expression with time.

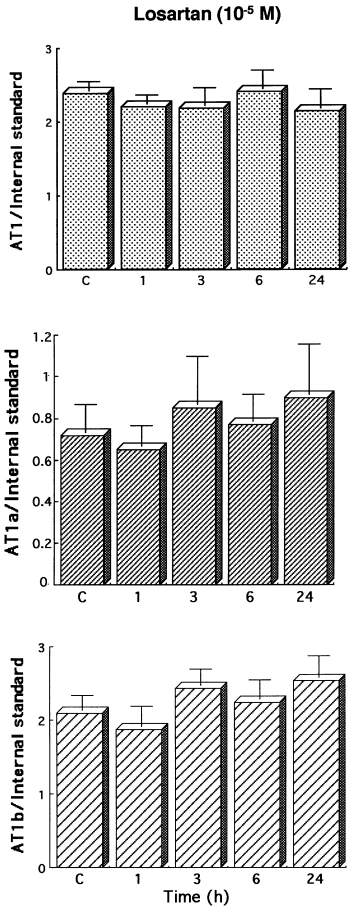

Fig. 3. Effect of losartan (10 μM) treatment on angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA levels in rat mesangial cells. Results are presented as the ratio of $[^3H]$ radioactivity for the angiotensin AT_1 receptor cDNA fragments over $[^3H]$ radioactivity for the internal standard. Mean ± S.E. of six experiments are given. There was no significant change of angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA expression with time.

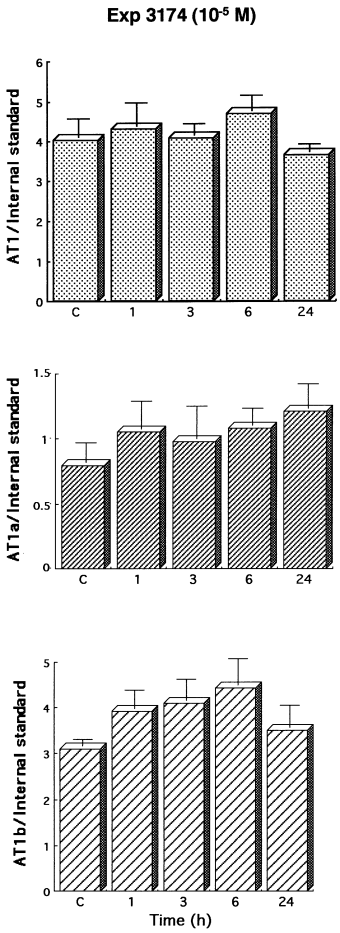

Fig. 4. Effect of EXP 3174 (10 μM) treatment on angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA levels in rat mesangial cells. Results are presented as the ratio of [^{3}H] radioactivity for the angiotensin AT_1 receptor cDNA fragments over [^{3}H] radioactivity for the internal standard. Means ± S.E. of 11 experiments are given. There was no significant change of angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA expression with time.

ment (−26.5%) (Fig. 6). There was also a marked decrease in angiotensin AT_{1B} receptor mRNA expression at 6 h (−36.5%) and 24 h (−31.2%). The decrease for angiotensin AT_{1A} receptor mRNA expression was significant only at 24 h (−22.1%).

3.3. Effects of angiotensin II, angiotensin AT_1 receptor antagonists and dexamethasone on angiotensin AT_1 receptor protein expression

Western blot analysis of angiotensin AT_1 receptor protein from rat mesangial cell homogenates using the rabbit polyclonal antibody showed a predominant band at the expected position of 42 kDa molecular weight. The receptor protein levels remain unchanged after exposure of the cells to angiotensin II (0.1 μmol/l), losartan (10 μmol/l) and EXP 3174 (10 μmol/l) during 48 h. In contrast, dexamethasone (1 μmol/l) and candesartan (10 μmol/l) produced a marked decrease compared to control (Fig. 7). Therefore, we examined the dose–effect relationship for candesartan. This antagonist was inactive on angiotensin AT_1 receptor protein expression at 0.1 μmol/l whereas concentrations of 1 and 10 μmol/l produced decreases of 35% and 65%, respectively (Fig. 8).

4. Discussion

Our study indicates that rat mesangial cells express predominantly the angiotensin AT_{1B} receptor subtype, in contrast with the total renal cortex and the proximal tubule which possess almost exclusively the angiotensin AT_{1A} receptor subtype (Du et al., 1995; Bouby et al., 1997). These results confirm those we reported in a previous study (Chansel et al., 1996) and are in agreement with the reports by Gasc et al. (1994) using in situ hybridization on

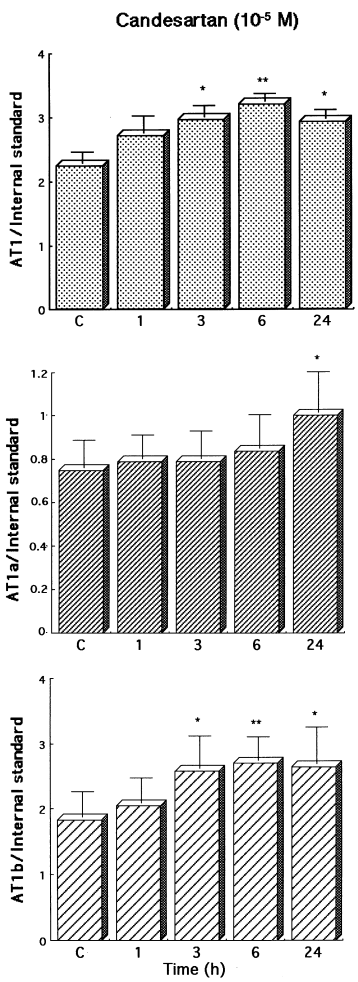

Fig. 5. Effect of candesartan (10 μM) treatment on angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA levels in rat mesangial cells. Results are presented as the ratio of [^{3}H] radioactivity for the angiotensin AT_1 receptor cDNA fragments over [^{3}H] radioactivity for the internal standard. Means ± S.E. of five experiments are given. There was a significant effect of candesartan on total angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA expression with time. * $p < 0.05$ and ** $p < 0.01$ vs. control (C).

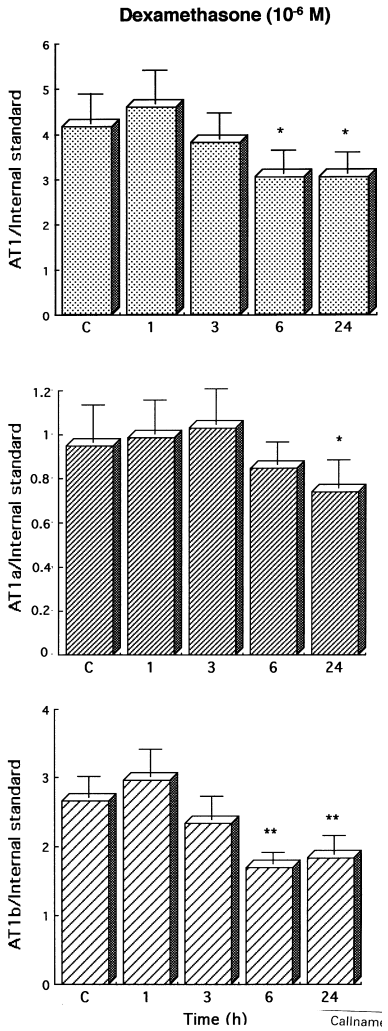

Fig. 6. Effect of dexamethasone (1 μM) treatment on angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA levels in rat mesangial cells. Results are presented as the ratio of [^{3}H] radioactivity for the angiotensin AT_1 receptor cDNA fragments over [^{3}H] radioactivity for the internal standard. Means ± S.E. of 10 experiments are given. There was a significant effect of dexamethasone on total angiotensin AT_1, AT_{1A} and AT_{1B} receptor mRNA expression with time. $^*p < 0.05$ and $^{**}p < 0.01$ vs. control (C).

rat renal sections and by Bouby et al. (1997) using RT-PCR on freshly dissected rat glomeruli.

Studies on the regulation by angiotensin II of the angiotensin AT_1 receptor mRNA expression in the kidney provided conflicting information. Makita et al. (1992) reported down regulation of angiotensin AT_1 receptor mRNA in rat mesangial cells exposed to 1 μmol/l angiotensin II for 6 h. We could not confirm these results in a previous study. Experiments, however, were performed with human mesangial cells and using Northern blot analysis instead of the quantitative PCR method (Chansel et al., 1994). In rats, Na^+ depletion did not change mRNA levels of angiotensin AT_{1A} and AT_{1B} receptors in the kidney (Kitami et al., 1992). In contrast, Sechi et al. (1996) reported that the angiotensin AT_1 receptor mRNA level was significantly

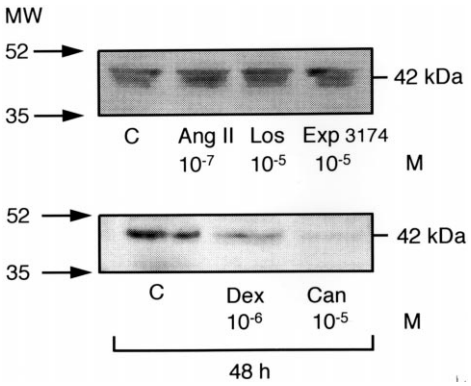

Fig. 7. Two representative immunoblots of angiotensin AT_1 receptor protein in rat mesangial cells. Cells were incubated for 48 h under control conditions (C) or in the presence of 0.1 μmol/l angiotensin II, 10 μmol/l losartan (LOS) or 10 μmol/l EXP 3174 in serum-deprived RPMI medium (upper blot). Incubation was also performed in the presence of 0.1 μmol/l dexamethasone (DEX) or 10 μmol/l candesartan (CAN) (lower blot). Migration and size of molecular weight markers are shown on the left allowing an approximate estimation of the molecular weight of the angiotensin AT_1 receptor to be done (42 kDa).

lower in rats fed with a low salt diet, a difference that was exclusively due to a decrease in the angiotensin AT_{1A} receptor mRNA subtype. This difference was not mediated by a change in angiotensin II plasma levels because angiotensin II infusion had no effect. Similarly, Harrison-Bernard et al. (1999) concluded that the renal angiotensin AT_1 protein and angiotensin AT_{1A} receptor mRNA levels were not different in rats infused or not with angiotensin II for 13 days. Taken together, most of these studies are not in favour of a direct control of angiotensin AT_1 receptor mRNA and protein in the kidney by angiotensin II levels via angiotensin AT_1 receptor activation. However, in many of them, angiotensin AT_{1A} and AT_{1B} receptor mRNA were

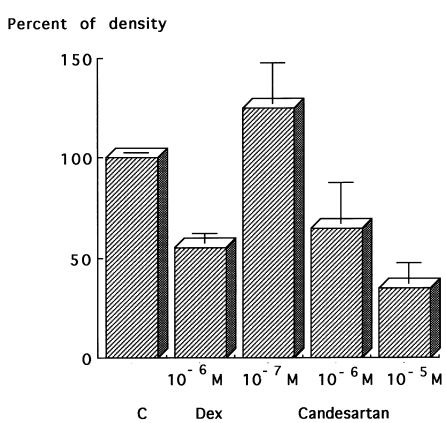

Fig. 8. Densitometric quantification of angiotensin AT_1 receptor protein in Western blot experiments. Rat mesangial cells were incubated for 48 h under control conditions, or in the presence of 1 μmol/l dexamethasone or increasing concentrations of candesartan (0.1–10 μmol/l). Band densities are expressed as percent of control. Means ± S.E. are indicated. There was a significant effect of dexamethasone and of 1 μmol/l and 10 μmol/l candesartan on angiotensin AT_1 receptor protein expression ($p < 0.05$).

not simultaneously examined in homogeneous defined renal cell types. This could explain the variability in the results observed since the two subtypes are differently distributed. The present study indicates that, in mesangial cells where both subtypes coexist, angiotensin AT_{1A} and AT_{1B} receptor mRNA levels and angiotensin AT_1 receptor total protein are not directly affected by angiotensin II within 24- or 48-h incubation, respectively. Therefore, one can conclude that the down regulation of the angiotensin AT_1 receptor protein observed in various kidney preparations after treatment by angiotensin II (Chansel et al., 1994; Amiri and Garcia, 1996) is likely to be due to post-transcriptional events and essentially to the increase of the internalization rate rather than to a decrease in angiotensin AT_1 receptor synthesis.

The intrinsic effects of angiotensin AT_1 receptor antagonists (in the absence of angiotensin II in the medium) on angiotensin AT_1 receptor mRNA expression have not been, to our knowledge, examined until now except some preliminary results included in a review on angiotensin II receptors in mesangial cells (Ardaillou et al., 1999). Most previous studies were performed in rats with different models of renal or vascular diseases that had been treated in vivo. Administration of losartan in uninephrectomized rats did not change angiotensin AT_1 receptor mRNA expression in the kidney (Iwai and Inagami, 1992), whereas the same agent upregulated angiotensin AT_1 receptor mRNA in control and sodium-depleted rats (Wang and Du, 1995). In contrast, candesartan cilexetil which is the prodrug of candesartan, downregulated angiotensin AT_{1A} and AT_{1B} receptor mRNA expression in the heart and the aorta (Kitami et al., 1992). This drug also decreased angiotensin AT_1 receptor mRNA content in the remaining kidney of rats with reduced renal mass (Li et al., 1999) and in balloon-injured rat carotid artery (Tazawa et al., 1999). We found, in the present study, no direct influence of losartan and its metabolite, EXP 3174, on angiotensin AT_1 receptor mRNA and protein expression, whereas similar treatments have been previously shown to decrease angiotensin AT_1 receptor density at rat mesangial cell surface (Chansel et al., 1994). In contrast, candesartan significantly stimulated mRNA expression for both angiotensin AT_1 receptor subtypes with a more marked effect on angiotensin AT_{1B} receptor, whereas it markedly inhibited the whole angiotensin AT_1 receptor protein expression. The main difference between these different angiotensin AT_1 receptor antagonists is the much greater affinity of candesartan for the angiotensin AT_1 receptor in vitro (Shibouta et al., 1993). Candesartan is an unsurmountable AT_1 receptor inhibitor which is not displaced from its binding site by an excess of angiotensin II. One may hypothesize that the long-term blocking of the angiotensin AT_1 receptor by candesartan is responsible for its particular effect when compared with other angiotensin AT_1 receptor antagonists such as losartan which displays an 80 times lower affinity for the angiotensin AT_1 receptor and behaves as a surmountable antagonist (Nishikawa et al., 1997). The dissociation between the angiotensin AT_1 receptor mRNA and protein responses suggests a predominant effect of candesartan at a post transcriptional stage. This effect of candesartan appears to be specific since it differs from those of the two other angiotensin AT_1 receptor antagonists studied, losartan and EXP 3174. It can, however, be compared with the stimulatory effect of converting enzyme inhibitors on the synthesis of angiotensin converting enzyme in endothelial cells (King and Oparil, 1992) which is another example of the influence of an inhibitor on the synthesis of its ligand. The marked inhibitory effect of candesartan on angiotensin AT_1 receptor protein expression is probably not implicated in the mechanism of its major therapeutic efficiency because the lowest concentration at which candesartan was active (1 μmol/l) is higher than the maximum plasma concentration of candesartan (100 ng/ml or 0.22 μM) observed in humans receiving the usual dose of the drug (Hoogkamer et al., 1998).

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