Agonist Regulation of Human $\beta_2$-Adrenergic Receptor mRNA Stability Occurs via a Specific AU-rich Element*

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Prolonged agonist stimulation of $\beta_2$-adrenergic receptors results in receptor down-regulation, which is closely associated with a reduction of the corresponding mRNA, an effect mediated in part by changes in mRNA stability. Transfection experiments with human $\beta_2$-adrenergic receptor cDNAs bearing or lacking the untranslated regions suggested that the essential agonist sensitivity of the mRNA resides within the 3' untranslated region. The importance of this region was further confirmed in gel shift experiments; cytosolic preparations from agonist-stimulated DDT1-MF2 smooth muscle cells caused a shift of $\beta_2$-adrenergic receptor mRNAs containing the 3' untranslated region. Progressive 3'-terminal truncations of the receptor cDNA led to the identification of an AU-rich element at positions 329–337 of the 3'-untranslated region as the responsible cis-acting element. Substitution of this motif by cytosine residues almost completely abolished mRNA down-regulation and inhibited the formation of the RNA-protein complex. Even though the $\beta_2$-adrenergic receptor AU-rich element showed two U → A transitions compared with the recently proposed AU-rich element consensus sequence, it revealed an almost identical destabilizing potency. Fusion of the $\beta_2$-adrenergic receptor 3'-untranslated region to the $\beta_2$-globin coding sequence dramatically reduced the half-life of the chimeric transcript in an agonist- and cAMP-dependent manner. This suggests that the agonist-induced $\beta_2$-adrenergic receptor mRNA destabilization is regulated by cAMP-dependent RNA-binding protein(s) via a specific AU-rich element.

Chronic stimulation of the $\beta_2$-adrenergic receptor ($\beta_2$AR)$^1$ results in a decrease of receptor responsiveness, a process called agonist-induced receptor desensitization (1, 2). Long term desensitization often involves a significant reduction of receptor numbers, which is termed receptor down-regulation. Several distinct molecular mechanisms affecting both mRNA and protein levels contribute to receptor down-regulation (2–4), which appear to be operative to varying extents in different cell lines. To date there is evidence that the expression of the $\beta_2$AR gene can be regulated at the level of transcription (5, 6), posttranscriptionally at the level of mRNA stability (7) or at the level of translation via a short peptide encoded within the 5' untranslated region (UTR) of the $\beta_2$AR gene (8).

Post-transcriptional mechanisms are of particular interest, since they participate in the stability and turnover of various highly labile mRNAs, such as granulocyte-macrophage colony-stimulating factor, interleukin-3, and the oncogenes c-fos and c-myc (9, 10). AU-rich elements (AREs) are often found in the 3' UTRs of these mRNAs and appear to be key determinants of their short half-lives, even if mRNA turnover does not strictly depend on these motifs. The optimal destabilization motif was recently suggested to be UUAUUUA(U/A)(U/A) (11, 12), but there is also evidence that an AUUUA pentamer need not be an integral part of a functional ARE (13). On the contrary, it appears that each ARE represents a combination of structurally distinct domains, such as AUUUA motifs, AU nonamers, and U-rich elements, and that it is the combination of these sequence elements that determines its ultimate destabilizing function (14). AREs appear to represent recognition sites for several cytoplasmic and nucleus-associated RNA-binding proteins, which mediate RNA degradation (15–19). Some of these proteins have been purified, but their precise roles in the regulation of mRNA stability remain unclear.

For the $\beta_2$AR mRNA, three binding proteins have been described so far: (i) the $\beta_2$-adrenergic receptor mRNA-binding protein ($\beta$ARB), a 35,000 cytosolic protein identified in hamster DDT1-MF2 smooth muscle cells (20); (ii) a 85,000 protein mediating $\beta$AR transcript destabilization in adult rat hepatocytes (21); and (iii) the M, 37,000 AU-rich element RNA-binding/degradation factor (AUF1), which has been shown to bind also $\beta$AR mRNA (22). Although AU-rich sequence motifs within the $\beta_2$AR 3'-UTR have been demonstrated to function as recognition sequences for these proteins in vitro (21–24), the exact nature of the particular cis-acting elements mediating $\beta$AR mRNA destabilization in vivo has not been established. During the preparation of this manuscript, Tholainkunnell and Malbon (25) reported the first characterization of such an element, a 20-nucleotide AU-rich domain with an unusual AUUUA hexamer core, which is obligatory for the destabilization of the hamster $\beta_2$AR mRNA. However, a sequence alignment revealed no equivalent within the human $\beta_2$AR transcript (26, 27), which in turn suggests that $\beta_2$AR mRNA stability is regulated via species-specific cis-acting elements.

In this study, we report the identification of a nonconsensus AU-rich nonamer within the $\beta_2$AR 3'-UTR as a critical determinant for the agonist-induced destabilization of the human receptor transcript and provide evidence that the participation of a RNA-binding protein and of cAMP are required for $\beta_2$AR mRNA down-regulation in vivo.

MATERIALS AND METHODS

Plasmid Construction—The $\beta_2$AR vectors used for transient transfections were constructed based on the plasmid pBC12BI-$\beta_2$ (26), from which a 1.95-kb fragment corresponding to the complete human $\beta_2$AR transcript was excised and inserted into the expression vector pcDNA3.

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The abbreviations used are: $\beta_2$AR, $\beta_2$-adrenergic receptor; UTR, untranslated region; ARE, AU-rich element; $\beta$ARB, $\beta_2$-adrenergic receptor mRNA-binding protein; AUF1, AU-rich element RNA-binding/degradation factor; HEK293 cells, human embryonic kidney cells; DIG, digoxigenin; PCR, polymerase chain reaction; bp, base pair(s).
alkaline capillary transfer (30). Single-stranded DNA probes were prepared in two steps. First, the respective region was amplified in a “standard” PCR. The resulting double-stranded DNA fragment served as a template in a second, asymmetric PCR that included only the 5′-primer. 20 μM DIG-11-dUTP (Boehringer Mannheim) was added for labeling along with 30 μM dNTPs. The primers used for the preparation of the β2AR probe were β2AR.seq.2 and β2AR.rev.4, spanning a 672-bp fragment immediately downstream of the start codon. Probes specific for α2-crystallin (468 bp, used as an internal standard) and β-globin (319 bp) were amplified using the primers cry.seq/cry.rev and hbg.seq/hbg.rev, respectively. Hybridization was done at 37 °C for 24–48 h in 50% formamide, 5 × SSC, 3 × Denhardt’s solution, 0.5% SDS, 0.2% sodium laurylsarcosinate, and 5% dextran sulfate. Chemiluminescent detection was performed using the DIG Luminescent Detection kit (Boehringer Mannheim). The signal intensity on the x-ray films was analyzed densitometrically.

In Vitro Transcription—Transcripts were generated from 1 μg of linearized template DNA in a total reaction volume of 20 μl in the presence of 1 unit/μl RNase inhibitor, 1 mM ATP/CTP/GTP, 0.65 mM UTP, 0.35 mM DIG-UTP, and 2 units/μl T7-RNA-polymerase (all reagents purchased from Boehringer Mannheim). Cotranscriptional capping was performed by using the cap analogue m(5)Gppp(5)G (New England Biolabs) in a concentration 10 times that of GTP. The cotranscriptional capping mixtures were incubated at 37 °C for 2 h. RNase-free DNase I (Boehringer Mannheim) was added to remove template DNA. The labeled transcripts were extracted twice with phenol and then once with chloroform and precipitated with ethanol.

Gel Shift Assay—After a 12-h treatment with either (-)-isoproterenol (10 μM) or vehicle, DDT-2MF smooth muscle cells were washed twice with ice-cold phosphate-buffered saline and scraped into 20 mM Heps, pH 7.5, 30 mM KCl, 1 mM dithiothreitol, 2.5 mM EDTA, 2.5 mM EGTA, 20 μg/ml benzamidine, 20 μM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.1% Nonidet P-40. Samples were sonicated for 20 s, centrifuged at 50,000 × g for 20 min, and aliquots containing 10 μg of protein were electrophoresed at 20–30 mA for 3 h on nondenaturing 4% polyacrylamide gels and blotted onto nylon membranes as described above. Signals were visualized using the DIG Luminescent Detection kit (Boehringer Mannheim).

RESULTS

Mapping of the Agonist-sensitive Region within the β2AR mRNA—Since in many cell types the β2AR mRNA down-regulation appears to be mediated by several different processes (2–4), we decided to use a transient transfection system to identify the agonist-sensitive region(s) within the β2AR transcript. For this purpose, HEK293 cells were chosen due to their very low level of endogenous βAR as well as the very high transcription efficiency compared with other cell lines. 48 h after the transfection of vectors bearing human β2AR cDNAs corresponding to the complete receptor transcript and to mutants lacking either one or both UTRs, respectively, the cells were stimulated for 12 h with 10 μM isoproterenol and the β2AR mRNA levels were quantified in Northern analyses (Fig. 1A). α2Crystallin, a widely expressed heat-shock protein, was used as an internal standard in all experiments. Cotransfection of a β-galactosidase encoding plasmid and subsequent staining of the cells revealed transcription efficiencies of about 90% in all samples (not shown), so that influences resulting from different transfection efficiencies of various constructs should be minimal. As shown in Fig. 1B, the β2AR wild-type transcript and the 3′-UTR deletion mutants were down-regulated upon agonist stimulation by 62 ± 8% and 68 ± 8%, respectively, compared with unstimulated controls. The presence of elements encoded within the 3′-UTR for transcript destabilization was further confirmed by the respective deletion mutant which showed only a small reduction of the β2AR mRNA level by 12 ± 8%. The mRNA concentration of the transcript covering only the coding sequence remained almost unchanged. These results suggested that the agonist sensitivity of the human β2AR

### Table I

List of the primers used in this study

| Primer      | Sequence                        | Position |
|------------|---------------------------------|----------|
| β2AR coding|                                 |          |
| β2AR seq.2 | 5′-GGGCAACCGCCGACGCCGAC-3′      | 4–23     |
| β2AR rev.4 | 5′-GGCCCTCCGAAAGCTCCGG-3′       | 678–659  |
| β2AR 3′-UTR|                                 |          |
| β2AR seq.4 | 5′-AGTATAGGCGGCCCCCCGCTCCG-3′  | 319–347  |
| β2AR seq.41| 5′-AGTATAGGGTTATTGCCTCG-3′     | 318–347  |
| β2AR seq.42| 5′-AGTATAGGCGGCTTCATCTCG-3′    | 318–347  |
| β2AR seq.43| 5′-AGTATAGGCGGATTCCATCTCG-3′   | 318–347  |
| βfus.seq.2 | 5′-CCACACGCGCCGAGCAGTTTCCTGAG-3′| 1–14     |
| βfus.rev.2 | 5′-CCATCTCAGTACTGCTACATTTT-3′  | 554–540  |
| α2-crystallin|                                 |          |
| cry.seq    | 5′-CCCTCCTTTCTTTTCATCCCTCC-3′  | 62–84    |
| cry.rev    | 5′-CCGACACGCTTCTCCATCGGCT-3′   | 500–359  |
| β-globin   |                                 |          |
| hbg.seq    | 5′-CACATGCTGACCTGACCTC-3′       | 4–17     |
| hbg.rev    | 5′-CGCTAAGTTCTCTACGAC-3′        | 315–295  |

(Invitro) Deletion constructs lacking either one or both untranslated regions were obtained by PCR amplification of the respective cDNA fragments from pBC12BI-β2AR 3′-UTR cDNAs to obtain polyadenylated mRNAs.

The mutations of the ARE at positions 329–337 of the β2AR 3′-UTR were introduced by PCR. 247-bp fragments covering the 3′-halves of the 3′-UTR were amplified using the mutagenesis primers β2AR seq.4, β2AR seq.41, β2AR seq.42, and β2AR seq.43, respectively, and βfus.rev (for all primers see Table I). These fragments were subsequently used as “reverse primers” in a second PCR together with βfus.seq.2. The resulting 571-bp mutated 3′-UTRs were fused to the β2AR coding sequence in the vector pcDNA3-β2AR 3′-UTR. Additionally, pGEM vectors bearing the mutated AREs were constructed analogous to β2AR 3′-UTR as templates for in vitro transcription.

For the generation of the β2AR deletion mutants, the fusion plasmid a 2.2-kilobase-pair fragment corresponding to the β-globin primary transcript was excised from pEGEM12-β-globin and inserted into pcDNA3. After that, a 540-bp Dral–XhoI fragment comprising the β-globin 3′-UTR was replaced by the 564-bp β2AR 3′-UTR excised from pcDNA3-β2AR 3′-UTR. The correctness of all constructs was confirmed by double-stranded DNA sequencing.

Cell Culture and Transfection—Hamster DDT-2MF smooth muscle cells and human embryonic kidney cells (HEK 293) were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (all purchased from Life Technologies, Inc.). Monolayer cultures were harvested at 60–70% confluence, and DDT-2MF suspension cultures were maintained at a cell density of about 5 × 10⁶ cells/ml. The calcium phosphate precipitation method (28) was used for transfection of HEK293 cells. Transfection efficiencies were determined by cotransfection of a β-galactosidase encoding plasmid, pSVβgal.

48 h after transfection the cells were stimulated with 10 μM (-)-isoproterenol (Sigma) or 10 μM forskolin (Sigma) for the time indicated. To block transcription and/or translation, 5 μM/ml actinomycin D (Roth) and/or 0.5 μg/ml Pseudomonas exotoxin A (Sigma), respectively, were added 30 min after the beginning of agonist stimulation. At the time indicated, the cells were harvested, washed twice with phosphate-buffered saline, and subjected to RNA analysis.

RNA Isolation and Northern Analysis—Total RNA was isolated by the AGPC extraction method (29), separated on formaldehyde gels, and subsequently transferred to nylon membranes (Qiagen) by downward
mRNA resides essentially within the 3' UTR.

Interaction of the Human \( \beta_2 \)-AR mRNA with a Binding Protein Identified in Hamster DDT1-MF2 Smooth Muscle Cells—In a recent study (31), we have demonstrated that the agonist-induced \( \beta_2 \)-AR mRNA down-regulation occurs in a cell type-specific manner. In DDT1-MF2 smooth muscle cells, it is caused predominantly at the posttranscriptional level via decreased mRNA stability. It has been shown for several highly labile mRNAs that destabilization motifs may function as recognition sequences for RNA-binding proteins (15–19). Therefore, we attempted to prove the existence of such a factor in DDT1-MF2 cells and to analyze a possible interaction with the human \( \beta_2 \)-AR transcript. DDT1-MF2 cells were grown in suspension cultures, and transcription was blocked by adding actinomycin D to the medium. After various incubation periods, the \( \beta_2 \)-AR mRNA was determined to be about 120 min, whereas in agonist-

The \( \beta_2 \)-AR mRNA, respectively, were incubated with cytosolic extracts prepared either from DDT1-MF2 control cells or cells stimulated with isoproterenol for 12 h. The samples were separated on nondenaturing polyacrylamide gels and transferred onto nylon membranes. After chemiluminescent detection, a protein-mRNA complex was only found if cytosolic fractions of stimulated cells were mixed with the 3'-half of the \( \beta_2 \)-AR mRNA (Fig. 3). The addition of exotoxin A to the cells to block de novo protein synthesis inhibited the formation of this complex. These observations are a further indication that the 3'-UTR contains elements critical for the stability of the human \( \beta_2 \)-AR mRNA. Furthermore, they provide evidence that binding protein(s) induced in hamster DDT1-MF2 cells can bind to the human transcript and are apparently involved in this regulation.

Identification of an AU-rich Destabilization Motif within the \( \beta_2 \)-AR 3'-UTR—For a more detailed characterization of the human \( \beta_2 \)-AR 3'-UTR, two truncation mutants lacking the 3'-terminal 157 bp and 313 bp of the receptor cDNA, respectively, were generated, and their degree of agonist-induced \( \beta_2 \)-AR mRNA down-regulation after transient transfection into HEK293 cells was determined (Fig. 4A). The mRNA level changes of the mutant lacking 157 bp were comparable with those of the wild-type receptor, whereas deletion of 313 bp completely abolished agonist-mediated down-regulation. The respective levels of \( \alpha_c \)-crystallin mRNA remained unchanged in all three cases. These results provide evidence that the region between positions 241 and 397 of the \( \beta_2 \)-AR 3' UTR is critical for receptor mRNA destabilization (Fig. 4B).

AU-rich elements have been shown to be key determinants for the destabilization of several highly regulated mRNAs (9, 10). Their consensus sequence has recently been proposed to be U/AUUAUA(U/A)(U/A) (11, 12). Therefore, we looked for elements consisting of at least nine consecutive adenosine or uridine residues within the \( \beta_2 \)-AR 3'-UTR. None of the four regions identified (Table II and Fig. 4B) exactly fits the proposed consensus sequence. Because of its location in the region shown to be critical for \( \beta_2 \)-AR mRNA destabilization, the motif UAUUAUAUU found at positions 329–337 was of special interest. The sequence differed from the consensus only at positions 2 and 5. To test the importance of this element for \( \beta_2 \)-AR mRNA stability, the wild-type sequence (Fig. 5, WT) was re-
placed by a stretch of nine cytosine residues (M), and the two constructs were transiently transfected into HEK293 cells as before. Upon agonist stimulation, the mutant β2AR mRNA levels, normalized for the respective values for CRY, were reduced to only 90 ± 8% of control levels compared with 35 ± 5% for the wild type (Fig. 5). Therefore, this element appears to be absolutely essential for the destabilization of the human β2AR transcript. Three additional ARE point mutants (M1–3, Table III) were generated to provide further insights in the minimal sequence requirements of this motif. In mutant M1, positions 2 and 5 were changed (A → U) so that the resulting ARE corresponded to the suggested consensus sequence (11, 12). The respective transcript shown an almost identical degree of down-regulation in HEK293 cells compared with the wild-type sequence, with a reduction to 32 ± 8% of the unstimulated control (Fig. 5). The flanking adenosine residues of the consensus sequence have been shown to be critical for the destabilizing potency of an ARE (11, 12). In mutant M3, the three central nucleotides were replaced by cytosines to investigate the function of the ARE core domain. A small but significant decrease in the respective β2AR mRNA levels to 75 ± 7% was observed (Fig. 5), demonstrating that at least for the β2AR ARE the core is not as essential as proposed for the consensus sequence (11, 12).

Additionally, we performed gel shift experiments to answer the question whether the protein(s) identified in cytosolic ex-
isoproterenol or forskolin further suggests that of transcript destabilization is almost the same using either sufficient to regulate mRNA stability in an agonist-dependent gene. To consider a possible participation of sequence motifs mechanism.

### Table I

| Number | Sequence     |
|--------|--------------|
| WT     | UAAUAUAAU    |
| M1     | UAAUUAAU     |
| M2     | CCCCCCUC     |
| M3     | UCCUACCUU    |

The wild-type sequence (WT) is included for comparison. The 13 h under all conditions, i.e. encoded within the 3'-UTR of the protein recognizing a nonconsensus AU-rich motif at positions 329–337 appears to be mediated, at least in part, by an RNA-binding mechanism.

### Stability of a Chimeric β-globin/β2AR 3'-UTR Transcript—To test the hypothesis of a β2AR-specific regulation of mRNA stability, we asked whether the elements within the β2AR 3'-UTR are sufficient to destabilize a normally stable gene. To consider a possible participation of sequence motifs beside the ARE, the complete human β2AR 3'-UTR was fused to the coding sequence of the human β-globin gene, and the half-life of the resulting chimeric transcript was measured in comparison with wild-type β-globin. To analyze whether the activation of the β2AR has an influence on the function of the cis-acting elements within the transcript, a vector harboring the β2AR cDNA was cotransfected together with the two β-globin constructs in HEK293 cells. 48 h after transfection, the cells were stimulated either with 10 μM isoproterenol or with 10 μM forskolin to directly activate the adenyl cyclase. The stability of the β-globin wild-type transcript remained unaffected by agonist stimulation. The β-globin mRNA half-lives were about 13 h under all conditions, i.e. in control cells as well as in cells stimulated with either isoproterenol or forskolin (Fig. 6 and Table IV). The exchange of the endogenous β-globin 3'-UTR against the respective region from the β2AR dramatically reduced the stability of the chimeric mRNA. In addition, its stability became β2AR agonist-dependent. Upon stimulation of the β2AR with isoproterenol, the half-life of the chimeric mRNA decreased from about 4 to 2.5 h. The same regulatory pattern was observed with forskolin, which reduced the half-life of the chimeric transcript from 4.8 to 3.3 h. Therefore, the elements encoded within the 3'-UTR of the human β2AR mRNA are sufficient to regulate mRNA stability in an agonist-dependent manner in a heterologous system. The finding that the degree of transcript destabilization is almost the same using either isoproterenol or forskolin further suggests that β2AR mRNA stability is essentially regulated by cAMP.

### DISCUSSION

The β2AR is a prototypical member of the large family of G-protein-coupled receptors and is subject to a complex regulatation by hormones and other signaling molecules (2–4). Distinct molecular mechanisms on both mRNA and protein levels, which may be operative to varying extents in different cell lines
and tissues, contribute to this regulation. Response elements specific for cAMP (CRE), glucocorticoids (GRE), and thyroid hormones (TRE) regulating β2AR gene transcription have been identified in the β2AR promoter region and the coding sequence (5, 6, 32–34). Here, we report the identification and functional characterization of a cis-acting element within the 3′-UTR of the human β2AR mRNA that is sufficient to cause destabilization of the mRNA.

Many highly labile mRNAs possess AREs within their 3′-UTRs, although rapid mRNA turnover does not strictly depend on these motifs (9, 10). In cytokine mRNAs, for example, Brown et al. (35) recently identified another class of destabilizing elements, which require at least one stem-loop (hairpin) in the secondary structure. Nevertheless, AREs are considered to be the predominant destabilization determinants.

Analyses using synthetic AU-rich sequences revealed that a nonamer with an AUUUA pentanucleotide core, UUAUUUAUU, at positions 329–337 is the critical element for ARE function (13). In recent years, evidence has accumulated that it is the combination of structurally and functionally distinct AU-rich domains that determines the ultimate destabilizing function of an ARE (14). Several AU-rich sequences have also been identified in the 3′-UTRs of G-protein-coupled receptors (22, 24), and binding of the three β2AR mRNA-specific proteins identified so far, βARB, P85, and AUF1, is selectively competed by poly(U) RNA (20–22). Furthermore, in vitro binding of βARB to the hamster β2AR mRNA requires both an AUUUUA pentamer and U-rich flanking domains (23). However, neither the hamster nor the human β2AR mRNAs (26, 27) possess any AU-rich consensus motifs within their 3′-UTR. Therefore, it is tempting to speculate that agonist-induced β2AR mRNA destabilization occurs via unique cis-acting elements.

Measurements of the human β2AR mRNA steady-state levels using mutants lacking either one or both UTRs predicted a predominant regulatory role for the 3′-UTR. In contrast, deletion of the 5′-UTR revealed a reduction of β2AR mRNA levels similar to that of the wild-type receptor. Although the human β2AR 3′-UTR is sufficient to significantly destabilize the β2AR mRNA when fused to the β2AR coding region, we cannot exclude the existence of additional determinants within the 5′-UTR that might contribute to mRNA stability. The importance of the β2AR 3′-UTR was further confirmed by the observation that a protein, whose synthesis was induced in DDT1-MF2 smooth muscle cells by the β2AR stimulation, selectively bound to the 3′-half of the receptor transcript. This supports data from studies (15–19) in which AREs also functioned as recognition motifs for RNA-binding proteins.

A more detailed characterization of the β2AR 3′-UTR identified an AU-rich nonamer, UUAUUUAUUA, at positions 329–337 as the critical element for β2AR mRNA regulation. Its substitution by a stretch of nine cytosine residues almost completely abolished mRNA down-regulation and inhibited the interaction with the β2AR mRNA-binding protein induced in DDT1-MF2 cells. Therefore, one may conclude that this motif represents a potent destabilization determinant. This motif differs from the consensus sequences at positions 2 and 5, which have both been shown to be important for the destabilizing potency of an ARE (11, 12). However, mutational analyses of this specific ARE revealed a potency identical to the consensus element. This shows that a functional ARE does not have to contain an AUUUA pentamer (13). In accordance with previous reports (11, 12), the flanking adenosine residues appear to constitute the most critical nucleotides for ARE function, since their substitution by cytosine residues was sufficient to abolish β2AR transcript destabilization. Surprisingly, the replacement of the three central nucleotides still allowed mRNA down-regulation by about 25%. Furthermore, a weaker interaction with the RNA-binding protein induced in DDT1-MF2 cells could be detected. A possible explanation for this unexpected result is that this mutant comprises a minimal sequence capable of functioning as an ARE, at least in the case of the human β2AR mRNA, in which an intact core domain is not required. Since the region, in which the ARE is embedded, also does not resemble the U-rich sequences found in other highly labile mRNAs (9, 10), the human β2AR mRNA stability appears to be regulated via a potent but nonconsensus ARE. This parallels a recent study (25), in which a 20-nucleotide AU-rich domain with an unusual AUUUAU hexamer core was identified as an obligate element for destabilization of the hamster β2AR mRNA. Therefore, β2AR mRNA stability seems to be regulated via species-specific cis-acting elements. Another possibility is that the deviations from the consensus can be compensated by other β2AR-specific elements, such as the additional AU-rich domains within the 3′-UTR or secondary structure elements.

On the other hand, the binding protein(s) observed in DDT1-MF2 cells upon β2AR stimulation does not discriminate between these sequence motifs. Therefore, one may assume that a rather general factor is responsible for β2AR transcript destabilization. Two β2AR mRNA-binding proteins, βARB and AUF1, have been detected in this cell line so far; the latter one was also identified in the human myocardium (20, 22). The binding affinity of AUF1 has recently been shown to correlate directly with the destabilizing potency of the respective ARE in vitro (36). The biochemical and functional relatedness of the two proteins initially led to the assumption that they might be identical, but immunological experiments recently suggested that they were distinct (22).

The analysis of the β2-globin/β2AR 3′ chimeric transcript confirmed that the regulation of the β2AR mRNA stability occurs in an agonist-dependent manner and requires the presence of an RNA-binding protein. Although the stability of the chimeric mRNA was only about one-third of the β2-globin wild-type transcript, stimulation with either isoproterenol or forskolin caused a further decrease of the mRNA half-life by almost a factor of 2. This suggests that the coordinated interplay between β2AR activation and the induction of specific binding protein(s) is required for efficient destabilization of the receptor transcript. The almost identical results with isoproterenol and forskolin show a predominant role for cAMP as a regulator of β2AR mRNA stability. The biochemical mechanisms mediating this cAMP-dependent regulation remain to be elucidated.

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