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Regulation of β_2-Adrenergic Receptor mRNA and Gene Transcription in Rat C_6 Glioma Cells: Effects of Agonist, Forskolin, and Protein Synthesis Inhibition

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SUMMARY

Incubation of rat C_6 glioma cells with β-adrenergic receptor (βAR) agonist or with agents that increase cAMP levels results in down-regulation of the β_2AR, as measured by the loss of radioligand binding sites. In the present study, the role of β_2AR mRNA expression and stability in the down-regulation of β_2AR sites in C_6 cells was examined. Isoproterenol or forskolin treatment decreased β_2AR mRNA levels in a time-dependent manner, with maximal loss of ~50% being observed after 2 hr. Pretreatment of the cells with a potent protein synthesis inhibitor, *Pseudomonas* exotoxin A, completely blocked isoproterenol- and forskolin-mediated down-regulation of β_2AR mRNA. Exposure to agonist did not significantly influence the half-life of β_2AR mRNA, which was ~60 min. In contrast, isoproterenol treatment for 2 hr significantly decreased the rate of β_2AR gene transcription, as determined by nuclear run-on analysis. Based on these results, we propose that agonist regulation of β_2AR mRNA in C_6 cells is mediated by activation of the cAMP system and occurs at the level of β_2AR gene transcription, not mRNA stability. In addition, the observed requirement for protein synthesis indicates that down-regulation of β_2AR mRNA may be mediated by expression of a repressor of β_2AR gene transcription.

It is well established that down-regulation of the β_2AR occurs in response to agonists or other agents that elevate cAMP levels (1, 2). Loss of receptors involves multiple mechanisms, including down-regulation of receptor mRNA (3, 4). In both hamster smooth muscle DDT_1MF-2 and mouse lymphoma S49 cells, β_2AR mRNA levels decrease by 40% over a 24-hr period in response to isoproterenol or forskolin (5, 6). In DDT_1MF-2 cells, this effect is accompanied by a decrease in β_2AR mRNA half-life but not gene transcription rate (7). In contrast, Collins *et al.* (8) reported that, in DDT_1MF-2 cells exposed to agonist or agents that elevate cAMP, there is first an increase in levels of β_2AR mRNA and gene transcription and then a decrease of receptor mRNA levels; no change in β_2AR mRNA half-life was reported in that study.

A similar biphasic change in β_1AR mRNA is observed when rat C_6 glioma cells are exposed to agonist or forskolin (9–11). We recently demonstrated that the initial rapid up-regulation of β_1AR mRNA is accompanied by an increased rate of gene transcription, whereas the subsequent slower down-regulation of receptor mRNA is correlated with a decreased rate of transcription, with no change in β_1AR mRNA stability (11). C_6 cells also express endogenous β_2AR (11, 12), and β_2AR mRNA levels are reported to be down-regulated by agonist treatment (9), although the roles of the cAMP system, mRNA half-life, and gene transcription rate have not been examined.

Because β_2AR mRNA expression may be under the control of different regulatory mechanisms in different cell types and tissues, we examined the mechanisms that mediate the regulation of β_2AR mRNA in C_6 glioma cells. We found that agonist or forskolin treatment decreased steady state levels of β_2AR mRNA and that this effect was accompanied by a decrease in β_2AR gene transcription rate but not mRNA stability. Moreover, inhibition of protein synthesis blocked the down-regulation of β_2AR mRNA, suggesting that the decrease in the transcription rate is mediated by induction of an inducible repressor. These results indicate that the mechanisms that underlie agonist regulation of β_2AR mRNA differ

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ABBREVIATIONS: βAR, β-adrenergic receptor(s); CRE, cAMP response element; ICER, inducible cAMP early repressor(s); bp, base pair(s); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

depending on the cell line under examination, and they suggest that different mechanisms may control the regulation of β_2AR mRNA expression in different tissues *in vivo*.

Experimental Procedures

Materials. [α-^{32}P]UTP (3000 Ci/mmol) and [α-^{32}P]CTP (800 Ci/mmol) were obtained from DuPont-New England Nuclear. Forskolin and actinomycin D were purchased from Calbiochem, *Pseudomonas* exotoxin A from List Biological Laboratories (Campbell, CA), and (−)-isoproterenol and (−)-alprenolol from Sigma. CGP 20712A and ICI 118,551 were generous gifts from Ciba-Geigy (Summit, NJ) and ICI (Macclesfield, UK), respectively. The rat β_2AR cDNA (13) was generously provided by Dr. J. C. Venter (Institute of Genomic Research, Gaithersburg, MD).

Cell culture. The culture of rat C_6 glioma cells was carried out as described previously (11). Cells were plated at 5–7 × 10^6 cells/175-cm^2 flask, in 50 ml of medium. After 4 days of culture the medium was changed to serum-free medium, and on the following day the cells were exposed to isoproterenol (1 μM) or forskolin (10 μM) for the times indicated. Where indicated, cells were also pretreated with exotoxin A (0.3 μg/ml) for at least 4 hr before addition of the stimulators. Cells (4–5 × 10^7/flask) were collected by removal of the medium and addition of serum-free medium buffered with 25 mM HEPES and containing 2 mM EGTA and 2 mM EDTA. The detached cells were centrifuged at 200 × *g* for 5 min, and the cell pellet was taken up in an ice-cold solution containing 4 M guanidine thiocyanate, 25 mM sodium acetate buffer, pH 6.2, and 0.5% 2-mercaptoethanol. The resulting suspension was frozen at −80° for subsequent RNA isolation.

RNA extraction. After homogenization of the cells in the buffered guanidine thiocyanate solution, total RNA was isolated by centrifugation at 150,000 × *g* at 20° for 21 hr, through a 5.7 M cesium chloride gradient (14). RNA was then resuspended in 0.3 M sodium acetate, pH 5.2, and precipitated with ethanol, and the concentration was determined by spectrophotometry at 260 nm.

Riboprobe and cRNA preparation. A 207-bp fragment of the rat β_2AR cDNA (positions +1099–1305) was amplified by polymerase chain reaction using the forward and reverse primers GGATTGCCTTCCAGGAGCTTCTG and GGCTAGGCACAGTACCTTGACAG, respectively, and was cloned into pBluescript II SK⁻ (Strategene). The cDNA was linearized by *Eco*RI digestion 5′ to the insert, and uniformly radiolabeled riboprobes corresponding to the antisense DNA strand were synthesized with T3 RNA polymerase, as described previously (11, 15). The specific activity of a typical riboprobe was ~1 × 10^9 dpm/μg. Unlabeled sense strand cRNA was also prepared from the same plasmid and was used as a hybridization standard. The plasmid was linearized 3′ to the DNA insert, and cRNA complementary to the riboprobe was synthesized using T7 RNA polymerase (16). Unlabeled sense strand was then purified, quantified by spectrophotometric analysis at 260 nm, and frozen in aliquots at −70°.

RNase protection assay. RNase protection analysis was carried out as described previously (11, 15). Briefly, 30-μg aliquots of total RNA were hybridized with ^{32}P-labeled riboprobe (10^5 cpm/sample) at 63° for 16–18 hr. The samples were then digested with RNase at 37° for 45 min. For the filtration assay, 10% trichloroacetic acid was added and then the samples were filtered through GF/C glass filters (Whatman). The filters were then extensively and sequentially washed with cold 5% trichloroacetic acid and then 95% ethanol and quantified by liquid scintillation counting. For polyacrylamide gel analysis, samples were treated in a similar manner, with modifications (11, 15), and then analyzed on 6% polyacrylamide/8 M urea denaturing gels. The gels were dried, and labeled bands were detected by autoradiography.

mRNA stability analysis. To determine the half-life of β_2AR mRNA, the cells were incubated with actinomycin D to block transcription, as described previously (11, 17). Cells were incubated in the absence or presence of isoproterenol as described above; actinomycin D (2 μg/ml) was then added to the medium and the cells were harvested at different times (0–120 min). Total cellular RNA was extracted at each of the time points, and β_2AR mRNA levels were quantified by the RNase protection assay as described above. The concentration of actinomycin was shown to inhibit RNA synthesis by >98% (11).

Nuclear run-on analysis. Nuclei were isolated by Dounce homogenization using the alternate protocol described by Greenberg and Bender (18), and nuclear run-on analysis was conducted as described previously (11). Briefly, nuclei were incubated for 30 min at 30° in a transcription mixture containing 1 mM unlabeled ATP, CTP, and GTP and 250 μCi of [α-^{32}P]UTP. The newly transcribed RNA was extracted as described previously (18). The radiolabeled RNA was denatured and hybridized (at 42° for 3 days) to β_2AR or cyclophilin cDNA immobilized on nitrocellulose membranes (5 μg/slot), in the hybridization buffer-N described previously (14), with the addition of 0.5% sodium dodecyl sulfate, 300 μg/ml salmon sperm DNA, and 20 μg/ml yeast tRNA. Filters were washed, dried, and subjected to autoradiography, with two intensifying screens, at −70° for 5 days. Quantitative results were obtained by densitometric scanning.

Other methods. Levels of intracellular cAMP were determined by radioimmmune assay (19).

Results

Characterization of β_2AR mRNA by RNase protection analysis. A 207-bp fragment of the rat β_2AR cDNA, corresponding to coding region positions +1099–1305, was subcloned into pBluescript, and ^{32}P-labeled antisense riboprobes were synthesized using T7 RNA polymerase. The radiolabeled riboprobe was hybridized with tRNA, sense strand cRNA synthesized with T3 RNA polymerase, or total RNA isolated from C_6 cells (Fig. 1). The riboprobe and sense cRNA contain additional vector sequences and are larger than the 207-bp fragment of the β_2AR coding region. The RNase-resistant hybrid resulting from hybridization with

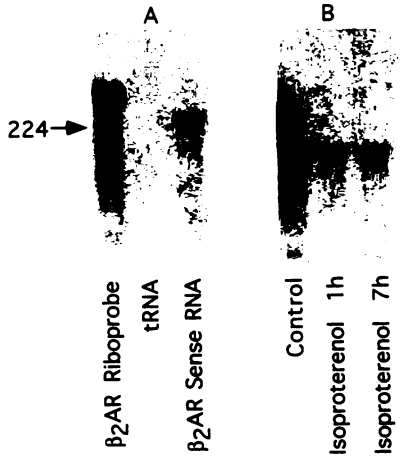

Fig. 1. RNase protection analysis of β_2AR mRNA by polyacrylamide gel electrophoresis. A, ^{32}P-labeled β_2AR riboprobe alone or RNase-protected fragments after hybridization, in solution, with 40 μg of tRNA or 60 ng of β_2AR sense strand are shown. B, The β_2AR riboprobe was hybridized with 30 μg of total RNA isolated from C_6 cells that had been incubated without (*Control*) or with isoproterenol (1 μM) for 1 or 7 hr, as indicated. The samples were hybridized, digested with RNase, and subjected to gel electrophoresis as described in Experimental Procedures. The gel was dried and the radiolabeled bands were visualized by autoradiography. The location of the 224-bp DNA marker is shown on the *left*.

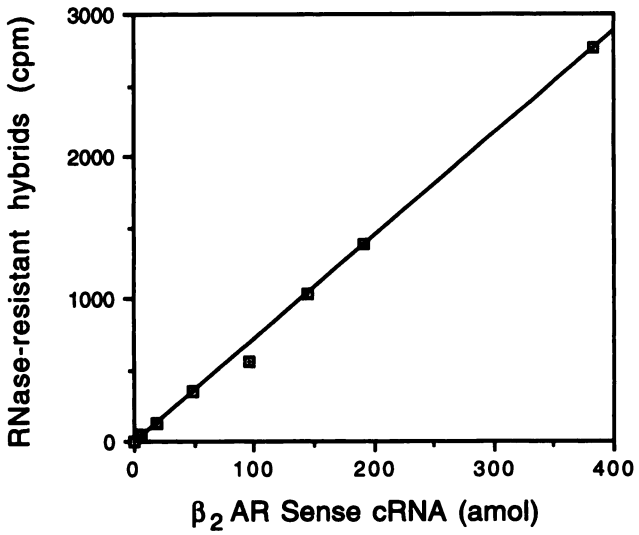

Fig. 2. Standard curve for determination of β_2AR mRNA levels by RNase protection and filtration analysis. ^{32}P-labeled β_2AR riboprobe was hybridized with different amounts of β_2AR sense strand cRNA (2–390 ng) as described in Experimental Procedures. The RNase-resistant hybrids were precipitated and then collected by filtration over glass fiber filters. After the filters were washed, the level of radiolabeled β_2AR hybrids was quantified by liquid scintillation counting.

total RNA from C_6 cells is 207 bp. The specificity of this probe was demonstrated by the lack of protected hybrids after hybridization with yeast tRNA (Fig. 1).

Distribution of β_2AR mRNA in C_6 cells and rat tissues. The absolute levels of β_2AR mRNA were determined by construction of a standard curve with different amounts of sense cRNA (Fig. 2); this also demonstrated that the level of radioactive RNase-protected hybrids was linear over a range of 2–390 amol of β_2AR sense cRNA. Levels of β_2AR mRNA in C_6 cells and several rat tissues were determined and compared with those of β_1AR mRNA (from Refs. 11 and 15). As shown in Fig. 3, levels of β_2AR and β_1AR mRNA ranged from 1 to 10 and from 0.5 to 5 amol/μg of total cellular RNA, respectively (Fig. 3). The relative levels of β_2AR and β_1AR mRNA and their ratios compare favorably with the levels of β_2AR and β_1AR determined by ligand binding studies (20–22). The C_6 cells used in these studies have ~70% β_1AR and 30% β_2AR (11, 12, 23), which is the same as the distribution of the respective receptor subtype mRNAs.

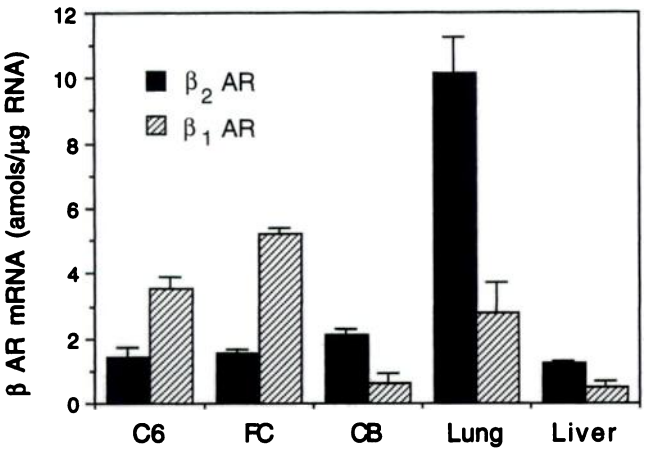

Fig. 3. Comparison of β_2AR and β_1AR mRNA levels in C_6 cells and in different rat tissues. The radiolabeled β_2AR riboprobe was hybridized in solution with 30 μg of total RNA isolated from C_6 cells or different rat tissues, including frontal cortex (*FC*), cerebellum (*CB*), lung, and liver. Levels of protected hybrids were quantified by filtration analysis and liquid scintillation counting. The absolute level of β_2AR mRNA in each tissue was determined by linear regression, using a β_2AR sense strand cRNA standard curve. The results are expressed as amol/μg of total RNA and are the mean ± standard error of one to four separate experiments, each analyzed in triplicate. Values for β_1AR mRNA levels are from the reports by Hosoda and Duman (15) and Hosoda *et al.* (11).

Isoproterenol- and forskolin-induced down-regulation of β_2AR mRNA in C_6 cells. Previous studies have demonstrated that incubation of C_6 cells with isoproterenol or forskolin, which stimulates cAMP formation, results in a coordinate, time-dependent, down-regulation of both βAR subtypes (11, 12). Whereas isoproterenol mediates a receptor loss of ~90% by 6 hr, forskolin causes only ~50% loss, indicating that receptor down-regulation is mediated, in part, by the cAMP system. Exposure of C_6 cells to isoproterenol (Figs. 1B and 4A) or forskolin (Fig. 4B) resulted in time-dependent down-regulation of β_2AR mRNA by ~50–60%. In addition, isoproterenol-induced down-regulation of β_2AR mRNA was dose dependent; the half-maximally effective concentration was ~5 nM, and the maximal concentration of agonist required for down-regulation of β_2AR mRNA was ~100 nM (Fig. 5).

Agonist stimulation of cAMP production was also time and dose dependent (Fig. 6). The concentrations of isoproterenol required for half-maximal (~10 nM) and maximal (~100 nM) stimulation of cAMP production were similar to those required for down-regulation of β_2AR mRNA. However, the time courses for these two events were different. This is not surprising, because receptor stimulation of cAMP production is a relatively rapid event, whereas down-regulation of receptor mRNA, which is dependent on activation of intracellular signal transduction pathways and is limited by the half-life (~60 min) of β_2AR mRNA (Fig. 7), proceeds more slowly. We have previously demonstrated that agonist treatment leads to activation of cAMP-dependent protein kinase in C_6 cells (11), which could represent the initiating event for down-regulation of β_2AR mRNA expression.

Effect of inhibition of protein synthesis on the regulation of β_2AR mRNA in C_6 cells. To examine the role of *de novo* protein synthesis in the down-regulation of β_2AR mRNA, C_6 cells were pretreated with the potent and selective protein synthesis inhibitor *Pseudomonas* exotoxin A before exposure to isoproterenol or forskolin (Fig. 4). Pretreatment with exotoxin alone increased levels of β_2AR mRNA by approximately 30% (1.45 ± 0.26 and 1.91 ± 0.14 amol/μg of RNA in control and treated cells, respectively, mean ± standard error). Moreover, exotoxin pretreatment completely blocked the down-regulation of β_2AR mRNA in response to either isoproterenol or forskolin (Fig. 4).

Effect of agonist treatment on β_2AR mRNA stability in C_6 cells. To further explore the mechanism(s) by which β_2AR mRNA is down-regulated, the influence of isoproterenol treatment on mRNA stability was examined. C_6 cells were incubated in the absence or presence of isoproterenol for 2 hr, actinomycin D (an inhibitor of DNA transcription) was then added to the medium, and cells were harvested at different times. The half-life of β_2AR mRNA, determined from the rate of β_2AR mRNA degradation in the presence of actinomycin D, reflects the stability of mRNA. As shown in Fig. 7, the half-life of β_2AR mRNA was ~60 min in control C_6

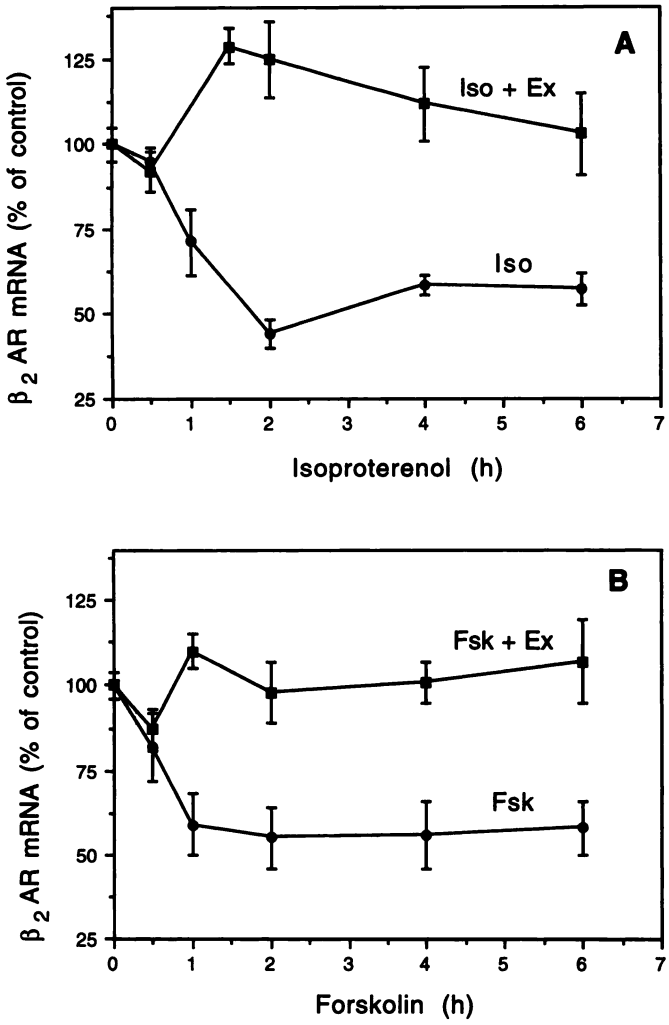

Fig. 4. Agonist- and forskolin-mediated regulation of β_2AR mRNA in rat C_6 glioma cells. C_6 cells were incubated with 1 μM isoproterenol (*Iso*) (A) or 10 μM forskolin (*Fsk*) (B) for the indicated periods of time. After the cells were collected, the levels of β_2AR mRNA were determined by RNase protection analysis as described in Experimental Procedures. In some experiments, cells were first treated for 16 hr with 0.3 μg/ml exotoxin A (*Ex*), to inhibit protein synthesis, and then exposed to stimulator. The results are presented as percentage of control and are the mean ± standard error of three or four separate determinations.

cells, and isoproterenol treatment did not significantly influence the half-life. Thus, regulation of mRNA stability does not appear to play a role in the down-regulation of β_2AR mRNA induced by agonist treatment. We have reported that β_1AR subtype mRNA has a similar half-life (61 min) in these same cells and that β_1AR mRNA half-life is not influenced by agonist treatment (11).

Agonist-mediated regulation of β_2AR gene transcription. To determine the influence of isoproterenol on the rate of β_2AR gene transcription, nuclear run-on analysis was conducted on nuclei isolated from control and isoproterenol-treated C_6 cells (Fig. 8). Briefly, radiolabeled nascent RNA transcripts were generated from the isolated nuclei and used for hybridization with β_2AR cDNA, which had been blotted onto nitrocellulose filters. As a control, cyclophilin cDNA was also blotted onto the nitrocellulose filters. Incubation with isoproterenol for 90 min significantly decreased levels of nascent β_2AR RNA transcripts but did not influence levels of cyclophilin gene transcription (Fig. 8).

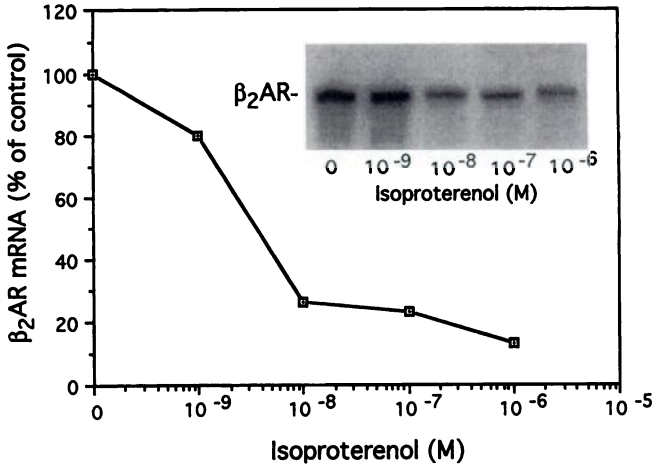

Fig. 5. Isoproterenol-mediated regulation of β_2AR mRNA in rat C_6 glioma cells. C_6 cells were incubated with the indicated concentration of isoproterenol for 2 hr, and levels of β_2AR mRNA were determined by RNase protection analysis and polyacrylamide gel electrophoresis as described in Experimental Procedures. The results are presented as percentage of control and are from a single experiment. *Inset*, autoradiogram of RNase-resistant β_2AR hybrids.

Discussion

Our results demonstrated that incubation of C_6 cells with isoproterenol decreased levels of β_2AR mRNA. This effect was mimicked by incubation with forskolin, indicating that down-regulation of β_2AR mRNA expression is mediated by activation of the cAMP system. Agonist-induced down-regulation of β_2AR mRNA is similar to that reported previously in C_6 cells (9), S49 cells (6), and DDT_1MF-2 cells (5, 8). In DDT_1MF-2 cells the down-regulation of β_2AR is preceded by a rapid transient elevation of β_2AR mRNA. This increase has been shown to result from activation of a CRE (8, 24); a CRE has been found in the promoters of the human, hamster, mouse, and rat β_2AR genes (24, 25). The lack of a transient elevation of β_2AR mRNA levels in C_6 cells is surprising, because we have observed a transient up-regulation of β_1AR mRNA in this cell line (11). One possibility is that the rate of β_2AR gene transcription is under a greater degree of negative control in C_6 cells than in other cell lines. This possibility is supported by the observation that incubation with a protein synthesis inhibitor increased basal β_2AR mRNA levels. These variations suggest that the mechanisms for regulation of β_2AR mRNA differ between cell lines and under different culture conditions.

Down-regulation of β_2AR mRNA in DDT_1MF-2 cells is reported to be mediated by decreased stability of receptor mRNA (7). The decreased β_2AR mRNA stability in DDT_1MF-2 cells is accompanied by induction of β_2AR mRNA-binding proteins that could mediate the destabilization of receptor mRNA (26). In the present study, the half-life of β_2AR mRNA was determined to be ~60 min in C_6 cells, shorter than that of 12 hr reported by Hadcock *et al.* (7) but similar to that reported by Collins *et al.* (8) in DDT_1MF-2 cells and by Kiely *et al.* (27) in C_6 cells. Incubation of C_6 cells with isoproterenol for 2 hr did not influence β_2AR mRNA half-life, suggesting that regulation of mRNA stability is not

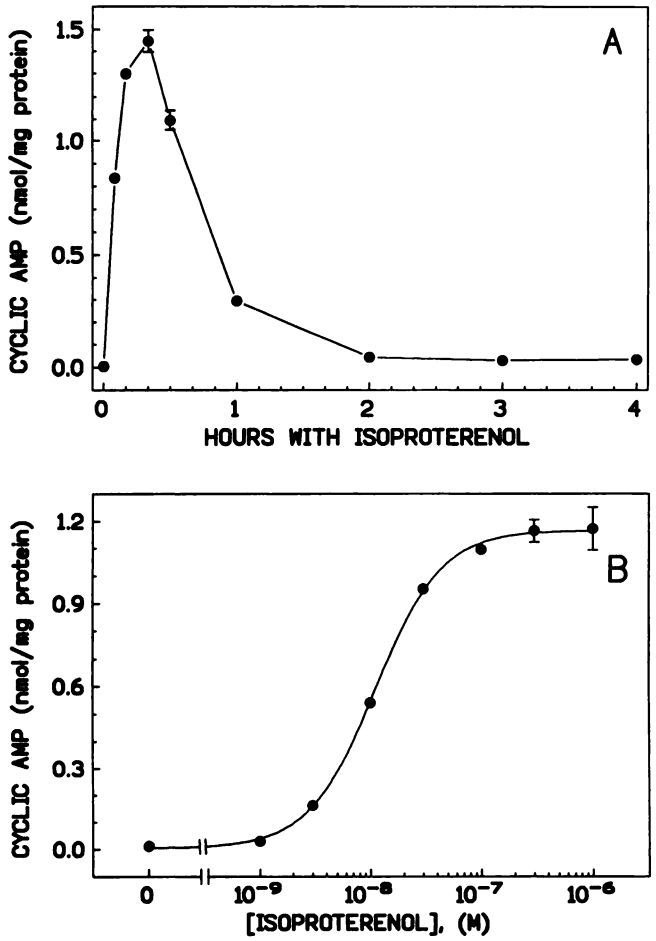

Fig. 6. Effect of isoproterenol on cAMP levels in rat C_6 glioma cells. A, C_6 cells were incubated in serum-free medium for 16 hr, stimulated with 1 μM isoproterenol for the indicated times, and assayed for intracellular cAMP and protein levels as described in Experimental Procedures. B, The procedure was the same as in A except that the cells were stimulated with increasing concentrations of isoproterenol for 20 min. Values are the mean ± standard error of triplicate determinations for a representative experiment.

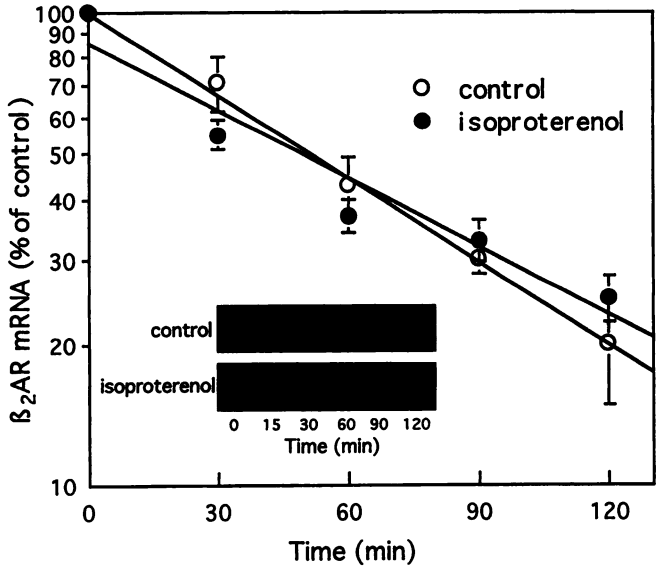

Fig. 7. Determination of β_2AR mRNA stability in control and agonist-treated rat C_6 glioma cells. C_6 cells were incubated in the absence (○) or presence (●) of 1 μM isoproterenol for 2 hr, at which time actinomycin D (2 μg/ml) was added to the medium. The cells were then further incubated for the indicated times and collected, and β_2AR mRNA levels were determined by RNase protection analysis. The results are expressed as percentage of control and are plotted on a logarithmic scale versus time. Values are the mean ± standard error of three separate experiments. *Inset*, representative autoradiogram of a β_2AR mRNA half-life experiment in which RNase-protected hybrids were analyzed by gel electrophoresis, as described in the legend to Fig. 1.

the mechanism by which agonist treatment decreases the expression of β_2AR mRNA in this cell line. Given that the half-life was determined in the same manner in all three studies, the variations in mRNA half-life observed in control and agonist-treated cells provide additional evidence that the mechanisms that regulate the levels of mRNA differ between cell lines and among cell lines cultured under different conditions.

The lack of effect of agonist treatment on β_2AR mRNA half-life suggests that down-regulation of receptor mRNA occurs at the level of gene transcription. To test this hypothesis, we carried out run-on analysis in nuclei isolated from control and isoproterenol-treated C_6 cells. We found that agonist treatment significantly decreased the rate of β_2AR gene transcription in C_6 cells, by ~25%, consistent with the hypothesis that gene transcription, and not mRNA stability, mediates the down-regulation of β_2AR mRNA in C_6 cells. The smaller magnitude of the change in transcription rate, relative to levels of β_2AR mRNA, could be a reflection of the technical complexity of the nuclear run-on assay (e.g., maintenance of the mechanisms that control basal and agonist-regulated levels of transcription elongation in nuclei isolated from different groups of cells). We have reported similar levels of regulation for β_1AR mRNA and gene transcription rates in C_6 cells after agonist treatment (11).

The mechanisms that underlie the down-regulation of β_2AR gene transcription by agonist treatment are not known. The observation that down-regulation of β_2AR mRNA in response to agonist or forskolin incubation is blocked by inhibition of protein synthesis suggests that induction of an inhibitory transcription factor may be involved. Some forms of modulatory CRE-binding proteins, referred to as ICER, are rapidly induced by activation of the cAMP system and are negative regulators of CRE-mediated gene transcription (28, 29). In this way, it has been suggested that ICER acts as a negative feedback regulator and turns off, or inhibits, those genes that are rapidly induced by stimulatory CRE-binding proteins (29). We have found that isoproterenol or forskolin treatments increase the expression of ICER in C_6 cells,[1] consistent with the possibility that ICER induction mediates the down-regulation of β_2AR gene expression. Blockade of ICER, or another repressor, could reveal CRE-mediated activation of β_2AR gene expression, like that reported for β_1AR (11). In fact, there was a tendency for levels of β_2AR mRNA to be increased by isoproterenol and forskolin treatments in the presence of the protein synthesis inhibitor (Fig. 4).

Regulation of receptor mRNA is one additional mechanism by which levels of β_2AR binding sites are regulated by agonist treatment, as well as a mechanism for heterologous regulation via other receptors that regulate the cAMP system. Moreover, it is important to point out that multiple

[1] L. R. Fitzgerald, C. A. Machida, P. H. Fishman, and R. S. Duman. Adrenergic regulation of ICER (inducible cAMP early repressor) and β_1-adrenergic receptor gene expression in C6 glioma cells. Manuscript in preparation.

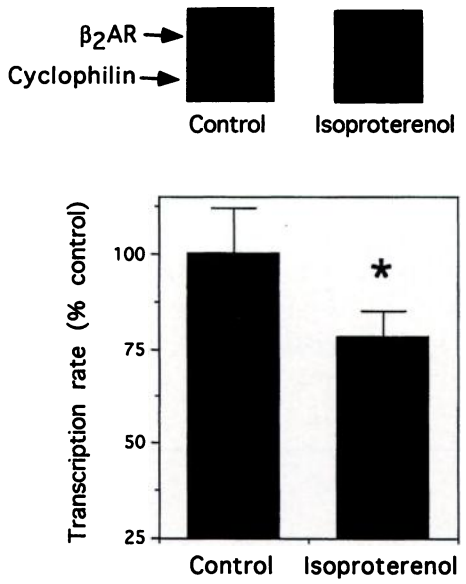

Fig. 8. Effect of agonist treatment on β_2AR gene transcription rate in rat C_6 glioma cells. C_6 cells were incubated in the absence or presence of 1 μM isoproterenol for 90 min, at which time cell nuclei were isolated and frozen at −80°. Transcription elongation was allowed to continue in the isolated nuclei in the presence of [^{32}P]UTP and unlabeled nucleotides, as described in Experimental Procedures. After elongation, the radiolabeled RNA was isolated and hybridized to β_2AR or cyclophilin cDNA (5 μg) immobilized on nitrocellulose filters. The resulting labeled filters were then washed and subjected to autoradiography for 2 days. The level of hybridized transcript in each band was quantified by densitometric scanning. *Top*, representative autoradiogram of β_2AR run-on analysis; *bottom*, quantitation of the results, expressed as percentage of control (mean ± standard error of three separate determinations). *, $p < 0.05$, relative to control, by χ^2 test.

mechanisms may be involved in the regulation of βAR mRNA levels in different cells and tissues. One mechanism elucidated in the present study, as well as our previous study (11), involves decreased rates of βAR gene transcription. A second mechanism appears to be decreased β_2AR mRNA stability (26). A third mechanism involves increased βAR gene transcription via activation of CRE-binding proteins (8, 11, 24). Identification of the different mechanisms underlying the regulation of β_2AR expression could prove useful in future studies to identify disorders associated with altered levels of these receptors and to develop strategies for correction of such disorders.

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