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Cloning and sequence analysis of the human brain β-adrenergic receptor

Evolutionary relationship to rodent and avian β-receptors and porcine muscarinic receptors

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Two cDNA clones, λ-CLFV-108 and λ-CLFV-119, encoding for the β-adrenergic receptor, have been isolated from a human brain stem cDNA library. One human genomic clone, LCV-517 (20 kb), was characterized by restriction mapping and partial sequencing. The human brain β-receptor consists of 413 amino acids with a calculated M_r of 46480. The gene contains three potential glucocorticoid receptor-binding sites. The β-receptor expressed in human brain was homology with rodent (88%) and avian (52%) β-receptors and with porcine muscarinic cholinergic receptors (31%), supporting our proposal [(1984) Proc. Natl. Acad. Sci. USA 81, 272–276] that adrenergic and muscarinic cholinergic receptors are structurally related. This represents the first cloning of a neurotransmitter receptor gene from human brain.

cDNA; Genomic sequence; Glucocorticoid regulatory site; Receptor evolution; Sequence homology; (Human)

1. INTRODUCTION

The structure and function of β-adrenergic receptors have been the subject of numerous investigations during the past decade [1,2]. Our own biochemical studies have suggested that β-adrenergic receptor subtypes are closely related proteins under both hormonal (glucocorticoid) and immunological regulation, findings that may have important implications in human disease [3–5].

Here we describe the cloning and sequencing of a human brain β-adrenergic receptor gene. This is the first report on the cloning of a neurotransmitter receptor gene from human brain. By investigating the structure and regulation of human neurotransmitter receptor proteins and genes we hope to establish a reference point for evolutionary studies of neurotransmitter receptors and to provide a means of elucidating the role of neurotransmitter receptors in human disease.

Our data, together with those of others [6,7], have revealed considerable conservation of β-receptor structure among human, rodent and avian species. Furthermore, comparison of β-receptor protein sequence data with those from pig brain muscarinic cholinergic receptors [8] has demonstrated a high degree of homology between these pharmacologically unrelated receptor classes. These findings confirm our earlier hypothesis that adrenergic and muscarinic cholinergic receptors

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share significant structural homology and strongly suggest that neurotransmitter receptors may be part of a multigene family that evolved from a common ancestral gene [9–11].

2. MATERIALS AND METHODS

2.1. *Screening of a human brain stem cDNA library*

A cDNA library (LMG2) of neonatal human brain stem constructed in λ-gt11 (constructed by C. Puckett, J. Kamholz and R.A. Lazzarini and kindly provided by Dr R. Lazzarini) was screened at a density of about 20000 plaques per 150 mm plate using the bacterial host strain Y-1090r$^-$ with an oligonucleotide probe (51-mer) prepared from the C-terminus (base pair 1384–1435) of the hamster lung β-adrenergic receptor cDNA sequence [6]. The probe was end-labeled by phosphorylation with [γ-^{32}P]ATP (spec. act. 3000 Ci/mmol) and T_4 polynucleotide kinase. Duplicate filters (Hybond-N, Amersham) were lifted from each plate, lysed with 0.5 M NaOH/1 M NaCl, neutralized with 1 M Tris (pH 7.4), dried and UV irradiated for 5 min. Hybridization was carried out for 12 h at 42°C in a solution containing 6 × SSC (1 × SSC = 0.15 M NaCl/0.015 M trisodium citrate, pH 7.0), 5 × Denhardt's solution, 100 μg/ml sonicated, heat-denatured salmon sperm DNA, 30% formamide (deionized) and 50 mM Na phosphate, pH 7.4. Subsequent to hybridization, the filters were washed with 6 × SSC/0.1% SDS for 10 min at room temperature followed by a 10 min wash in 6 × SSC at 20°C. Filters were then dried and autoradiographed overnight using Kodak X-Omat AR5 film at −70°C. Two positive clones, λ-CLFV-108 and λ-CLFV-119, were isolated from 500000 recombinants. Their inserts were purified and subsequently subcloned into M13mp18/19 and pUC9/19 for sequence analysis and restriction mapping, respectively.

2.2. *Screening of a human genomic leucocyte library*

A human genomic leucocyte DNA library with a library base of 8.8 × 10^5 plaques constructed in λ-EMBL3 (Clontech, Palo Alto) was screened with the cDNA clone CLFV-108 (radiolabeled to a specific activity of 1.3 × 10^8 dpm/μg DNA by nick-translation) at a density of 25000 plaques per 150 mm plate using the bacterial host strain NM538. Six positive clones, LCV-501, -508, -509, -510, -517 and -520 were isolated from 500000 plaques. The insert of clone LCV-517 was purified and further digested with the restriction enzyme *BglII* and the resulting fragments subcloned into M13 for sequence analysis.

3. RESULTS AND DISCUSSION

Screening of the human brain stem cDNA library with a C-terminal oligonucleotide probe under conditions of low stringency yielded two hybridizable clones, designated CLFV-108 and CLFV-119. Sequence analysis of the two clones showed that CLFV-108 (1.2 kb) and CLFV-119 (1.1 kb) contained considerable homology with the hamster lung β-receptor sequence [6]. In order to study the genomic organization of the β-receptor gene we screened a human genomic leucocyte DNA library with the radiolabeled cDNA clone CLFV-108. Six hybridizable clones were detected and one (designated LCV-517) was purified for restriction mapping and partial sequencing (fig.1). Sequence analysis of this genomic clone revealed sequence identity to cDNA clones CLFV-108 and 119. The CLFV-108 sequence starts at base pair 170 of the 5′-coding region and extends about 30 bp into the 3′-untranslated region while the CLFV-119 sequence started further downstream at bp 750 of the coding region and contained nearly the whole 3′-untranslated region including the polyadenylation site (fig.1). These data indicate that there are no introns in the coding or in the 3′-untranslated region of this particular gene. However, we cannot rule out the possibility of introns in the 5′ region. LCV-517 also contains at least bp −177 of the 5′-flanking region up to about 50 bp beyond the polyadenylation signal in the 3′-untranslated region.

The most 5′ initiation codon (nucleotides −101 to −99) is followed by a TGA termination codon 57 nucleotides downstream (nucleotides −44 to −42). A second initiation codon (nucleotides 1–3) is in the same reading frame as the first initiation and termination codons. The nucleotides surrounding the second initiation codon are nearly identical to the consensus eucaryotic initiation sequence CC(A/G)CCAUGG [12] while those surrounding the first initiation codon are not similar

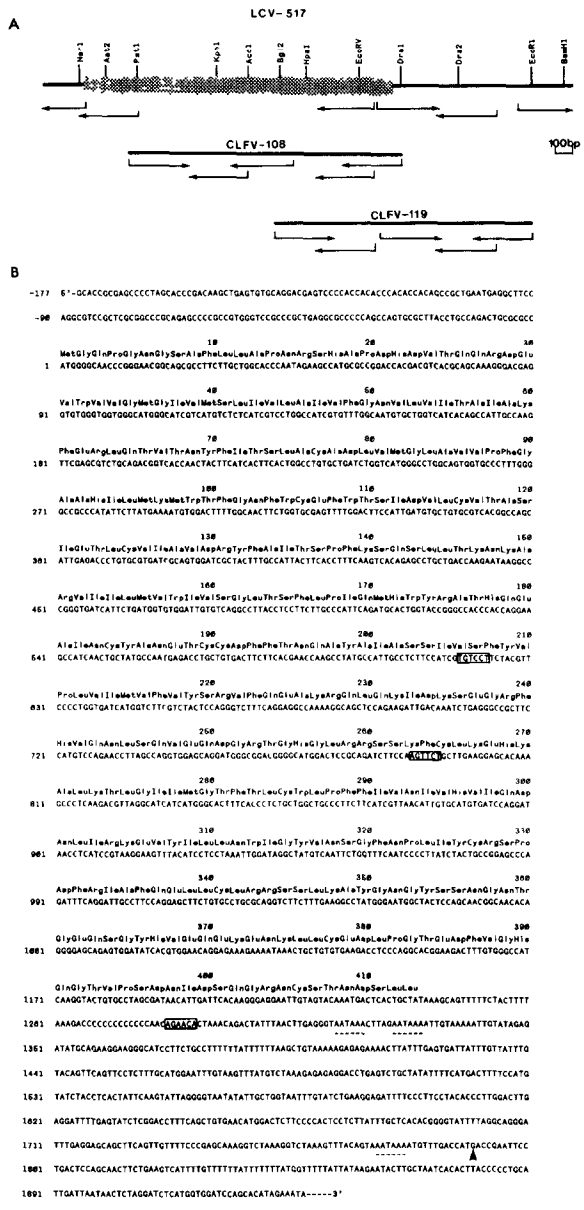

Fig.1. Nucleotide sequence and deduced amino acid sequence of the human brain β-adrenergic receptor. (A) Partial restriction endonuclease map and nucleotide sequencing strategy for the genomic clone LCV-517 and the two cDNA clones CLFV-108 and CLFV-119. The protein coding region is marked by the stippled box. The extent and direction of sequence determination are indicated by horizontal arrows. In most cases, oligonucleotides made from overlapping sequences were used as primers for sequence determination. DNA sequencing was performed using the dideoxynucleotide chain termination method of Sanger [24] and M13 bacteriophage vectors mp18 and mp19. (B) Nucleotide sequence and deduced amino acid of the cDNA clones CLFV-108 and CLFV-119 and the genomic clone LCV-517. The glucocorticoid-responsive elements are boxed. The three possible polyadenylation signals in the 3′-flanking region are marked (– – –). The polyadenylation site in clone CLFV-119 is indicated by the arrow.

to the consensus sequence. The open reading frame coding for the 19 amino acids upstream from the proposed translation initiation site is not unique to the human brain β-receptor gene. Similar open reading frames have been reported in the mRNA for hamster lung β-receptor [6], pig brain muscarinic receptor [8] and human estrogen receptor [13]. It is not known if any of these open reading frames are translated.

The open reading frame defined by the initiating ATG (nucleotides 1–3) to the translation termination codon TAA (nucleotides 1240–1242) codes for a protein of 413 amino acids. The calculated M_r of this polypeptide is 46480 compared with M_r 46860 for the hamster lung β-receptor [6], M_r 54078 for the turkey erythrocyte β-receptor [7] and M_r 51416 for the pig brain muscarinic receptor [8]. The human brain β-receptor has two potential glycosylation sites (Asn-X-Ser/Thr) at Asn_6 and Asn_{15} which are conserved in the hamster lung β-receptor and the pig brain muscarinic receptor. Only the former is conserved in the turkey erythrocyte β-receptor. In addition, the human brain β-receptor has three additional sites of potential glycosylation at Asn_{244}, Asn_{405} and Asn_{909}. The latter two sites are conserved in the hamster lung β-receptor. Which, if any, of these sites is actually glycosylated in the human brain β-receptor is not known.

Comparison of the resulting protein sequence of the human brain β-receptor to those of turkey and hamster (fig.2) shows high homology. The extent of sequence identity of human brain β-receptor with hamster lung and turkey erythrocyte β-receptors and pig brain muscarinic receptor is 88, 51 and 31%, respectively. When the homology is calculated considering favored amino acid substitutions the figures are 92, 68 and 46%, respectively. The sequence identity among all three

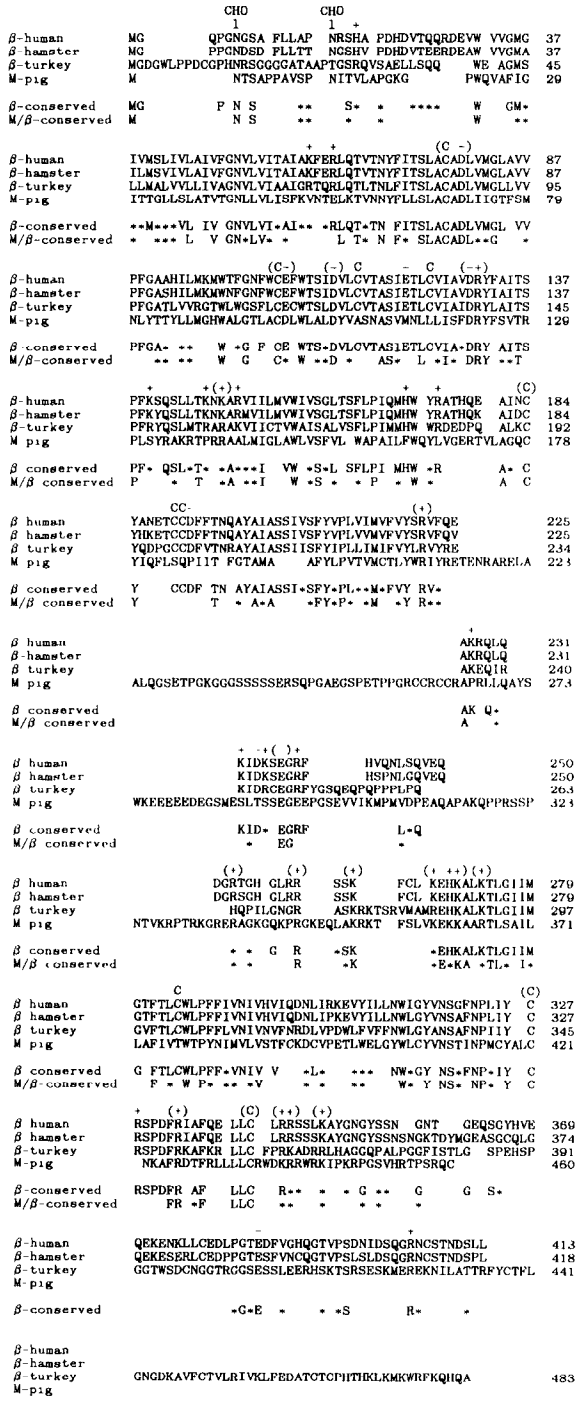

Fig.2. Comparison of the deduced amino acid sequences of the β-adrenergic receptor from human brain, hamster lung [6] and turkey erythrocyte [7] and muscarinic receptor from pig brain [8]. The sequences were aligned with gaps introduced to display maximum homology by the GAP program of the University of Wisconsin Genetics Computer Group [25] which uses the algorithm of Needleman and Wunsch [26]. The alignment was made without bias toward putative structural domains of the molecules. Those amino acids which are conserved in all three β-adrenergic proteins (β-conserved) or all four proteins (M/β-conserved) are shown below the sequences, indicated by the letter for that amino acid or by an asterisk if favored substitutions are present. Favored amino acid substitutions, as defined by Dayhoff [27], are those which belong to one of the following groups: (S,T,P,A,G), (M,I,L,V), (F,Y,W), (N,D,E,Q), (H,R,K) or (C). Charged groups and cysteines which are conserved in all three β-adrenergic proteins are shown above the sequences. Those which are conserved in all four proteins are enclosed in parentheses. The two potential glycosylation sites (Asn-X-Ser/Thr) at Asn_6 and Asn_{15} are indicated.

β-receptors is 49%, increasing to 67% when favored amino acid substitutions are considered. The degree of identity of the three β-receptors and the pig brain muscarinic receptor sequence is 20%, increasing to 39% when favored amino acid substitutions are considered. There are six segments of 10 amino acids or longer which are conserved in all three β-receptor sequences and one of these is also conserved in the pig brain muscarinic receptor. The three β-receptors have 10 conserved cysteines, 8 conserved prolines and 38 conserved charged groups. Of these, 5 cysteines, 5 prolines and 20 charged groups are also conserved in the pig brain muscarinic receptor. The conserved cysteines are particularly interesting in light of our earlier data demonstrating the presence of a free sulfhydryl group in the ligand-binding site of the dog heart β-receptor as well as other evidence for the involvement of disulfides in the structural maintenance and activation of the receptor [14,15]. The conserved prolines and charged groups may also be involved in the maintenance of a requisite receptor structure.

The hydropathy profile, based upon the analysis of Kyte and Doolittle [16], of the human brain β-receptor is nearly identical to that of the hamster lung β-receptor [6] and has features similar to those of the turkey β-receptor [7], the pig brain muscarinic receptor [8] and the rhodopsins from human [17], cow [18], sheep [19,20], fruitfly [21] and bacteria [22] (not shown). The overall homology of these proteins is shown in table 1.

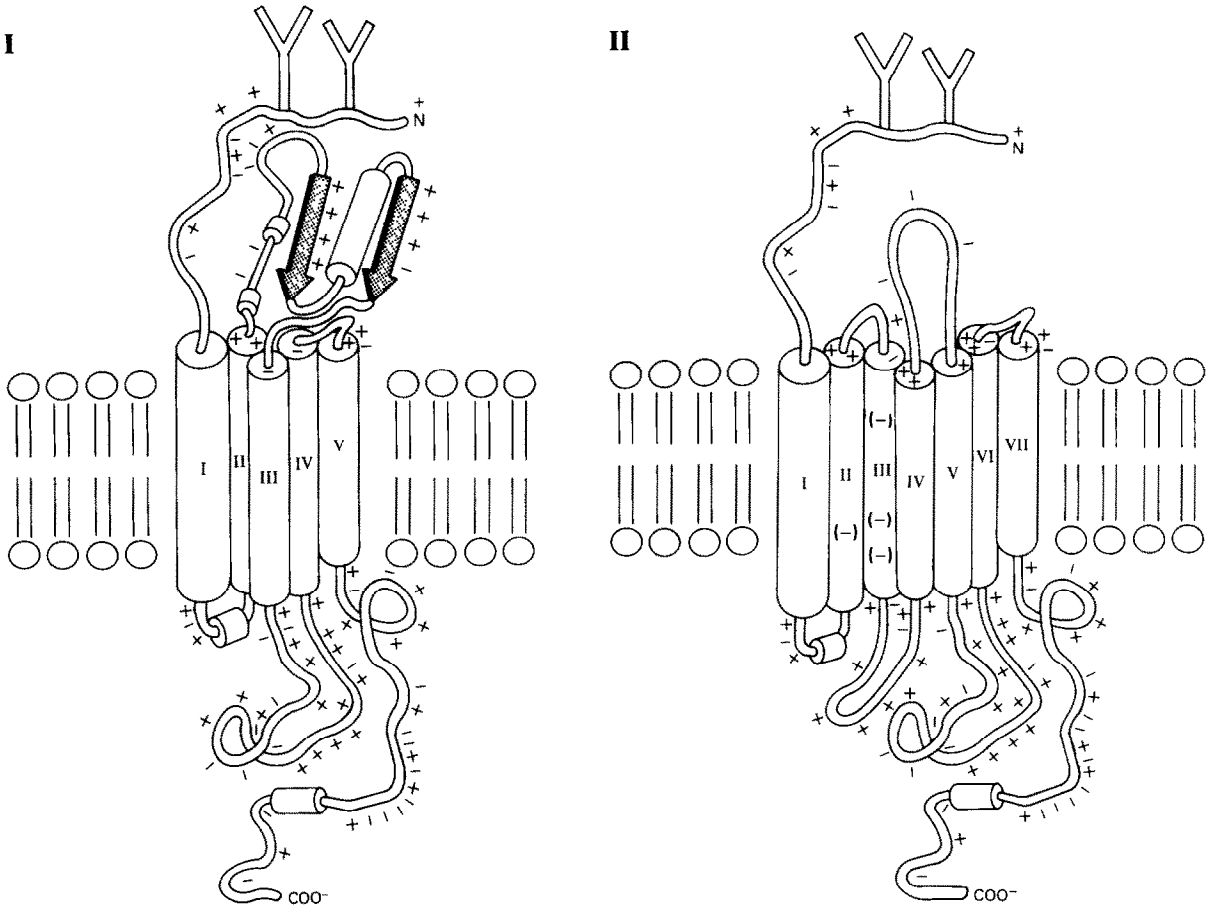

Fig.3. Alternative models for the polypeptide organization of the β-adrenergic receptor with respect to the lipid bilayer. The charges shown are those of the human brain receptor. Sites of potential glycosylation are indicated.

Models containing seven-transmembrane-spanning regions have been proposed for the β-receptors and the muscarinic receptor based upon the similarity of these profiles to that of bacteriorhodopsin, a protein for which several lines of evidence support the notion of seven-transmembrane-spanning segments. However, this model (fig.3) contains only minimal extracellular domains from which a high-affinity, stereoselective ligand-binding site must be formed. Fig.3 compares the seven-transmembrane model of the human β-receptor to a five-transmembrane model which moves negative charges from presumed transmembrane segments and places them extracellularly, allowing for a putative ligand-binding domain with a greater degree of secondary structure.

Three potential glucocorticoid receptor-binding sites or glucocorticoid-responsive elements (GRE) are found in the human β-receptor gene sequence, two within the coding region (nucleotides 617–622 and 788–793) and one closely following the stop codon (nucleotides 1282–1287). All three sites match the GRE consensus sequence (T/A)GT(T/C)CT although the third site is found in the antisense strand in the reverse orientation. There is precedent for GREs located within genes, and in the flanking untranslated regions in both the same and antisense orientation ([23] and references contained within). Thus, all three GRE sites observed in the human brain β-receptor gene may be of functional importance, consistent with our previous data on glucocorticoid induction of human β-adrenergic receptor synthesis [3].

It is not clear whether the gene we have isolated encodes for a β_1- or β_2-adrenergic receptor. The higher homology seen between our gene and the

Table 1

Percent homology of β-adrenergic, muscarinic and opsin proteins

		MAChR	β-AR			Opsin					
		Pig	Human	Hamster	Turkey	Human-G	Human-R	Human-B	Cow	Sheep	Fruit-fly
	Human	31									
β-AR	Hamster	31	88								
	Turkey	21	52	50							
	Human-G	20	22	21	19						
	Human-R	20	19	22	20	96					
	Human-B	18	19	20	21	43	42				
Opsin	Cow	22	18	25	23	43	42	44			
	Sheep	22	24	27	27	43	42	42	96		
	Fruitfly	21	21	21	22	30	31	27	27	25	
	Bacteria	20	19	19	19	19	19	20	19	19	19

Percent homology was calculated by the GAP program of the University of Wisconsin Genetics Computer Group [25] which uses the algorithm of Needleman and Wunsch [26]. Protein sequences were obtained from the following sources: β-AR: human brain (this study), hamster lung [6], turkey erythrocyte [7]; pig brain muscarinic receptor [8]; opsin: human green, red and blue [17], cow [18], sheep [19,20], fruitfly [21], bacteria [22]

hamster lung β_2-receptor as compared to the turkey erythrocyte 'β_1-like' receptor could imply that we have cloned a human β_2-receptor gene. On the other hand, the degree of homology could merely reflect species variation and evolution of these proteins. Restriction maps of our human genomic clones indicate the presence of additional β-receptor-related sequences that hybridize with CLFV-108 under high stringency conditions. These data suggest either a genetic basis for β-receptor heterogeneity and/or possible introns in additional β-receptor genes.

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