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The rat β3-adrenergic receptor gene contains an intron

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We report that the rat β3-adrenergic receptor (β3-AR) gene has an intron. The intron starts with an in-frame stop codon with the result that unspliced transcripts will encode a C-terminal truncated protein. The reported protein sequences of mouse and human β3-AR were both deduced from genomic DNA sequences. Given the heterogeneity at the C-termini of the otherwise highly similar rat, mouse and human sequences, we discuss the intriguing possibility that the β3-AR gene of the latter two species also contain an intron near the extremity of the open reading frame. A β-adrenergic receptor (β-AR) cDNA we have cloned from rat colonic tissue which has a sequence essentially identical to that previously reported for the rat adipose β3-AR cDNA [(1991) J. Chem. 266, 24053], encodes the spliced version of the β3-AR.

β3-Adrenergic receptor; Intron; Polymerase chain reaction

1. INTRODUCTION

Activation of β-adrenergic receptors (β-AR) by agonists leads to a stimulation of adenylyl cyclase through the interaction of the receptor with a G-protein termed G_s (stimulatory guanine nucleotide binding protein) [1]. The sub-classification of β-AR into β1 and β2 subtypes is based on the relative potency of the naturally occurring catecholamines, epinephrine and norepinephrine. β-1 responses are equally sensitive to these two agonists; β2 responses are more potently stimulated by epinephrine [2,3]. During the past few years, evidence has accumulated indicating that, in addition to the β1- and β2-adrenoceptors, a further subtype may exist. The most prominent example is the rat adipocyte β-adrenoceptor, originally classified as typically β1 [3]. However, pharmacological studies have shown incongruities with respect to this characterization; (i) unexpected low potencies of non-selective [4] and β1-selective [5] antagonists, and (ii) the failure to confirm the β1-receptor properties with non-catecholamine agonists [6]. In the rat, besides the adipose tissues [3], atypical β-adrenoceptors have been found in the heart [7], and the colon [8,9]. Recently, a correlation between colonic motility inhibition and adipocyte lipolysis was found for phenylethanolaminotetralines, selective inhibitors of colonic motility, indicating that the rat colonic and adipose atypical β receptors are similar [10].

The existence of these three subtypes has now been confirmed by molecular biology techniques [11]. Cell lines expressing the different subtypes of the cloned β-AR genes have been constructed to study structure–function relationships. In contrast to previous observations with CHO cells transfected with β1- and β2-AR genes, there were noticeable pharmacological differences between the β3-ARs expressed on transfected CHO cells and the atypical β-adrenoceptors [12–15]. That raised questions concerning the molecular diversity of atypical β-adrenoceptor proteins, and/or the specificity of the transduction process in CHO cells. We report here the cloning of the rat colonic atypical β-AR cDNA and we confirm, at the molecular level, the similarity between the adipose and colonic atypical β-ARs. In addition, we have looked for the presence of intron(s) in the rat β3-AR gene and demonstrate that: (i) both the rat adipocyte and colonic cDNAs represent a spliced form of the β3-AR gene, and (ii) the spliced and unspliced transcripts will encode receptor proteins with different C-termini.

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2. MATERIALS AND METHODS

2.1. *Construction of a cDNA library*

Total RNA was extracted from colonic tissue 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 using the primer-adapter procedure [18] and the pTZ18 vector (Pharmacia). Approximately 1.5×10^6 primary recombinants were analyzed as described by Grunstein and Hogness [19]. DNA sequences were determined by the method of Sanger [20].

2.2. *Polymerase chain reaction (PCR)*

Degenerated primers (D3 and D6) derived from nucleotide sequences corresponding to transmembrane segments III and VI of G-protein-coupled receptors (see Fig. 1) were used to amplify rat genomic DNA as described by Libert [21]. Amplified DNAs were cloned into pTZ19 vector (Pharmacia) and the recombinant clones were analyzed by in situ colony hybridization using ^{32}P-labeled oligonucleotides specific for β1-, β2- and β3-AR genes.

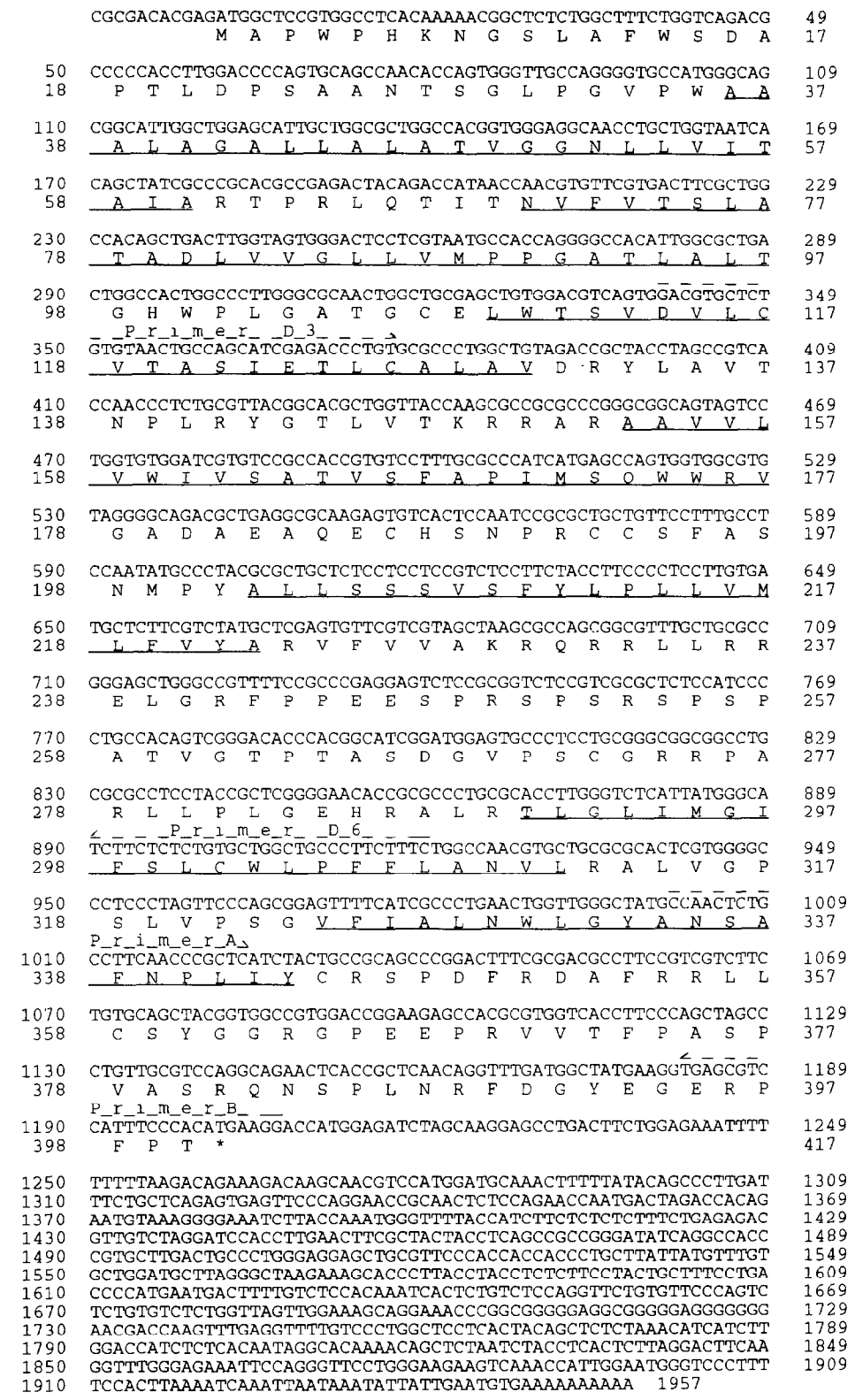

Fig. 1. Nucleotide and predicted amino acid sequences of rat colonic β3-AR cDNA. The presumptive transmembrane domains are underlined. Primers used for PCR are marked by dashed lines with arrows.

cDNAs were reverse transcribed from total RNA then treated with DNAse prior to subjection to PCR. Specific primers (A and B) derived from the β3-AR cDNA (Fig. 1) were used to amplify (30 cycles) rat genomic DNA and cDNAs using the *Taq* DNA polymerase. Cycle conditions were 1.5 min at 93°C, 1.5 min at 60°C and 2 min at 72°C. Aliquots of the amplified DNA were analyzed by Southern blot.

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b3-AR.Rat / cDNA      .V F I A L N W L G Y A N S A F N P L I Y C R S P D F R D A F R R L L C S Y G G R G
                      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
b3-AR.Mouse/Gene      .V F I A L N W L G Y A N S A F N P V I Y C R S P D F R D A F R R L L C S Y G G R G
                        : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
b3-AR.Human/Gene      .A F L A L N W L G Y A N S A F N P L I Y C R S P D F R S A F R R L L C R C G R R L

b3-AR.Rat / cDNA      P E E P - - - - R V V T F P A S P V A S R Q N S P L N R F D G Y E G E R P F P T
                      : : : :         :   : : : : : : : :     : :     : : : :
b3-AR.Mouse/Gene      P E E P - - - - R A V T F P A S P V E A R Q S P P L N R
                      :   : :         :       : :           : :   : :       :
b3-AR.Human/Gene      P P E P C A A A R P A L F P S G V P A A R S S P A Q P R L C Q R L D G
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Fig. 2. Aligment of rat, mouse and human C-terminal β3-ARs. Doubled dots indicate identities. The arrow shows the C-terminus of the rat β3-AR deduced from the genomic sequence.

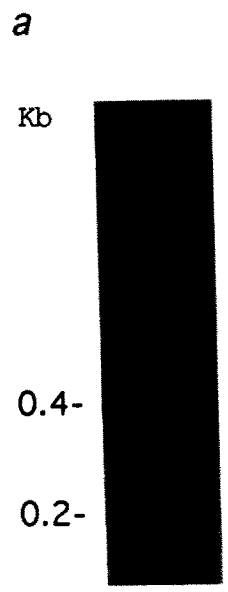
3. RESULTS AND DISCUSSION

3.1. *PCR, cDNA cloning and sequencing*

Rat genomic DNA was prepared and amplified by PCR using degenerated primers matching the transmembrane domains III and VI of G-protein-coupled receptors as described elsewhere [21]. The resulting amplified DNAs were cloned into the pTZ19 vector and the recombinant clones containing β-AR-related DNA sequences were identified by in situ colony hybridization. Nucleotide sequence determination revealed that only β1-, β2-, and β3-AR gene fragments were amplified, with no other β-AR related DNA sequence being detectable. These fragments were used to isolate β1-, β2- and β3-AR cDNAs from a rat colonic cDNA library. Fig. 1 shows the 1,957 nucleotide sequence of a β3-AR cDNA. The putative encoded protein shows the classical structural features of G-protein-coupled receptors and an almost complete identity with the adipose β3-AR [12]. These results confirm the similarity of adipose and colonic atypical β-ARs.

3.2. *PCR identification, cloning and sequencing of an intron*

The rat and mouse β3-AR proteins are almost identical (95%) but differ at the C-terminal end, a region shown to be important for β2-AR desensitization [22]. The mouse protein [15] is 12 amino acids shorter than the rat receptor (Fig. 2). Considering that the mouse β3-AR was deduced from a genomic sequence, whereas the rat β3-AR is encoded by the cDNA, we thought that the difference could be related to a splicing event rather than a difference between species. In order to investigate the rat β3-AR genomic sequence, we performed PCR experiments with rat genomic DNA and rat colonic cDNA. A pair of oligonucleotides matching the cDNA as shown in Fig. 1 (primers A and B) served as primers.



b

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                - - - Primer A - - - - ->
1   CCAACTCTGCCTTCAACCCGCTCATCTACTGCCGCAGCCCGGACTTTCGCGACGCCTTCC  60
      N  S  A  F  N  P  L  I  Y  C  R  S  P  D  F  R  D  A  F  R

61  GTCGTCTTCTGTGCAGCTACGGTGGCCGTGGACCGGAAGAGCCACGCGTGGTCACCTTCC 120
      R  L  L  C  S  Y  G  G  R  G  P  E  E  P  R  V  V  T  F  P

121 CAGCTAGCCCTGTTGCGTCCAGGCAGAACTCACCGCTCAACAGGTAGGCGACGCAGGCAG 180
      A  S  P  V  A  S  R  Q  N  S  P  L  N  R  *

181 AGGACTGGAGTCTGGGTGGGGACACCTCTGTCTCTATTTTTGAGTTTGAGGGTTGGGGGA 240

241 GGAGAAGGTGTAGACAGGGCTTTTGTCTCGAGAGGACAGAAAAGGAGTAAGAACAGAATC 300

301 GGGATCTAGGGCCCTTCCTTTTATTGGATCCAATCCCTGGGTCTGAGGCAAAGGAGGAAA 360

361 GGGAAATTTGTTCACCTTGGGACCAGGTGAGCCCCACAGGTTTCTGCCAGCAGGTTTCTG 420

421 ACCTCTCTGGTTGCCTCTAGTTTGGATCTTTTTAGTTCTATTCTCCAGGCGCCCAGGTAT 480

481 CACTAACTTGTCTGGGACATCCATAGACAGCAATGGACATGTCAAGTCCTCTGCCTCAGT 540

                                          <- - - Primer B - - - -
541 TCCGCTTTCTTTCAAGGTTTGATGGCTATGAAGGTGAGCGTCCATTTCCCACATGAAGG 599
                F  D  G  Y  E  G  E  R  P  F  P  T  *
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Fig. 3. (a) Southern blot analysis. Lane A, amplified DNA fragments from genomic DNA; lane B, amplified DNA fragments from cDNAs. (b) Nucleotide sequence of the β3-AR DNA segment amplified from genomic DNA. Primers used are marked by dashed lines with arrows. The arrows indicate the exon–intron junctions.

The amplified DNA fragments were analyzed by Southern blot (Fig. 3a). From the cDNA, a 207 bp amplified DNA fragment was obtained as expected (Fig. 3a, lane B), whereas a larger fragment was amplified from the genomic DNA (Fig. 3a, lane A). The amplified genomic fragment was cloned and sequenced (Fig. 3b).

The rat β3-AR gene has an additional 393 bp located between positions 1,164–1,165 of the cDNA. This intron starts with a stop codon which interrupts the ORF of the cDNA, shortening by 12 amino acids the C-terminus of the protein encoded by the cDNA. Interestingly, from the rat and mouse β3-AR genomic sequences almost identical proteins are deduced sharing the same C-terminus. Moreover, the homology of sequence between both genes is maintained (87%) further downstream from the stop codon (Fig. 2), indicating the probability of an intron in the mouse β3-AR gene as well.

This result raises interesting questions.

(i) What is the biological significance of this intron which may give rise to spliced variants encoding different forms of the receptor? The use of alternate splicing to generate different forms of G-protein-coupled receptors was first described for the dopamine D_2 receptor [23] and more recently for the mouse somatostatin receptor [24]. We are presently investigating the presence of spliced variants in rat tissues.

(ii) What are the pharmacological differences between the two forms of the rat β3-AR? The C-terminus of the β2-AR has been shown to be involved with receptor desensitization through the phosphorylation of hydroxyl amino acid sites [22]. At least one phosphorylation site is present among the 12 amino acids missing at the C-terminus of the receptor encoded by genomic sequences. We are also investigating the pharmacological properties of these two possible forms of rat β3-AR.

(iii) What of the human β3-AR, the sequence of which was deduced from genomic sequences. In spite of high homologies between the human, rat and mouse receptors, their C-terminal sequences are quite different (Fig. 2). It is clearly important to dispose of a human β3-AR cDNA to be able to determine the protein sequence of β3-AR actually expressed in tissues. As for the rat β3-AR gene, two forms of receptor might exist due to the presence of an intron and splicing events. This would probably explain the pharmacological differences found between the human β3-AR expressed on CHO cells transfected with the β3-AR and atypical β-AR from adipose and colonic tissues [14].

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