A cAMP Response Element in the β2-Adrenergic Receptor Gene Confers Transcriptional Autoregulation by cAMP*

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The rate of transcription of the β2-adrenergic receptor gene is increased in response to β-adrenergic agonist stimulation of the receptor at the cell surface. This effect is mediated by stimulation of adenyl cyclase and elevation of intracellular cAMP. We have previously shown that this responsiveness to cAMP resides in the 5'-flanking region of the human β2-adrenergic receptor gene (Collins, S., Bouvier, M., Bolanowski, M. A., Caron, M. G., and Lefkowitz, R. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4853–4857). A 34-base pair sequence derived from the β2-adrenergic receptor promoter region (~70 to ~37 base pairs), containing the sequence GTACGTCA, confers responsiveness to cAMP when present in either orientation 5' to the thymidine kinase promoter on the chloramphenical acetyltransferase reporter gene. Overexpression of the catalytic subunit of protein kinase A fully substituted for forskolin in inducing expression through this sequence, indicating that the cAMP induction is mediated through this kinase. Mutations within the GTACGTC sequence completely abolished the stimulation.

A 43-kDa transcription factor (cAMP response element-binding protein) confers cAMP responsiveness through binding to specific sequences. In gel mobility shift assays, purified cAMP response element-binding protein bound to the 34-base pair oligonucleotide from the β2-adrenergic receptor gene with an affinity similar to that for the well-characterized cAMP response element from the human glycoprotein hormone α-subunit gene, and failed to bind to mutated elements. Thus, positive autoregulation of the β2-adrenergic receptor gene appears to occur through receptor-mediated stimulation of adenyl cyclase, with consequent activation of cAMP response element-binding protein and stimulation of β2-adrenergic receptor gene transcription. These results demonstrate a novel mechanism by which a receptor (β2-adrenergic receptor) stimulatory for adenyl cyclase can exert positive feedback regulation on its own expression.

The family of G-protein coupled receptors regulate intracellular concentrations of many second messenger molecules, thus performing a pivotal role at the cell membrane in the transduction of extracellular signals. The β2-adrenergic receptor (β2-AR) is a prototypic member of the G-protein coupled-receptor family which is linked to the stimulation of adenyl cyclase and the elevation of cAMP levels (1). The diverse regulatory and metabolic effects of cAMP are mediated through the catalytic subunit of the cAMP-dependent protein kinase, and have been shown to include the regulation of gene expression (2–4). While activation of the cAMP pathway frequently leads to an increase in the rate of transcription of target genes, changes in mRNA stability have also been noted (5–7). The transcriptional response of several genes to cAMP has been localized to a distinct DNA sequence termed the cAMP response element (CRE) (8–11). Most CREs contain a variation of the palindromic sequence motif TGACGTCA, which is recognized by a 43-kDa phosphoprotein (cAMP response element-binding protein, CREB) present in many cells. This protein has been purified (12), cloned (13, 14), and its ability to stimulate the transcription of target genes is modified by phosphorylation (15), thus linking ligand-receptor interactions at the cell surface with the regulation of gene expression.

We have previously shown (16) that cAMP stimulates transcription of the β2-AR gene either as a consequence of agonist stimulation of the receptor-adenyl cyclase complex or when intracellular levels of cAMP are elevated in response to forskolin and the phosphodiesterase inhibitor IBMX. These earlier studies showed that the 5'-flanking region from the human β2-AR gene could confer cAMP responsiveness to a reporter gene. Since this promoter construct (pL.4CAT) (Fig. 1A) contained the sequence GTACGTC, which bears significant homology to the CRE consensus sequence TGACGTC, we investigated whether this sequence motif functions in the β2-AR promoter as a CRE. Accordingly oligonucleotides were synthesized spanning this sequence with and without specific mutations, and their ability to confer cAMP responsiveness to a thymidine kinase (tk) promoter-CAT construct (Fig. 1B) was tested. Results from these studies support the conclusion that the β2-AR sequence GTACGTC is a CRE. In addition, a purified preparation of the CREB transcription factor binds the wild type, but not mutant β2-AR CRE in gel mobility shift assays. Taken together these studies establish a mechanism by which cAMP promotes β2-AR gene expression.

**MATERIALS AND METHODS**

**Plasmid Constructions**—A reporter plasmid (pL.4CAT) (Fig. 1A) containing the β2-AR 5'-flanking region cloned upstream from the

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The abbreviations used are: β2-AR, β2-adrenergic receptor; CRE, cAMP response element; CREB, cAMP response element-binding protein; bp, base pairs; tk, thymidine kinase; CAT, chloramphenical acetyltransferase; Mt, metallothionein; IBMX, isobutylmethylxanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
coding sequence for the chloramphenicol acetyltransferase (CAT) gene was constructed as previously described (16). Oligonucleotides from the β2-adrenergic receptor region corresponding to position -70 to -57 bp (Fig. 1B), and mutant versions of this sequence were synthesized and purified on 8 μm uca, 12% polyacrylamide gels run in Tris-borate/EDTA buffer, and annealed in TE buffer. These blunt-ended double-stranded oligonucleotides were cloned into the NruI site of the plasmid pTSGN (henceforth called "tGCaT"). This plasmid contains sequences -769 to +75 bp of the herpes simplex virus thymidine kinase gene linked to CAT coding sequences. Recombinant clones were selected to contain a single copy of each oligonucleotide in both orientations, and are designated β2(+)-CAT and β2(-)-CAT.

A double-stranded 18-bp oligonucleotide from the promoter for the human glycoprotein hormone α-subunit was also cloned into the NruI site of tGCaT to produce α18-CAT. This construct contains only one copy (from -146 to -129 bp) of the CRE from the human α-subunit gene (10). The presence of the oligonucleotides and the integrity of all clones were verified by DNA sequencing.

Expression vector plasmids containing the mouse metallothionein 1 promoter (Mt-1) (17) cloned onto the cDNA for the α form of the mouse cAMP-dependent protein kinase catalytic subunit (MtC), or a mouse-human hybrid β-globin gene (MtGlobin) were prepared as described by Dr. G. Stanley McKnight (University of Washington, Seattle, WA), and their construction is detailed elsewhere (18, 19).

Cell Culture and Transfections—JEG-3 cells and C6 glioma cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and glucose (4.5 mg/ml). Transfections were performed using calcium phosphate precipitates (20) containing a total of 12 μg of plasmid DNA/100-mm dish. This amount of plasmid consisted of a 10-μg mixture of reporter plasmids, expression vectors, and nonspecific plasmid DNA as indicated for individual experiments, plus 2 μg of pUClac19 (a Rous sarcoma virus-promoter driven β-galactosidase expression vector) as an internal control for transfection efficiency. Cells were incubated with precipitates for 5 h, washed twice in serum-free medium, and then supplemented with fresh medium. Cyclic AMP levels were stimulated in cells by the addition of 10 mM forskolin and 0.2 mM of the phosphodiesterase inhibitor IBMX 12 h prior to harvest. For co-transfection experiments with MtC and MtGlobin, 100 μM zinc was added to the cultures 12 h prior to harvest to induce expression from the Mt-1 promoter. Since there was no detectable effect of MtGlobin in the presence or absence of zinc on the expression of the CAT reporter plasmids used in these studies the "control" condition in these experiments is defined as the level of CAT activity expressed in the presence of MtGlobin without zinc.

CAT and β-Galactosidase Assays—Cells were harvested 40 h after transfection and extracts were prepared by three cycles of freeze-thawing (40). Oligonucleotides described (21). A digest of cell extracts was assayed in duplicate. For CAT assays, 120–150 μg of cell extract protein from C6 glioma and 80–100 μg of cell extract from JEG-3 were used in a final volume of 125 μl as previously described (16). Acetylated and nonacetylated forms of [3H]chloramphenicol were separated by thin layer chromatography. After autoradiographic visualization, spots were cut from the plate and counted in a liquid scintillation counter. Protein concentrations were determined by the method of Bradford (22).

β-Galactosidase assays were performed as described (23) using ~25 μg of protein from JEG-3 cells, and the CAT activity of each sample was normalized for transfection efficiency by β-galactosidase activity.

In C6 glioma cells the β-galactosidase expression vector pUClac19 was transiently expressed, and for these experiments CAT activity is expressed per μg of extract protein.

Gel Shifts—The oligonucleotides used as probes were annealed and labeled at the 5’-ends using T4 polynucleotide kinase and [γ-32P]ATP (>8000 Ci/mmol). The double-stranded form was purified over a 15% polyacrylamide gel, eluted, precipitated with ethanol, and resuspended in TE buffer. Half of the oligonucleotide preparation was run over a 15% polyacrylamide gel, eluted, precipitated with ethanol, and resuspended in TE buffer. The coupling efficiency was about 60% as estimated by following the radioactive oligonucleotide multimer. The affinity matrix was then stored in TE buffer, 0.02% sodium azide. Directly before use the resin was equilibrated with 10 column volumes of column buffer (CB, 20 mM HEPES-KOH, pH 7.8, 12.5 mM MgCl₂, 1 mM EDTA, 20% (v/v) glycerol, 0.1% (v/v) Nonidet P-40, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) containing 50 mM KCl.

Purification of CREB from JEG-3 Cells—JEG-3 nuclear extract was prepared as described elsewhere (11) and 160 μg were fractionated on heparin-Sepharose. The CREB containing fractions were heated for 10 min to 70 °C and the denatured proteins pelleted by centrifugation (10,000 X g for 10 min at 4 °C). The soluble proteins were diluted with CB to 50 mM KCl final concentration and applied to the affinity matrix at a flow rate of approximately 20 ml/h. The column was washed with 10 volumes of CB, 100 mM KCl and the proteins eluted with CB containing 0.1 μg/ml poly(d(C1-C1))poly(d(C1-C1)) and stepwise increases in KCl concentrations (200 mM, 400 mM, 600 mM, 800 mM KCl, 10 column volumes/step, 1 column volume/fraction). The wash and elution steps were performed at a flow rate of approximately 50 ml/h. The fractions containing CREB were pooled and dialyzed against 100 volumes of CB, 50 mM KCl overnight with two buffer changes. This CREB pool was subjected to a second purification cycle on the affinity matrix as described above. This enriched CREB was stored as described by Singh and Kaups (24).

Data Analysis—Oneand two-way analysis of variance were run to compare treatment groups and constructs, followed by a Newman-Keuls test. A p<0.05 was considered significant.

RESULTS

In previous studies (16) we showed that the 5’-flanking region from the human β2-Ar gene could confer cAMP responsiveness to a reporter gene. Inspection of this promoter fragment (−1335 to +85) revealed the sequence GTACGTCA (−57 to −50 bp; Fig. 1A), which closely resembles the CRE consensus TGACGTCA (25). Constructs containing a single copy (from −146 to −129 bp) of the CRE from the human α-subunit gene (10). The presence of the oligonucleotides and the integrity of all clones were verified by DNA sequencing.

A schematic diagram of the β2-Ar promoter-CAT construct is shown in Fig. 1A. The location and sequence of the putative CRE at −57 to −50 bp relative to the start of transcription (raised arrow in the figure) was a single copy of a 34-bp oligonucleotide encompassing the β2-Ar CRE (large letters) plus flanking sequences (smaller letters) that were inserted upstream of the thymidine kinase (tk) promoter in either the forward (+) or reverse (−) orientation relative to the direction of transcription (raised arrow) to generate the CAT reporter constructs β2(+) -CAT and β2(−)-CAT.
copy of a 34-bp oligonucleotide from the human β2-AR promoter (−70 to −37 bp) were cloned upstream of the thymidine kinase promoter in either the forward (β2(+)-CAT) or reverse (β2(−)-CAT) orientation (Fig. 1B). Following transfection into C6 glioma cells they were analyzed for their ability to respond to elevated levels of intracellular cAMP following the addition of forskolin and IBMX (Fig. 2). An equivalent construct (α18-CAT) containing a single copy of one of the CREs from the promoter of the common α-subunit of the human glycoprotein hormones (10) was included. While there was essentially no stimulation of CAT activity from the parent vector tkCAT by cAMP, a 3-4-fold induction of CAT activity was observed with the 1335-bp β2-AR promoter-CAT construct p1.4CAT, as previously described (16). For the constructs β2(+)-CAT and β2(−)-CAT containing the β2-AR oligonucleotide, CAT activity was stimulated 5-6-fold, and was equal to the activity obtained for α18-CAT. Thus, the sequence from the β2-AR-containing the 8-bp element GTACGTCA is able to confer cAMP inducibility to a heterologous basal promoter, and may be responsible for the cAMP-dependent stimulation of β2-AR transcription.

The cAMP-dependent protein kinase has been shown to regulate the expression of genes containing a CRE (2-4) and to modulate the transcriptional enhancing activity of CREB by phosphorylation (12, 15). We thus decided to evaluate the role of the cAMP-dependent protein kinase pathway in the CAMP-mediated induction of β2(+)−CAT and β2(−)-CAT. This pathway has been studied in detail in the JEG-3 human choriocarcinoma cell line (4, 11, 26, 27), and these cells have served as a source of CREB protein (11, 13). These CAT reporter plasmids were co-transfected into human placental JEG-3 cells with an expression vector (MtC) containing the catalytic subunit of cAMP-dependent protein kinase under the control of the mouse metallothionein-I (Mt-1) promoter. Control transfections included an analogous Mt-1 plasmid directing the expression of an irrelevant gene product, β-globin (MtGlobin).

In the presence of MtGlobin, the parent plasmid tkCAT shows a negligible increase in response to forskolin (Fig. 3A). By contrast, CAT activity for β2(+)−CAT and β2(−)-CAT was significantly elevated, and equivalent to the 4-fold stimulation observed for α18-CAT. Thus, promoters containing a palindromic CRE (α18-CAT) or a similar sequence from the β2-AR are stimulated equally by CAMP in both C6 glioma cells (which express and regulate β2-AR), and in JEG-3 cells (a model cell system for cAMP-regulated genes and the CREB transcription factor).

When the CAT reporter plasmids were co-transfected with the cAMP-dependent protein kinase catalytic subunit MtC, equally dramatic elevations in CAT activity were observed for α18-CAT, β2(+)−CAT, and β2(−)-CAT following the induction of the Mt-1 promoter alone or in combination with forskolin (Fig. 3B). Thus, expression of the cAMP-dependent protein kinase catalytic subunit eliminated the requirement for forskolin. While overexpression of the cAMP-dependent protein kinase catalytic subunit produced modest increases in the parent vector tkCAT and other promoters lacking a CRE (data not shown), these effects were not significant in comparison to α18-CAT, β2(+)−CAT, and β2(−)-CAT. These data indicate that this 34-bp segment of the β2-AR promoter serves a functionally equivalent role to the CRE in α18-CAT, where the stimulation of transcription in response to cAMP proceeds by the cAMP-dependent protein kinase pathway (4). Collectively, these results suggest that the same pathway could be responsible for the cAMP-dependent increase in transcriptional activity of the β2-AR promoter.

To test this possibility directly, mutations were generated in the putative β2-AR CRE sequence GTACGTCA. This mutation, shown in Fig. 4A (β2-AR mut), completely disrupts the highly conserved GTGCA motif found in many CREs (25). This mutant oligonucleotide was cloned into tkCAT in both (+)- and (−)-orientations, and each was evaluated functionally for the ability to activate transcription in response to forskolin (Fig. 4B).
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Fig. 4. Mutation of the GTACGTCA motif of the β2-AR CRE. A, sequences of the wild type and mutant versions of the β2-AR oligonucleotide cloned into tkCAT as shown in Fig. 1. Forward (+) and reverse (−) orientations of each construct are indicated. B, stimulation of CAT activity by forskolin following transfection of the constructs into JEG-3 cells. Transfection mixtures included 2 μg of the CAT reporter plasmids and 2 μg of pUC19 plus 10 μg of MgtGlobin as carrier DNA. The results shown are the average of four to six experiments (*, p < 0.001).

Following transfection into JEG-3 cells, CAT activity from the wild type constructs (β2-AR wild type) was stimulated ~4-fold by forskolin (Fig. 4B). By contrast, mutations within the GTACGTCA motif (β2-AR mutant) completely abolished forskolin stimulation of promoter activity. These results establish that the β2-AR GTACGTCA motif is a functional regulatory element that can confer cAMP responsiveness to a heterologous promoter.

Finally, the role of the CREB transcription factor in cAMP induction of β2-AR gene transcription is indicated in the experiment shown in Fig. 5A. CREB was purified from JEG-3 cells and tested for its ability to bind to the 32P-labeled wild type and mutant β2-AR oligonucleotides in DNA mobility shift assays. Incubation of the wild type β2-AR sequence (β2-AR wild type) with increasing amounts of CREB generates a single complex (arrow) which is absent from control incubations lacking CREB. By contrast, no binding of CREB to the corresponding mutant oligonucleotide (β2-AR mutant) was detected. The relative affinity of CREB for the β2-AR CRE was determined in competition binding studies and compared with the affinity of CREB for the CRE from the α-subunit gene (Fig. 5B). Increasing amounts of unlabeled oligonucleotide for either the β2-AR CRE (β2-AR wild type) or the α-subunit CRE reduced the binding of CREB to the β2-AR wild type sequence to the same extent, indicating that the affinity of CREB for these sequences is essentially identical. By contrast, no reduction in CREB binding to the wild type sequence was observed with equivalent amounts of the β2-AR mutant sequence. Similarly, in the presence of the AP-1 binding site from the human collagenase gene (−76 to −61 bp, colT TRE) (28), CREB binding to the β2-AR wild type CRE was unaffected. Competition with this sequence was performed to insure that the binding activity in the protein preparation is, in fact, CREB and not AP-1, since it is known (29) that AP-1 can bind weakly to CREs. Taken together, the data from Figs. 4 and 5 show that CREB factor binding correlates with the ability of forskolin to stimulate CAT activity from these constructs, and it suggests that transcriptional up-regulation of the β2-AR gene by cAMP occurs through this CREB/CRE interaction.

Fig. 5. Binding of purified CREB to the β2-AR CRE. A, binding of CREB to the wild type and mutated CRE sequences. The 34-bp double-stranded radiolabeled oligonucleotides containing the CRE from the β2-AR promoter (β2-AR wt) or the corresponding mutant (β2-AR mut) were incubated with the indicated amounts of partially purified CREB protein from JEG-3 cells. The resulting complexes were resolved on a nondenaturing polyacrylamide gel and visualized by autoradiography. B, specificity of the β2-AR CRE-CREB complexes. The radiolabeled β2-AR wild type oligonucleotide was incubated with partially purified CREB protein from JEG-3 cells in the presence of increasing amounts of unlabeled competitor oligonucleotides. The competitors were the wild type and mutated CRE from the β2-AR promoter (β2-AR wt and β2-AR mut), the 18-bp CRE from the human chorionic gonadotropin α-subunit gene (αCG CRE), and the region between −76 bp and −61 bp of the human collagenase gene (coll TRE) containing the binding site for the AP-1 protein complex. All four competitors were used at 5, 15, 50, and 150 × molar excess over the probe, as indicated by .

Fig. 6. Conservation of the CRE motif in the promoters of the three mammalian β2-AR genes cloned to date. The sequence GTACGTCA is underlined, while the homology between these three sequences extends beyond this 5-bp region.

DISCUSSION

In these studies we have extended our previous observations (16) that transcription of the β2-AR gene is stimulated by the second messenger cAMP. We previously noted the existence of an 8-bp sequence (GTACGTCA) in the β2-AR promoter with homology to the consensus sequence for the cAMP responsive enhancer, TGACGTCA. Our current results establish the presence of a CRE in the 5'-flanking region of the human β2-AR gene which is recognized by the CREB transcription factor. The sequence from the human β2-AR is able to confer cAMP responsiveness to a heterologous promoter, while mutations that disrupt this motif are inactive. Moreover, the ability of this sequence to promote cAMP stimulation is independent of its orientation, a property now consid-
er a basic feature of transcriptional enhancer elements.

While CREs within cAMP responsive genes appear to contain a variation of the palindromic TGACGTCA element, the CGTCA sequence is the most highly conserved aspect (25). It appears that the CRE need not be a perfect palindrome, and this 5-bp motif is sufficient for cAMP stimulation via CREB. Accordingly, we mutated this region of the β2-AR sequence to assess its role in cAMP stimulation (β2-AR mut in Fig. 4A). In the case of the genes for vasoactive intestinal peptide (30) and proenkephalin (31), experiments have suggested that two closely spaced motifs which both contain CGTCA comprise the CRE. Since this 5-bp sequence can be found downstream from the GTACGTCA in the β2-AR promoter at 41 bp in reverse orientation, we also deleted these 5 bases from the β2-AR sequence to generate a double mutant (not shown). The results from CAT assays indicate that cAMP stimulation of the β2-AR CRE is already completely abolished by the first mutation (β2-AR mutant) in the GTACGTCA region alone, with no further reduction observed for the double mutant (data not shown). Therefore, the β2-AR sequence, in agreement with the majority of CREs, consists of a single element containing the conserved CGTCA sequence.

In comparing the potency of the β2-AR CRE to the palindromic human chorionic gonadotropin α-subunit CRE for mediating cAMP responsiveness, we found that these two CREs are equivalent by both a functional test (CAT activity) and by physical interaction of the sequences with purified CREB. In both C6 glia cells and JEG-3 cells the activities of p14CAT, β2(+)-CAT, and β2(-)-CAT are essentially identical. It is interesting to note that when the 1355-bp β2-AR promoter construct p14CAT is expressed in C6 glia cells, both the stimulation by forskolin and the overall basal expression of this construct are lower than the expression of β2(+)-CAT and β2(-)-CAT. Possibly, this observation reflects the low level of expression of the β2-AR gene in C6 glia cells (32). We have also found little to no expression of p14CAT when transfected into JEG-3 cells, a cell line in which there is no detectable expression of the β2-AR. Thus, the possibility exists that elements required for tissue-specific expression reside further upstream in the promoter of the β2-AR gene.

Expression of the catalytic subunit of cAMP-dependent protein kinase was able to substitute completely for forskolin in stimulating CAT activity from the β2(+)-CAT and β2(-)-CAT reporter constructs. This finding is consistent with previous studies demonstrating that the cAMP-dependent protein kinase catalytic subunit can stimulate CAT activity from promoters that contain a CRE (2-4). Such studies, in conjunction with biochemical evidence for cAMP-dependent protein kinase-mediated phosphorylation of CREB (12, 15), support a model in which post-transcriptional activation and CRE-binding of CREB leads to increased transcription of cAMP-regulated genes. It has been further demonstrated for some genes (11, 33, 34) that, even in the absence of elevated levels of cAMP, the CRE serves an important role in maintaining basal transcription rates.

The ability of cAMP to stimulate transcription of the β2-AR gene can serve important regulatory roles. As the receptor population is dynamic and turning over, new synthesis must be maintained to replace receptors which have been degraded. The CRE/CREB interaction defined in these studies could function in the maintenance of basal promoter activity, the degree of which would be dependent upon the ambient cellular levels of cAMP and cAMP-dependent protein kinase. On the other hand, as we have previously shown (16), acute elevations of cAMP following activation of a stimulatory receptor such as the β2-AR provoke a significant rise in the rate of β2-AR gene transcription. The receptor itself is a substrate for cAMP-dependent protein kinase phosphorylation, and this phosphorylation appears to play a role in the process of receptor desensitization, and possibly in the internalization and degradation of the receptor (35, 36). This rapid and transient increase in β2-AR expression may provide the cell with a compensatory mechanism for maintaining receptor number and hormonal responsiveness. The generality of this mode of regulation is suggested by the presence of comparable CRE sequences in the 5′-flanking regions of the three mammalian β2-AR genes cloned to date (Fig. 6; Rets. 37-39). This fact, together with emerging evidence that other members of the G-protein-coupled receptor family may be similarly modulated by cAMP (40), suggests a common autoregulatory transcriptional control mechanism that involves the same second messenger cascades that these receptors activate.

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REFERENCES

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