Down-Regulation by cAMP of Angiotensin II Type 2 Receptor Gene Expression in PC12 Cells

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The rat angiotensin II type 2 receptor (AT2-R) gene was isolated, and cis-regulatory regions in its 5'-flanking area were analyzed. Primer extension and RNase protection analyses revealed a single transcriptional initiation site at the position 24 bp downstream of the TATA box. The 5'-flanking region of AT2-R contained several cis-regulatory elements, such as AP-1, AP-2, C/EBP, NF-1, NF-IL6, NF-κB, and glucocorticoid- and cAMP-responsive elements (CRE). The treatment of PC12 cells with dibutyryl cAMP caused a marked decrease (90%) in the AT2-R mRNA level, which was blocked by the inhibitor of protein kinase A and did not require new protein synthesis. The protein level was also reduced 84% after a 24-h exposure to cAMP and the binding affinity was unchanged. The half-life of the AT2-R mRNA decreased −66% by cAMP as compared with control (18.4 ± 0.4 h). Deletion and mutation analyses of the 5'-flanking region (1.2 Kb) revealed that there were one negative (−1,199 to −739) and two positive cis-regulatory regions (−739 to −436 and −59 to +45), and that the CRE motif located at −426 repressed (−23%) the promoter activity of the rat AT2-R gene. The region between −59 and +45 containing TATA box and AP-2 site accounted for 70% of the promoter activity. These findings indicate that the promoter activity of the rat AT2-R gene is modulated by several cis-regulatory regions and that cAMP markedly downregulates the expression of the AT2-R mainly by inducing AT2-R mRNA destabilization rather than CRE-mediated inhibition of the gene transcription. Thus, humoral factors that transduce cAMP as an intracellular signal may modulate AT2-R-mediated function of Ang II by reducing AT2-R expression. (Hypertens Res 1996; 19: 271-279)

Key Words: angiotensin II receptor, AT2 receptor, cAMP responsive element, gene transcription, mRNA stability

Angiotensin II (AngII), a potent vasoactive peptide, exerts a wide variety of physiological functions including cardiovascular regulation, fluid volume homeostasis, neuroendocrine regulation, and cellular growth (1). At present AngII receptors are classified into two major subtypes, designated as type 1 receptor (AT1-R) and type 2 receptor (AT2-R) (2). Most of the well-known AngII functions are mediated by the AT1-R, whereas there is little information reported concerning the roles of AT2-R (3). Recently, several laboratories have cloned rat (4-7), mouse (8, 9), and human (10-12) cDNAs and genes encoding the AT2-R. Ichiki et al. (13) and Hein et al. (14) have recently found in mice lacking the AT2-R that the AT2-R is involved in the maintenance of systemic blood pressure and responsiveness of the cardiovascular system to Ang II. The AT2-R has an antiproliferative effect on neointima formation following vascular injury (15) and on endothelial cells prepared from the myocardium (16). In addition, it was shown that the AT2-R contributes to induction of apoptosis (17, 18) and modifies phosphotyrosine phosphatase activity (4, 19, 20) and voltage sensitive ion currents (20, 21). The AT2-R is abundantly and widely expressed in fetal tissues (22, 23) and its expression is activated in skin wounds (24) or neointima following vascular injury (15). These findings suggest an involvement of AT2-R in control of the cardiovascular system as well as an important role of AT2-R in growth and development.

In vitro binding studies using cell lines, such as mouse R3T3 fibroblasts (25) and PC12W cells (26) or primary cultures of ovarian granulosa cells (18)
and neonatal cardiomyocytes (27) showed that the AT2-R protein is substantially expressed in these cells. Although Leung et al. reported that cAMP suppressed the AT2-R protein expression in PC12W cells (26), the molecular mechanism for cAMP-induced regulation has not been defined. The gene structures and the 5'-flanking sequences of the mouse (28, 29) and human (30) AT2-R, in which putative cis-regulatory elements involved in regulation by growth factors, cytokines or glucocorticoids are located, have been characterized; however, the cAMP responsive element (CRE) was not present in the mouse and human AT2-R genes. Although Kobayashi et al. (6) and Koike et al. (7) reported the nucleotide sequences in the promoter region of the rat AT2-R gene, neither characterization of cis-regulatory elements nor promoter analysis was performed. In this study, we isolated the gene encoding the rat AT2-R and found that a putative CRE motif is present in the 5' flanking region. cAMP markedly downregulated AT2-R mRNA and protein levels in PC12 cells, in which cAMP-induced destabilization of AT2-R mRNA, rather than CRE-mediated inhibition of gene transcription, played a more important role in the down-regulation mechanism by cAMP.

Methods

Library Screening and DNA Sequencing

A Lambda DASH II rat genomic library prepared from Sprague Dawley male testis (Strategene Inc., La Jolla, CA) was screened using a rat AT2-R cDNA fragment (31). Positive clones were purified and characterized by restriction endonuclease mapping and Southern blotting as previously described (32). The specific restriction fragments subcloned into the pBluescript II vector (Stratagene) were sequenced in both strands by dideoxy chain termination.

RNA Analysis

Primer extension reactions and RNase protection assays were carried out with 40 μg and 10 μg of total RNA from PC12 cells, respectively, as previously described (32, 33). For the analysis of AT2-R mRNA a 30-nucleotide-long primer designed from the 5' end of the exon 1 (position +39 to +68) was synthesized (Fig. 2). For RNase protection analysis, the 5'-flanking region between -352 and +48 was subcloned into pBluescript (Stratagene), and used for the probe. After the probe was hybridized with RNA at 52°C overnight, it was digested with RNase-A (2.1 U/ml, U.S. Biochemical Corp.) and RNase-T1 (0.21 U/ml, U.S. Biochemical Corp.) and analyzed on a 6% sequence gel, as previously described (33).

Cell Culture

PC12 cells were generously provided by Dr. Eva J Neer (Harvard Medical School, Boston, MA) and cultured using the plates precoated with poly-L-lysine (1%, Sigma, St. Louis, MO) as previously described (32).

[125I] AngII Binding Assay and Northern Blotting

The receptor binding assays were performed using the membrane fractions as previously described (32). The Bmax and Kd values were determined by Scatchard analyses (32, 34). The cDNA fragment encoding the whole part of the coding region was obtained by reverse transcriptase and polymerase chain reaction (PCR) using the total RNA from PC12 cells as previously reported (31) and was used as a probe. The used filters were boiled and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (35). The obtained mRNA signals were counted with a densitometer.

Transcript Stability Analysis

The confluent PC12 cells were incubated for 24 h in serum-depleted medium and then exposed to dibutyryl cAMP (1 μmol/l) in the presence of actinomycin D (5 μmol/l). After various times of incubation, total RNA was isolated from individual dishes and the disappearance of mRNA abundance was determined by Northern blotting (27, 35).

Construction of AT2-R Luciferase Expression Vectors and a Mutation in the CRE

The 1,245 bp EcoRI-Hind III fragment (from -1,199 to +46 relative to the transcription initiation site) was fused with the 5' end of the luciferase reporter gene (pGL3, Promega). Sequential deletion mutants of the 5' flanking region were prepared by using Exonuclease III (double-stranded Nested Deletion Kit, Pharmacia), as previously reported (36). The 5'-end of the deletion mutant was determined by reading the sequence. A mutation in the CRE was created by the PCR overlapping extension mutagenesis method, as previously described (37). Briefly, two DNA fragment having overlapping ends were first amplified from 1,245 bp EcoRI-Hind III fragment, using two sets of primers. The primers had the following sequences: A, 5'-CTGACC-AAAATGGTTCG-3' (sense; nt -469 to -452); B, 5'-ATTGTAGCAATTCTACTTCTACCTCC-3'; C, 5'-AGTAGAATTGCTACCAT-3' (sense); D, 5'-GAAAGCTTT ACAATTCTACTTCTACCTCC-3' (antisense, nt +50 to +32). Primer B contained a mutated CRE in the 3' end and primers B and C were designed to overlap at the 5' end (underlined). These respective PCR products were mixed and amplified again with primers A and D. The resultant PCR product was subcloned into pGEM-T vector (Promega) and sequenced to confirm the nucleotides of the mutated CRE.

DNA Transfection and Luciferase Assay

PC12 cells, rat A10 vascular smooth muscle cells, and rat Sol 8 skeletal muscle cells were prepared in a 6-cm tissue culture dish. On the day of transfection, the medium was changed to fresh medium and incubated for 2 h at 37°C. The cells were then transfected with AT2-R promoter-luciferase (5 μg) and pRSV β-galactosidase (2 μg) genes (32) for 24 h using LIPOFECTAMINETM Reagent according to the manufacturer's instructions (GIBCO BRL).
followed by incubation with fresh medium for 24 h. Subsequently, the cells were exposed to 1 μmol/l dibutyryl cAMP for 24 h in the serum-depleted medium and then washed twice with phosphate buffered saline and lysed in 150 μl Reporter Lysis Buffer (Promega). After a single freeze/thaw cycle, 50 μl of lysate was used for luciferase activity assay in a 1,253 luminometer (Bio-Orbit Inc.). The assay was initiated by adding 100 μl of Luciferase Assay Reagent (Promega) to the cell lysate, and peak luminescence was measured 1 min after mixture. The luciferase activity was normalized for transfection efficiency by β-galactosidase activity and for cell density by protein concentration (32).

Reagents and Statistical Methods
All reagents were purchased from Sigma Chemical Co. unless otherwise indicated below. The results are expressed as means ± SE. Analysis of variance and the Fisher’s PLSD test were used for multi-group comparisons. Differences with p < 0.05 were considered to indicate statistical significance.

Results

Gene Structure, Transcriptional Start Site and 5’-Flanking Sequence of Rat AT2-R
Two positive clones were isolated from ~10⁶ clones and analyzed further by restriction enzyme mapping and Southern blotting, which indicated that these clones were identical. The ~6 Kb band, after the clone was digested with EcoRI, was hybridized to the probe and then sequenced. The complete sequencing of this fragment revealed that the entire coding region is not interrupted by the intron and is contained in exon 3. There are 156 nucleotides of the intron between exons 1 and 2 (68 bp) and 1,182 nucleotides between exons 2 and 3 (2,728 bp). The exon-intron boundary completely matched the GT-AG rule (data not shown). This gene structure of the rat AT2-R was in good agreement with a recent report (7).

Figure 1A shows the primer extension experiments using total RNA isolated from PC12 cells. The primer was designed from the position +39 to +68 relative to the transcriptional initiation site (+1) (Fig. 2). A single extended band was detected by the primer extension, which corresponded to the position 24 bp downstream from the TATA box. No extended signals were observed when yeast tRNA was used as control. To confirm the result by the primer extension, we performed RNase protection analyses (Fig. 1B). The used riboprobe was an antisense RNA run off from the 5’-flanking region between −352 and +48. If transcription begins at the position 24 bp downstream from the TATA box, as the primer extension data suggest, this probe would protect 48 nucleotides. Figure 1B shows a protected band running at approximately 48 bp. Koike et al. (7) have also recently characterized the transcription initiation site of the rat AT2-R gene only by means of primer extension assays, but the initiation site corresponded to the position that we determined in this study using both RNase protection and primer extension assays.

To identify the sequences of putative cis-regulatory elements that might be involved in the control of rat AT2-R gene expression, the 1.2 Kb of the 5’-flanking region was analyzed with a computer program (TFD 7.2). As Fig. 2 shows, there were several cis-regulatory sequences for the known transcriptional factors (38), including AP-1 (−954 and −176) and AP-2 (−54) binding sites, CCAAT box (−1,052 and −887), and TATA box (−31). The presence of the binding sites for C/EBP (−449 and −334), NF-IL6 (−449), and NF-κB (−813, −657}
and -149) suggests that cytokines or cell growth and differentiation may be involved in the transcription of the AT2-R gene. PEA3 and NF1 binding sites are located at -636, and -133 and -105, respectively, suggesting that the expression of this gene is modified by growth factors or serum (38). The glucocorticoid responsive element (GRE, -1,073 and -654) and the CRE (-426) were present in the promoter region, which suggests the sensitivity of this gene to glucocorticoid and cAMP (38). CArG box and MEF-2 binding sites were found at -1,038 and -268, respectively. These transcriptional factors have been reported to participate in the coordinate regulation of genes during myogenesis (37), in agreement with the findings that the AT2-R is expressed in skeletal muscles in the fetus (22) or myocytes in the developing and neonatal heart (34, 39). Although the C/EBP, AP-2 and PEA3 binding sites are also present in the mouse (28, 29) and human (30) AT2-R genes, the CRE motif was found only in the 5' flanking region of the rat AT2-R gene, suggesting that the tran-

![Fig. 2. Structure of the 5'-flanking region of the rat AT2-R gene. The transcriptional initiation site is indicated by the arrow and marked position “+1”. GATA motif, potential AP-1, AP-2, NF-1, C/EBP, NF-IL6, NFκB, PEA3, MEF-2, v-Myb, and c-Myb binding sites, GRE and CRE sequences, and CArG, CAAT, and TATA boxes are underlined. The primer used for primer extension analysis is indicated by the double underline. Exon 1 was boxed.](image-url)
scriptional regulation by cAMP may be specific to the rat AT2-R gene. Therefore, we examined the effect of cAMP on the rat AT2-R mRNA and protein levels using the PC12 cells.

**Regulation of Rat AT2-R mRNA and Protein Levels by cAMP**

Northern blot analyses indicated that PC12 cells express a single size (−3.5 Kb) of AT2-R mRNA as previously reported (4, 5) (Fig. 3). We have recently reported that serum-deprivation upregulates the AT2-R mRNA level in the PC12 cells: the AT2-R mRNA level reached a maximal increase after 24 h incubation with serum-depleted medium and remained at a similar level for another 24 h and then gradually declined (35), in agreement with the protein level in R3T3 cells (25). Therefore, the effect of cAMP was examined using confluent PC12 cells incubated for 24 h in serum-depleted medium. The exposure to the cAMP analogue induced a marked decrease in AT2-R mRNA levels: the decrease was significant 6 h after exposure to cAMP analogue and the maximal decrease (90%) was observed after 24 h and sustained for up to 48 h. The activities of lactate dehydrogenase and creatinine kinase were not detectable in medium in which cAMP-treated PC12 cells were cultured for 24 h, indicating that the inhibitory action of cAMP analogue is not due to its toxic effect on PC12 cells (data not shown).

To further elucidate the mechanism of the cAMP effect, PC12 cells were incubated with cAMP and H-89, a selective inhibitor of protein kinase A. H-89 blocked the inhibitory effect of cAMP (Fig. 3), indicating that the cAMP effect is modulated by protein kinase A. To examine whether these inductions require new protein synthesis, the cells were pretreated with cycloheximide for 4 h in serum-depleted medium followed by the addition of dibutyryl cAMP. Coincubation of cycloheximide with dibutyryl cAMP did not affect the repression by dibutyryl cAMP (Fig. 3).

We previously reported that the PC12 cells exclusively express the AT2-R protein with a single binding site and a high binding affinity (32, 35). We found that the receptor density (Bmax) and binding affinity (Kd) were 453± 13 fmol/mg protein and 0.54± 0.03 nmol/l (n=5), respectively, in cells incubated for 24 h in serum-depleted medium, and that these Bmax and Kd values did not change significantly after a subsequent 24 h incubation in the same medium (Bmax: 428 ± 9, Kd: 0.54 ± 0.03). The 24 h exposure to dibutyryl cAMP caused a marked (−84%) decrease in the AT2-R density (72 ± 3 fmol/mg protein, p<0.01, n = 5) without affecting the binding affinity (Kd = 0.53 ± 0.03 nmol/l), confirming the previous observation at the protein level that cAMP downregulates the AT2-R numbers in the PC12W cells (26).

**Effect of cAMP on AT2-R mRNA Stability**

We have recently shown that the half-life of the AT2-R mRNA levels in the confluent PC12 cells is not influenced by serum deprivation (35). The change in AT2-R mRNA half-life was examined by inhibiting the gene transcription by treatment with actinomycin D. The half-life (18.4± 0.4 h, n = 5) of confluent PC12 cells incubated for 24 h in serum-depleted medium was decreased to 6.3 ± 0.3 h (66%, p<0.01, n = 5) by dibutyryl cAMP (Fig. 4).

**Promoter Analyses and Characterization of cis-Regulatory Element for cAMP Responsiveness**

A portion of the AT2-R gene from −1,199 to +45 was serially deleted toward the 3' end and inserted immediately upstream of the luciferase reporter gene. The results of transfection of these deletion

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**Fig. 3. Northern blot analyses of cAMP-induced changes of rat AT2-R mRNA levels in PC12 cells.** The confluent PC12 cells were serum-depleted for 24 h and then exposed to dibutyryl cAMP (1 μmol/l) in the same medium. Cycloheximide (5 μg/ml) and H-89 (30 μmol/l), an inhibitor for protein kinase A, were added 4 h and 0.5 h prior to exposure to dibutyryl cAMP, respectively. AT2-R mRNA levels are expressed relative to GAPDH mRNA levels and shown as % changes of values at 0 h. The results are shown as the means ± SE of four separate experiments with duplicate determinations in each experiment. *p<0.01 vs. the value in 0 h. CHX, cycloheximide.
mutants into PC12 cells revealed that 1.2 Kb of the 5’ flanking region had sufficient promoter activity, whereas transfection into the rat A10 vascular smooth muscle cells and rat Sol 8 skeletal muscle cells showed no significant promoter activity (data not shown).

Deletion from -1,199 to -739 resulted in a significant increase (~30%) in promoter activity, suggesting that there is a negative cis-regulatory element (NRE). In this region, two CAAT boxes, AP-1 and GATA-1 binding sites, were located (Fig. 2).

Interestingly, Ichiki et al. reported that the mouse AT2-R gene also contains the NRE between -1,497 and -874 when transfected into PC12W cells (28), suggesting that the rat and mouse AT2-R genes harbor the NRE active in PC12 cells in similar promoter regions. Successive deletion from -739 to -469 resulted in a -55% decrease in the luciferase activity, indicating that a positive cis-regulatory element is present in this region, which contributes about 36% of the promoter activity. There were no significant changes in the luciferase activities between -469 and -59. Further deletion from -59 to +45, in which AP-2 site and TATA box are contained, caused a marked reduction to the basal level in the relative luciferase activity, although this region did not modulate the function in the A10 and Sol 8 cells. These findings demonstrate the presence of a strong positive cis-regulatory element between -59 and +45 which accounts for about 70% of the promoter activity and is responsible for the differential expression of the rat AT2-R gene. The DNA segment between -47 and +56 of the mouse AT2-R gene also contributes about 70% of the promoter activity in the PC12W cells (28), indicating that the proximal part of the AT2-R gene promoter is important for basal and cell-specific expression.

The rat AT2-R gene, unlike the mouse and human AT2-R genes, contained both CRE (AGACGTCA, 1 bp mismatch) at -426 and AP-2 site (GGCTCCCC, 2 bp mismatches) at -53. These promoter elements are reported to be involved in cAMP-induced transcription (38). As shown in Fig. 5, the promoter activities of the 5’ flanking regions (AT2R1199, AT2R739, and AT2R469) were modestly (20 to 25%) inhibited by dibutyryl cAMP, whereas progressive deletion mutants that contained the AP-2 site, but not the CRE, did not confer cAMP sensitivity on the AT2-R gene. To further elucidate the role of the CRE, we created a mutant CRE (wild AGACGTCA → AGACCGGA) (37). The results showed that the 5’ flanking region (ΔAT2R469) containing the mutated CRE was not responsive to exposure to the cAMP analogue, suggesting that the CRE located at -426 be involved in the cAMP-mediated repression of rat AT2-R gene transcription.

**Discussion**

The present study demonstrates that the rat AT2-R gene consists of three exons (exon 1, 68 bp; exon 2, 60 bp; and exon 3, 2,768 bp), and that its entire coding region and 5’ non-coding regions are included in the third exon. The transcriptional initiation site of the rat AT2-R was distinct from those of the mouse (28, 29) and human (30) AT2-R, despite their high homology (96% and 91%, respectively, in the 250 bp upstream sequences from TATA boxes). The initiation sites of the mouse AT2-R gene were located 2 and 18 bp downstream of the TATA box, as determined only by primer extension analyses (29). The initiation site of the human AT2-R was
proposed to be 33 bp downstream of the TATA box, although the mRNA analyses were not performed (30). Koike et al. (7) reported the nucleotide sequences of the promoter region of the rat AT2-R gene and characterized the transcriptional initiation site only by primer extension assay. In the present study analyzing the initiation site using both RNase protection and primer extension analyses, we determined the position to be 24 bp downstream of the TATA box.

As Fig. 2 shows, the 5' flanking region of rat AT2-R contains several cis-regulatory elements, such as AP-1, AP-2, C/EBP, NF-IL6, NF-1, NF-xB, PEA3, GRE, and CRE, suggesting that the rat AT2-R gene transcription may be regulated by phorbol ester, intracellular calcium level, cytokines, growth factors, glucocorticoids, or cAMP (38). We (27, 35) and others (40) recently demonstrated that growth factors, glucocorticoids, and cytokines downregulate the AT2-R expression in PC12 cells and R3T3 fibroblasts. Leung et al. reported that the addition of dibutyryl cAMP reduced the rat AT2-R protein expression in PC12W cells, a substrain of PC12 cells (26). In this study, we extended the observations on the protein level by Leung et al. (26) and found that cAMP downregulates AT2-R expression by inhibiting both the gene transcription and mRNA stability without the requirement of de novo protein synthesis.

Although the molecular mechanisms of cAMP-induced transcription are complex and not completely determined, it has been suggested that there are two groups of cAMP-responsive genes (41): one contains genes in which the stimulatory action of cAMP is mediated by a promoter element with the consensus CRE sequence T(G/T)ACGTCA (42), and the other includes genes that are activated by both cAMP and protein kinase C. This cAMP response sequence, CCCC(A/T)GGC, is termed AP-2 binding site (43). The rat AT2-R gene contains both CRE and AP-2 site in the 5' flanking region. It should be noted that the CRE sequence is present only in the rat AT2-R gene, but not in the human and mouse AT2-R genes, since the CRE sequence (AGACGTCA) in the rat AT2-R gene has been altered to AGACTTCA in the human gene (30) and AGACCTCA in the mouse gene (28, 29). The AP-2 sequences were commonly located at similar
positions for the rat, mouse, and human AT2-R genes. The deletion and mutation analyses of the 5' flanking region using a luciferase reporter gene system revealed that the CRE motif, rather than the AP-2 site, is involved in the cAMP-induced repression of rat AT2-R gene transcription. The CRE is known to be a binding site for a number of nuclear factors that belong to the CREB (CRE-binding protein)/ATF (activating transcription factor) family of transcription, in which the CRE-binding factors can be functionally divided into activators and repressors (44). Examples of the first group are CREB, CRE modulator τ (CREM), and ATF-1, while repressors include the CREM isoforms CREMα, -β and -γ and inducible cAMP early repressor (ICER), and these transcription factors occupy the CRE site by forming homo- or heterodimers (44). Characterization of CRE-binding factors will be a subsequent step in determining which repressor factors regulate the cAMP sensitivity of the rat AT2-R gene.

The rate of mRNA degradation is another important regulatory mechanism in the control of gene expression. cAMP has been demonstrated not only to regulate the rate of transcription of the gene, but also to affect the stability of mRNA (45, 46). The mRNA level of the rat AT1a-R gene is also down-regulated by cAMP, but the molecular mechanism for this regulation remains to be determined (47). The present findings show that the half-life of AT2-R mRNA was reduced about 66% by a cAMP analogue. Given that AT2-R mRNA levels were down-regulated 90% by cAMP and cAMP-induced changes in AT2-R mRNA half-life and gene transcription were about 66% and 23% respectively, it is suggested that AT2-R mRNA destabilization, rather than transcriptional regulation, plays an important role in cAMP-induced down-regulation of AT2-R gene expression. Since down-regulation of the AT2-R by cAMP is also observed in mouse R3T3 fibroblasts (data not shown), it is conceivable that the mouse AT2-R gene, which has no CRE motif, is regulated by cAMP in a post-transcriptional mechanism.

Although the signal transduction pathway and physiological significance of the AT2-R have not been well defined yet, studies using mice lacking the AT2-R (13, 14) have shown that AT2-R has an anti-AT1-R effect on the maintenance of systemic blood pressure. The AT2-R also has an antiproliferative action on neointima formation following vascular injury (15) and on coronary endothelial cells (16). The AT2-R is abundantly and widely expressed in fetal tissues (22, 23), and its expression is activated in skin wounds (24) and neointima following vascular injury (15). These findings suggest a physiological involvement of AT2-R in control of the cardiovascular system as well as an important role in growth and development. We characterized the cis-elements responsible for the regulation of rat AT2-R gene transcription, cell-specific expression and cAMP responsiveness. However, little is known about the molecular mechanism by which AT2-R expression is developmentally regulated. Promoter analyses using a suitable cell system derived from fetal tissue or an in vitro transfer system would allow us to begin to characterize the cis-elements or trans-acting factors for the developmental regulation of the AT2-R gene.

Abbreviations

Angiotensin II (Ang II), angiotensin II type 1 and type 2 receptors (AT1-R, AT2-R), Dubellco's modified Eagle's medium (DMEM), fetal calf serum (FCS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cAMP responsive element (CRE), glucocorticoid responsive element (GRE), polymerase chain reaction (PCR).

References


