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Molecular cloning of a human β3-adrenergic receptor cDNA

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We report the molecular cloning of a β3-adrenergic receptor [β3-AR] cDNA from human brown adipose tissue. The cDNA-encoded protein is identical to the previously cloned β3-AR but with 6 additional amino acids at the C-terminus. The C-terminus is shared by the β3 receptors expressed in human neuroblastoma cells [SK-N-MC] [Mol. Pharmacol 42 (1992) 964–970]. Furthermore, using a polymerase chain reaction strategy we have cloned and sequenced the β3-AR introns Sequence analysis demonstrates that the human β3-AR gene comprises at least 3 exons and 2 introns and that the most abundant β3-AR transcripts encode a protein with an exon 3-derived C-terminus. Interestingly, although a similar organization has been found in rodent genes, the rat β3-AR transcripts encode a receptor with an exon 2-derived C-terminus.

Intron; Alternative splicing; Polymerase chain reaction

1. INTRODUCTION

The existence of atypical β-adrenergic receptors (β-ARs) was postulated to explain the non-β1- and non-β2-adrenergic responses of rat brown and white adipose tissues [1]. Since then, β-ARs, pharmacologically similar to those of adipocytes, have been found in colon [2,3], ileum [4], gastric fundus [5], heart [6] and skeletal muscle [7] of rodents. Subsequently, the molecular cloning of a gene related to the β1- and β2-AR genes termed β3, in human [8] and rodent [9 12], has confirmed the existence of another β-AR with a similar tissue distribution to that of the atypical β-ARs. Nevertheless, the pharmacological characteristics of the cloned rat or human β3-AR genes expressed by transfected CHO cells have raised a question concerning the relationship between atypical β- and β3-ARs [13]. Elements of answer to that question were brought since the rodent and human β3-AR genes have been shown to contain intron(s) [12,14]. The splicing of intron(s) results in rodent as in SK-N-MC, a human neuroblastoma cell line, in the expression of a β3-AR with several more amino acids at the C-terminus than receptors encoded by unspliced transcripts [14]. Interestingly, based on pharmacological evidence, the SK-N-MC β3-AR more closely resembles that of the cloned rat β3-AR than that of the cloned human β3-AR expressed from an unspliced version of the gene [15]. Therefore, the pharmacological discrepancies with the rat β-AR reported for the cloned human β3-AR might be related to its truncated C-terminus. To address the question of the primary structure of the human β3-AR expressed in normal human tissues we have cloned the β3-AR cDNA from human brown adipose tissue. Furthermore, using our cDNA sequence, we have determined the primary structure of the human β3-AR introns.

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2. MATERIALS AND METHODS

2.1. *Construction and screening of a cDNA library*

Total RNA from infant brown adipose tissue was prepared by using the acid guanidinium isothiocyanate phenol chloroform method [16]. Poly(A)RNA was isolated from total RNA by oligo(dT)-cellulose chromatography as described by Aviv and Leder [17]. The cDNA library was constructed in the vector pTZ18 from Pharmacia using the primer-adapter procedure [18]. 2×10^6 primary recombinants were analyzed as described by Grunstein and Hogness [19] using a labeled probe derived from genomic sequences. DNA sequences were determined by the method of Sanger [20].

2.2. *Polymerase chain reaction (PCR)*

Specific primers derived from the cDNA sequence (positions 1,299–1,320 and 1,741–1,717) were used to amplify human genomic DNA as described by Bensaid [12]. The PCR product was blunt-ended, digested by *Sst*I and inserted between the *Sma*I and the *Sst*I sites of the M13 mp19 phage for cloning and sequencing.

3. RESULTS

Ten β3-AR cDNAs were isolated from a human brown adipose tissue cDNA library. Fig. 1 shows the sequence of one of the β3-AR cDNAs obtained which represents the complete sequence of the β3 transcript. The longest open reading frame found encodes a protein of 419 residues. The putative protein shows the classical structural features of G protein-coupled receptors and a complete identity with the β3-AR previously

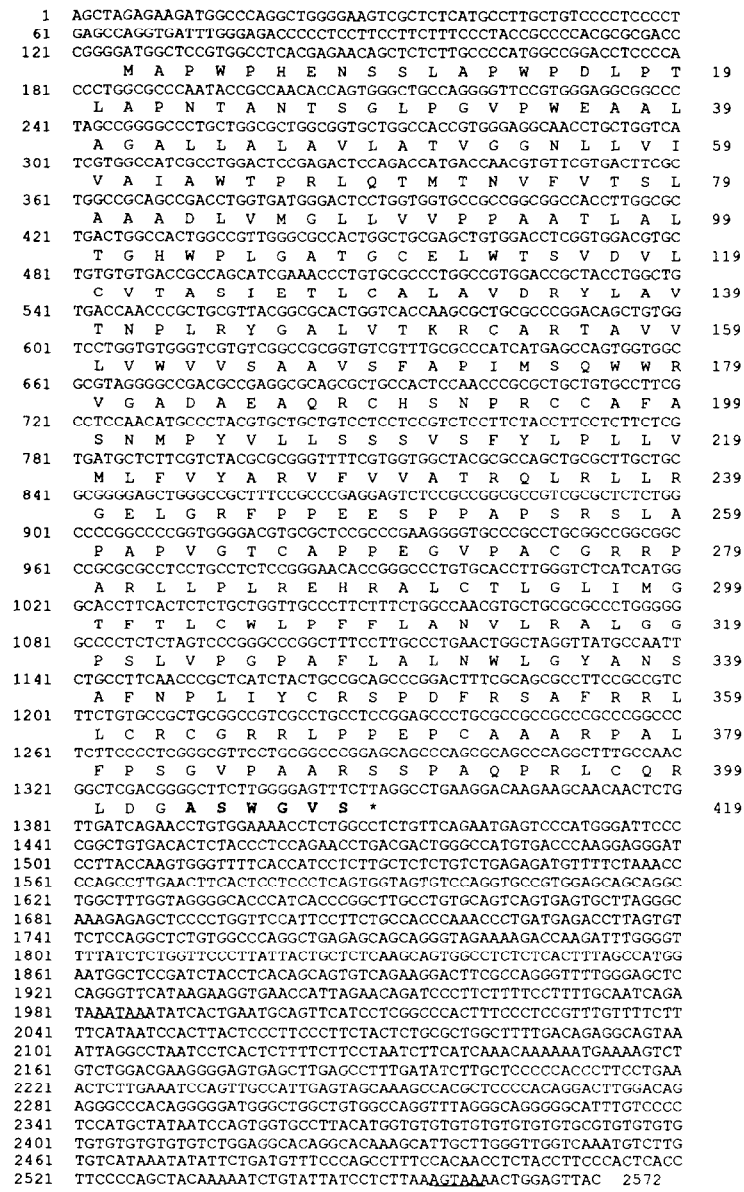


Fig. 1. Nucleotide and predicted amino acid sequences of the human β3-AR cDNA. Polyadenylation sites are underlined. The bold amino acids indicate the additional residues compared to the previously published receptor.

deduced from genomic DNA sequences [8], but with 6 additional amino acids at the C-terminus. Sequence analysis of the 3′ untranslated region of the β3-AR cDNA identified 2 polyadenylation sites. Comparison of the ten isolated β3-AR cDNAs demonstrated that the two polyadenylation sites are used to the same extent (see Fig. 1). Furthermore, a screening of the cDNA library using a common β probe identified only one β1-AR and no β2-AR cDNAs out of 2×10^6 primary recombinants, indicating a preponderance of β3- over β1-AR transcripts.

An alignment of genomic and cDNA sequences shows a divergence after the position 1,332 of the cDNA. In order to determine the structure of the spliced DNA fragment, we performed a polymerase chain reaction (PCR) experiment on genomic DNA using as primers a pair of oligonucleotides matching the positions 1,299–1,320 and 1,741–1,717 of the cDNA sequence. A comparison between the amplified DNA and cDNA sequences revealed a 1,025 bp intron (Fig. 2a, positions 33–1,057). Its splicing from primary transcripts makes the exons 1 and 3 contiguous in β3 mRNAs (Fig. 2b). A search for potential splice junctions and open reading frame(s) within the intron revealed a potential exon at the positions 796–859. Interestingly, this exon encodes 12 amino acids which share 50% homology with rat and mouse β3-AR protein C-termini. Therefore, as recently reported for the rodent β3-AR genes [14], we postulated

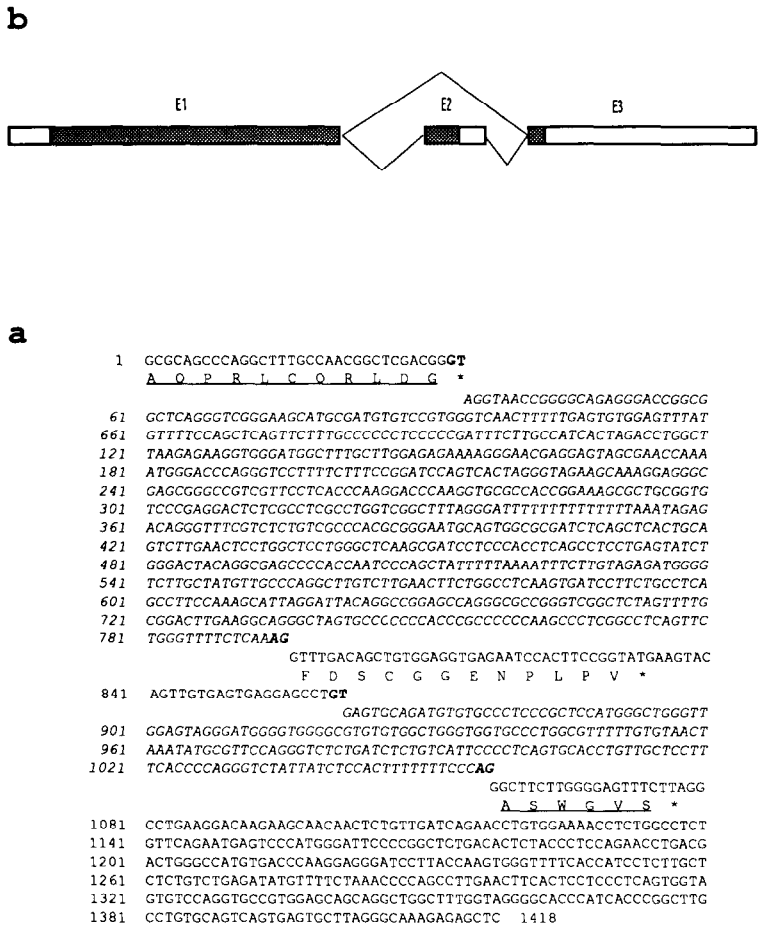


Fig 2. Structure of the human β3-AR gene. (a) Nucleotide and amino acid sequences of exon/intron junctions of the human β3-AR gene beginning with Ala[391]. Bold characters indicate donor splice sites. Intron sequences are in italic. Underlined residues represent the carboxyl-terminal tail of the cDNA. (b) Schematic representation of the human β3-AR gene, with mature mRNA as blocks and the coding sequence filed. E, exon.

that the human gene contains 3 exons and 2 introns (Fig. 2b) and as represented on the scheme of Fig. 2b, an alternative splicing can generate receptor isoforms with, respectively, 12 and 6 additional amino acids at their C-terminus with respect to the deduced protein from the unspliced RNA sequence.

4. DISCUSSION

We report here the molecular cloning of a human brown adipose tissue β3-AR cDNA and the discovery of introns within the gene. At the genomic level, the coding sequence of the β3-AR cDNA is interrupted by a DNA fragment of 1,025 bp. This intron starts with an in-frame stop codon with the result that unspliced transcripts will encode a C-terminal truncated protein.

The previously reported human β3-AR was deduced from genomic DNA sequences [8] and is therefore 6 amino acids shorter than the sequence derived from the cDNA. This C-terminal-truncated human β3-AR expressed in CHO cells shares with the cloned rat β3 receptor a low affinity for naturally occurring catecholamines but differs regarding the potencies and efficacies of noncatecholamine 'atypical' agonists and 'typical' antagonists [13]. A recent finding indicates that these differences are related to the 'missing' carboxyl-terminal tail of the CHO-expressed human β3-AR. Since, the β3-AR expressed in the human neuroblastoma cell line SK-N-MC which has the same C-terminus as the cDNA-encoded receptor [14], more closely resembles the cloned rat β3-AR than the CHO-expressed truncated human β3-AR [15]. To directly demonstrate this point, we have stably transfected CHO cells with the human β3-AR cDNA and are presently investigating the pharmacological characteristics of the CHO-expressed full-length human β3-AR. The C-terminus of the β2-AR has been shown to be involved with the desensitization process through the phosphorylation of specific sites by the β-AR kinase [21,22]. In rat, the β3-AR fails to undergo functional desensitization during short-term exposure [23] or down-regulation of receptor expression during long-term exposure [24] to iso-

properenol, a non-specific agonist. Moreover, recent work reported different coupling properties with G proteins of two isoforms of the prostaglandin E receptor with different carboxyl-terminal domains and demonstrated the implication of the C-terminus in signaling from both a G protein to a receptor and from a receptor to a G protein [25]. In rodent as in man, alternative splicing of $\beta 3$ primary transcripts can generate at least three isoforms of receptors with different C-termini (Fig. 2b). The polymerase chain reaction was used to determine the existence and the tissue distribution of $\beta 3$ splice variants. Our results support the idea that alternative splicing might take place in some tissues (manuscript in preparation). It is therefore of importance to evaluate the pharmacological differences between these $\beta 3$-AR isoforms and especially receptor desensitization and the down-regulation of receptor expression.

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