

Expansion of the α_2 -adrenergic receptor family: Cloning and characterization of a human α_2 -adrenergic receptor subtype, the gene for which is located on chromosome 2

(catecholamines/G protein/ α_2 -adrenoceptor/[3 H]yohimbine binding/polymerase chain reaction)

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ABSTRACT Pharmacologic, biochemical, and genetic analyses have demonstrated the existence of multiple α_2 -adrenergic receptor (α_2 AR) subtypes. We have cloned a human α_2 AR by using the polymerase chain reaction with oligonucleotide primers homologous to conserved regions of the previously cloned α_2 ARs, the genes for which are located on human chromosomes 4 (C4) and 10 (C10). The deduced amino acid sequence encodes a protein of 450 amino acids whose putative topology is similar to that of the family of guanine nucleotide-binding protein-coupled receptors, but whose structure most closely resembles that of the α_2 ARs. Competition curve analysis of the binding properties of the receptor expressed in COS-7 cells with a variety of adrenergic ligands demonstrates a unique α_2 AR pharmacology. Hybridization with somatic cell hybrids shows that the gene for this receptor is located on chromosome 2. Northern blot analysis of various rat tissues shows expression in liver and kidney. The unique pharmacology and tissue localization of this receptor suggest that this is an α_2 AR subtype not previously identified by classical pharmacological or ligand binding approaches.

The catecholamines, epinephrine and norepinephrine, mediate various physiological effects in different tissues by binding to different subtypes of adrenergic receptors (β_1 , β_2 , α_1 , α_2). The adrenergic receptors are currently classified according to their unique pharmacology. This classification has been both confirmed and expanded by the cloning of the genes for these receptors (1–7). While the genes encoding these receptors are distinct, they are quite homologous and are members of the growing family of guanine nucleotide-binding regulatory protein (G protein)-coupled receptors (8). This family also contains the muscarinic cholinergic, substance K, lutropin-choriogonadotropin, thyrotropin, serotonin, and dopaminergic receptors, as well as rhodopsin.

Heterogeneity of α_2 -adrenergic receptors (α_2 ARs) has been demonstrated in several pharmacological studies by showing different rank orders of potency for several compounds at α_2 AR binding sites (9–12). Accordingly, three subtypes have been proposed (α_{2A} , α_{2B} , α_{2C}) based on the differential potencies of prazosin, oxymetazoline, yohimbine, ARC 239, and chlorpromazine. Other evidence for multiple α_2 AR subtypes includes the demonstration of pre- and postsynaptic receptor localizations (13, 14). In addition, our laboratory has shown that a probe made from the human platelet α_2 AR gene recognizes, at moderately high stringency, three different genes by Southern blot analysis of DNA from somatic cell hybrids (1). Each of these genes localizes to a different human

chromosome: 2, 4, or 10 (α_2C2 , α_2C4 , and α_2C10). The human platelet α_2 AR gene is located on chromosome 10. The gene for a second α_2 AR, which has been cloned from a human kidney cDNA library, is located on chromosome 4. We now report the cloning of a human α_2 AR whose gene is located on chromosome 2.†† The pharmacological properties of this receptor as well as its tissue distribution by Northern blot analysis suggest that the heterogeneity of this set of receptors may extend beyond the three pharmacologically defined subtypes.

MATERIALS AND METHODS

Polymerase Chain Reaction (PCR) Cloning. Oligonucleotide primers (primer A, 5'-GTGCAAGCTTGCACCTCGTCGATCGTGCATCTGTGNGC-3'; primer B, 5'-CCCAAAGAGCTCAGCCAGCACAAAGGTGAAGCG-3') corresponding to identified α_2 AR consensus sequences were synthesized on an Applied Biosystems 380 B DNA synthesizer and purified on a 16% (wt/vol) denaturing polyacrylamide gel or by HPLC using an Applied Biosystems Aquapore RP-300 column and a 5–95% acetonitrile gradient (15). The primers were designed with the restriction endonuclease linkers *Hind*III and *Sac* I at the 5' ends to facilitate subcloning. Sheared human genomic DNA (5 μ g) was amplified with 1 μ M primers in 10 mM Tris-HCl, pH 8.3/50 mM KCl/1.5 mM MgCl₂/0.01% gelatin/200 μ M each dATP, dCTP, dGTP, TTP/10% dimethyl sulfoxide/2.5 units of *Thermus aquaticus* DNA polymerase (*Taq* DNA polymerase) (Perkin-Elmer/Cetus) (16). The amplification profile was run for 25 cycles: 2 min at 92°C, 2 min at 45°C, and 5 min at 72°C.

Genomic Library Screening. A human placental genomic library in EMBL-3 SP6/T7 (2.5 \times 10⁶ recombinants; Clontech) was screened with the 900-base-pair (bp) PCR product labeled with ³²P by nick-translation. Duplicate nitrocellulose filters were hybridized in 6 \times SSC (1 \times SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0)/0.2% polyvinylpyrrolidone/0.2% Ficoll/0.2% bovine serum albumin/0.1% sodium pyrophosphate/0.1% SDS/100 μ g of sheared salmon sperm DNA per ml at 42°C for 24 hr. Filters were then washed in 0.2 \times SSC at 55°C, and exposed at -70°C with Kodak X-OMAT film with Cronex enhancing screens.

Expression. To facilitate the construction of the expression vector, the initiator methionine codon of α_2C2 was changed to an *Nco* I methionine codon. This was accomplished by synthesizing a 40-bp double-stranded oligonucleotide linker that matched the α_2C2 sequence with *Nco* I and *Sfi* I

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Abbreviations: G protein, guanine nucleotide-binding protein; α_2 AR, α_2 -adrenergic receptor; PCR, polymerase chain reaction.
†† The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34041).

restriction sites. This was ligated to the 1310-bp *Sfi*I/*Hind*III α_2 C2 restriction fragment and the *Nco*I/*Hind*III-cut mammalian expression vector pBC12BI β_2 (1). The resulting construct, pBC α_2 -C2, contained 40 bp of the 5' untranslated region of the human β_2 -adrenergic receptor adjacent to the Rous sarcoma virus promoter, followed by 1350 bp of open reading frame, and 182 bases of 3' untranslated region from the α_2 C2 clone. The construct was transfected into COS-7 cells by the DEAE-Dextran method (17).

Ligand Binding. COS-7 cells were harvested 48 hr after transfection. Culture flasks (75 cm²) were rinsed with 5 ml of TME solution (50 mM Tris-HCl/10 mM MgCl₂/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride, pH 7.4). Cells were then scraped and lysed in TME with a Brinkman homogenizer (model PT10/35, setting 5 for 5 sec). Membranes were washed once and stored at -70°C until use. Approximately 50 fmol of receptor per 0.25 ml of assay mixture was incubated in TME at 25°C for 1 hr with [³H]yohimbine (79.1 Ci/mmol; 1 Ci = 37 GBq). Assays were placed on ice, filtered onto Whatman GF/C membranes, and washed with ice-cold buffer. For competition curve analysis, each assay contained 5 nM [³H]yohimbine. Nonspecific binding was determined for saturation curve analysis with 10 μ M phentolamine. Data were analyzed by computer with an iterative nonlinear regression program (18).

DNA Sequencing. Single-stranded DNA template was made from pTZ18R and pTZ19R (Pharmacia). Nucleotide sequence analysis of both strands was performed by the dideoxynucleotide chain-termination method (19, 20) by primer extension with T7 DNA polymerase (Sequenase; United States Biochemical).

Southern Blotting. Genomic DNA was extracted from fresh human kidney by standard techniques (21). DNA (10 μ g) was cut with *Pst*I and run in a single lane on a 1% agarose gel. The

gel was blotted onto nitrocellulose and hybridized in 50% formamide/5 \times SSC/1 \times Denhardt's solution/20 mM sodium phosphate, pH 6.5/100 μ g of sheared salmon sperm DNA per ml/10% dextran sulfate/2 \times 10⁶ cpm of ³²P-labeled probe per ml at 42°C for 12 hr. The blot was then washed in 0.2 \times SSC/0.1% SDS and exposed for 3 days.

Northern Blot Analysis. Total cellular RNA was isolated from fresh Sprague-Dawley rat tissues by the guanidinium isothiocyanate/cesium chloride method (22). Neonatal lungs were excised within 24 hr after birth. Poly(A)⁺ RNA was selected by using two cycles of oligo(dT)-cellulose chromatography. After denaturation by glyoxylation, the RNA was fractionated by agarose gel electrophoresis and transferred to membranes.

Pharmacological Agents. Sources of drugs were as follows: phentolamine (CIBA-Geigy); SKF 104078 (Smith Kline & French); prazosin and UK 14304 (Pfizer Diagnostics); rau-wolscine (Roth, Karlsruhe, F.R.G.); WB 4101 and *p*-aminoclonidine (Research Biochemicals, Natick, MA); corynanthine, oxymetazoline, epinephrine, and norepinephrine (Sigma); and [³H]yohimbine (New England Nuclear).

RESULTS AND DISCUSSION

The PCR was used to identify an α_2 AR subtype. A similar method to isolate clones for several members of the G protein and G protein-coupled receptor families has recently been used (23, 24). First, regions of amino acid identity between the previously cloned human α_2 ARs, C4 and C10, were identified. Then, oligonucleotides corresponding to the conserved nucleotide sequences were synthesized and used to amplify sheared human genomic DNA. A pair of such oligonucleotide primers (Fig. 1) representing the sense strand encoding the third transmembrane domain of α_2 C4 (nucleotides 402-431), and the antisense strand encoding the car-

	GCAGG CGCGG CTGGG GCGAA ACGTG CGAGC TGAGC GGGCG CAAGG TCCTC CGCGC CTCCT TTAAG AACCG GCCCA GCCCG	-349
	GCCCG CGCCC CCAGA GCGTA CGGCA TCCCG GTGGC GGAGG GCGCG ACTTT CTCGG GTCCC GGGCG GGACG GGGAG GCGCG CGGGA CAACT TGGGA AACCT CTCTG GGGCG	-239
	GACGG CAGGG ACCCC GGGCA CCGTG GAGGA GGATG TAGGA GGGCG GCTGC TGGTC CTGGG TGTTG CCGAC CTCCT AGGCC CCGCT CGTCC AGGCC ATGGG GCTCC AGCGC	-129
	CTCTG CGCGC CCGGA GGGCG GACGC TCTTG TCTAG CCGAG CCGGG CAGCG CTGTG GTCCA CGGTG CGCAC TGGGG GGGCA GCGCT CCCTC TGCCC ACCTC CCGCC CCGTC	-1
ATG	GAC CAC CAG GAC CCC TAC TCC GTG CAG GCC ACA GCG GCC ATA GCG GCG GCC ATC ACC TTC CTC ATT CTC TTT ACC ATC TTC GGC AAC GCT CRG GTC ATC	102
M	D H Q D P Y S V Q A T A A I A A A I T F L I L F T I F G N A L V I	
CTG	GCT GTG TTG ACC AGC CGC TCG CTG CGC GCC CCT CAG AAC CTG TTC CTG GTG TCG CTG GCC GCC GCC GAC ATC CTG GTG GCC ACG CTC ATC ATC CCT TTC	204
L	A V L T S A R A P A P A L V L V T L V T C G A A D I L V A T I L I I P F	
	Primer A: -----G-----	
TGC	CTG GCC AAC GAG CTG CTG GGC TAC TGG TAC TTC CGG CGC ACG TGG TGC GAG GTG TAC CTG GCG CTC GAC GTG CTC TTC TGC ACC TCG TCC ATC CTC GTC CAC	306
S	L A N E L L G Y W Y F R R T W C E V Y L A L D V L F C T S S I V H	
	-----N-----	
CTG	TGC GCC ATC AGC CTG GAC CGC TAC TGG GCC GTG AGC CGC GCG CTG GAG TAC AAC TCC AAG CGC ACC CCG CGC CGC ATC AAG TGC ATC ATC ACT GTG	408
L	G A I S A R E V N R A V S R L F E Y N S K R T P R R I K C I T L A L P V	
TGG	CTC ATC GCC GCC GTC ATC TGG CTG CCG CCC CTC ATC TAC AAG GGC GAC CAG GGC CCC CAG CCG CGC GGG CGC CCC CAG TGC AAG CTC AAC CAG GAG GCC	510
W	L I A A V I S L P P L I Y K G D Q G P Q P R G R P Q C K L N Q E A	
TGG	TAC ATC CTG GCC TCC AGC ATC GGA TCT TTC TTT GCT CCT TGC CTC ATC ATG ATC CTT GTC TAC CTG CGC ATC TAC CTG ATC GCC AAA CGC AGC AAC CGC	612
S	W Y I L A S F L A P F A P L I M I Y L R I Y L R I Y L A K R S A N R	
AGA	GGT CCC AGG GCC AAG GGG GGG CCT GGG CAG GGT GAG TCC AAG CAG CCC CGA CCC GAC CAT GGT GGG GCT TTG GCC TCA GCC AAA CTG CCA GCC CTG GCC	714
R	G P R A K G G P G Q G E S K Q P R P D H G G A L A S A K L P A L A	
TCT	GTG GCT TCT GCC AGA GAG GTC AAC GGA CAC TCG AAG TCC ACT GGG GAG AAG GAG GAG GGG GAG ACC CCT GAA GAT ACT GGG ACC CGG GCC TTG CCA CCC	816
V	P A S A R E V N R A V S R L F E Y N S K R T P R R I K C I T L A L P V	
AGT	TGG GCT GCC CTT CCC AAC TCA GGC CAG GGC CAG AAG GAG GGT GTT TGT GGG GCA TCT CCA GAG GAT GAA GCT GAA GAG GAG GAA GAG GAG GAG GAG	918
S	W A A L P N S G Q G Q K E G V C G A S P E D E A E E E E E E E E	
GAG	GAA GAG TGT GAA CCC CAG GCA GTG CCA GTG TCT CCG GCC TCA GCT TGC AGC CCC CCG CTG CAG CAG CCA CAG GGC TCC CGG GTG CTG GCC ATC CTA CGT	1020
E	E E C E P Q A V P V S P A C S A C S P P L Q Q P L Q G S R V L A T L R	
	Primer B: -----A-----	
GCC	CAG GTG CTC CTG GGC AGG GGC GTG GGT GCT ATA GGT GGG CAG TGG TGG CGT CGA AGG GCG CAC GTG ACC CGG GAG AAG GCG TTC ACC TTC GTG CTG GCT	1122
G	Q V L L G R G V G A I G G Q W W R R R A H V T R E K R F T F V L A	
GTG	GTC ATT GGC GTT TTT GTG CTC TGC TGG TTC CCC TTC TTC TTC AGC TAC AGC CTG GGC GCC ATC TGC CCG AAG CAC TGC AAG GTG CCC CAT GGC CTC TTC	1224
V	V I G V F V L C W F F F F F S Y S L G A I C P K H C K V P H G L F	
CAG	TTC TTC TGG ATC GGC TAC TGC AAC AGC TCA CTG AAC CCT GTT ATC TAC ACC ATC TTC AAC CAG GAC TTC CGC CGT GGC AGG ATC CTG TGC	1320
Q	F W I G Y C N S S L N P V I Y T I F N Q D P R R A F R R I L C	
CGC	CCG TGG ACC CAG ACG GCC TGG TGA GCGCC CTTGC GCTGC CCTGC TGGGT TGGTG CCGTG GCGCC GGGGT CACCC TGCTT CTCTG CTTGC TGTTG GTGGC TGCCT	1433
R	P W T Q T A W	
	CCCTT GGGCT TTCTG CTCCC TGCCC AGATC CTGTA GGCCCT CATCT TAGGA ACCCC TTGGG AGGGG TGGGC AGGGG GGCTG CTAGC AAGGG TCCCA GTGAA GCTTC CCCTT	1543
	GCCGG CTTAG CTGTG GGGGA CCCCT TCTCC ACCCT CTCCC TGAGC ACAGG CCGAT GGAGG TGGTT CAAAT CCTCT GGAAC ATAGC CAAGA CCAGG AGAAG AGAGA GCAC	1653

FIG. 1. Nucleotide and deduced amino acid sequence of the α_2 C2 human genomic clone. PCR oligonucleotide primers are located and mismatched nucleotides are indicated.

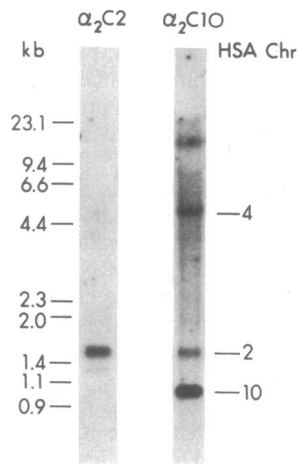


FIG. 2. Southern blot of *Pst* I-digested human genomic DNA, hybridized with the α_2C2 PCR product and with a 0.95-kb *Pst* I fragment of α_2C10 . The positions of the molecular size standards are indicated on the left. Chromosomal localizations are indicated on the right. The 0.95-kb fragment corresponds to α_2C10 , the 5.9-kb fragment corresponds to α_2C4 , and the 1.6-kb fragment corresponds to α_2C2 . An additional unidentified fragment is seen at 1.3 kb with the α_2C2 probe and at 16 kb with the α_2C10 probe.

boxyl end of the third cytoplasmic loop of α_2C4 (nucleotides 1505–1526), was able to amplify a 900-bp fragment of DNA that was recognized by a probe made from the coding region of α_2C4 . In addition, the labeled PCR fragment hybridized to a 1.6-kilobase (kb) fragment on a Southern blot of *Pst* I-digested human genomic DNA (Fig. 2). We have previously shown that a 1.6-kb *Pst* I fragment is identified with a probe from α_2C10 and that this 1.6-kb fragment localizes to human chromosome 2 (1). Hybridization of the PCR product with human somatic cell hybrids (25) showed that the gene is in fact located on chromosome 2 (data not shown).

The PCR fragment was subcloned into pTZ18R and sequenced. The deduced amino acid sequence contained regions of extensive homology to α_2C10 and α_2C4 (Fig. 3) in the putative second intracellular loop, fourth transmembrane domain (α_2C10 , 62%; α_2C4 , 38%), fifth transmembrane domain (α_2C10 , 73%; α_2C4 , 81%), and portions of the third cytoplasmic loop adjacent to the membrane. However, the majority of the putative third cytoplasmic loop was divergent from the previously cloned α_2ARs . Since there was extensive homology to the α_2ARs , yet also extensive divergence and a different chromosomal localization, the PCR fragment appeared to encode an α_2AR subtype that we refer to here as α_2C2 .

The α_2C2 amplified DNA fragment was then used to probe a human genomic library to obtain a full-length clone. After screening at high stringency, a 15-kb clone was isolated that contained a 1332-bp open reading frame uninterrupted by introns and 3000 bases of 5' untranslated region. Fig. 1 shows the nucleotide and deduced amino acid sequence of the genomic clone. The open reading frame encodes a protein of 450 amino acids. Hydropathy analysis (26) of this amino acid sequence indicates that there are seven hydrophobic clusters of 20–25 residues, each separated by stretches of hydrophilic residues. This pattern is similar to that observed with the other members of the G-protein-coupled receptor family, in which the hydrophobic residues are thought to span the plasma membrane, and the hydrophilic stretches project from the membrane (27, 28). Thus, according to this topographic model, the amino terminus and three loops would extend into

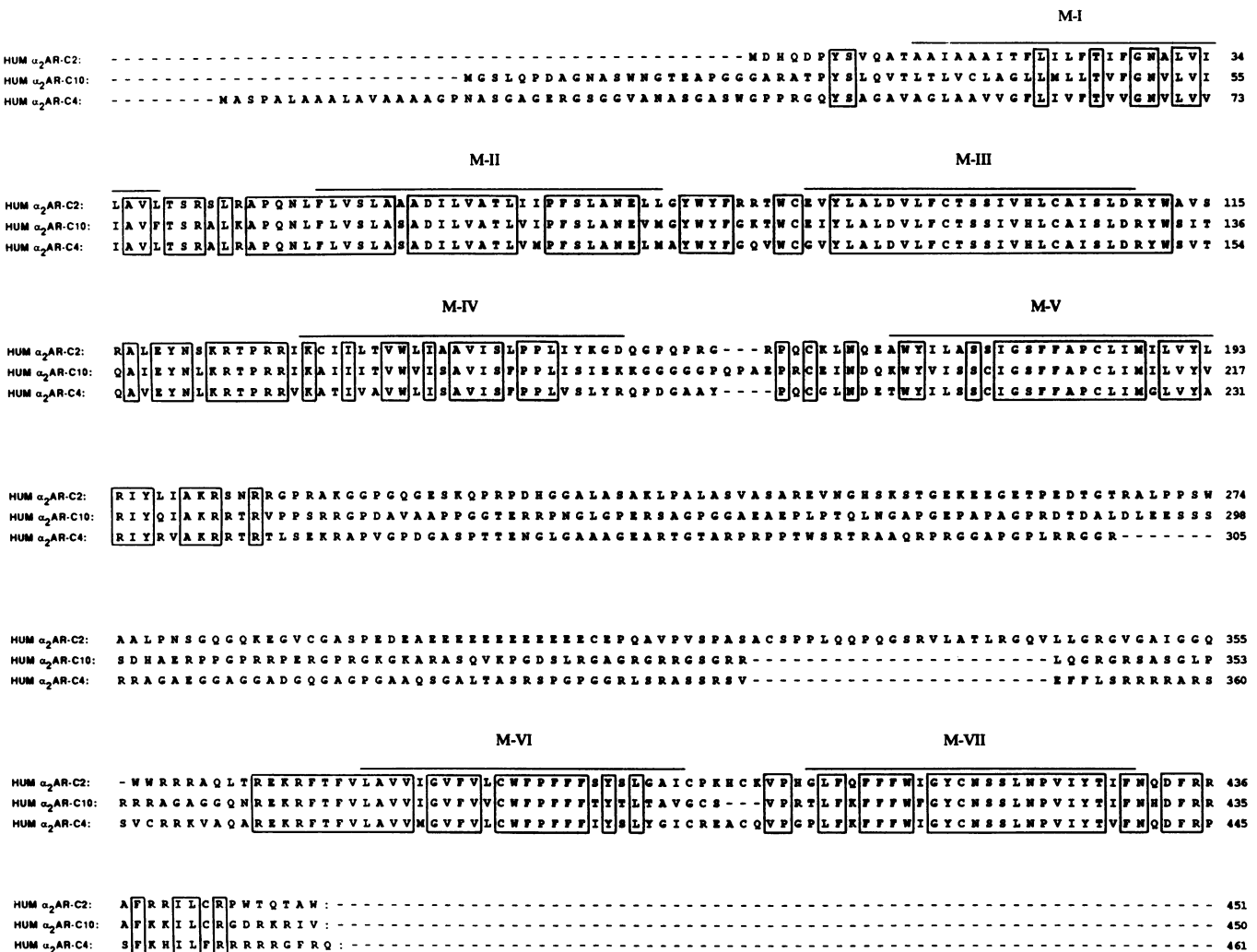


FIG. 3. Amino acid sequence alignment of the three human α_2ARs . Residues identical for all three receptors are boxed.

the extracellular space, and the carboxyl terminus and three remaining loops would extend into the cytoplasm.

The putative transmembrane domains contain the highest concentrations of amino acids homologous to those in other G-protein-coupled receptors. Comparison of the deduced amino acid sequence of our clone with that of the other members of the G-protein-coupled receptor family shows that the highest homology is with the α_2 ARs. Amino acid identities with α_2 C2 in the putative transmembrane domains of each human adrenergic receptor are as follows: α_2 C4, 75%; α_2 C10, 74%; β_2 , 36%; β_1 , 39%; β_3 , 39%; α_{1B} , 45% (D. A. Schwinn, personal communication). Fig. 3 shows the deduced amino acid alignment of the three human α_2 ARs. The transmembrane domains are strikingly conserved, while the amino terminus, the carboxyl terminus, and the third cytoplasmic loop represent the most divergent domains.

The putative topography of α_2 C2 closely resembles that of α_2 C10 and α_2 C4 in that it has a long third intracellular loop and a short carboxyl terminus. Multiple serine and threonine residues present in these domains may be sites for phosphorylation by regulatory kinases such as β -adrenergic receptor kinase, which has been shown to phosphorylate the human platelet α_2 C10 (29). The carboxyl terminus contains a conserved cysteine (residue 432), which appears to be a site of palmitoylation in the family of G-protein-coupled receptors (30).

The amino terminus of α_2 C2 is among the shortest (12 amino acids) of the members of the G-protein-coupled receptors thus far cloned and lacks potential sites for N-linked glycosylation. An α_2 AR that appears not to be glycosylated has been described in neonatal rat lung (31). Fig. 4 shows that the α_2 C2 probe does not detect a mRNA species in rat neonatal lung, even though the rat neonatal tissue used contained ≈ 300 fmol of α_2 AR binding per mg of protein (data not shown). Moreover, in this same tissue we were able to detect mRNA species for other adrenergic receptors (α_{1B}) and actin (data not shown). Therefore, it appears that while α_2 C2 is without potential sites of N-linked glycosylation in the amino terminus and extracellular loops, it is not equivalent to the rat neonatal lung receptor. The canine putative G-protein-coupled receptors RDC7 and RDC8, whose functions are unknown, also have similar short amino termini that lack glycosylation sites (24). The relationship between α_2 C2 and these receptors is at present unknown. Since α_2 C2 was cloned from a genomic library we cannot totally exclude the possibility that an intron exists somewhere in the amino terminus. However, the biochemical demonstration of a nonglycosylated α_2 AR (31) and the existence of two canine putative G-protein-coupled receptors with short amino termini lacking N-linked glycosylation sites indicate that while the amino terminus of α_2 C2 is unusual, it is probably not unique.

To examine the ligand binding characteristics of α_2 C2, a construct containing the entire 1532-bp coding region and 182 bp of 5' untranslated region was inserted into the mammalian expression vector pBC12BI and was used to transiently transfect COS-7 cells. COS-7 cells transfected with the vector

containing the α_2 C2 receptor sequence were able to bind the α_2 AR antagonist [3 H]yohimbine in a saturable manner with high specific activity (2 pmol per mg of protein) and affinity (K_d , 9.5 nM). Nontransfected COS-7 cells exhibited no specific binding. Binding competition curves showed that the potency series for agonists [*p*-aminoclonidine > (-)-nor-epinephrine \approx (-)-epinephrine > (+)-epinephrine] and antagonists (yohimbine \approx rauwolscine > prazosin > corynanthine) exhibited α_2 AR specificity and stereoselectivity. Table 1 shows the K_i values for various adrenergic compounds determined simultaneously for the three cloned human α_2 ARs. It is apparent that the pharmacology of α_2 C2 is unique. Rauwolscine, corynanthine, WB 4101, and epinephrine appear to be useful in distinguishing α_2 C2 from α_2 C4, while prazosin and *p*-aminoclonidine distinguish α_2 C2 from α_2 C10. Oxymetazoline is the most useful drug as it discriminates between all three α_2 ARs, having approximately a 10-fold difference in affinity for each receptor.

The antagonist SKF 104078 appears to discriminate between the pre- and postsynaptic α_2 ARs, as it has moderate affinity for the postsynaptic site (K_b , 70–150 nM), but low affinity for the presynaptic site (K_b , 3–30 μ M) (32). The relatively high affinity for the compound SKF 104078 (K_i , 118 nM) suggests that α_2 C2, like α_2 C4 and α_2 C10, is a postsynaptic receptor. This notion fits with the distribution of α_2 C2 as assessed by Northern blot analysis of various rat tissues shown in Fig. 4. mRNA for the receptor is clearly detected in adult rat liver and kidney.

The relatively high affinity for prazosin (K_i , 293 nM) and low affinity for oxymetazoline (K_i , 1506 nM) suggests that α_2 C2 is an α_2 B-like receptor. However, the absence of an α_2 C2 mRNA species in the cerebral cortex or neonatal lung, two model tissues for the α_2 B receptor, suggests that this receptor is not the pharmacologically defined α_2 B. In fact we cannot clearly place α_2 C2 into any of the presently defined α_2 AR subtypes.

While the present pharmacological classification is useful, it is not sufficient. The isolation of distinct but related genes constitutes the most sensitive way to classify receptors. The expression of these single gene products in various cell systems will allow for detailed biochemical, molecular, and pharmacological analyses of the receptors and their coupled signal transduction pathways. These studies in conjunction with those of gene structure and receptor tissue distribution should lead to a more accurate and useful classification.

Recently, two preliminary reports have appeared describing the cloning of other α_2 ARs (33, 34). Available information

Table 1. Competition by α -adrenergic ligands for the binding of [3 H]yohimbine to membranes from COS-7 cells transfected with either pBC α_2 -C2, pBC α_2 -C4, or pBC α_2 -C10

	K_i , nM		
	C2	C4	C10
Agonists			
(-)-Epinephrine	1851	318	1671
(+)-Epinephrine	8422	ND	ND
(-)-Norepinephrine	1265	606	3677
Oxymetazoline	1506	125	13.2
<i>p</i> -Aminoclonidine	120	97	31
Antagonists			
Corynanthine	1002	182	1188
Phentolamine	9.2	14.4	6.2
Prazosin	293	67.7	2237
Rauwolscine	11	2.1	7.1
SKF 104078	105	41	97
WB 4101	132	13	47

The results shown are representative of two experiments, each done simultaneously for all three receptors. The expression vectors pBC α_2 -C4 and pBC α_2 -C10 were constructed as described (2). ND, not determined.

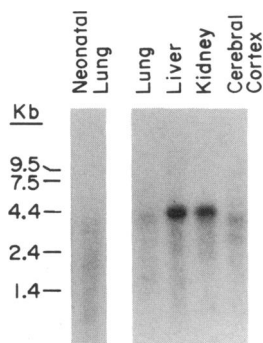


FIG. 4. Northern blot of rat tissues probed with the α_2 C2 PCR fragment. Each lane contains 10 μ g of poly(A) $^+$ selected RNA. After hybridization, the filters were washed successively in $2\times$ SSC/0.1% SDS at room temperature and $0.1\times$ SSC/0.1% SDS at 55°C, and exposed at -70°C for 5 days.

about these clones suggests that they are different from α_2C10 , α_2C4 , and α_2C2 .

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