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Decreased β_2-Adrenergic Receptor mRNA Expression in Receptor-Deficient S49 Lymphoma Cells

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SUMMARY

Many variants of the S49 mouse lymphoma cell have been isolated along the pathway of cyclic AMP generation and response. Two such variants, β^p and β^d, were isolated by Johnson and colleagues and described in 1979 [*Mol. Pharmacol.* **15**:16–27 (1979)]. The β^p and β^d variants express one half and one quarter, respectively, of the wild-type number of β_2-adrenergic receptors. This observation has now been extended through the use of DNA-excess solution hybridization. Using this exquisitely sensitive technique for quantitation of gene and mRNA, we have been able to demonstrate that the β_2-adrenergic receptor-deficient variant cells contain the same quantity of the β_2-adrenergic receptor gene as the wild-type cells. In contrast, the β_2-adrenergic receptor-deficient variant cells express reduced quantities of β_2-adrenergic receptor-specific mRNA. The amount of β_2-adrenergic receptor-specific mRNA correlates very well with the reduction in receptor expression in these cells. Both gene and mRNA in the wild-type and variant cells appear to be the same size, as judged by Southern and Northern analysis. Thus, the diminution of β_2-adrenergic receptors in the β^p and β^d variants appears to reflect primarily the relative paucity of gene transcripts in the variant cells. These data imply that variations in cellular content of β_2-adrenergic receptor mRNA, which may occur among closely related cells, is one explanation for differences in receptor number.

The β_2-adrenergic receptor has been cloned and sequenced from several species, including mouse, hamster, and human (1–3). In each species, the β_2-adrenergic receptor is encoded by a single intronless gene. This β_2-adrenergic receptor is the prototypical member of a gene family characterized by partial sequence homology, seven clusters of hydrophobic amino acids, consensus sequences for glycosylation in the amino-terminal region and phosphorylation in the carboxyl-terminal region, and a shared functional requirement for a G protein (4). Other members of this family include the other adrenergic receptors, the retinal photoreceptors, the opsins and rhodopsin, muscarinic cholinergic receptors, and several other types of G protein-linked receptors (5). By homology with bacterial rhodopsin (6), the seven clusters of hydrophobic amino acids are postulated to span the membrane. Glycosylation has been confirmed by direct experiment (7–9). Phosphorylation of the carboxyl-terminal region leads to a diminution of activity and is likely to be involved in desensitization (10, 11). The core polypeptide has a molecular weight of about 44,000.

Evidence from several laboratories suggests that the β_2-adrenergic receptor is subject to transcriptional control from a variety of inputs, including cyclic AMP (12–14). Known transcription-regulating elements are located upstream of the open reading frame (15, 16). In spite of this accumulation of knowledge concerning the β_2-adrenergic receptor structure and function, the detailed cellular and molecular mechanisms that regulate transcription of the β_2-adrenergic receptor genes and the subsequent translation of receptor mRNA still remain to be elucidated. One system that has proved valuable in elucidating the functional aspects of receptor regulation is the S49 T lymphoma cell line. Together with variants along the pathway of cyclic AMP generation and response, the S49 cells have yielded much information concerning the β_2-adrenergic receptor-G_s protein-adenylyl cyclase system (17). Two of the S49 cell variants, namely, β^p and β^d, are characterized by decreased expression of β_2-adrenergic receptors. The β^p variant expresses approximately 50% and the β^d variant expresses approximately 25% of the wild-type complement of β_2-adrenergic receptors (18). To define more precisely the lesion in these variant cells, we have investigated the correlation between mRNA and β_2-adrenergic receptor expression in the S49 wild-type and the β_2-adrenergic receptor-deficient variant cells.

Materials and Methods

Cell culture. S49 lymphoma cells were grown at 37° in Dulbecco's modified Eagle's medium supplemented with 10% horse serum

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ABBREVIATIONS: G protein, GTP-binding protein; HEPES, N-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; kb, kilobases; SSPE, 0.18 M NaCl, 10 mM $NaPO_4$, 1 mM EDTA, pH 7.7.

(GIBCO), in an atmosphere of 10% CO_2 in air. Cell number was maintained at not more than 10^6 cells/ml, at which density they were harvested for experimental use. Cell growth and size distribution were monitored routinely on a Coulter counter model ZB-1. Only cultures that were ≥90% viable (as judged by size distribution) and had a normal doubling time (18–20 hr) were used in these studies.

Radioligand binding. All radioligand binding studies were conducted using intact cells. Cells were harvested and washed twice in phosphate-buffered saline before resuspension in binding medium (Dulbecco's modified Eagle's medium supplemented with 0.1% (w/v) bovine serum albumin and 20 mM HEPES, pH 7.4). Approximately 10^6 cells were incubated with radioligand for 1 hr in a volume of 0.5 ml at 37° in a shaking water bath. (−)-[^{125}I]Iodocyanopindolol (≈2000 Ci/mmol; Amersham) was used to label the S49 cell β_2-adrenergic receptor. Nonspecific binding was defined as that which remained on inclusion of 1 μM (±)-propranolol (Ayerst, New York) in the binding reaction. Incubation was terminated by the addition of 10 ml of 20° wash buffer (120 mM NaCl, 20 mM Tris·HCl, pH 7.4) and filtration over Whatman GF/C filter paper on a Brandel cell harvester, followed by two washes. Bound counts were measured on a Packard γ-spectrometer at 85% efficiency. Data were fit by nonlinear regression analysis to a rectangular hyperbola model on a personal computer running GraphPAD (GraphPAD Software, San Diego, CA).

Isolation of DNA and Southern blot analysis. High molecular weight genomic DNA was isolated from S49 cells by the method of Herrmann and Frischauf (19). Approximately 2×10^8 cells were incubated overnight at 37° on a rocking platform, in 10 ml of 50 mM Tris·HCl, pH 9.0, 100 mM EDTA, 200 mM NaCl, 1% (w/v) SDS, 500 μg/ml proteinase K. After phenol extraction, the DNA solution was dialyzed overnight against 20 mM Tris·HCl, pH 7.4, 1 mM EDTA. One tenth volume of 3 M sodium acetate and 2 volumes of ethanol were added to the dialysate, the solution was mixed, and the DNA precipitate was collected by spooling. Genomic DNA was digested to completion with *Hind*III (150 units of enzyme/50 μg of DNA, for 2 hr). The digested DNA was precipitated with ethanol, and 20 μg were fractionated on a 1% (w/v) agarose gel. The agarose gel was blotted onto Nytran (Schleicher and Schuell, Keene, NH), according to the manufacturer's directions. The Nytran was prehybridized, after baking at 65° for 1 hr, with 50% (v/v) formamide, 5× Denhardt's reagent (0.1% ficoll, 0.1% polyvinylhyrrolidine, 0.1% bovine serum albumin), 0.1% (w/v) SDS, 200 μg/ml denatured salmon sperm DNA, 6× SSPE, (1.08 M NaCl, 60 mM $NaPO_4$, 6 mM EDTA, pH 7.7), for 4 hr at 42°. Nick-translated probe was prepared by labeling of a 1.3-kb fragment of mouse genomic DNA, cloned into pUC8, with [α-^{32}P]dCTP. This fragment included the majority of the β_2-adrenergic receptor coding sequence, extending from 107 bases 5′ to the coding sequence and terminating 97 bases short of the 3′ end of the coding sequence. The probe was added in the same medium, at a radioisotopic concentration of approximately 10^6 cpm/ml, immediately after being boiled for 10 min. Incubation was continued for 16 hr at 42°. The blot was washed in two changes of 6X SSPE, 1% SDS, for 15 min at room temperature, followed by two washes in 1X SSPE, 1% SDS, for 30 min at 37° and two washes in 0.1X SSPE, 1% SDS, for 30 min at 42°.

Isolation of RNA and Northern blot analysis. Total cellular RNA was prepared by a guanidinium isothiocyanate/lithium chloride method. S49 cells were harvested and washed twice in phosphate-buffered saline by centrifugation, at 4°. Nucleic acids were precipitated overnight by incubation in 1.4 M guanidinium isothiocyanate, 2.25 M LiCl, 2.5% (v/v) β-mercaptoethanol, at 4°. The insoluble material was separated by centrifugation for 20 min at 20,000 × *g*. RNA was extracted from the pellet by incubation in 1 mM EDTA, 0.2% (w/v) SDS, 20 mM Tris·HCl. The remaining precipitate was re-extracted, and the pooled supernatants were deproteinated before precipitation of the RNA. Total cellular RNA was fractionated on a 1% (w/v) agarose-1.1% (v/v) formaldehyde gel and blotted onto Nytran, according to the manufacturer's directions. The Nytran was probed as described above for Southern blot analysis.

Preparation of the solution hybridization probe. A 17-base oligonucleotide, ATATTGACAATGAAGAA, spanning region 1082–1098 of the mouse β_2-adrenergic receptor, as described by Allen *et al.* (20), was synthesized by the Peptide and Oligonucleotide Synthesis Facility at the University of California, San Diego. Ten picomoles of the oligonucleotide primer were hybridized to 3 pmol of a single-stranded M13mp18 clone containing a 2.2-kb mouse β_2-adrenergic receptor genomic fragment complementary to the message (20). The primer and template were annealed in a volume of 20 μl containing 500 mM NaCl, 50 mM $MgCl_2$, and 50 mM Tris·HCl. This mixture was heated to 65° and allowed to cool to 30° over a period of 30 min. The extension reaction included 2 μl of the annealed primer-template mix in a solution that also contained 10 μg/ml bovine serum albumin, 10 mM dithiothreitol, 0.3 mM dATP, 0.3 mM ddTTP, 50 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; DuPont), 110 μCi of [α-^{32}P]dGTP (3000 Ci/mmol; DuPont), and 2 units of Klenow enzyme (Bethesda Research Laboratories). The reaction was incubated at 37° for 30 min before termination by boiling for 2 min. The extended probe was purified by electrophoresis on a 10% polyacrylamide-8 M urea denaturing gel run at 300 V. The reaction products were visualized by brief exposure to Kodak XAR film. The full length probe, a 35-mer, ATATTGACAATGAAGAAGGGCAGCCAGCAGAGGGT, was excised and eluted in 1% SDS, 5 mM EDTA, 10 mM Tris·HCl, pH 7.5, during a 2-hr incubation in a 65° shaking water bath.

Solution hybridization assay. mRNA and genomic DNA samples were quantitated by hybridization in solution with the radiolabeled probe described above. Total RNA or genomic DNA (50–200 μg) was assayed in a final volume of 30 μl. The genomic DNA was prepared for assay by three passes through a 22-gauge syringe needle to reduce its viscosity, followed by boiling for 2 min to separate the two strands. The hybridization reaction was carried out for 20 hr at 68° in 610 mM NaCl, 1 mM $MgCl_2$, 5 mM EDTA, 10 mM Tris·HCl, pH 7.5, with between 104 and 2×10^4 cpm of probe. Paraffin oil (approximately 100 μl) overlaid the reaction, to prevent evaporation. Single-stranded probe was digested by the subsequent addition of 8 units of S1 nuclease in 1 ml of 300 mM NaCl, 30 mM sodium acetate, 3 mM $ZnSO_4$, 100 μg/ml boiled and sheared herring sperm DNA. The temperature was adjusted to 55°, and incubation was continued for an additional hour. The reaction was terminated by addition of 100 μl of 6 M trichloroacetic acid and filtration over Whatman GF/C filters that were subsequently washed three times with 1% (w/v) trichloroacetic acid and 3% (w/v) sodium pyrophosphate and once with 95% ethanol. Precipitated counts were measured on a γ-spectrometer and were related to M13 β_2-adrenergic receptor standards that were similarly treated.

Quantitation. Protein was quantitated by the method of Peterson (21), using bovine serum albumin as standard. DNA and RNA were quantitated by E_{260}. Data representative of results obtained in three experiments are shown unless noted otherwise. The standard errors, which are ≤ 5% of the mean, have been omitted for clarity.

Results

The S49 variant cells, β^p and β^d, were isolated by Johnson *et al.* (18) in 1979. To ensure that the variant cells have retained their original phenotype over the intervening years, radioligand binding studies were carried out in order to quantitate their expression of β_2-adrenergic receptors. Saturation binding isotherms were performed on intact cells using the high affinity radiolabeled β-adrenergic receptor-specific antagonist [^{125}I] iodocyanopindolol (Fig. 1). We found that the β_2-adrenergic receptor-deficient variant cells expressed their original phenotypes, β^p having 42 ± 13% of wild-type (four experiments) and β^d having 15 ± 4% of the wild-type (four experiments) complement of β_2-adrenergic receptors (Fig. 1). In addition, as was also noted by Johnson *et al.* (18), there was little difference between the dissociation constant for radioligand in the three

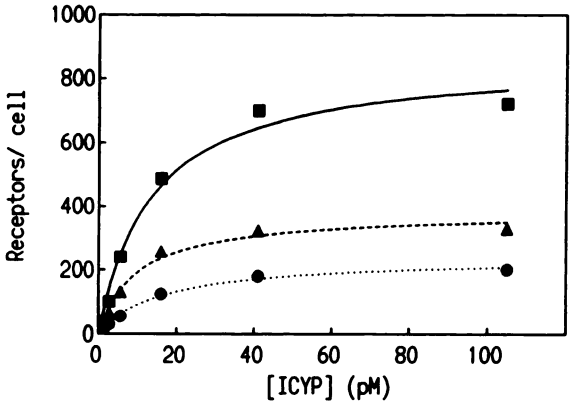

Fig. 1. Binding isotherm of [^{125}I]iodocyanopindolol to S49 cells. Approximately 10^6 cells were incubated with the indicated concentrations of [^{125}I]iodocyanopindolol (*ICYP*), in the presence or absence of an additional 1 μM (±)-propranolol to define specific binding. A representative result of six experiments is shown for wild-type (■), β^p (▲), and β^d (●) S49 cells. Wild-type S49 cell receptor expression was 1865 ± 968 receptors/cell (six experiments), corresponding to approximately 10,000 cpm of [^{125}I] iodocyanopindolol specifically bound. A summary of the differences in β_2-adrenergic receptor expression between wild-type and β^p and β^d variants is given in Table 1.

cell types (13 ± 1, 10 ± 0, and 16 ± 1 pM for wild-type, β^p, and β^d cells, respectively; four experiments).

In an attempt to ascertain the site of action of the lesion(s) responsible for diminished receptor expression in the variant cells, the relative expression of both the gene and the mRNA coding for the β_2-adrenergic receptor was estimated both qualitatively by Southern and Northern blot techniques and quantitatively by DNA-excess solution hybridization. Each of the two methods, blotting and solution hybridization, used a different probe. The probe used for Southern and Northern blot analyses was a nick-translated 1.3-kb fragment of mouse genomic DNA that included the majority of the β_2-adrenergic receptor coding sequence. The solution hybridization studies utilized a single-stranded 35-mer that was derived from the middle of the β_2-adrenergic receptor coding sequence.

Examination of the amount of β_2-adrenergic receptor gene in the S49 wild-type and variant cells showed that the lesion giving rise to the β_2-adrenergic receptor-deficient variant cells is not related to gene dosage. A genomic *Hind*III digest from each of the three cell types was blotted onto Nytran and probed with a nick-translated mouse β_2-adrenergic receptor probe (Fig. 2). The amount of gene coding for the β_2-adrenergic receptor appeared identical in wild-type and β_2-adrenergic receptor-deficient variant β^p and β^d S49 cells. Furthermore, the size of the *Hind*III β_2-adrenergic receptor fragment appeared identical in all three cell types, indicating that there has been no detectable deletion of the gene. The conservation of amount of β_2-adrenergic receptor gene expressed in the wild-type and variant cells that was noted qualitatively on Southern blot analysis was confirmed quantitatively by DNA-excess solution hybridization (Fig. 3).

In contrast to the situation with the β_2-adrenergic receptor gene itself, we were able to measure a marked diminution of β_2-adrenergic receptor-specific mRNA in the β^p and β^d variant S49 cells, compared with that in the wild-type S49 cells. Northern blot analysis of total RNA was undertaken using the same nick-translated probe as that used for the Southern blots (Fig. 4). There was a striking decrease noted in the abundance of β_2-

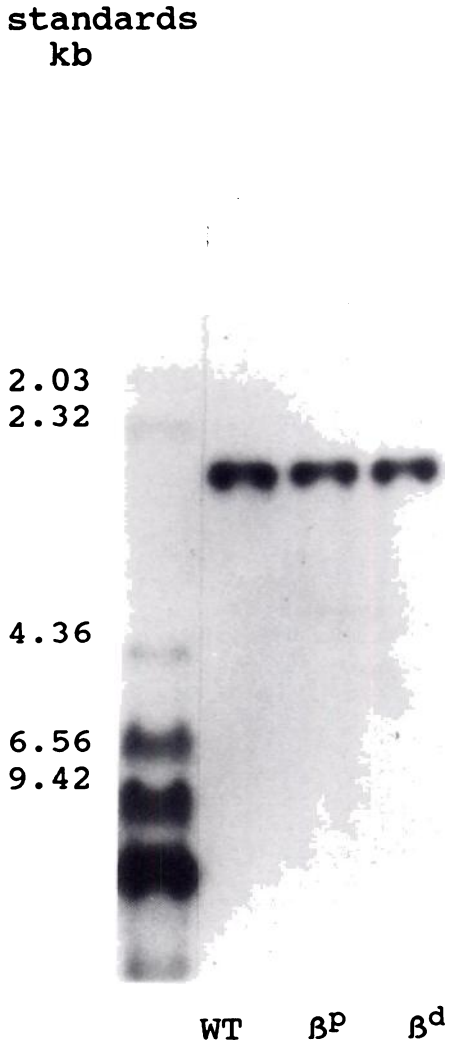

Fig. 2. Southern blot analysis of S49 genomic DNA. High molecular weight genomic DNA from the S49 wild-type (*WT*) and β^p and β^d variants was digested to completion with *Hind*III. Twenty micrograms of the digested DNA were fractionated on a 1% agarose gel, together with size standards prepared by *Hind*III digestion of wild-type phage λ DNA, and blotted onto Nytran. The blot was probed with approximately 10^6 cpm/ml nick-translated probe prepared from a 1.3-kb fragment of mouse genomic DNA that encompassed the majority of the mouse β_2-adrenergic receptor.

adrenergic receptor-specific mRNA. Despite the difference noted in message abundance, the same length transcript appeared to be expressed in each of the three cell types. It appeared from the results of Northern blots that the amount of β_2-adrenergic receptor-specific mRNA might be closely correlated to the expression of β_2-adrenergic receptors in the S49 variant cells. A more quantitative analysis of the message levels was needed to confirm this observation. DNA-excess solution hybridization was used to quantitate β_2-adrenergic receptor-specific mRNA levels in a manner very similar to that used to determine levels of the β_2-adrenergic receptor-specific gene (Fig. 5).

Comparison of the levels of β_2-adrenergic receptor mRNA with those of the β_2-adrenergic receptor itself in wild-type and β_2-adrenergic receptor-deficient β^p and β^d S49 cells showed that they bore a striking correlation (Table 1). Although these observations do not prove that the diminished expression of β_2-

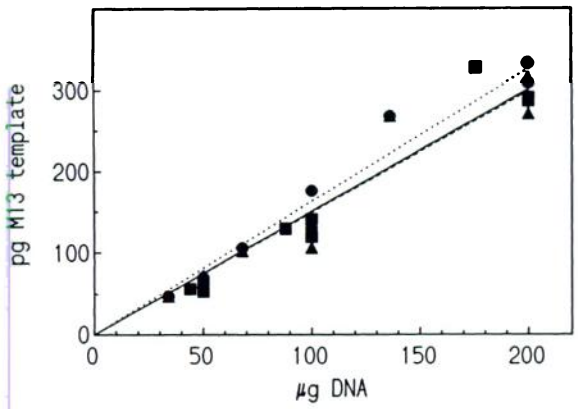

Fig. 3. Quantitation of β_2-adrenergic receptor-specific genomic DNA by DNA-excess solution hybridization. Genomic DNA was prepared from the S49 wild-type and variant cells. β_2-Adrenergic receptor-specific DNA in wild-type and variant cells was quantitated by protection of a ^{32}P-radiolabeled 35-mer against nuclease digestion. Quantitation of β_2-adrenergic receptor gene is expressed relative to the M13 template. The accumulated results from three experiments are shown for wild-type (■), β^p (▲), and β^d (●) S49 cells. Two hundred micrograms of DNA bound 3000–5000 cpm of probe.

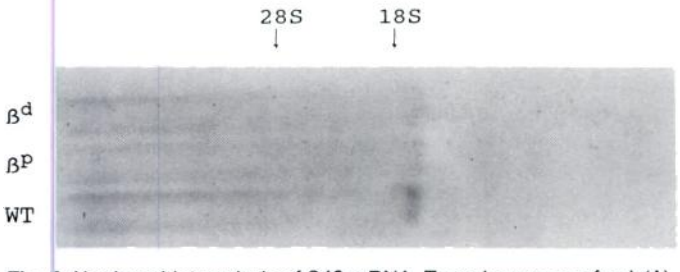

Fig. 4. Northern blot analysis of S49 mRNA. Ten micrograms of poly(A)-enriched RNA from the S49 wild-type (*WT*) and β^p and β^d variants were fractionated on a 1% agarose-formaldehyde gel, together with size standards prepared by *Hind*III digestion of wild-type phage λ DNA, and blotted onto Nytran. The blot was probed with a nick-translated probe prepared from a 1.3-kb fragment of mouse genomic DNA that encompassed the majority of the mouse β_2-adrenergic receptor.

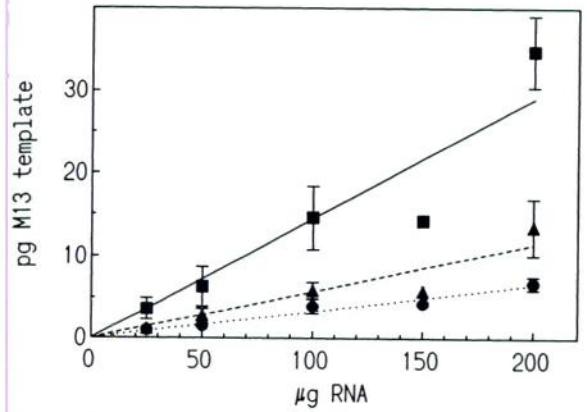

Fig. 5. Quantitation of β_2-adrenergic receptor-specific mRNA by solution hybridization. mRNA was prepared from the S49 wild-type and variant cells. β_2-Adrenergic receptor-specific mRNA was quantitated by protection of a ^{32}P-radiolabeled 35-mer against nuclease digestion. Quantitation of β_2-adrenergic receptor mRNA is expressed relative to the M13 template. The accumulated results from three experiments are shown for wild-type (■), β^p (▲), and β^d (●) S49 cells. Each *point* is the mean ± standard deviation from three separate experiments, except for the 150-µg point (one experiment) and the 200-µg point (two experiments). Two hundred micrograms of wild-type RNA bound 300–500 cpm of probe.

TABLE 1

Levels of β-adrenergic receptors in wild-type and variant cells

The gene coding for the β_2-adrenergic receptor, the mRNA, and the receptor itself were quantitated as described in the text and as shown in Figs. 1, 3, and 5. The interexperimental variability was considerably greater than the intraexperimental variability. A comparison of the results obtained within each experiment is presented here as the mean ± standard deviation percentage, compared with wild-type S49 cells. The results of three separate experiments were pooled for quantitation of the gene and mRNA, and the results of six separate experiments were pooled for quantitation of the β_2-adrenergic receptor.

	Expression		
	Wild-type	β^p	β^d
		%	
Gene	100	102 ± 5	110 ± 4
mRNA	100	40 ± 3	24 ± 5
β_2-Adrenergic receptor	100	42 ± 10	20 ± 8

adrenergic receptors in the β^p and β^d variant cells is caused by diminished expression of β_2-adrenergic receptor mRNA, this hypothesis seems highly likely.

The number of mRNA molecules coding for the β_2-adrenergic receptor should be estimable from these data. In practice, however, it is difficult to determine these numbers precisely, due to uncertainty in the estimation of cellular RNA content. The solution hybridization probe has a specific activity of 39,000 Ci/mmol on the reference date of the radiolabeled nucleotides, because the probe was extended by addition of 18 nucleotides, 13 of which were radiolabeled [^{32}P]dCTP and [^{32}P]dGTP (3,000 Ci/mmol). At 85% counting efficiency, this corresponds to a probe specific activity of 7.3×10^{16} cpm/mmol (3.9×10^4 Ci/mmol × 2.2×10^{12} dpm/Ci × 85% counting efficiency). The binding of radiolabeled probe to the single-stranded M13 β_2-adrenergic receptor template in the standard curves is asymptotic. However, the initial slope of the curves is approximately 14,000 cpm bound/ng of M13 β_2-adrenergic receptor template. The size of the single-stranded template is 9.45 kb, corresponding to a mass of 3.06×10^6 g/mol. Fourteen thousand counts/min of probe correspond to 190 amol of probe, yet 330 amol of template standard are required to bind this amount of probe. Thus, the probe is approximately 60% efficient at binding template. RNA extracted from S49 wild-type cells binds approximately 200 cpm (2.7 amol) of probe/100 µg of RNA. The highest yield of RNA from the S49 cells was 100 µg/10^7 cells. This corresponds to less than one copy of β^2-adrenergic receptor mRNA/wild-type S49 cell.

Discussion

The two receptor-deficient variants, β^p and β^d, were first reported in 1979 by Johnson *et al.* (18). The β^p variant cells were isolated from wild-type S49 mouse lymphoma cells by a selection process that exploited the sensitivity of T lymphocytes to killing by high endogenous concentrations of cyclic AMP. S49 cells were treated with a β-adrenergic agonist, terbutaline, in the presence of phosphodiesterase inhibitor (18). The first round of selection yielded the naturally occurring β^p variant, which expressed approximately a 50% complement of β_2-adrenergic receptors. The second round of selection, which utilized mutagenesis, was expected to yield a variant, β^d, that would be completely devoid of β_2-adrenergic receptors, but this was not the case. Once again, the number of receptors was halved. Although the β^p and β^d variants possess fewer β_2-adrenergic receptors than the wild-type S49 cells, these receptors are, nonetheless, functionally normally (18).

In view of the manner in which the β^p and β^d variants were isolated, it is probable that there are at least two distinct lesions in the β^d cells, one of which occurs in the β^p cells. In this manuscript, we demonstrate that, in both the β^p and β^d variants, the decreased expression of β_2-adrenergic receptors is correlated with decreased steady state expression of β_2-adrenergic receptor-specific mRNA.

We have not yet defined the lesion in the β^p and β^d variants that is responsible for the decreased mRNA expression that must result from either a change in the rate of transcription or a change in the stability of the mRNA. Our preliminary attempts to perform nuclear run-off experiments to assess the rates of transcription have proved unsuccessful, presumably due to the low levels of mRNA expression in the variant cells.

It is attractive to speculate that at least one of the variants is the result of a mutation in a locus involved in regulating transcription of the β_2-adrenergic receptor gene. The promoter region of the β_2-adrenergic gene encompasses several putative regulatory elements of transcription and appears to be highly conserved across species (15). Alteration of any of these elements may be responsible for either or both of the two lesions resulting in decreased β_2-adrenergic receptor mRNA expression. Understanding of the mechanisms involved in transcriptional control of the β_2-adrenergic receptor genome may be facilitated by comparisons between the S49 wild-type and the receptor-deficient variant cells.

A correlation between β_2-adrenergic receptor mRNA and β_2-adrenergic receptor expression has been reported in several settings. In most cases, it appears that β_2-adrenergic receptor expression is correlated with corresponding changes in β_2-adrenergic receptor mRNA expression. For example, when Chinese hamster fibroblast cells were transfected with human β_2-adrenergic receptor DNA, multiple clones were isolated in which β_2-adrenergic receptor expression varied over a 200-fold range. In these clones, there was, nevertheless, good correlation between the amounts of β_2-adrenergic receptor-specific mRNA and β_2-adrenergic receptor expression (22). Likewise, in Chinese hamster ovary cells transfected with hamster β_2-adrenergic receptor DNA, there was also a 200-fold variation of β_2-adrenergic receptor expression in the isolated clones, but there was a good correlation between β_2-adrenergic receptor-specific mRNA and β_2-adrenergic receptor expression (23). Another scheme that can be added to this list is that stable variants of the S49 T lymphoma cell demonstrate close correlation between β_2-adrenergic receptor mRNA and β_2-adrenergic receptor expression.

Exposure of cells to glucocorticoids, β_2-adrenergic agonists, and dibutyryl-cAMP has been noted to alter β_2-adrenergic receptor expression. Glucocorticoids have been found to elevate β_2-adrenergic receptor expression in some cells, and this has been correlated with an increase in β_2-adrenergic receptor-specific mRNA expression (13, 24). In addition, it has been shown that this increase in β_2-adrenergic receptor-specific mRNA expression is due to an enhanced rate of transcription (14) and is apparently regulated by glucocorticoid responsive elements in the 5′ noncoding region of the gene (16).

Down-regulation of the β_2-adrenergic receptor by β_2-adrenergic agonists is widely observed. Agonist-stimulated down-regulation of β_2-adrenergic receptor has been correlated with a decrease in β_2-adrenergic receptor-specific mRNA in S49 cells (25). Down-regulation of β_2-adrenergic receptors has also been associated with decreased expression of β_2-adrenergic receptor mRNA upon chronic exposure of DDT1 cells to agonist (26, 27). Down-regulation of both receptors and mRNA in these cells is blocked by addition of β_2-adrenergic antagonists (26). In contrast, however, brief (30-min) exposure of DDT1 cells to epinephrine has been reported to elevate β_2-adrenergic receptor-specific mRNA, through an increase in the rate of transcription, with no change in β_2-adrenergic receptor expression (27). A cyclic AMP-responsive element was localized to the proximal promoter region of the β_2-adrenergic receptor gene that is presumed to be responsible for the transcription-enhancing activity of β_2-adrenergic agonists (27).

Thus, in several settings, including stably transfected cells expressing β_2-adrenergic receptors and after glucocorticoid treatment, increased β_2-adrenergic receptor expression appears to be correlated with an increased number of β_2-adrenergic receptor mRNA transcripts. In the setting of agonist-induced β_2-adrenergic receptor down-regulation, the situation is more complex, with reports of both increases and decreases in the steady state levels of β_2-adrenergic receptor-specific mRNA levels.

Organization of the β_2-adrenergic receptor genome has been described in human and hamster (2) and mouse chromosomes (3). The gene is located on chromosome 5 in human cells (2). It is not duplicated in any cell that has been examined. Perhaps the most surprising finding that we report here is the low level of β_2-adrenergic receptor mRNA expression in S49 cells. We find less than one β_2-adrenergic receptor mRNA/cell. Such a low level of mRNA expression is not altogether surprising. S49 wild-type cells express approximately 10^3 β_2-adrenergic receptors/cell. Previous observations in our laboratory have indicated that the β_2-adrenergic receptor is an extremely long lived protein, with a half-life in excess of 20 hr in several cells (28, 29). In the case of the S49 cell, the rate of β_2-adrenergic receptor synthesis has been estimated to be relatively slow, requiring many hours (30). This low level of receptor synthesis could be sustained by a proportionately lower number of β_2-adrenergic receptor mRNA transcripts. In addition, receptor expression varies throughout the cell cycle in S49 cells (31). Conceivably, this is due to variation in the amount of β_2-adrenergic receptor mRNA that is expressed at different stages in the cell cycle. The sensitive technique of solution hybridization may prove to be of further value in helping to define regulation of the β_2-adrenergic receptor mRNA in these cells.

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