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Long-term agonist exposure induces upregulation of β_3-adrenergic receptor expression via multiple cAMP response elements

(adenylyl cyclase/downregulation/desensitization/adipocyte/transcription)

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ABSTRACT **During continuous stimulation by agonist, β_1- and β_2-adrenergic receptors (ARs) undergo processes that lead to decreases in receptor expression. This receptor downregulation serves to limit the cellular cAMP response during chronic agonist exposure. In the recently described third subtype of the βAR, denoted β_3AR, we found four potential cAMP response elements in the 5′ flanking region, suggesting that expression of this receptor might be positively regulated by agonists. These elements were cloned into the vector $pA_{10}CAT_2$, which contains a chloramphenicol acetyltransferase reporter gene, and transiently expressed in VERO cells. Three of these elements, TGACTCCA, TGAGGTCT, and CGAGGTCA (located 518, 622, and 1125 bases upstream of the β_3AR coding block, respectively) were found to increase transcription of the chloramphenicol acetyltransferase gene in response to cAMP analogues and agents that increase intracellular cAMP. 3T3-F442A cells, when differentiated into the adipocyte phenotype by insulin, expressed β_3AR, and nuclear runoff studies from such cells confirmed cAMP enhancement of β_3AR mRNA transcription. In these cells, β_3AR mRNA increased in response to exposure to the β_3AR agonist isoproterenol and remained elevated during exposures of up to 24–30 hr. During prolonged exposure to agonist, no downregulation of β_3AR expression in 3T3-F442A cells occurred. Indeed, β_3AR expression increased during agonist exposure to ≈165% of basal expression. In marked contrast, β_1AR expression declined by ≈70% in response to chronic agonist exposure. These studies reveal a subtype-specific prolonged transcriptional regulation of a βAR gene by the end product of its signal transduction pathway. Thus, the β_3AR undergoes a paradoxical increase in receptor expression during chronic agonist exposure.**

Three β-adrenergic receptor (βAR) subtypes have been described. These receptors all couple to the stimulatory guanine nucleotide binding protein G_s, and during agonist occupancy, serve to increase intracellular cAMP. During exposure to agonist, the β_1- and β_2ARs display the process of desensitization, which serves to limit the cellular response despite the continuous presence of agonist (1–3). The early phases of agonist-promoted desensitization of the β_2AR, occurring within seconds of agonist exposure, are mediated by phosphorylation of the receptor by the cAMP-dependent protein kinase (PKA) and the βAR kinase (2). After exposure to agonist for more prolonged time periods, on the order of hours, an even more profound desensitization is typically observed. The prominent mechanism during long-term agonist desensitization of the β_1- and β_2ARs is the loss of cellular receptors (2, 4). During this process, which is termed receptor downregulation, a loss of 50–70% of the receptors after 24 hr of agonist exposure is not uncommon. Downregulation of β_2AR has been proposed to be due to several potential processes including receptor degradation, mRNA degradation, and a decreased transcription of mRNA (5–7). Phosphorylation of βAR, which is the hallmark of short-term agonist-promoted desensitization, is not required for long-term downregulation of β_2AR expression (5).

To our knowledge, no studies have examined whether the recently cloned β_3AR (8), which is the "atypical" βAR of adipocytes and several other metabolic tissues, undergoes agonist-promoted downregulation. There is considerable interest as to how the β_3AR is regulated, since agonists for this receptor appear to have antidiabetic, thermogenic, and antiobesity properties (8–10). The physiologic and pharmacologic characteristics of this receptor have been reviewed (8–10). We have noted (11) that the 5′ flanking region of the β_3AR possesses multiple potential transcriptional regulatory elements, including four potential cAMP response elements (CREs). This suggests the possibility that β_3AR expression might be positively regulated by the end product of its signal transduction pathway, cAMP. We considered that such a process might serve to minimize agonist-promoted downregulation of the β_3AR. We therefore examined each of the four potential CREs in the 5′ flanking region of the β_3AR for functional activity and then assessed their role in long-term agonist-promoted regulation of β_3AR mRNA and receptor expression.

MATERIALS AND METHODS

Cell Culture. VERO cells were grown as monolayers in Dulbecco's modified Eagle's medium with 5% (vol/vol) fetal calf serum in a 95% air/5% CO_2 atmosphere at 37°C. 3T3-F442A fibroblasts were grown as monolayers in the same medium supplemented with 50 mM sodium bicarbonate containing 10% (vol/vol) bovine serum in an atmosphere of 10% CO_2/90% air. Under these conditions, these cells maintained the characteristic fibroblast morphology (12), did not express β_3AR, and had no detectable β_3AR mRNA (13). Nearly confluent cells were differentiated into the adipocyte phenotype by replacing the serum with fetal calf serum and supplementing the medium with insulin at 1.0 μg/ml (12). After differentiation to the adipocyte phenotype (which required 7–12 days), these cells were found to contain β_3AR mRNA and expressed the receptor at ≈40 fmol/mg of protein (13). In experiments with agonist exposure, 3T3-F442A cells were incubated with fresh medium containing 100 μM isoproterenol, 0.2 mM isobutylmethylxanthine (IBMX), and 0.1 mM ascorbic acid at 37°C in 10% CO_2/90% air for the indicated

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Abbreviations: AR, adrenergic receptor; PKA, protein kinase A or cAMP-dependent protein kinase; CRE, cAMP response element; IBMX, isobutylmethylxanthine; CAT, chloramphenicol acetyltransferase; ^{125}I-CYP, ^{125}I-labeled cyanopindolol.

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Table 1. Location and sequence of potential CREs in the 5′ flanking region of the β_3AR

Location	Sequence	Name
518–525	TGACtcCA	p518
622–629	TGAgGTCt	p622
955–962	TcACaTCA	p955
1125–1132	cGAgGTCA	p1125
Classic CRE motif	TGACGTCA	—

Four potential CREs in the 5′ flanking region of the β_3AR are shown, with uppercase type indicating identity with the classic CRE motif (ref. 14). The locations represent the number of nucleotides upstream of the β_3AR coding block.

times. Control cells received fresh medium containing ascorbic acid only.

Constructs. The 5′ flanking region of the human β_3AR contains four octomers within 1320 bases of the coding block, each being a variation of the classic CRE (14) palindromic motif TGACGTCA (Table 1). The complete sequence of this region has been reported elsewhere (11).¶ The potential functional activities of these four sequences were explored by ligation of the appropriate segment into the vector $pA_{10}CAT_2$ (15), which contains the simian virus 40 early promoter and a chloramphenicol acetyltransferase (CAT) reporter gene. Oligonucleotides were synthesized for each strand containing a centrally located putative CRE motif, with 15 bases of flanking β_3AR sequence on either side including an engineered *Bgl* II site used for ligation into $pA_{10}CAT_2$. After annealing the two oligonucleotides, the duplex was ligated into the $pA_{10}CAT_2$ vector at the *Bgl* II site, which is ≈200 bases on the 5′ side of the simian virus 40 promoter. In other constructs, the simian virus 40 enhancer, also cloned upstream of its promoter, was utilized as a control. Transformations were carried out in *Escherichia coli* HB101 and screened for recombinant molecules and the orientation of the inserts by paired PCR (16), and the sequence was verified by dideoxynucleotide sequencing.

Transfections and CAT Assays. Transient transfections of VERO cells were carried out in 100-mm dishes by using 10 μg of supercoiled DNA and calcium phosphate precipitation. DNA remained on the cells for 15 hr, and then the medium was replaced. Fifteen hours later some cells were treated with various agents that increase intracellular cAMP including the phosphodiesterase inhibitor IBMX (0.2 mM), forskolin (100 μM), or the cell-permeable cAMP analogs dibutyryl-cAMP (1 mM) or 8-bromo-cAMP (1 mM) in fresh medium. Control cells received medium alone. Incubations with drugs were carried out for 18 hr, at which time the cells were washed and scraped and extracts were prepared (15). Samples of cell extracts were then assayed for CAT activity by incubation with 0.05 μCi of [^{14}C]chloramphenicol (1 Ci = 37 GBq) and acetyl coenzyme A (15). The reaction was stopped by addition of ethyl acetate, the organic phase was lyophilized, and the product was dissolved in ethyl acetate. Acetylated and nonacetylated products were spotted on silica gel sheets and resolved by ascending thin layer chromatography in a 95:5 (vol/vol) chloroform/methanol environment. The sheets were then exposed to Kodak XAR-5 film overnight.

Radioligand Binding. ^{125}I-labeled cyanopindolol (^{125}I-CYP) was utilized in radioligand binding studies as described (17–19). To assess the effects of agonist exposure on β_3AR expression, 3T3-F442A cells in monolayers were incubated in medium with 100 μM isoproterenol, 0.2 mM IBMX, and 0.1 mM ascorbic acid for the indicated times. The plates were then placed on ice and washed five times with 15 ml of ice-cold phosphate-buffered saline. Cells from one-half of the plate were then scraped with a rubber policeman, transferred to a centrifuge tube, and lysed with 5 mM Tris·HCl, pH 7.40/2 mM EDTA, and membranes were prepared for ligand binding as described (20). Briefly, broken cells were centrifuged for 20 min at 40,000 × *g*. The pellet was resuspended in 2 ml of the above buffer, homogenized with a Polytron for 10 sec at 50% maximal speed, diluted 1:8 with additional buffer, and centrifuged again. This pellet was resuspended in binding buffer (75 mM Tris·HCl, pH 7.4/12.5 mM $MgCl_2$/2 mM EDTA). The remaining one-half of the intact cells on the plate were utilized for extraction of RNA as described below. As has been reported by others (8), we found that the K_d for ^{125}I-CYP binding to the β_3AR was ≈500 pM. Therefore, to monitor the β_3AR receptor density of 3T3-F442A cells (adipocyte phenotype), membranes were incubated with 1.2 nM ^{125}I-CYP in the absence (total binding) or presence (nonspecific binding) of 1 mM isoproterenol for 2 hr at 37°C. These assays included 100 μM GTP (to inhibit retained agonist binding), 1 mM ascorbic acid (to inhibit isoproterenol oxidation), and 4 μM desipramine [to reduce nonspecific binding (8)]. Reactions were terminated by dilution with ice-cold 10 mM Tris buffer, and bound ligand was separated by rapid filtration over glass fiber filters. Specific binding was defined as the difference between total and nonspecific binding and was normalized for protein. In the adipocyte phenotype, most (>90%) of the βARs present are β_3ARs (13). The fibroblast form of 3T3-F442A cells possess only β_1AR (21). The β_1AR density of nondifferentiated 3T3-F442A cells was determined in the same manner as above, except that 300 pM ^{125}I-CYP was utilized in the binding assays due to the higher affinity of this receptor for the radioligand.

Quantification of mRNA. Total RNA was extracted from the remaining adhered cells (see above) by a guanidinium thiocyanate method (22). Quantification of mRNA from cells exposed to agonist was performed by a modification of an RNA-PCR method, which has been described and validated elsewhere (13, 23). We utilized r*Tth* DNA polymerase (Perkin–Elmer/Cetus), which in the presence of Mn^{2+} acts as a reverse transcriptase, to produce template-specific cDNA (24). This product was then amplified by a two-step PCR, where r*Tth* polymerase acts as a DNA polymerase in the presence of Mg^{2+} (24). The primers utilized were 5′-GCTCCGTGGCCTCACAG-3′ (sense) and 5′-CTCGGCATCTGCCCCTA-3′ (antisense), which are identical to reported sequences of the mouse β_3AR (13, 25) encoding regions of the N terminus and second extracellular loop, respectively, and provided a single specific product of the expected molecular weight. No β_1- or β_2AR signals were obtained using these β_3AR primers with RNA from tissues rich in the β_1- or β_2AR subtypes (see *Results*). The antisense oligonucleotide was also utilized as the primer for the reverse transcriptase reaction. The PCR mixture consisted of 2.0 mM $MgCl_2$, 0.15 μM of primers, all four NTPs (each at 200 μM), 5 units of polymerase, and 0.25 μl of [α-^{32}P]dCTP, which was utilized to quantitate the product. The same procedure as described above was used for quantitation of mouse β-actin mRNA, which did not vary significantly with agonist exposure, and was used as a control. These primers were 5′-CTGAAGTACCCCATTGAACA-3′ (sense) and 5′-GCAGCTCAGTAACAGTCCGC-3′ (antisense). The PCR products were electrophoresed on 1.4% agarose gels and visualized by ethidium bromide staining, the bands were excised, and radioactivity was measured in a scintillation counter. Typically, 150,000 cpm represented basal levels of β_3AR mRNA from untreated cells. In preliminary experiments, we found that by using 20–360 ng of total RNA and 30 cycles of the PCR, a linear quantification of mRNA was obtained (see *Results*). Routinely, 250 ng of total RNA was utilized for all experiments. Results are expressed as a percentage of the basal β_3AR mRNA from untreated cells.

¶GenBank accession no. M62473.

Nuclear Runoff Assays. Nuclei from differentiated 3T3-F442A cells exposed to medium alone or medium with 100 μM isoproterenol and 0.2 mM IBMX for 1 hr were isolated (26) and incubated for 30 min at 30°C with 1.25 mM ATP, 1.25 mM GTP, 1.25 mM CTP, and 0.25 mCi of [^{32}P]UTP (3000 Ci/mmol; 1 Ci = 37 GBq) per sample. Total RNA was isolated from unincorporated nucleotides over Sephadex columns, dried, and dissolved in hybridization solution. One million counts per ml were allowed to hybridize to human β_3AR (in pGEM-4Z), human γ-actin (in pBr, positive control), and pUC (negative control) DNA at 65°C. Filters were washed at 50°C, dried, scanned, and quantified using a Betagen radioscanner and imaging device (Betagen, Waltham, MA).

Data Analysis. Radioligand binding data were analyzed by iterative least squares techniques as described (18). Data are presented as the mean ± SEM.

Materials. ^{125}I-CYP, ^{35}S-labeled dATP, [α-^{32}P]dCTP, and [^{14}C]chloramphenicol were from New England Nuclear. [α-^{32}P]UTP was from ICN. VERO cells were a gift from S. Bachenheimer (University of North Carolina), $pA_{10}CAT_2$ was a gift from John Brady (National Institutes of Health), and 3T3-F442A cells were from H. Greene (Harvard University). Other reagents were from standard commercial sources.

RESULTS AND DISCUSSION

A consistent feature of long-term exposure of β_1- or β_2ARs to agonists is a decrease in cellular receptor expression as determined by ligand binding assays. Such agonist-promoted downregulation has been documented in whole animal (27) and human (28) studies as well as studies utilizing cells in culture (5, 6). Although the extent of receptor loss may vary with tissue or cell type, agonist-promoted downregulation of β_1- or β_2AR is an invariable finding in mammalian cells. In experiments where such studies were performed, β_1- or β_2AR mRNA has also been found to be decreased after prolonged agonist exposure (5, 6, 29), confirming that regulation of transcription and the stability of mRNA may play important roles in the process of β_1- and β_2AR downregulation. Interestingly, within the 5′ flanking region of the β_2AR a single functional CRE has been documented (30, 31). An early transient increase in cellular β_2AR mRNA that has been reported by some (30, 31) but not all (29) observers during agonist exposure to this receptor does not, however, result in detectable increases in receptor expression, and downregulation of β_2AR expression proceeds (6, 29–31).

We examined the 5′ flanking region of the human β_3AR and found four potential CREs (11), each having at most two mismatches with the classic consequence sequence (Table 1). To discern which, if any, confer increases in rates of transcription, we cloned these motifs into $pA_{10}CAT_2$, which utilizes CAT as a reporter gene, transfected these constructs into VERO cells, and exposed them to various agents. Fig. 1 shows the results of these experiments. As shown, p518, p622, and p1125 (named according to the number of bases 5′ of the β_3AR coding block) all markedly enhanced transcription of the CAT gene in response to cAMP. The potential CRE p955 was found to be unresponsive to cAMP. The above functionally active CREs were also, to a variable extent, responsive in the antisense orientation as well. Although 8-bromo-cAMP was routinely utilized as the stimulatory agent in these experiments, IBMX, dibutyryl-cAMP, and forskolin also activated these three CREs (data not shown). To assess whether these CREs affected the rate of transcription of the β_3AR mRNA, we performed nuclear runoff studies with isolated nuclei from differentiated 3T3-F442A cells that had been incubated with medium alone or medium containing 100 μM isoproterenol and 0.2 mM IBMX for 1 hr. These results revealed an ≈50% increase in the rate of transcription

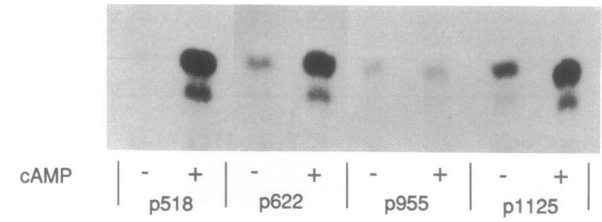

FIG. 1. CAT assay autoradiographic data for the four putative CREs (p518, p622, p955, and p1125) cloned into the CAT plasmid $pA_{10}CAT_2$. The numerical designations (e.g., p518) refer to the distance of the potential CRE upstream of the β_3AR open reading frame. The data represent samples from cells incubated with medium alone (lanes −) and samples from cells incubated with 8-bromo-cAMP (lanes +). VERO cells were transfected and then treated with medium alone or medium with 1 mM 8-bromo-cAMP. Expression of the CAT gene was assayed by the ability to acetylate radiolabeled chloramphenicol, which was resolved by ascending chromatography and visualized by autoradiography. The acetylations depicted in this figure show marked increases in transcription imparted by three of the four potential CREs. A single experiment is shown that is representative of four performed.

of β_3AR mRNA from nuclei of cells so exposed (untreated, 1000 ± 120 cpm; treated, 1500 ± 100 cpm).

We then examined the net effect of this cAMP-mediated increase in transcription on cellular levels of β_3AR mRNA and on receptor expression during prolonged *in vivo* agonist exposure. 3T3-F442A fibroblast cells, after undergoing differentiation to an adipocyte phenotype by incubation with fetal calf serum and insulin (12), expressed β_3AR at ≈40 fmol/mg (13). (Other clones of the 3T3 cell line, such as the 3T3-L1, did not appear to express the β_3AR after differentiation). β_3AR mRNA, however, was in low abundance in 3T3-F442A cells, and Northern blots with poly(A)-selected mRNA were not adequate for quantitative analysis. This has also been observed with multiple tissues by others (8). We therefore developed a method of quantitating mRNA by use of the reverse transcriptase capacity of the r*Tth* polymerase to generate a template-specific cDNA, which was then amplified by the polymerase activity of this enzyme in a series of PCRs. Fig. 2 shows the specificity and sensitivity of this method. As can be seen in Fig. 2*A*, the choice of primers used in this RNA-PCR method provides a specific signal for the β_3AR, but not the β_1- or the β_2AR. Fig. 2*B* shows that the method employed provides a linear and quantitative measurement of β_3AR mRNA when using a wide range of total RNA starting material.

To assess the effects of agonist exposure on cellular β_3AR mRNA levels, we exposed differentiated 3T3-F442A cells to 100 μM isoproterenol plus 0.2 mM IBMX for the indicated times, prepared total RNA, and then quantitated β_3AR mRNA as outlined above. As shown in Fig. 3, an increase in β_3AR mRNA, detectable within several hours of agonist exposure, was found. These elevated levels continued for up to 24–30 hr of agonist exposure. While we consistently found that after prolonged exposure β_3AR mRNA was always increased (≈145%), the earlier time points often revealed variability. We found that the time of the first clear increase in β_3AR mRNA varied considerably, ranging from 0.5 to 6 hr. With these same cells, we also determined changes in receptor density, to assess whether the observed changes in mRNA resulted in altered expression of the receptor. As shown in Fig. 4, β_3AR expression increased during prolonged agonist exposure, reaching 165 ± 9% of the basal levels. This agonist-promoted increase in β_3AR expression of differentiated 3T3-5442A cells is in sharp contrast to that which was found with the β_1AR of the undifferentiated phenotype, which showed a characteristic decrease in receptor number

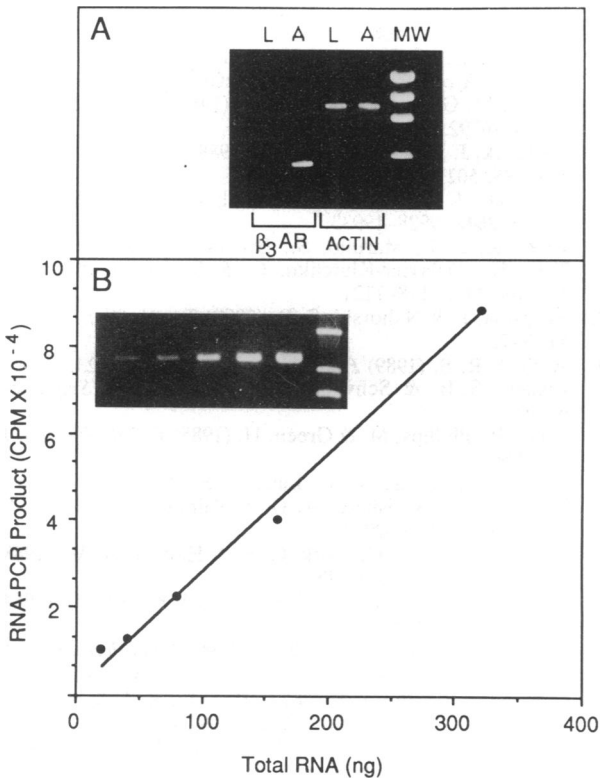

FIG. 2. Specificity and sensitivity of the RNA-PCR protocol used to quantitate changes in β_3AR mRNA from differentiated 3T3-F442A cells. (*A*) Electrophoresis of products from RNA-PCR performed with total RNA from mouse lung (L) and 3T3-F442A adipocytes (A). The two lanes on the left were from reaction mixtures that utilized β_3AR primers, with β_1- and β_2AR-rich lung, showing no discernible product. 3T3-F442A adipocytes expressing β_3AR displayed a single product of the expected size. The two lanes on the right were from reaction mixtures that utilized mouse β-actin primers as a control, and both exhibited a product of the predicted size. The far right lane (MW) contains ϕX174 DNA digested with *Hae* III. The four bands represent DNA lengths of 603, 872, 1078, and 1353 base pairs, respectively. (*B*) Sensitivity and linearity of the RNA-PCR method used to quantitate β_3AR mRNA. Serial dilutions of adipocyte total RNA ranging from 20 to 360 ng were reverse-transcribed, and the resulting cDNA was amplified over 30 cycles. [^{32}P]dCTP was incorporated into the products, which were electrophoresed on a 1.4% agarose gel and visualized by ethidium bromide staining (*Inset*). The bands were excised, and radioactivity was measured to reveal a linear correlation ($R^2 = 0.99$) between the amount of starting material and the PCR product.

(to 31 ± 12% of basal levels) during agonist exposure under the same conditions.

We have demonstrated here a unique form of catecholamine-regulated expression of a βAR subtype. Classically, agonist exposure to β_1- or β_2AR subtypes has led to events that serve to limit the receptor-mediated response either by phosphorylation of the receptor during short-term agonist exposure or by downregulation of receptor expression during long-term exposure (1–4). Such "negative feedback" has been considered to be a protective effect, serving to blunt the cellular response to continuous agonist exposure. In contrast, we have shown here that cells expressing the β_3AR fail to undergo a decrease in receptor number after prolonged agonist exposure and, in fact, display increases in number over time. The increase in receptor expression found here is accompanied by increases in β_3AR mRNA. To assess whether this agonist-promoted increase in cellular mRNA was due to enhanced transcription, we evaluated potential CREs in the 5′ flanking region of the β_3AR. Three of these potential CREs, when inserted into the $pA_{10}CAT_2$ vector,

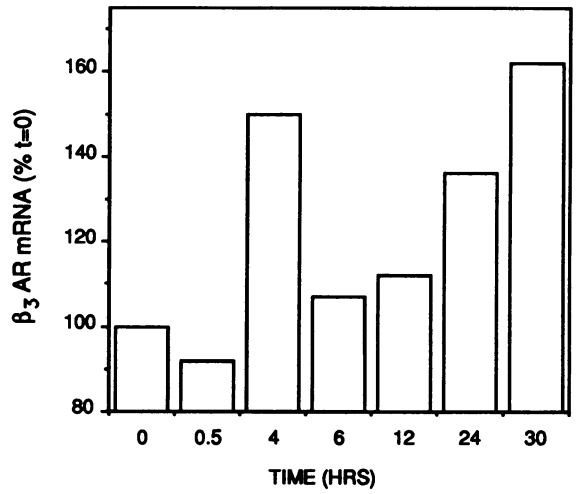

FIG. 3. Changes in β_3AR mRNA during exposure to agonist. Differentiated 3T3-F442A cells expressing β_3AR were exposed in monolayers for the indicated times to 100 μM isoproterenol, 0.2 mM IBMX, and 0.1 mM ascorbic acid. Total RNA was prepared, and a quantitative RNA-PCR was performed using primers for the β_3AR (see text and Fig. 2). Data are normalized to the amount of mRNA in control ($t = 0$) cells. A single experiment is shown where β_3AR mRNA was elevated after as long as 30 hr of incubation with isoproterenol. In four such experiments, β_3AR mRNA was found to be increased to 145 ± 9% of control levels after 24 hr of agonist exposure.

expressed in VERO cells, and exposed to cAMP analogs or agents that raise intracellular cAMP, showed enhanced transcription of the CAT reporter gene. Nuclear runoff studies with 3T3-F442A cells exposed to agonist confirmed increased β_3AR mRNA transcription.

An additional factor that ultimately affects receptor expression is receptor degradation. Although little is known of the determinants of this process with βAR, it appears that phosphorylation of the β_2AR by PKA slightly enhances the ultimate extent of receptor loss (5). Interestingly, the deduced amino acid sequence of the β_3AR (8) has no consensus

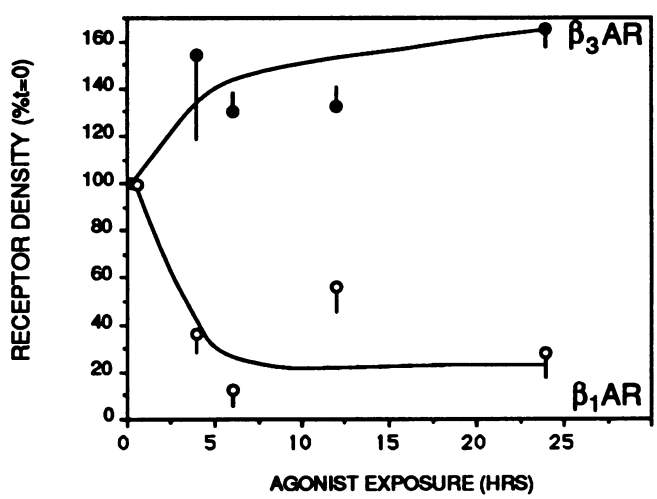

FIG. 4. Effects of agonist exposure on β_3AR and β_1AR expression in 3T3-F442A cells. Cells in monolayers were exposed to 100 μM isoproterenol, 0.2 mM IBMX, and 0.1 mM ascorbic acid for the indicated times and washed, and membranes were prepared. Receptor density was determined by ^{125}I-CYP binding. Results from four experiments are shown. Untreated cells had a β_1AR density of 14.3 ± 3 fmol/mg, which decreased to 31 ± 12% of control levels after 24 hr of agonist exposure. In contrast, β_3AR density (which was 39 ± 10 fmol/mg in untreated cells) increased during agonist exposure to 165 ± 9% of control levels.

sequence for PKA phosphorylation. We do not, however, believe that this lack of a PKA substrate is responsible for our findings. PKA-mediated phosphorylation of the β_2AR does not appear to be a dominant mechanism of agonist-mediated downregulation, as has been shown in studies with the β_2AR of S49 kin$^-$ cells (which lack PKA), HC-1 cells (which lack the catalytic unit of adenylyl cyclase), or recombinant β_2AR in which the PKA sites have been altered (for review, see ref. 5). It appears that the cellular requirements for downregulation are present in 3T3-F442A cells, as we demonstrated a marked decrease in β_1AR expression during agonist exposure to cells of the undifferentiated phenotype (Fig. 4). Although our current study does not address other potential mechanisms that may be in play during agonist exposure, our data reveal that β_3AR regulation is clearly different than that of β_1- and β_2ARs and that a transcriptional mechanism is at least one component of such regulation.

The current study provides evidence that long-term agonist-mediated regulation of βAR expression is subtype-specific. Both β_1- and β_2ARs undergo agonist-dependent decreases in receptor expression during long-term agonist exposure (29, 32). Our findings of upregulation of β_3AR expression are consistent with several *in vivo* studies performed at a time when the identity and the molecular structure of the "atypical adipocyte" βAR was not known (33, 34). During chronic exposure to drugs targeted toward these receptors, low-affinity GDP binding to the atypical βAR in brown adipose tissue increased by several fold, whereas that to the high-affinity site (presumably the β_1AR) did not (31). In these same studies, the antidiabetic and thermogenic responses elicited by these compounds underwent progressive enhancement during chronic agonist exposure (33, 34).

One recurrent theme in some adenylyl cyclase-coupled signal transduction systems is that increased levels of cAMP mediate changes in relevant mRNA levels and/or expressed proteins, which tend to direct the system toward a compensatory lowering of cAMP. For example, increased intracellular cAMP induces an increase in mRNA of a cAMP phosphodiesterase (35), an enzyme that serves to decrease cAMP by digestion. Similarly, increased intracellular cAMP causes an increase in α_2AR mRNA and α_2AR expression (36). The α_2AR inhibits adenylyl cyclase by coupling to the inhibitory guanine nucleotide binding protein G_i, and in settings where intracellular cAMP is increased, the resulting increase in receptor expression would serve to enhance α_2AR-mediated inhibition of adenylyl cyclase. Finally, as discussed earlier, chronic agonist exposure to β_1- or β_2AR (which couple to the stimulatory guanine nucleotide binding protein G_s and increase adenylyl cyclase) results in decreases in β_1- and β_2AR mRNA and receptor expression, thus blunting further receptor-mediated increases in cAMP. In marked contrast to the above scenarios, our current findings with the β_3AR reveal a unique transcriptional control of β_3AR by the end product of the signal transduction cascade, which results in increased receptor expression.

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