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Identification of an additional gene belonging to the α_2 adrenergic receptor family in the human genome by PCR

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We here describe the cloning of an additional gene, called α_2-1.8, which is similar to the previously cloned human α_2-adrenergic receptor located on chromosome 4. The α_2-1.8 gene was identified by using the polymerase chain reaction with primers specific for sequences in transmembrane regions 2 and 5 of the previously isolated human α_2-C4 and α_2-C10 adrenoceptor genes, which are localized on chromosomes 4 and 10, respectively. The new gene was identified by amplifying the 1.8 kb size fractionated region of *Pst*I restriction cut human genomic DNA. The previously cloned α_2-C10 and α_2-C4 genes were recovered at their expected locations, 0.96 and 5.9 kb, respectively. We have identified 387 bases of the new α_2-1.8 gene, and its sequence is identical to the previously described α_2-C4 gene, but is distinct from the α_2-C10 and α_2-C2 genes. Our results demonstrate that the α_2-C4 adrenergic receptor exists in more than one copy in the human genome.

α_2-Adrenergic receptor; Two copy gene; Polymerase chain reaction

1. INTRODUCTION

The diversity of catecholamine effects are mediated by proliferating a multitude of receptors which all belong to a superfamily of G-protein coupled receptors [1]. The catecholamine receptors have previously been classified according to pharmacological criteria into several types such as α_1, α_2, β_1, β_2, D_1 and D_2 [2–4]. These receptors elicit their responses by coupling to membrane bound G-protein which regulate the activity of different cellular effectors. For example, a distinct G-protein links β-adrenoceptors in a stimulatory fashion to adenylate cyclase so that stimulation of the β-adrenoceptor will lead to increased formation of cAMP. Other examples are α_1-adrenoceptors which mediate breakdown of phospholipids by activating specific phospholipases via G-protein linked pathways [5]. Recently, gene cloning techniques have revealed that there exist many more distinct genes coding for catecholamine receptors than were initially assumed from the pharmacological classifications. For example, in humans distinct genes coding for 3 different β-adrenoceptors [6–8], 3 different α_2-adrenoceptors [9–11] and 2 dopamine receptors [12,13] have been identified. The three α_2-adrenergic receptors have been named α_2-C2, α_2-C4 and α_2-C10 due to their location on chromosomes 2, 4 and 10 of the human genome. These genes may be identified in Southern blot analysis of *Pst*I cut DNA as bands at 1.6, 5.9 and 0.95 kb [9,11] and their sequences are distinct albeit with great homologies.

All adrenergic receptors show amino acid sequence patterns common to all other G-protein coupled receptors which is consistent with a topology of the receptor proteins spanning the cell membrane 7 times. Moreover, the membrane spanning regions show the

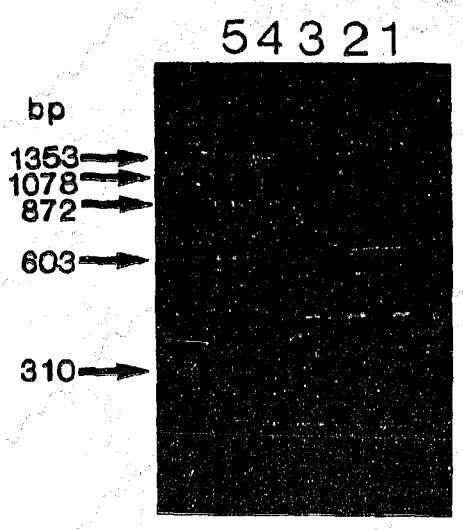

Fig. 1. Ethidium bromide-stained agarose gel showing PCR amplified products obtained by using size fractionated *Pst*I cut DNA and primers designed from conserved nucleotide sequences encoding the 2nd and 5th transmembrane regions of human α_2-C4 and α_2-C10 adrenoceptor genes. Shown are products obtained from DNA fragments of 0.96 kb (lane 1), 1.8 kb (lane 2), 5.9 kb (lane 3) and 3.5 kb (lane 4). Lane 5 shows Øx174/*Hae*III standard fragments.

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greatest sequence similarities which has made it possible to clone additional members of the G-protein coupled receptor family by using homologous primers in the PCR [14]. Using PCR we now report the identification of an additional human α_2-adrenoceptor gene which is similar to the α_2-adrenoceptor gene previously cloned from kidney cDNA and found to be located on chromosome 4 [10].

2. MATERIALS AND METHODS

2.1. *Oligonucleotides*

The oligonucleotide primers used were specific for the previously cloned α_2-C4 and α_2-C10 and designed from their membrane spanning segments 2 and 5 and selected from regions being identical for both receptors. The primers were designed with restriction endonuclease linkers *EcoR*I and *Bam*HI at the 5' ends to facilitated cloning of amplified fragments:

```
                                        II
Alpha-2 C2     A  A  D  I  L  V  A  T  L  I  I  F  F  S  L  A  N  E  L  L  G  Y  W  Y  F  R  R
Alpha-2 C2     GCC GCC GAC ATC CTG GTG GCC ACG CTC ATC ATC CCT TTC TCG CTG GCC AAC GAG CTG CTG GGC TAC TGG TAC TTC CGG CGC
Alpha-2 1.8    S  A  D  I  L  V  A  T  L  V  M  F  F  S  L  A  N  E  L  M  A  Y  W  Y  F  G  Q
Alpha-2 1.8    TCG GCC GAC ATC CTG GTG GCC ACG CTG GTC ATG CCC TTC TCG TTG GCC AAC GAG CTC ATG GCC TAC TGG TAC TTC GGG CAG
Alpha-2 5.9    S  A  D  I  L  V  A  T  L  V  M  F  F  S  L  A  N  E  L  M  A  Y  W  Y  F  G  Q
Alpha-2 5.9    TCG GCC GAC ATC CTG GTG GCC ACG CTG GTC ATG CCC TTC TCG TTG GCC AAC GAG CTC ATG GCC TAC TGG TAC TTC GGG CAG
Alpha-2 0.96   S  A  D  I  L  V  A  T  L  V  I  F  F  S  L  A  N  E  V  M  G  Y  W  Y  F  G  K
Alpha-2 0.96   TCG GCC GAC ATC CTG GTG GCC ACG CTC GTC ATC CCT TTC TCG CTG GCC AAC GAG GTC ATG GGC TAC TGG TAC TTC GGC AAG
               Primer 1

                                                III
Alpha-2 C2     T  W  C  E  V  Y  L  A  L  D  V  L  F  C  T  S  S  I  V  H  L  C  A  I  S  L  D
Alpha-2 C2     ACG TGG TGC GAG GTG TAC CTG GCG CTC GAC GTG CTC TTC TGC ACC TCG TCC ATC GTG CAC CTG TGC GCC ATC AGC CTG GAC
Alpha-2 1.8    V  W  C  G  V  Y  L  A  L  D  V  L  F  C  T  S  S  I  V  H  L  C  A  I  S  L  D
Alpha-2 1.8    GTG TGG TGC GGC GTG TAC CTG GCG CTC GAT GTG CTG TTT TGC ACC TCG TCG ATC GTG CAT CTG TGT GCC ATC AGC CTG GAC
Alpha-2 5.9    V  W  C  G  V  Y  L  A  L  D  V  L  F  C  T  S  S  I  V  H  L  C  A  I  S  L  D
Alpha-2 5.9    GTG TGG TGC GGC GTG TAC CTG GCG CTC GAT GTG CTG TTT TGC ACC TCG TCG ATC GTG CAT CTG TGT GCC ATC AGC CTG GAC
Alpha-2 0.96   A  W  C  E  I  Y  L  A  L  D  V  L  F  C  T  S  S  I  V  H  P  C  A  I  S  L  D
Alpha-2 0.96   GCT TGG TGC GAG ATC TAC CTG GCG CTC GAC GTG CTC TTC TGC ACG TCG TCC ATC GTG CAC CCG TGC GCC ATC AGC CTG GAC

                                                                                IV
Alpha-2 C2     R  Y  W  A  V  S  R  A  L  E  Y  N  S  K  R  T  P  R  R  I  K  C  I  I  L  T  V
Alpha-2 C2     CGC TAC TGG GCC GTG AGC CGC GCG CTG GAG TAC AAC TCC AAG CGC ACC CCG CGC CGC ATC AAG TGC ATC ATC CTC ACT GTG
Alpha-2 1.8    R  Y  W  S  V  T  Q  A  V  E  Y  N  L  K  R  T  P  R  R  V  K  A  T  I  V  A  V
Alpha-2 1.8    CGC TAC TGG TCG GTG ACG CAG GCC GTC GAG TAC AAC CTG AAG CGA ACA CCA CGC CGC GTC AAG GCC ACC ATC GTG GCC GTG
Alpha-2 5.9    R  Y  W  S  V  T  Q  A  V  E  Y  N  L  K  R  T  P  R  R  V  K  A  T  I  V  A  V
Alpha-2 5.9    CGC TAC TGG TCG GTG ACG CAG GCC GTC GAG TAC AAC CTG AAG CGA ACA CCA CGC CGC GTC AAG GCC ACC ATC GTG GCC GTG
Alpha-2 0.96   R  Y  W  S  I  T  Q  A  I  E  Y  N  L  K  R  T  P  R  R  I  K  A  I  I  I  T  V
Alpha-2 0.96   CGC TAC TGG TCC ATC ACA CAG GCC ATC GAG TAC AAC CTG AAG CGC ACG CCG CGC CGC ATC AAG GCC ATC ATC ATC ACC GTG

Alpha-2 C2     W  L  I  A  A  V  I  S  L  P  P  L  I  Y  K  G  D  Q  G  P  Q  P  R  G  -  -  -
Alpha-2 C2     TGG CTC ATC GCC GCC GTC ATC TCG CTG CCG CCC CTC ATC TAC AAG GGC GAC CAG GGC CCC CAG CCG CGC GGG  -  -  -
Alpha-2 1.8    W  L  I  S  A  V  I  S  F  P  P  L  V  S  L  Y  R  Q  P  D  G  A  A  Y  -  -  -
Alpha-2 1.8    TGG CTC ATC TCG GCC GTC ATC TCC TTC CCG CCG CTG GTC TCG CTC TAC CGC CAG CCC GAC GGC GCC GCC TAC  -  -  -
Alpha-2 5.9    W  L  I  S  A  V  I  S  F  P  P  L  V  S  L  Y  R  Q  P  D  G  A  A  Y  -  -  -
Alpha-2 5.9    TGG CTC ATC TCG GCC GTC ATC TCC TTC CCG CCG CTG GTC TCG CTC TAC CGC CAG CCC GAC GGC GCC GCC TAC  -  -  -
Alpha-2 0.96   W  V  I  S  A  V  I  S  F  P  P  L  I  S  I  E  K  K  G  G  G  G  G  P  Q  P  A
Alpha-2 0.96   TGG GTC ATC TCG GCC GTC ATC TCC TTC CCG CCG CTC ATC TCC ATC GAG AAG AAG GGC GGC GGC GGC GGC CCG CAG CCG GCC

                                                                    V
Alpha-2 C2     R  P  Q  C  K  L  N  Q  E  A  W  Y  I  L  A  S  S  I  G  S  F  F  A  P  C
Alpha-2 C2     CGC CCC CAG TGC AAG CTC AAC CAG GAG GCC TGG TAC ATC CTG GCC TCC AGC ATC GGA TCT TTC TTT GCT CCT TGC  390 bp
Alpha-2 1.8    -  P  Q  C  G  L  N  D  E  T  W  Y  I  L  S  S  C  I  G  S  F  F  A  P  C
Alpha-2 1.8    -  CCG CAG TGC GGC CTC AAC GAC GAG ACC TGG TAC ATC CTG TCC TCC TGC ATC GGC TCC TTC TTC GCT CCC TGC  387 bp
Alpha-2 5.9    -  P  Q  C  G  L  N  D  E  T  W  Y  I  L  S  S  C  I  G  S  F  F  A  P  C
Alpha-2 5.9    -  CCG CAG TGC GGC CTC AAC GAC GAG ACC TGG TAC ATC CTG TCC TCC TGC ATC GGC TCC TTC TTC GCT CCC TGC  387 bp
Alpha-2 0.96   E  P  R  C  E  I  N  D  Q  K  W  Y  V  I  S  S  C  I  G  S  F  F  A  P  C
Alpha-2 0.96   GAG CCG CGC TGC GAG ATC AAC GAC CAG AAG TGG TAC GTC ATC TCG TCG TGC ATC GGC TCC TTC TTC GCT CCC TGC  399 bp
                                                                Primer 2
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Fig. 2. Alignment of the nucleotide and deduced amino acid sequences for α_2-C2 as well as the PCR products obtained from 1.8 (α_2-1.8), 5.9 (α_2-5.9) and 0.96 (α_2-0.96) kb DNA. The DNA sequence of α_2-5.9 is completely identical to the published sequence of α_2-C4 [10]. The sequence of α_2-0.96 is identical to the published sequence of α_2-C10 [11], except for three amino acids marked with boxes. These differences cannot be attributed to PCR-artifacts as we have observed them in 2 completely different experiments. Sequences representing membranes spanning membranes II–V are marked with bars above sequence. The underlined sequences indicate primer 1 and 2 used for the PCR. Gaps, shown as dashes, are inserted to maximize homologies.

Primer 1: CCGAATTCGGCCGACATCCTGGTGGC-CACGCPrimer 2: CCGGATCCAGGGAGCGAAGAAGGAGC-CGATGCA

2.2. PstI digestion, amplification, cloning and sequencing

One μg of human genomic DNA was cut to completion with *PstI* and electrophoresed on 1% agarose gel. The bands at the positions 0.96 kb, 1.8 kb, 3.5 kb and 5.9 kb were cut and extracted using Geneclean (Bio 101, USA). The eluted DNA was cut once again with *PstI* electrophoresed as above, and bands excised from respective positions and the DNA extracted using Geneclean. Two cycles of *PstI* digestion and electrophoresis were performed in order to ensure the exact size of the DNA.

Aliquots of the above prepared size fractionated DNA samples were subjected to PCR using the above-mentioned primers. The PCR was performed with Gene Amp DNA amplification reagent kit from Perkin Elmer Corp., USA and the thermal profile used was 93°C - 30 s × 1; 93°C - 30 s, 60°C - 30 s, 72°C - 40 s × 30; 72°C - 5 min × 1. Twenty percent of each PCR sample was analyzed by electrophoresis as shown in Fig. 1. The rest of the material was used for ligating into PGEM 7Z f(+) vector and subsequent sequencing using the dideoxy chain termination method [15].

3. RESULTS AND DISCUSSION

Oligonucleotides which represented conserved nucleotide sequences encoding the 2nd and 5th transmembrane regions of human α_2-C4 and α_2-C10 adrenoceptor genes were used as primers in amplification of *PstI* cut size fractionated human genomic DNA. Using this approach we amplified 387 bp of the α_2-C4 gene from 5.9 kb DNA fragments (α_2-5.9), as well as 399 bp of the α_2-C10 gene from 0.96 kb DNA fragments (α_2-0.96) (Figs 1 and 2). These results are fully consistent with previously published sequence and Southern blot data for α_2-adrenoceptor genes [10,11]. The new α_2-1.8 gene (387 bp) was amplified from 1.8 kb DNA fragment and its sequence was found to be identical to the α_2-C4 gene (Figs 1 and 2). In order to ensure that the new gene was not a product of carryover from the 5.9 kb fragment that had not been fully separated on the agarose gel, the *PstI* digestion and electrophoresis of DNA was repeated twice before performing the PCR. Moreover, the DNA of 3.5 kb fragments, which is expected to be a non-specific region for α_2-adrenoceptors, did not yield any PCR products (Fig. 1). Furthermore, the whole experiment was repeated twice starting from genomic DNA and identical sequencing results were obtained in both experiments. The reason that we in the present study were unable to identify the α_2-C2 gene that was recently described [9] is that there is a single base mismatch at the 3′ end of primer 2 used in this study, as compared to the α_2-C2 sequence.

The new α_2-1.8 gene shows distinct sequence differences with the α_2-C10 and α_2-C2 genes, but has almost identical sequence to the α_2-C4 gene (Fig. 2). The α_2-C4 gene has been previously cloned from human kidney cDNA. This gene was said to be localized on chromosome 4 and a 5.9 kb fragment of *PstI* cut human genomic DNA [10]. The α_2-1.8 gene is localized on the 1.8 kb fragments of *PstI* cut human DNA and it shows sequence identity to the gene that we amplified from 5.9 kb fragments of *PstI* cut DNA in the present study. As the two genes have identical sequences, but different localizations on human genomic DNA, it is concluded that there are two different copies of the same α_2-adrenoceptor gene present in the human genome.

It is not presently understood what the physiological relevance of the presence of several α_2-adrenoceptor genes in the genome is. The products of the α_2-C2, α_2-C4 and α_2-C10 genes, when expressed in vitro, show differing abilities to bind catecholamines and drugs [9]. It appears that all these genes are expressed in vivo but with grossly different distributions in the tissues [9–11]. Moreover, a variety of functional and ligand binding studies have indicated the presence of several pharmacological subtypes of α_2-adrenoceptors with different distributions in both human and animal tissues [16–21]. Part of the functional and binding data is consistent with the molecular biology data but there are also results which indicate that there might possibly exist still more species of α_2-adrenoceptors than those already cloned [9]. Moreover, in a recent study it was reported that four different mRNAs could be detected in Northern blot analysis of rat tissues when α_2-adrenoceptor genes were used as probes, possibly supporting this contention [22]. Very little is presently known about the regulation of α_2-adrenergic gene expression in vivo. Such information should be of value to further the understanding of the functional importance of different α_2-adrenoceptor genes. The elucidation of the promoter regions for the α_2-adrenoceptor genes will constitute a first step towards the understanding of the regulation of their expression. The presence of multiple copies of the same α_2-adrenoceptor gene in the human genome must be taken into account in such an analysis.

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