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Characterization of the Human β_3-Adrenergic Receptor Gene

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SUMMARY

Comparison of the rodent and human β_3-adrenergic receptor cDNAs with the respective genomic sequences has revealed unexpectedly that these genes contain two protein-coding exons. The rat gene was cloned recently and was found to contain three exons and two introns. In the present report, the human β_3 receptor gene was characterized and was found to consist of two exons and a single intron. Sequence analysis of the human β_3 receptor gene identified regions in the intron that were homologous to the second exon and second intron of the rat gene. It appears that both species utilize homologous 5′ donor sites in the first intron and 3′ acceptor sites of the final exon. However, splicing signals within the human intron that are homologous to the second exon of the rat gene are not used. Nuclease protection assays of tissue RNA and polymerase chain reaction-amplified cDNA demonstrated conclusively that β_3 receptor mRNA, containing two protein-coding exons, is expressed in human adipose and intestinal tissues. The pharmacological properties of the full length human β_3 receptor were determined for the first time in Chinese hamster ovary cells, where catecholamine agonists activated adenylyl cyclase with low potency. The β_3 receptor agonists CGP 12177 and BRL 37344 also activated adenylyl cyclase. CGP 12177 was 10–15 times more potent than either isoproterenol or BRL 37344 in stimulating adenylyl cyclase activity. These pharmacological properties differed somewhat from those reported previously for Chinese hamster ovary cells expressing the truncated receptor. However, direct comparison indicates that it is unlikely that the amino acid sequence derived from the second exon can account for these differences.

β-Adrenergic receptors are members of the family of G protein-coupled receptors, which all contain the hallmark of seven transmembrane regions. The three β-adrenergic receptor subtypes that have been cloned are related structurally and functionally and appear to have evolved from a common ancestral gene (1). The rodent and human β_3 receptor genes contain a continuous open reading frame that encodes a protein with seven membrane-spanning regions, and these genes were assumed to consist of a single protein-coding exon (1, 2). We recently reported, however, that the rat, mouse, and human β_3-adrenergic receptors genes each contain two protein-coding exons (3).

The unexpected discovery that the β_3 receptor gene is a split gene raises numerous unresolved issues. For example, the exon/intron organization of the human gene has not been clarified, nor is it known whether this organization has been conserved between rodents and humans. Furthermore, because the human β_3 receptor gene contains a second protein-coding exon, the β_3 receptor protein is larger than previously supposed. Previous analyses of the functional properties of the recombinant human β_3 receptor (2, 4, 5) were performed on an incomplete protein and it is not known whether the terminal six-amino acid sequence derived from the newly discovered second exon alters the functional properties of the human β_3 receptor. However, the pharmacological properties of the β_3 receptor natively expressed in SK-N-MC cells appear to differ significantly from those reported for the receptor expressed in CHO cells transfected with only the first exon of the human β_3 receptor gene (6).

In the present report, we have used a human β_3 receptor cDNA probe to clone and characterize the full human β_3 receptor gene. These data indicate that exon/intron organization of the β_3 receptor gene differs in humans and in rats, owing to the differential use of homologous splicing signals. Additionally, the pharmacological properties of the full length human β_3 receptor were characterized for the first time. These data indicate that the pharmacological properties of the full length receptor differ from those reported originally (2, 4).

Materials and Methods

Cloning of the human β_3 receptor gene. Standard molecular cloning techniques were used, as described by Sambrook *et al.* (7). The cloning of a partial human β_3 receptor cDNA (p184) was described previously (3). This cDNA clone, encoding the terminal 70 amino acids of the receptor and containing 657 bp of 3′ nontranslated sequence, was labeled with random primers and used to screen a human genomic library (Stratagene, La Jolla, CA), to isolate the full length human β_3

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ABBREVIATIONS: CHO, Chinese hamster ovary; PCR, polymerase chain reaction; ICYP, (−)-^{125}I-cyanopindolol; CGP, CGP 12177; BRL, BRL 37344; bp, base pair(s); kb, kilobase(s); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

receptor gene. The genomic clone was then extensively mapped with restriction enzymes and partially sequenced by using the dideoxynucleotide chain termination technique (Sequenase, United States Biochemicals, Cleveland, OH). All nucleic acid sequences in this report were confirmed on both strands.

Expression of the full length and truncated human β_3 receptors in CHO cells. The DNA encoding the full length (408-amino acid) human β_3 receptor was constructed as follows. The 2.4-kb *Pst*I-*Bam*HI fragment of the human β_3 receptor gene was excised from the phage DNA and cloned into pGEM 4z (Promega, Madison, WI) to yield p200. The *Bgl*II-*Bam*HI fragment of p200 was partially digested with *Taq*I and the 1.8-kb fragment was ligated to an oligonucleotide linker containing the following (+)- and (−)-strands: 5′-CGACGGGGCTTC TTGGGGAGTTTCTTAGGT-3′ and 5′-CTAGACCTAAGAAACTC CCCAAGAAGCCCCGT-3′, respectively. This linker contains the *Taq*I restriction site, the protein-coding sequence of exon 2, and an engineered *Xba*I restriction site on the 3′ end. The ligated fragments were recleaved with *Bgl*II and *Xba*I, gel-isolated, and ligated to the *Bam*HI/*Xba*I sites in pGEM 7z to form p201. The *Eco*NI-*Xba*I fragment of p201 was cloned into the filled *Hind*III and *Xba*I sites of the mammalian expression vector pRc/CMV (Invitrogen, San Diego, CA) and the engineered sequence was verified. The resulting construct (p202) begins 64 bp before the translation initiation codon and contains the entire coding sequence (408 amino acids) of the human β_3 receptor.

We previously found that the 5′ donor splice signals that are present at the beginning of the first intron of the human β_3 receptor pre-mRNA are used by CHO cells. Thus, CHO cells transfected with constructs containing these splice signals can express mRNA in which the first exon is inappropriately spliced to unknown sequences derived from the vector or integration site. Therefore, to express the 402-amino acid protein that is encoded by the first exon of the human β_3 receptor, it is necessary to eliminate the 5′ donor splice signal. To do so, the 711-bp *Sac*II-*Taq*I fragment of p200 was isolated and ligated to a double-stranded oligonucleotide linker with the following (+)- and (−)-strands: 5′-CGACGGATAGGT-3′ and 5′-CTAGACCTATCCGT-3′, respectively. This linker contains the *Taq*I and *Xba*I restriction sites and the final codons of the first exon, in which the codon for Gly^{402} was changed to GGA to eliminate the donor splice signal. The resulting DNA was recleaved with *Sac*II and *Xba*I and cloned into the *Sac*II-*Xba*I sites of p202 to form p207.

CHO cells were transfected with p202 or p207 plasmids by the $CaPO_4$ precipitation technique, and stable transformants were selected in the presence of G418 as described previously (8).

Analysis of β_3 mRNA expression in human tissues. Total RNA (10 μg) was reverse transcribed, as described previously (3), with the oligonucleotide primer 5′-TCTAGATCAACAGAGTTGTTGCTTCT TGTCC-3′, which is complementary to mRNA derived from the second exon of the human β_3 receptor. One tenth of the resulting cDNA was amplified by PCR with the aforementioned primer and the primer 5′-TGCGAATTCTGCCTTCAACCCGCTC-3′, which is sequence found near the end of the first exon (1). PCR was performed as described previously (3). Briefly, samples were denatured at 94° for 2 min, annealed at 63° for 1.5 min, and extended at 72° for 2 min. After 15–20 cycles, 0.5–5 μl of the reaction were removed and the PCR products were identified by nuclease protection assay (3). In some experiments nuclease protection assays were performed directly on total RNA, without prior amplification by PCR. The cRNA probes used in the protection assay were derived from genomic DNA (p174) or cDNA (p192). The probe derived from p174 spans the first exon/intron junction (3), whereas p192 is the *Eco*RI-*Nco*I subclone of p184 (3) and spans the junction of exon 1 and exon 2.

RNA from colon and adipose tissue was from sources described previously (3). RNA from CaCO and T-84 cells and human ileum was generously supplied by D. Rao (University of Illinois).

Southern blot analysis of human and rat genomic DNA. Rat and human genomic DNA (Promega) was digested with the indicated restriction enzymes, and the resulting fragments were resolved by gel electrophoresis and transferred to nitrocellulose. Blots were then hybridized with rat or human β_3 receptor cDNA probes (6, 8) that had been labeled with [^{32}P]dCTP using random primers. The rat and human cDNAs used were homologous sequences that encoded the predicted fourth through seventh transmembrane regions of each receptor (8). After overnight hybridization at 42°, blots were rinsed and then subjected to three 30-min washes at 55° in 0.1× standard saline citrate (15 mM NaCl, 1.5 mM trisodium citrate) containing 0.1% sodium dodecyl sulfate. After autoradiography, blots were stripped and reprobed, so that each blot was hybridized to probes from both species.

Adenylyl cyclase assay. Adenylyl cyclase activity was determined by the method of Salomon (9). Culture medium was removed and cells were washed in phosphate-buffered saline and then harvested in 25 mM HEPES buffer, pH 8.0, containing 2 mM $MgCl_2$ and 1 mM EDTA. Cells were lysed and centrifuged at 48,000 × g for 15 min to obtain crude membranes. Membrane pellets were resuspended by homogenization and used directly or frozen at −80° until used. Freezing did not affect activity. Membranes (5–15 μg of protein) were preincubated for 15 min at 4° with the specified drugs in a volume of 40 μl. Adenylyl cyclase reactions were initiated by addition of substrate mixture and were terminated after 30 min at 30°. Concentration-response data were analyzed by nonlinear regression analysis with a one-site mass action equation (Enzfitter; Elsevier Biosoft). Statistical comparison of drug potency and efficacy with the full length and truncated human β_3 receptors was performed by two-way analysis of variance.

Radioligand binding assay. Cells were harvested as described above and membranes were resuspended in 75 mM Tris, pH 7.4, 12.5 mM $MgCl_2$, 2 mM EDTA, 1 mM ascorbic acid. Saturation analysis was performed with concentrations of ICYP (DuPont NEN, Boston, MA) ranging from 65 pM to 4 nM, and 1 mM isoproterenol was used to define nonspecific binding. For competition studies, 100 μM desmethylimipramine (2) was included in the incubation to reduce nonspecific binding. Competition analysis of CGP could not be performed because this compound displaced ICYP binding in nontransfected CHO-K1 cells. Incubations were carried out in a volume of 150 μl for 1 hr at 30° and were terminated by vacuum filtration over glass fiber filters. K_i values were calculated from IC_{50} values, which were determined by nonlinear regression analysis of two or three experiments, each performed in triplicate.

Results

We previously reported the cloning of the 3′ end of the human β_3 receptor cDNA from SK-N-MC neuroblastoma cells (3). Comparison of the cDNA with the published genomic sequence (1) demonstrated that the human β_3 gene, like the rodent genes, contained at least one intron and two exons. To determine the structure of the human β_3 receptor gene, a human genomic DNA library was screened with the 3′ end of the human β_3 receptor cDNA (3). Shown in Fig. 1A is a restriction map of the human β_3 receptor gene clone that was obtained. Fig. 1B illustrates the exon/intron organization of the human β_3 receptor gene, as deduced by comparison with the β_3 receptor cDNA (3). The first exon of the human gene contains a large open reading frame that encodes 402 amino acids and includes all of the predicted seven transmembrane regions. The single intron interrupts the protein-coding sequence six amino acids from the carboxyl terminus and is approximately 1 kb long (Fig. 1C). Like the first intron of the rodent genes (3), the human intron begins after the second base within the codon and the 5′ donor splice site occurs within the sequence context GTAG, which, if unspliced, would contain an in-frame translation termination codon. As expected from the cDNA, a canonical 3′ acceptor site was found before the second exon. The second exon contains the codons for the final six amino acids

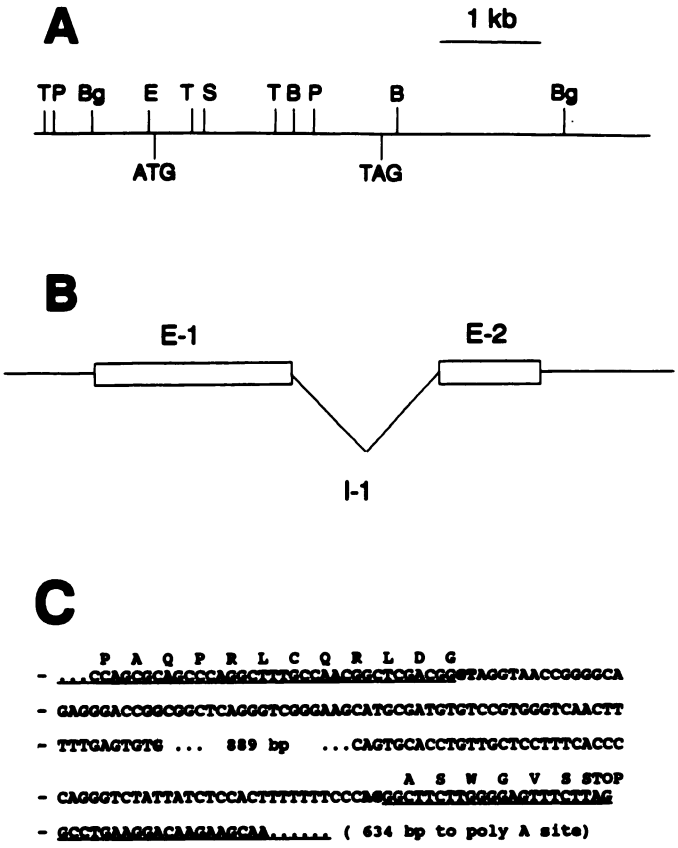

Fig. 1. Structure of the human β_3 receptor gene. A, Restriction map of the human β_3 receptor gene, indicating location of the translation initiation (ATG) and termination (TAG) codons. *T*, *Taq*I; *Bg*, *Bgl*II; *E*, *Eco*NI; *S*, *Sac*II; *B*, *Bam*HI; *P*, *Pst*I. B, Schematic diagram of the organization of the human β_3 receptor gene. *E*, exon; *I*, intron. C, Amino acid and nucleic acid sequences of exon/intron junctions of the human β_3 receptor gene. The illustrated sequence of the first intron begins with Pro^{391}. Exons are *underlined* and splice signals are in *bold*.

previously described (3) and nontranslated sequence to the polyadenylation signals.

The overall organization of the human β_3 receptor gene differs from that of the rat gene (3), which contains three exons and two introns (Fig. 2, *top*). As reported previously (8), the human and rat genes are highly (80%) homologous in the coding block of the first exon. The nucleic acid homology between the rat and human β_3 receptor genes then declines rapidly near the end of the first exon, before the donor splice site. In this regard, the donor splice site of the first intron is displaced by 42 bp between species (i.e., within Arg^{388} of the rat and within Gly^{402} of the human).

The position of the first intron and the differential exon/intron organization of the human and rat β_3 receptor genes raised questions of whether the newly discovered introns and exons were in fact homologous. To address these questions, the nucleic acid sequences of the rat and human genes were aligned, and two regions of significant homology were noted. One was found at the first exon/intron junction (Fig. 2, *region A*), where the 5′ ends of the introns are about 55% homologous over the first 200 bp. Thereafter, the homology between the rat and human genes declines, and it appears that the human gene contains about 350 bp of intervening sequence that is not present in the rat gene. The second region of significant homology was detected immediately surrounding the final exon

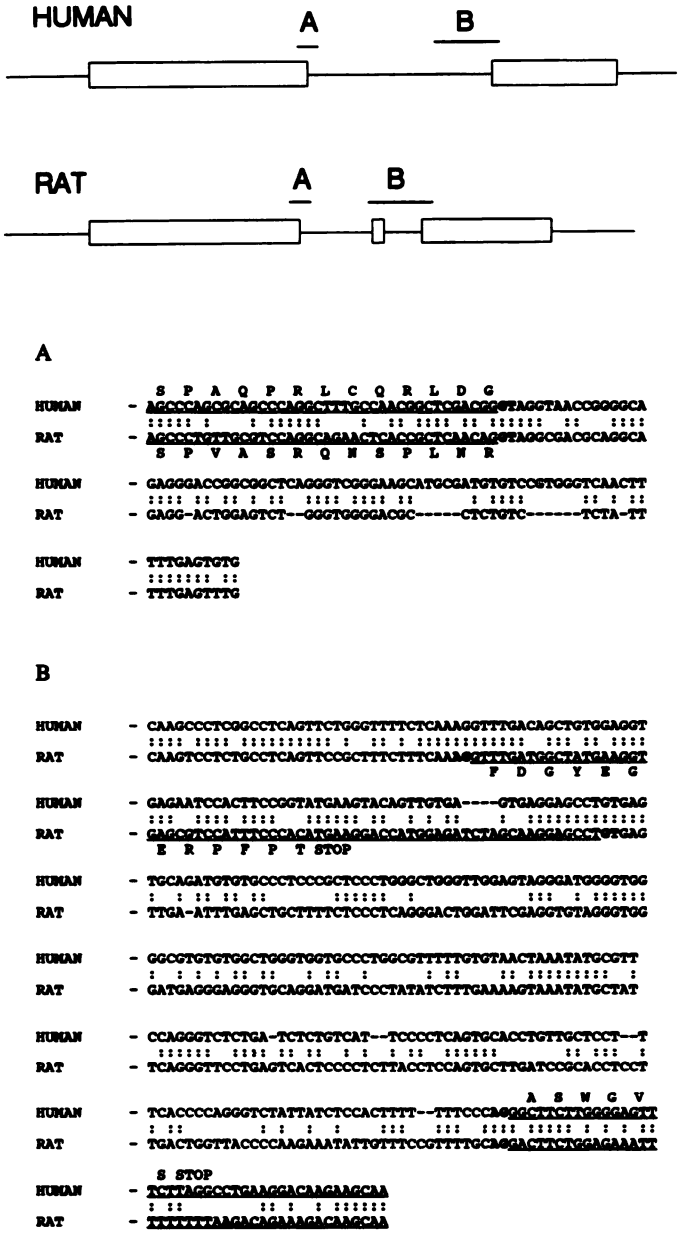

Fig. 2. Comparison of the human and rat β_3 receptor genes. *Top*, comparison of exon/intron organization. *Overlined regions A* and *B* correspond to sequences aligned below. *Bottom*, nucleic acid alignment of the first exon/intron junction (*A*) and the region containing the second and third exons of the rat gene (*B*). Exons are *underlined* and splice signals are in *bold*. The amino acid sequence of protein-coding regions is given in single-letter code.

(Fig. 2, *region B*). Sequence in the distal one third of the human intron is about 60% homologous to the second exon and intron of the rat gene. Although the human gene contains sequence that is similar to the second exon and intron of the rat gene, these splicing signals do not appear to be used in the human gene (3). Consequently, the final protein-coding sequence of the human gene is homologous to the third exon of the rat gene, which encodes the 3′ nontranslated region, whereas the final protein-coding sequence of the rat gene is homologous to sequence in the first intron of the human gene. The 3′ nontranslated sequences of the human and rat β_3 receptor genes are similarly sized (657 and 736 bp, respectively); however, the overall homology between them is very low.

Based upon differences in the pharmacological properties of the cloned rat and human β_3-adrenergic receptors, it has been suggested that these receptors are not species homologues (5). To examine this proposition, restriction fragments of human genomic DNA were probed with a rat cDNA probe, and vice versa. If the cloned rat and human β_3 genes are species homologues, then a probe derived from either gene should identify the same unique restriction fragments of a given species. As shown in Fig. 3, the rat and human probes separately identified the same unique restriction fragments from human genomic DNA. Analogous results were obtained with rat genomic DNA (data not shown). These data strongly indicate that the rat and human β_3 receptor genes are related more closely to each other than they are to any other rat or human gene.

In rats, the β_3 receptor is abundantly expressed only in adipose tissues (8). Whether the β_3 receptor is expressed in humans, however, is controversial. Previously, analysis of β receptor mRNA in human adipose tissue with a sensitive solution hybridization assay clearly identified β_1 receptor mRNA, but β_3 receptor transcripts were absent at the level of detection (3). An assay based upon the PCR was developed to greatly increase the sensitivity of the assay. RNA from human omental adipose tissue was reverse transcribed and the resulting cDNA was amplified with (+)- and (−)-strand primers corresponding to sequences in the first and second exons, respectively. RNA from SK-N-MC cells was also included as a positive control and to provide a rough comparison of the relative abundance of β_3 transcripts in these cells and in human fat. After amplification, PCR products were identified by nuclease protection assay using cRNA probes that distinguish cDNA containing both exons from unspliced/genomic DNA. The probes were derived from genomic DNA (p174) or cDNA (p192), and each contains sequence that is complementary to the terminal 194 nucleotides of exon 1. Thereafter, the p174 probe is complementary to the first intron, whereas the p192 probe is complementary to the second exon. PCR-amplified β_3 receptor cDNA from human omental adipose tissue protected only 194 nucleotides of the gene-derived probe but protected 247 nucleotides of the cDNA-derived probe (Fig. 4). The size difference of the protected probes is due to the presence of sequence from exon 2 in the amplified cDNA and demonstrates clearly that human adipose tissue expresses β_3 receptor mRNA that is spliced as described in Fig. 1. We previously reported that β_3 mRNA could not be directly detected in human colon by nuclease protection assay. However, β_3 transcripts could be detected in the colon after PCR amplification, and these transcripts, like those in adipose tissue, were found to contain two protein-coding exons (Fig. 4, *right*).

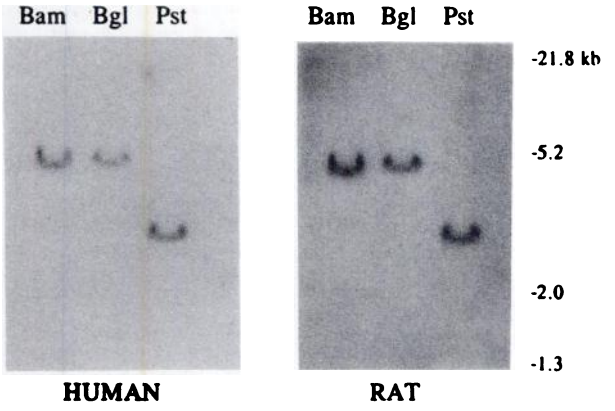

Fig. 3. Relationship between the human and rat β_3 receptor genes. Human genomic DNA (5 μg) was digested with *Bam*HI (*Bam*), *Bgl*II (*Bgl*), or *Pst*I (*Pst*), and the resolved fragments were hybridized to ^{32}P-labeled rat or human cDNA probes. Shown are autoradiograms of the same blot sequentially hybridized to the human (*left*) and rat (*right*) probes. The sizes of standards are indicated on the *far right*. Both probes recognized the same unique fragments of human genomic DNA.

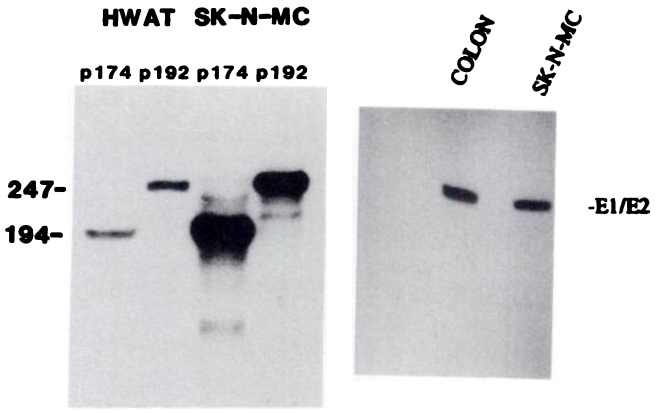

Fig. 4. Detection of β_3 receptor mRNA in human adipose tissue. *Left*, RNA from human omental adipose tissue (*HWAT*) or SK-N-MC cells was reverse transcribed and the resulting cDNA was amplified as detailed in Materials and Methods. The resulting PCR products were identified by nuclease protection assay. Shown is an autoradiogram of labeled cRNA probes that were protected from nuclease digestion by amplified cDNA. The sizes of the cRNA probes that were protected from digestion were exactly as predicted for mRNA containing both exons described in Fig. 1, as clearly indicated by the greater size of the p192-derived probe. *Right*, qualitative detection of RNA from human colon or SK-N-MC cells. RNA was amplified as described above, and PCR products were identified by nuclease protection assay with p192-derived probe. Under the conditions used the amplfied DNA was in molar excess over the probe, so the results are not indicative of the relative abundance of RNA in the starting material. The protected fragment is indicative of cDNA containing both exons 1 and 2 (*E1/E2*).

The presence of β_3 receptor mRNA in the colon prompted an examination of whether β_3 mRNA could be detected in human intestinal cells without prior amplification by PCR. RNA from human intestinal cell lines (CaCO and T-84), as well as from human ileum and colon, was subjected to nuclease protection assay with the p192 (cDNA) probe. β_3 receptor mRNA was clearly present in the human ileum, and barely detectable levels were found in the CaCO cell line (Fig. 5). This assay was performed under quantitative conditions (i.e., the probe was present in excess of cellular β_3 mRNA), and it is clear that levels in the human ileum are substantially lower than those found in SK-N-MC cells.

SK-N-MC cells were previously found to contain small amounts of unspliced β_3 receptor RNA (3). This RNA species was not detected by the PCR technique described above (Fig. 4). The reasons for this failure are not completely known; however, unlike the mRNA species containing both exons, the unspliced form is not enriched by oligo(dT) chromatography, nor can it be amplified by the rapid amplification of complementary ends technique.[1] Additionally, the abundance of the unspliced form can vary greatly among SK-N-MC cultures (e.g., Fig. 5 and Ref. 3). These observations suggest that the unspliced RNA seen previously may represent primary transcripts or aberrantly processed β_3 receptor RNA.

[1] J. G. Granneman and K. N. Lahners, unpublished observations.

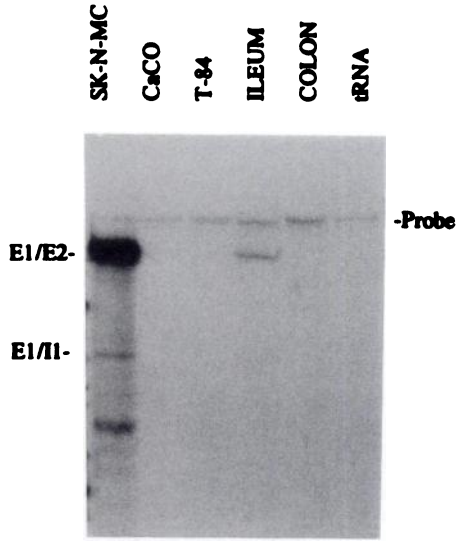

Fig. 5. Direct detection of β_3 mRNA in human intestinal and neuroblastoma cells. Total RNA (25–50 μg) was analyzed by probe-excess nuclease protection assay with a probe derived from p192. Shown is an autoradiogram (4-day exposure) of protected fragments. *Probe*, undigested probe; *E1/E2*, fragments indicative of mRNA containing both exons; *E1/I1*, fragments indicative of unspliced RNA or genomic DNA.

TABLE 1

Effects of β receptor agonists on adenylyl cyclase activity in membranes of CHO cells that express the full length (408-amino acid) human β_3 receptor

Values are means ± standard errors of the number of experiments given in parentheses.

Binding agonist	K_{act}	Intrinsic activity (relative to isoproterenol)	Inhibition of ICYP, K_i
	μM		μM
Isoproterenol	5.4 ± 0.8 (9)	1	2.6 ± 2.3 (3)
Norepinephrine	48 ± 13 (9)	0.79 ± 0.09	11.0 ± 0.2 (2)
Epinephrine	56 ± 22 (3)	0.72 ± 0.09	ND[a]
BRL	7.3 ± 1.0 (9)	0.55 ± 0.05	0.9 ± 0.4 (3)
CGP	0.51 ± 0.12 (9)	0.59 ± 0.04	ND

[a] ND, not determined.

CHO cells were stably transfected with p202, to study the pharmacological properties of the full length (408-amino acid) human β_3 receptor. Catecholamine agonists activated adenylyl cyclase with a potency order of isoproterenol > norepinephrine ≥ epinephrine (Table 1). We also analyzed two additional agonists that have been useful in characterizing the β_3 receptor (Table 1). BRL and CGP were both partial agonists, with similar intrinsic activities (about 60%), relative to isoproterenol. However, CGP was 10–20 times more potent than BRL in activating adenylyl cyclase. ICYP bound to the β_3 receptor in CHO cell membranes with an estimated K_d of 920 ± 200 pM (mean ± standard error, three experiments). Competition data were in good agreement with the functional analysis, indicating that the β_3 receptor has a relatively low affinity for catecholamines.

The pharmacological properties of the full length receptor differed somewhat from those determined in previous analyses (2, 4), which apparently were performed on a receptor with an incomplete carboxyl tail. In this regard, recent work showing that truncation of the carboxyl tail greatly alters the pharmacological properties of the avian β receptor (10) prompted us to examine whether the protein-coding sequence contributed by the second exon influences the ability of full and partial agonists to activate the β_3 receptor. For this purpose, CHO cells were stably transfected with DNA constructs encoding the full or truncated human β_3 receptor (see Materials and Methods) and adenylyl cyclase assays were performed in parallel. The potencies and efficacies of various agonists in activating adenylyl cyclase are given in Table 2. No significant differences between receptors were found either in the potency of the various drugs or in the maximal stimulation of adenylyl cyclase by the full agonist isoproterenol. The intrinsic activity of the partial agonists appeared to be slightly greater in membranes of CHO cells expressing the full length receptor. However, this trend was not consistently found in other clones, nor was it present in 293 cells expressing the recombinant receptors (data not shown).

Discussion

Comparison of the cDNA sequence (3) with the published genomic sequence (1) demonstrated that, contrary to expectation, the human β_3 receptor gene is a split gene. In the experiments described above we used a cDNA probe containing the final exon of the human β_3 receptor to complete the cloning of the full gene and to determine its exon/intron structure. Additionally, we have examined the pharmacological properties of the full length receptor for the first time and determined whether the additional, novel, protein-coding sequence could account for important discrepancies in the literature.

Organization of human and rat β_3 receptor genes. The β-adrenergic receptor subtypes are members of the family of G protein-coupled receptors. The high degree of homology among these receptors and the absence of introns in the human and hamster β_2 receptor genes (11) indicate that the β receptor subtypes evolved by duplication of a processed gene. If so, then it would appear that the β_3 receptor gene acquired intron(s) and additional exon(s) sometime after the duplication of the ancestor gene. The presence of homologous regions within the introns of the rat and human β_3 receptor genes leaves little doubt that these basic elements were present in the ancestral gene before the divergence of humans and rats.

The human β_3 receptor gene was found to contain two exons and a single intron, in contrast to the rat gene, which contains three exons and two introns (3). Although the organization of the gene differs between species, the single human intron contains sequences that are clearly homologous to the second exon and second intron of the rat gene. Analysis of these sequences indicates that both species utilize homologous 5′

TABLE 2

Effects of β receptor agonists on adenylyl cyclase activity in membranes of CHO cells expressing the full length (408-amino acid) or truncated (402-amino acid) human β_3 receptor

Values are means ± standard errors of five experiments.

Agonist	K_{act}		Intrinsic activity (relative to isoproterenol)	
	β_3-408	β_3-402	β_3-408	β_3-402
	μM			
Isoproterenol	6.7 ± 1.5	6.5 ± 0.7	1	1
Norepinephrine	59 ± 23	34 ± 8	0.75 ± 0.13	0.54 ± 0.02
BRL	6.6 ± 1.5	11.2 ± 1.4	0.59 ± 0.07	0.46 ± 0.07
CGP	0.55 ± 0.19	0.72 ± 0.23	0.62 ± 0.05	0.53 ± 0.05

donor splice signals in the first intron and 3′ acceptor signals of the final exon. However, because the splicing signals related to the second exon of the rat gene are not functional in the human gene, this gene is composed of two exons, rather than the three exons found in the rat. Furthermore, the size and origin of the final protein-coding sequence differ between species (Fig. 2, *region B*).

On the basis of pharmacological differences between the rat and human β_3 receptors, it was recently suggested by Liggett (5) that the rodent and human β_3 genes that have been cloned are not species homologues. Analysis of the intron sequences of the human and rat genes, however, indicates that these genes are closely related. Indeed, probes derived from either the human or rat β_3 gene identify the same unique restriction fragments of human genomic DNA (Fig. 3), indicating that these genes are related more closely to each other than to any other human or rat gene. Furthermore, the human and murine β_3 genes have been localized to homologous chromosomal regions (12). Taken together, these observations provide strong evidence that the rodent and human β_3 genes that have been cloned to date are the authentic species homologues.

The human and rodent β_3 receptor genes each contain a large open reading frame, encoding seven membrane-spanning regions, followed by a 5′ donor splice site within the sequence GTAG. This sequence is ambiguous because it contains both the 5′ donor splice signal (GT) and an in-frame translation termination codon (TAG). In this regard, Machida *et al.* (13) noted that the continuous homology between the human and rat β_1 receptor genes is interrupted by a 99-bp segment that begins with the same sequence (GTAG) and ends with a consensus 3′ donor splice signal, suggesting that this element is an intron. We have cloned rat β_1 receptor cDNA (14); however, both sequence analysis and nuclease protection analysis indicate that these splicing signals are not used in adult rats.[1] Nevertheless, taken together these observations suggest that the rat β_1 receptor gene may once have contained an intron as well. Comparison of the human and rat β_3 receptor genes indicates that there are species differences in the utilization of splicing signals and raises the possibility that a functional intron might be found in the β_1 receptor genes of other species.

Expression of the β_3 receptor in humans. In contrast to β_3 receptor expression in rodents, the question of whether humans normally express the β_3 receptor has been controversial. We previously performed direct quantitative analysis of β receptor mRNA in human adipose tissue (3) and found that β_1 receptor mRNA was readily detected but β_3 receptor mRNA was below the detection limit of the assay (about 4 copies/cell) in the same samples. In the present study we were able to detect β_3 receptor mRNA in adipose tissue after reverse transcription and PCR amplification and to further show that this mRNA contains two protein-coding exons. Taken with our previous studies (3, 15), these data indicate that the steady state levels of β_3 receptor mRNA in human fat are much lower than those found in the rat. The reasons for the comparatively low expression level found in human adipose tissue are unclear; however, the low level of β_3 receptor mRNA suggests that expression may be limited to a subset of cells, possibly brown adipocytes (16), within omental tissue. It seems likely that the genetic elements that are important in conferring abundant fat-specific expression in rodent fat tissue may not have been conserved in humans. In this regard, it may be significant that only 6 of 12 bp of a nuclear factor-1-like binding site found in the first intron of the rat gene (3) are conserved in the human gene.

β_3 receptor mRNA containing two exons was also clearly detected in the human intestine. Indeed, β_3 transcripts in the ileum were present in amounts that could be detected without prior amplification by PCR. These data and very recent work from Krief *et al.* (16) refute the recent claim by Thomas and Liggett (17) that humans do not express the β_3 receptor mRNA. The conclusions of the latter study were based upon the failure to amplify β_3 receptor cDNA from numerous tissues of a single individual. In contrast, we have found that the abundance of β_3 receptor mRNA in the human ileum is sufficient to be detected by solution hybridization without prior amplification by PCR and that these levels are comparable to those found in the rat (8). Functional responses to β_3 receptor agonists have been observed throughout the rodent gastrointestinal tract (18–20), although steady state levels of β_3 mRNA are much lower in the intestine than in adipose tissue (8). These observations indicate that quantitative differences in the tissue levels of β_3 mRNA may not be predictive of functional activity. Thus, it would seem inappropriate to conclude that the β_3 receptor plays no role in human tissues on the basis of low steady state levels of β_3 mRNA or upon a failed attempt to amplify β_3 cDNA from a single individual (17).

Pharmacological properties of the full length and truncated receptors expressed in CHO cells. As mentioned in the introduction, all previous characterizations of the pharmacological properties of the recombinant human β_3 receptor used cells transfected with incomplete DNA constructs. Furthermore, the nature of the recombinant proteins expressed in the previous studies is uncertain. On one hand, it is possible that these cells expressed proteins that were truncated due to the presence of an in-frame stop codon at the end of the first exon. On the other hand, it is possible that these cells produced unanticipated fusion proteins owing to the use by CHO cells of the splicing signals present in these constructs (3). Given these considerations, it is not surprising that important pharmacological discrepancies exist in the two independent reports that have characterized the human β_3 receptor with adenylyl cyclase assays (4, 5). We therefore felt it important to establish the pharmacological properties of the complete human β_3 receptor and to determine whether the amino acid sequence derived from the second exon could account for the discrepancies in the literature.

Like the rat β_3 receptor (8), catecholamine agonists exhibited low potency with the full length human β_3 receptor. In addition, the present data indicate that BRL is equipotent with isoproterenol but 15 times less potent than CGP. This potency order was similar to that reported by Liggett (5) for the incomplete receptor but differed from that reported by Fève *et al.* (4), who found that BRL was 4–6 times more potent than isoproterenol or CGP in adenylyl cyclase assays. The reasons for this discrepancy are unclear; however, direct comparison of the full length and truncated receptors indicates that it is not due to the presence of sequence from the second protein-coding exon.

In summary, the present report demonstrates that the human β_3 receptor gene contains two exons. Differences in the intron/exon organization of the rat and human β_3 genes can be understood in terms of differential use of homologous splicing signals related to the second exon of the rat gene. Despite these

differences in the organization of the rat and human genes, all available data indicate that they are true species homologues. β_3 receptor mRNA can be detected in human adipose and intestinal tissue. Lastly, pharmacological analysis indicated that catecholamines have low affinity for the full length human β_3 receptor and that CGP was the most potent and efficacious "atypical" agonist tested.

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