Angiotensin Regulates Endothelin-B Receptor in Rat Inner Medullary Collecting Duct

Norman L.M. Wong and Joseph K.C. Tsui

Our recent studies showed that endothelin ($ET)_B$ receptors are downregulated in congestive heart failure. These changes in ET_B receptor density can be prevented by angiotensin-converting enzyme inhibitors, suggesting a possible role for angiotensin. Using isolated inner medullary collecting ducts (IMCD), we examined the possibility that angiotensin-induced downregulation of ET_B receptors is accompanied by a decrease in ET_B receptor mRNA. Binding studies showed that overnight incubation with angiotensin II induced a downregulatiion of ET_A and ET_B receptors' density in IMCD by 39% and 29%, respectively. This downregulation in ET receptor density was abolished when IMCD was coincubated with angiotensin II and its receptor antagonist saralasin. Furthermore, when the cells were exposed to phorbol myristate acetate (PMA), it resulted in a reduction in ET_A and ET_B receptor binding sites by 41% and 34%, respectively, suggesting the involvement of protein kinase C (PKC). In isolated IMCD, ET-1 induced an increase in cyclic guanosine monophosphate (cGMP) accumulation (705 ± 63 to 1,015 ± 88 fmol/μg protein/5min, $P < .01$), and the ET-1–induced accumulation was attenuated in the presence of angiotensin II (641 ± 45 to 809 ± 46 fmol/μg protein/5min, $P < .01$). Using competitive polymerase chain reaction (PCR) method, we also observed downregulation of ET_A and ET_B receptors mRNA in IMCD treated with angiotensin II (ET_A, 1.09±0.11 *v* 0.77 ± 0.07 amol/μg of total RNA, $P < .01$; ET_B, 14.80 ± 1.95 *v* 8.65 ± 0.67 amol/μg of total RNA, $P < .01$). The addition of a PKC inhibitor abolished the downregulation of ET_A and ET_B receptor mRNA induced by angiotensin II (ET_A, 1.25 ± 0.07 *v* 1.19 ± 0.06 amol/μg of total RNA, not significant [NS]; ET_B, 14.36 ± 0.83 to 13.68 ± 0.64 amol/μg of total RNA, NS). These results suggest that angiotensin II-induced downregulation of ET_A and ET_B receptors mRNA is mediated by a mechanism involving PKC.

ENDOTHELIN (ET) IS A 21 amino acid peptide isolated from endothelial cells and has been shown to have a potent vasoconstrictor activity. It exists in 3 isoforms, namely ET-1, ET-2, and ET-3,[1,2] and mediates their biologic effects in the mammalian kidney by 2 subtypes of receptors, the ET_A and ET_B receptors. Both receptor subtypes are present in the inner medullary collecting duct (IMCD) cells.[3-5] It is now recognized that ET plays an important role in many organ systems and in a variety of physiologic events. In the kidney, the IMCD cells produce large amounts of ET, suggesting that this peptide plays an autocoid role in this segment of the nephron.[6] Supporting this notion is a study performed in rat IMCD that showed ET inhibits vasopressin-stimulated water flux through the ET_B receptors.[7]

In vitro studies suggested that ET-1 binding sites are regulated by angiotensin II.[8] It is known that phosphorylation of the G protein-receptor on serine and threonine residues is involved in agonist-induced receptor desensitization. Durieu-Traumann et al[9] showed that forskolin and dibutryl cyclic adenosine monophosphate (cAMP) reduce the binding capacity of ET receptors in rat astrocytoma C6 cells. The results of Asada et al[10] suggested that the ET-1–induced downregulation of ET_B receptors mRNA in ROS 17/2 rat osteosarcoma cells may be partly mediated through the increase in cAMP level secondary to the activation of the phosphoinositide signaling pathway. Studies by Takemoto et al[11] with the rat cortical collecting duct cells showed that arginine vasopressin (AVP) rapidly downregulates the ET_B receptor through a protein kinase A (PKA)-dependent pathway. Roubert et al[8] reported that angiotensin II and AVP downregulate ET receptors in vascular smooth muscle cells by a mechanism involving protein kinase C (PKC). Furthermore, Cozza and Gomez-Sanchez[12] have shown that PKC activation decreases surface ET receptors' numbers by receptor internalization. The observation that PKA and PKC modulate ET receptors suggests the existence of multiple regulatory mechanisms that may be specific to cell type or receptor subtypes.

We have shown previously that the density of ET_B receptors in the IMCD of heart failure hamsters is significantly reduced.[13] It is known that angiotensin II levels are elevated in heart failure. The hypothesis that ET receptor downregulation because of increased angiotensin II levels was supported by the finding that angiotensin-converting enzyme inhibition restored ET receptor density to normal. This reduction in ET_B receptors density may be related to the increased circulating angiotensin II levels. These data suggest that angiotensin II regulates the density of ET_B receptors in the IMCD.

The present study investigates the mechanisms by which angiotensin II regulates ET receptor density in the IMCD of the kidney. Our results provide evidence that both PKA and PKC pathways are involved in the regulation of ET_B receptors in the IMCD.

MATERIALS AND METHODS

Isolation of IMCD

Male Wistar rats weighing 250 to 300 g purchased from Charles River Breeding laboratories (Wilmington, MA) were anesthetized with sodium phenobarbital (50 mg/kg intraperitoneal [IP]). Kidneys were

From the Department of Medicine, University of British Colombia, Vancouver, British Columbia, Canada.

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Address reprint requests to Norman L.M. Wong, PhD, Department of Medicine, Vancouver Hospital and Health Sciences Center, Koerner Pavilion, 2211 Wesbrook Mall, Vancouver, British Columbia V6T 1Z3.

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removed from these animals and bisected with a scalpel in ice-cold phosphate-bufferd saline (PBS). IMCD were isolated from the papillary tissues of the kidney by a method that was previously described.[14] Briefly, the papillae were isolated, minced, and digested for 30 minutes in 4 mL of 37°C RPMI-1640 medium containing 1.5 mg/mL of collagenase. The digestion process was terminated by adding 4 mL of RPMI-1640 medium containing 10% fetal calf serum (FCS). The sample was then centrifuged for 3 minutes at 1,000 rpm, and the supernatant was discarded. The pellet was resuspended in 2 mL of RPMI-1640 containing 10% FCS and was centrifuged again for another 5 minutes at 1,000 rpm. The resulting pellet was resuspended in 10 mL of RPMI-1640 containing 10% FCS and fractionated in percoll (specific gravity, 1.07) for 20 minutes at 2,000 rpm. Papillary collecting duct cells were found at the top of the percoll layer. These cells were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) for histologic examination. They formed low columnar tubular structures with distinct cell borders, but they had no brush borders. They also stained positively for an epithelial membrane antigen (EMA) (Dakao, Santa Barbara, CA) and low molecular weight cytokeratin (LMWCK) (Enzo, New York, NY), but negative for high molecular weight cytokeratin (HMWCK). Biochemical studies showed that these cells could be stimulated by AVP and atrial natriuretic factor (ANF) to generate cAMP and cyclic guanosine monophosphate (cGMP), respectively, confirming these cells were of collecting duct origin.

Receptor Binding Studies

The IMCD cells were homogenized with a Caframo Stirrer (Wiarton, Ontario) for 2 minutes at 4°C and centrifuged at 1,000 rpm for 5 minutes at 4°C. The sediment containing cellular debris, unbroken cells, and nuclei were discarded. The supernatant was kept in Tris-Tyrode buffer (1%) and subjected to a centrifugation of 15,000 rpm for 20 minutes at 4°C. The resulting supernatant was discarded and the pellet resuspended with 8.5 mL of Tris-Tyrode (1%). This suspension was sonified at low speed for 5 seconds while on ice. A 20-μL aliquot of this suspension was taken for protein assays by the Lowry method.

The binding reactions began by adding 100 μL of IMCD homogenate with increasing concentration of ET-1 (0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 ng/20 μL) and 100 μL of ^{125}I-ET-1 ($\approx$ 100,000 cpm). To quantitate the distribution of ET_B recetpors, ET-1 competitive binding studies were performed by incubating membrane protein and ^{125}I-labelled ET-1 with 100 nmol/L of BQ123 that blocks the binding of ET-1 to the ET_A receptor. Nonspecific binding was defined as binding as the radioactivity bound in the presence of 1 μmol/L of ET-1. At the end of the reaction, bound and unbound labeled peptides were separated by goat rabbit gamma globulin (100 μL) in the presence of 10% normal rabbit serum (50 μL). After adding 1 mL of 5% polyethylene glycol 8000, the mixture was left at room temperature for 15 minutes before centrifuging at 3,000 rpm for 30 minutes at 4°C. The supernatant was aspirated by vacuum suction, and the pellet radioactivity was counted by an LKB minigamma counter (Turku, Finland). These counts were entered into the computer Ligand Program of Munson and Rodbard[15]to calculate the dissociation constant (Kd) and maximum binding capacity (B_{max}) of ET receptors for ET-1.

cGMP Accumulation Studies

cGMP accumulation studies were performed by a method described previously by Luk et al.[16] In brief, 100 μL of freshly isolated IMCD was incubated at 37°C for 15 minutes in 400 μL of Tris -buffer. A total of 50 μL of isobutylmethylxanthine (IBMX; 1 mmol/M) was added 2 minutes later with ET-1 (10^{-7} mol/L), and the mixture was incubated for 5 minutes. The tubes were then placed into an ice bath and the reaction stopped by the addition of 10 μL of 100 mmol/L EDTA solution. Samples were frozen at -80°C for 15 minutes and thawed to rupture cell membranes, allowing the release of soluble cGMP. After vigorous mixing, the freezing, thawing, and mixing procedures were repeated. Addition of 300 μL of Tris-EDTA (50 mmol/L Tris, 4 mmol/L EDTA) buffer brought the final volume to 0.5 mL, and the sample was centrifuged at 300 rpm for 30 minutes followed by acetylation. The supernatant was retained for cGMP measurement by radioimmunoassay and the pellet for protein content by Lowry method.

Quantitative Reverse Transcriptase-Polymerase Chain Reaction

Competitive reverse transcriptase-polymerase chain reaction (RT-PCR) was used to quantify the levels of mRNA for ET_B receptors. Known amounts of competitor DNA molecules are added to each PCR and the amount of target cDNA present in the sample is determined from the added competitor, which gives an equimolar amount of PCR products as the target cDNA. A single set of primers to amplify both target cDNA and an added competitor of known concentration were used. Competitors for ET_A were synthesized with the sense primer 5′-TTC GAC CCC CTA ATT TGG-3′ and the antisense primer 5′-CTG TGC TGC TCG CCC TTG TAT GAT GCC GAC GGA CGA CTT C-3′ producing a 380-bp fragment. The competitive PCR for ET_A was performed with the sense primer 5′-TTC GAC CCC CTA ATT TGG-3′C and the antisense primer 5′-CTG TGC TGC TCG CCC TTG TA-3′ producing a 556-bp fragment. Competitors for ET_B were synthesized with the sense primer 5′- TTA CAA GAC AGC CAA AGA CT-3′ and the antisense primer 5′-CAC GAT GAG GAC AAT GAG ATA GCA GCA CAA ACA CGA CTT A-3′ producing a 427-bp fragment. The competitive PCR for ET_B was performed with the sense primer 5′-TTA CAA GAC AGC CAA AGA CT-3′ and the antisense primer 5′-CACGAT GAG GAC AAT GAG AT- 3′, and the length of the amplicon was 564 bp. PCR was performed with GeneAMP PCR System 2400 (Perkin-Elmer, Norwalk, CT) in a total volume of 50 μL containing 2 μL of cDNA, 2 μL of competitor, 20 pmol of each primer, 100 μmol/L dNTPs, 10 mmol/L Tris-HCL, 0.75 mmol/L $MgCl_2$, and 1.25 U of Taq DNA polymerase (GIBCO-BRL, Gaithersburg, MD). Amplification was performed as follows: step 1, 95°C $\times$ 2 minutes; step 2, 30 cycles of 95°C $\times$ 30 seconds, 60°C $\times$ 30 seconds, 72°C $\times$ 1.5 minutes; and step 3, 72°C $\times$ 2 minutes. After completion of PCR, an aliquot of the reaction mixtures was electrophersed on 2% agarose gels followed by staining with ethidium bromide. The appropriate bands were scanned and quantitated by computer densitometry.

Experimental Protocol

Preliminary experiments were performed to determine the optimum experimental condition by testing different incubation times (4, 6, 17, and 24 hours) and various amount of angiotensin II (10^{-6} to 10^{-11} mol/L). We found that IMCD cells isolated from both kidneys of each rat when incubated in RPMI-1640 medium containing 10% FCS at 37°C for 17 hours gave the best results. Time control experiments were performed to be sure that ET-1 binding was not affected by overnight incubation and that any changes detected in ET-binding with treated IMCD cells were drug-induced. In all of the studies performed, IMCD cells from 1 kidney of each animal were treated with angiotensin II (10^{-11} mol/L) and those from the contralateral kidney served as time controls. IMCD cells were incubated with angiotensin II (10^{-11} mol/L) or saralasin (10^{-9} mol/L) or with a combination of angiotensin II and saralasin before ligand binding studies were performed. To study the possible role of PKC involvement in regulating ET receptors, binding studies were performed in IMCD cells preincubated in PMA (10^{-6} mol/L). Samples were collected after incubation for binding assays, PCR, and cGMP accumulation studies.

Data Analysis

All data were expressed as means $\pm$ standard error of the mean (SEM). Analysis of variance and Student's *t* tests were used to compare

Table 1. Summary of the Binding Results of ET in the Presence of Different Amounts of Angiotensin II

Ang II	Total Control	Total Ang II	ET_A Control	ET_A Ang II	ET_B Control	ET_B Ang II
10^{-7} mol/L (n = 5)	1,939 ± 74	1,650 ± 153	390 ± 66	292 ± 64	1,549 ± 132	1,358 ± 134
P value		<.02		<.06		<.001
10^{-9} mol/L (n = 6)	2,554 ± 231	1,824 ± 287	524 ± 96	346 ± 57	2,030 ± 142	1,508 ± 234
P value		<.04		<.05		<.04
10^{-11} mol/L (n = 6)	2,186 ± 451	1,381 ± 301	475 ± 162	167 ± 37	1,711 ± 324	1,214 ± 267
P value		<.01		<.01		<.01

NOTE. *P* value compared differences between control and angiotensin II-treated IMCD cells.
Abbreviation: Ang, angiotensin.

differences between groups. A *P* value of less than .05 was accepted as significant.

RESULTS

Binding Studies

Time control studies. The binding of ET-1 to isolated IMCD cells from the right and left kidneys of each rat after being incubated at 37°C for 17 hours was comparable (2,023 ± 356 *v* 2,009 ± 502 fmol/mg protein, n = 4). Incubation did not affect the densities of ET receptor subtypes (ET_A, 392 ± 42 *v* 290 ± 86 fmol/mg protein; ET_B, 1,631 ± 358 *v* 1,719 ± 339 fmol/mg protein). Receptor affinity (K_d) was also stable after 17 hours of incubation (total ET, K_d:1.74 ± 0.53 *v* 1.56 ± 0.32 nmol/L; ET_B, Kd: 1.29 ± 0.37 *v* 1.28 ± 0.27 nmol/L).

Effects of angiotensin II on ET-1 binding to IMCD Table 1 summarizes the effect of different amounts of angiotensin II on ET-1 binding. It can be seen that 10^{-11}mol/L of angiotensin II induces reduction in ET-1 binding comparable to that with the use of a higher concentration of angiotensin II. Thus, 10^{-11} mol/L of angiotensin II was selected for all subsequent protocols, and the results of these studies are shown in Fig 1. Overnight incubation of IMCD cells with angiotensin II (10^{-11} mol/L) reduced the B_{max} of ET receptors from 2,186 ± 451 to 1,381 ± 301 fmol/mg protein ($P < .01$, n = 6). In the presence of angiotensin II, ET_B and ET_A receptors density decreased from 1,711 ± 324 to 1,214 ± 267 fmol/mg protein ($P < .01$) and 475 ± 162 to 167 ± 37 fmol/mg protein ($P < .01$), respectively. These reductions in receptors' density were prevented when saralasin (10^{-7} mol/L), a specific angiotensin II receptor antagonist, was added into the incubation media (2,446 ± 207 fmol/mg protein, n = 6). There were also no changes in the densities of the receptor subtypes (ET_A, 559 ± 222 fmol/mg protein; ET_B, 1,887 ± 201 fmol/mg protein, n = 6).

Studies were performed in IMCD cells to explore the possible role of PKC in downregulating ET receptors density (Fig 2). IMCD cells were incubated with PMA (10^{-6} mol/L). The results of these studies showed that PMA mimicked the effects of angiotensin II in reducing ET receptors' density (2,495 ± 211 to 1,394 ± 247 fmol/mg protein, $P < .01$, n = 6). These data also showed that PMA treatment downregulated ET_B receptor density from 1,767 ± 148 fmol/mg protein to 1,097 ± 179 fmol/mg protein ($P < .01$, n = 6) and ET_A receptors from 728 ± 156 to 297 ± 72 fmol/mg protein ($P < .01$, n = 6).

cGMP Accumulation Studies

The results of ET-induced cGMP accumulation are shown in Fig 3. After ET-1 was added into the incubation medium, higher cGMP accumulations were noted in both groups. In the angiotensin II incubated cells, an attenuated response to ET-induced cGMP accumulation was noted (control, 704 ± 63 to 1,015 ± 88 fmol/μg protein/5min, $P < .01$, n = 6; angiotensin II, 641 ± 45 to 809 ± 46 fmol/μg protein/5min, $P < .01$, n = 6). These results complement the data collected with the binding studies.

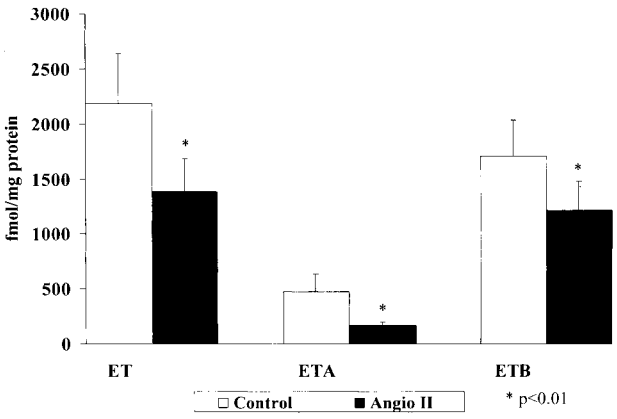

Fig 1. Effect of angiotensin II on ET receptor density is shown. Distribution of ET_A and ET_B receptors was determined with BQ123 (100 nmol/L). Each column is the mean ± SEM of 6 experiments.

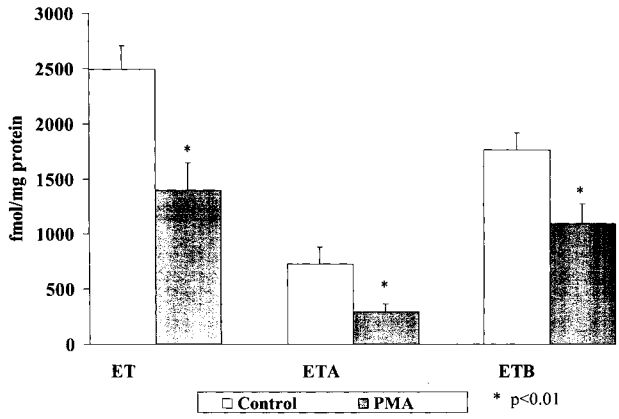

Fig 2. Effect of PMA on ET receptor density is shown. Distribution of ET_A and ET_B receptors was determined with BQ123 (100 nmol/L). Each column is the mean ± SEM of 6 experiments.

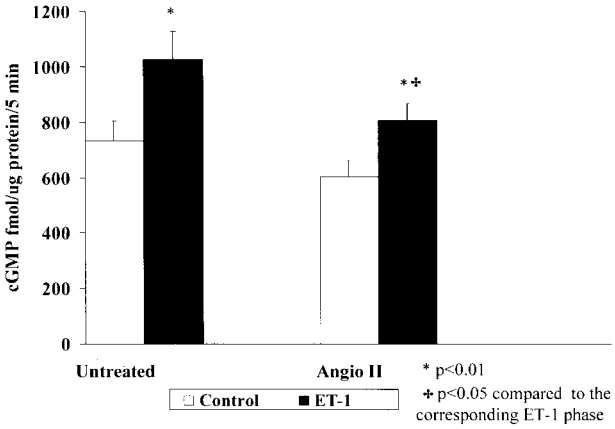

Fig 3. Effect of angiotensin II on ET-1–stimulated guanosine 3′,5′-cyclic monophosphate (cGMP) accumulation in IMCD cells of rats is shown. Each column is the mean ± SEM of 6 experiments.

Effect of Angiotensin II on the Expression of ET_A and ET_B Receptors mRNA in the IMCD

The effects of angiotensin II on the expression of ET_A and ET_B receptors mRNA in the IMCD were evaluated by competitive RT-PCR. The results are graphically shown in Fig 4. The expressions of ET_A and ET_B receptors mRNA were significantly decreased in IMCD incubated with angiotensin II (ET_A, 1.09 ± 0.11 *v* 0.77 ± 0.07 amol/μg total RNA, $P < .01$, n = 6; ET_B, 14.80 ± 1.95 *v* 8.65 ± 0.67 amol/μg total RNA, $P < .01$, n = 6). However, when IMCD was incubated with both angiotensin II and PKC inhibitor, RO-31-8220, the expression of ET_A and ET_B receptors mRNA did not differ between the control and the experimental groups (ET_A, 1.25 ± 0.07 *v* 1.19 ± 0.06 amol/μg total RNA, NS, n = 6; ET_B, 14.36 ± 0.83 *v* 13.68 ± 0.64 amol/μg total RNA, NS, n = 6).

DISCUSSION

We previously reported that ET_B receptors were downregulated in the IMCD of cardiomyopathic hamsters.[13] It is of interest to note that when these cardiomyopathic hamsters were treated with angiotensin-converting enzyme inhibitor, the downregulation of ET_B receptors in the IMCD was abolished. These results suggested to us that the downregulation of ET_B receptors in the kidney of cardiomyopathic hamsters can be due the action of angiotensin II. Supporting this idea is that circulating angiotensin II levels are elevated in congestive heart failure.

These studies were performed to conclusively determine whether a link exists between angiotensin II and ET in the kidney. Incubation of IMCD cells with angiotensin II showed a significant downregulation of ET receptors when compared with controls without angiotensin II. Eighty percent of the ET receptors were of the B variety, while the A subtype made up the remainder. There was substantial reduction of both A and B subtypes after angiotensin II treatment further corroborating that angiotensin II does cause downregulation of ET receptors. These findings are in agreement with that of Roubert et al[8] whose work showed a downregulation of ET-1 binding sites by angiotensin II in vascular smooth muscle cells of rat thoracic aorta. Coincubation of rat IMCD cells with angiotensin II and angiotensin nonspecific receptor antagonist, saralasin, prevented downregulation of ET receptors in this study. These results definitely show that angiotensin II is an important participant in the regulation of ET receptor expression.

In the present study, we showed that angiotensin II reduces ET-1 binding to both ET_A and ET_B receptors in the IMCD. This is different from that seen with AVP, which inhibits ET-1 binding to ET_B, but has no effect on ET-1 binding to ET_A.[14] Because downregulation of ET_B receptors after AVP treatment involved the cAMP-PKA axis, this suggests that different regulatory mechanisms exist for the 2 receptor subtypes. It is known that the ET_B receptor subtypes possess a PKA- and activated phosphorylation sequence site in the third intracellular loop, whereas ET_A lacks such a motif.[11] The correspondence domain in the ET_A receptor is occupied by 2 consensus sequences for PKC phosphorylation. These differences in amino acid sequences between ET_A and ET_B receptors in the third intracellular loop suggested a different regulatory pathway for modulating ET receptor subtypes functions. This can explain the results observed in the present study. Addition of angiotensin II into the cultured media stimulates the activation of PKC, which in turn, leads to downregulation of both ET receptor subtypes. Studies were also performed with phorbol esters to confirm the participation of protein kinase C. In these studies, we were able to mimic the effect of angiotensin with phorbol esters, which provides further evidence that PKC is involved in the angiotensin II-induced downregulation of ET receptors. This agrees with previous report on smooth muscle cells, which also suggested that angiotensin II-induced downregulation of ET receptors by activation of PKC.[8]

The angiotensin II-induced downregulation of ET_B receptors can occur by a mechanism secondary to angiotensin II-stimulated ET-1 release. Studies by Emori et al[17,18] had shown that angiotensin II stimulates the release of ET-1 from cultured bovine carotid artery endothelial cells through receptor-mediated mobilization of intracellular calcium and activation of

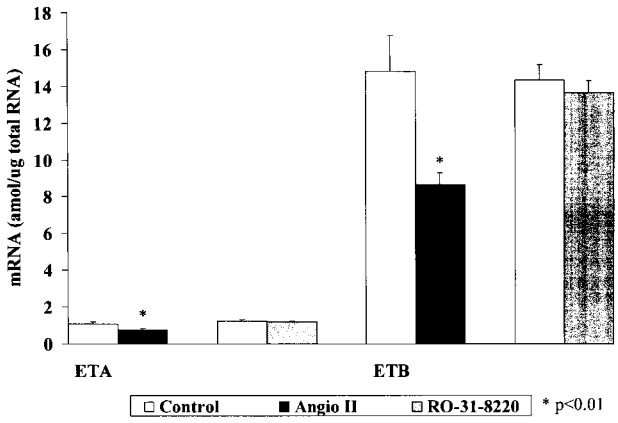

Fig 4. Effect of angiotensin II and RO-31-8220 on the expression of ET_A and ET_B mRNA in the IMCD cells of rats is shown. Each column is the mean ± SEM of 6 experiments.

PKC. The increase in ET-1 synthesis induced by angiotensin II can cause a decrease in receptor density, because it is known that vascular smooth muscle cells respond to excessive ET levels by downregulating the expression of its receptors. The ET-1–induced downregulation of ET_B receptor may be promoted by an increase in cAMP formation that was mediated by ET_A receptors. This possibility remains to be examined.

A growing number of genes has been found to be regulated by activation of PKC.[19] Studies were performed to determine if the downregulation of ET_A and ET_B receptors levels measured by ligand binding method was due to changes in the rate of gene transcription. In the present study, we showed a signification downregulation of the ET_A and ET_B receptors mRNA in IMCD cells after exposure to angiotensin II for 17 hours. This angiotensin II-induced reduction in ET receptors mRNA was abolished by coincubation with RO-31-8220, a selective PKC inhibitor, suggesting a PKC-mediated effect. These results suggested that vasoactive peptides modulate each other by regulating gene expression and protein synthesis. Another possibility for the downregulation of ET receptors mRNA is changes in mRNA stability, which remains to be examined.

The effects of ETs in causing cGMP accumulation in the kidney has been shown in renal epithelial cells and glomeruli.[20,21] The formation of cGMP after ET stimulation depends on the binding of ET to its receptors, guanyl cyclase activity, phospodiesterase activity, and substrate availability. In this study, we measured ET-induced cGMP accumulations in IMCD cells preincubated with and without angiotensin II. Our results showed that preincubation with angiotensin II attenuated the ET-1–induced cGMP accumulation. There are several possible explanations for the observation. One possibility is that angiotensin II prevents the binding of ET-1 to its receptors, which could account for the attenuated response of the angiotensin II-treated IMCD to ET-1–induced cGMP accumulation. This is unlikely in view of our results from the ligand binding studies that showed a downregulation of ET receptor subtypes can be induced with PMA. The attenuated response in the angiotensin II-treated IMCD can be due to phosphorylation of the ET_B receptors leading to downregulation of ET_B receptors. Another possibility includes activation of phosphodiesterase activity by angiotensin II, but this is doubtful, because experiments were performed in the presence of IBMX, a phosphodiesterase inhibitor. These cGMP measurements provide further support to our ligand binding results suggesting that angiotensin II induces downregulation of ET_B receptors.

In summary, our studies showed that angiotensin II causes a downregulation of ET receptors of both the A and B subtype in the IMCD cells of rats. The ET receptor distribution in rat IMCD was composed of approximately 80% B subtype and 20% A subtype. Both ET receptors subtypes were downregulated after angiotensin II treatment. Cells coincubated with angiotensin II and saralasin did not exhibit this downregulation. On the other hand, incubation with PMA caused a downregulation of ET receptor subtypes, suggesting this downregulation is mediated by PKC. This downregulation of ET receptors by angiotensin was supported by cGMP measurements that showed an attenuated response to ET-1–induced cGMP accumulation in IMCD cells pretreated with angiotensin II. Using a competitive PCR method, we also showed that angiotensin II downregulates the expression of ET receptor subtypes mRNA that is mediated by PKC. These experiments illustrate that angiotensin II is involved in the regulation of ET receptor expression.

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