and 1.02 kb in size as determined by comparison with comigrating DNA size markers (Fig. 6A, lanes 3 and 6).

Significance of triple helix formation in large DNA. Although triple-stranded structures of polynucleotides were discovered decades ago, the biological significance has remained obscure. Such triplexes were proposed to be involved in processes like regulation of gene expression, maintenance of folded chromosome conformations, chromosome condensation during mitosis, and induction of local conformational changes in B-DNA (*35–37*). The work reported here demonstrates that homopurine-homopyrimidine double helical tracts can be recognized within large DNA by triple helix formation under physiological conditions. Homopyrimidine oligonucleotides and their analogs equipped with efficient DNA cleaving moieties at the 5′ end could become useful tools in chromosome analysis, gene mapping, and isolation. Moreover, as molecular biology defines specific disease states at the DNA level, a chemotherapeutic strategy of "artificial repressors" based on triple helix–forming DNA analogs becomes a possibility.

REFERENCES AND NOTES

1. H. O. Smith, *Science* **205**, 455 (1979).
2. P. Modrich, *Crit. Rev. Biochem.* **13**, 287 (1982).
3. D. Schwartz and C. R. Cantor, *Cell* **37**, 67 (1984).
4. G. F. Carle, M. Frank, M. V. Olsen, *Science* **232**, 65 (1986).
5. P. B. Dervan, *ibid.*, p. 464.
6. J. K. Barton, *ibid.*, **233**, 727 (1986).
7. M. Bruist, S. J. Horvath, L. E. Hood, T. A. Steitz, M. I. Simon, *ibid.* **235**, 777 (1987).
8. J. Sluka, M. Bruist, S. J Horvath, M. I. Simon, P. B. Dervan, *ibid.*, in press.
9. R. P. Hertzberg and P. B. Dervan, *Biochemistry* **23**, 3934 (1984).
10. R. S. Youngquist and P. B. Dervan, *J. Am. Chem. Soc.* **107**, 5528 (1985).
11. G. Felsenfeld, D. R. Davies, A. Rich, *ibid.* **79**, 2023 (1957).
12. A. M. Michelson, J. Massoulié, W. Guschlbauer, *Prog. Nucleic Acids Res. Mol. Biol.* **6**, 83, (1967).
13. G. Felsenfeld and H. T. Miles, *Annu. Rev. Biochem.* **36**, 407 (1967).
14. M. N. Lipsett, *Biochem. Biophys. Res. Commun.* **11**, 224 (1963).
15. ———, *J. Biol. Chem.* **239**, 1256 (1964).
16. F. B. Howard, J. Frazier, M. N. Lipsett, H. T. Miles, *Biochem. Biophys. Res. Commun.* **17**, 93 (1964).
17. J. H. Miller and H. M. Sobell, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1201 (1966).
18. A. R. Morgan and R. D. Wells, *J. Mol. Biol.* **37**, 63 (1968).
19. J. S. Lee, D. A. Johnson, A. R. Morgan, *Nucleic Acids Res.* **6**, 3073 (1979).
20. S. Arnott and P. J. Bond, *Nature (London) New Biol.* **244**, 99 (1973).
21. S. Arnott and E. Selsing, *J. Mol. Biol.* **88**, 509 (1974).
22. S. Arnott, P. J. Bond, E. Selsing, P. J. C. Smith, *Nucleic Acids Res.* **3**, 2459 (1976).
23. K. Hoogsteen, *Acta Cryst.* **12**, 822 (1959).
24. W. Saenger, *Principles of Nucleic Acid Structure*, C. R. Cantor, Ed. (Springer-Verlag, New York, 1984).
25. G. D. Knorre and V. V. Vlassov, *Prog. Nucleic Acid. Res. Mol. Biol.* **32**, 291 (1985), and references therein.
26. G. B. Dreyer and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 968 (1985).
27. C. F. Chu and L. E. Orgel, *ibid.* **82**, 963 (1985).
28. B. L. Iverson and P. B. Dervan, *J. Am. Chem. Soc.* **109**, 1241 (1987).
29. J. S. Taylor, P. G. Schultz, P. B. Dervan, *Tetrahedron* **40**, 457 (1984).
30. R. Glaser and E. J. Gabbay, *Biopolymers* **6**, 243 (1968).
31. The *p*H values are not corrected for temperature or different ethylene glycol percentage and are given for the tenfold concentrated buffer solutions at 25°C.
32. T. Povsic and P. B. Dervan, unpublished observations.
33. G. R. Cassani and F. J. Bollum, *Biochemistry* **8**, 3928 (1969).
34. A. J. Raae and K. Kleppe, *ibid.* **17**, 2939 (1978).
35. A. R. Morgan, *Trends Biochem. Sci.* **4**, N244 (1979).
36. R. C. Hopkins, *Comments Mol. Cell. Biophys.* **2**, 153 (1984).
37. K. W. Minton, *J. Exp. Pathol.* **2**, 135 (1985).
38. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
39. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
40. The pDMAGIO plasmid was constructed by Mendel and Dervan [D. Mendel and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 910 (1987)].
41. M. W. Van Dyke and P. B. Dervan, *Science* **225**, 1122 (1984).
42. The concentrations of the single-stranded oligodeoxynucleotides determined with the use of the following ε values (260 mm) for each base: 15400 (A), 11700 (G), 7300 (C), and 8800 (T).

43. Supported by grants from the National Institutes of Health (GM 35724) and the Ralph M. Parsons Foundation, and a fellowship (to H.E.M.) from the Swiss National Science Foundation.

8 July 1987; accepted 21 September 1987

Cloning, Sequencing, and Expression of the Gene Coding for the Human Platelet α_2-Adrenergic Receptor

B. K. KOBILKA, H. MATSUI, T. S. KOBILKA, T. L. YANG-FENG, U. FRANCKE, M. G. CARON, R. J. LEFKOWITZ, J. W. REGAN

The gene for the human platelet α_2-adrenergic receptor has been cloned with oligonucleotides corresponding to the partial amino acid sequence of the purified receptor. The identity of this gene has been confirmed by the binding of α_2-adrenergic ligands to the cloned receptor expressed in *Xenopus laevis* oocytes. The deduced amino acid sequence is most similar to the recently cloned human β_2- and β_1-adrenergic receptors; however, similarities to the muscarinic cholinergic receptors are also evident. Two related genes have been identified by low stringency Southern blot analysis. These genes may represent additional α_2-adrenergic receptor subtypes.

A VARIETY OF NEUROTRANSMITTER AND HORMONE RECEPTORS elicit their responses via biochemical pathways that involve transduction elements known as guanine nucleotide regulatory (G) proteins (*1*). Among these are several types of receptors for epinephrine (adrenaline) which are termed adrenergic receptors. These adrenergic receptor subtypes are of particular interest because they are coupled to each of the major second messenger pathways that are known to be linked through G

B. K. Kobilka, H. Matsui, T. S. Kobilka, M. G. Caron, R. J. Lefkowitz, and J. W. Regan are at the Howard Hughes Medical Institute, Departments of Medicine, Biochemistry, and Physiology, Duke University Medical Center, Durham, NC 27710. T. L. Yang-Feng and U. Francke are at the Department of Human Genetics, Yale University School of Medicine, P.O. Box 333, New Haven, CT 06510. Correspondence should be sent to R.J.L. at Box 821, Duke University Medical Center, Durham, NC 27110.

proteins. Thus the β_1- and β_2-adrenergic receptors stimulate adenylate cyclase, the α_1-adrenergic receptors stimulate breakdown of polyphosphoinositides, and the α_2-adrenergic receptors inhibit adenylate cyclase (*2*).

Understanding ligand binding and the coupling of these receptors to specific biochemical pathways depends on determining the structures of the members of this family of related receptors. To this end we recently reported the molecular cloning of the gene and complementary DNA (cDNA) for the hamster (*3*) and human (*4, 5*) β_2-adrenergic receptors and the cDNA for the human β_1-adrenergic receptor (*6*), all of which stimulate adenylate cyclase. An avian β-adrenergic receptor, which also stimulates adenylate cyclase (*7*), and two subtypes of muscarinic cholinergic receptors, which are coupled to stimulation of polyphosphoinositide turnover and the inhibition of adenylate cyclase, have also been cloned (*8–10*). The deduced structures of these receptors reveal that they are homologous to each other and to the visual light pigment rhodopsin (*11*).

We now report the molecular cloning, sequencing, and expression of the gene for the human platelet α_2-adrenergic receptor. It is now possible to compare the structures of receptors that bind the same endogenous ligands but are known to be coupled to different G proteins and that have opposite effects on the adenylate cyclase system.

Cloning strategy. Our approach to cloning the α_2-adrenergic receptor gene entailed the synthesis of oligonucleotide probes corresponding to amino acid sequences obtained from the purified protein. These probes were then used to screen a human genomic DNA library. The decision to use a genomic library was influenced by several factors. Chief among them is that introns do not interrupt the coding sequences of the genes for the β_2-adrenergic receptor (*3, 4*), the M2 muscarinic receptor (*10*), and G-21 (*12*), another as yet functionally unassigned member of this gene family. Thus the likelihood of obtaining the complete coding sequence from a genomic library appeared good.

Human platelet α_2-adrenergic receptors were purified, and peptides were obtained with either cyanogen bromide or cyanogen bromide plus enzymatic cleavage (*13*). Peptides were isolated by a combination of gel filtration and reversed phase liquid chromatography, and their amino acid sequences were determined (Fig. 1). On the basis of the sequence of peptide 1, two overlapping oligonucleotide probes (39 nucleotides in length) were constructed with the use of codon bias data for humans (*14*) (Fig. 2). These probes were labeled and then used to screen 10^6 recombinants from a commercially available human genomic library (Clonetech) by plaque hybridization analysis. Three clones that strongly hybridized to both probes [stringency 0.5× standard sodium citrate (SSC) at 65°C] were isolated. The three clones, each of which contained an insert of genomic DNA of approximately 15 kb, were identical and contained, on the basis of Southern blot analysis, a 5.5-kb Bam HI fragment that also hybridized to both probes. (Fig. 3A). The sequences of the oligonucleotide probes matched the sequence of the gene in 36 out of 39 and in 33 out of 39 positions for probes 1 and 2, respectively. The deduced amino acid sequence (Fig. 3B) beginning with the first ATG (methionine) in this open reading frame and extending to the termination codon codes for a protein that is 450 amino acids in length. Hydrophobicity analysis (*15*) revealed seven clusters of hydrophobic residues of more than 20 amino acids in length that could represent membrane spanning regions of the receptor (Fig. 4, brackets). The presence of the deduced amino acid sequences of the four peptides isolated from the purified receptor protein (Fig. 3B) supports the conclusion that this gene codes for the human platelet α_2-adrenergic receptor.

Structural comparisons. Comparison of the deduced amino acid sequence of the α_2-adrenergic receptor with that of the human β_2-adrenergic receptor (*5*), the human β_1-adrenergic receptor (*6*), the human G-21 protein (*12*), and the porcine cerebral M1 (*8*) and

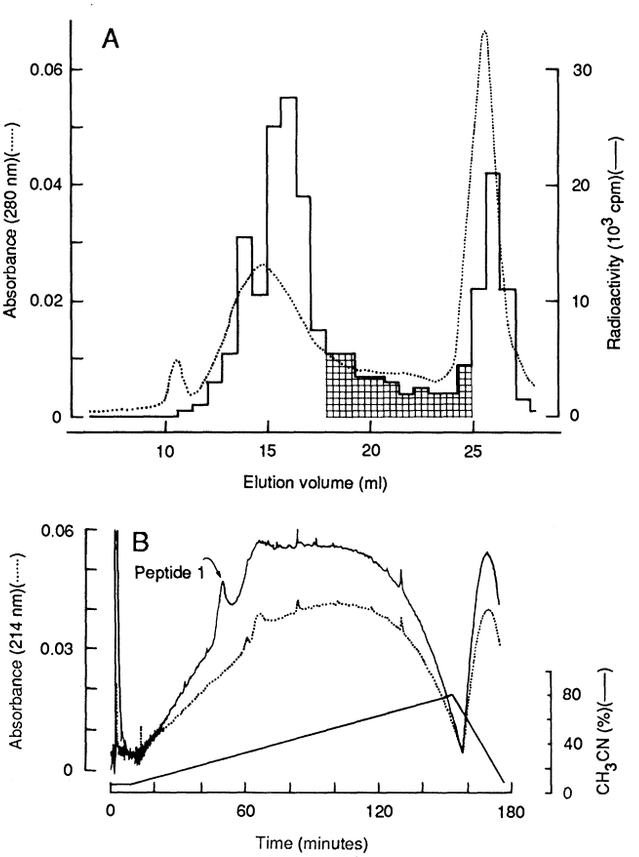


Fig. 1. Isolation of peptide 1 by gel filtration and by reversed-phase liquid chromatography. Approximately 900 pmol of α_2-adrenergic receptors were purified to a specific activity of ~8 nmol per milligram of protein from about 1400 units of outdated human platelets by a combination of affinity (*32*), heparin-agarose, and wheat germ–agarose chromatography (*33*). The receptors were covalently labeled with the α_2-adrenergic photoaffinity probe [^{3}H]SKF 102229 (*34*) and were then dialyzed in an Amicon stirred cell by repeated concentration and dilution with 30 percent acetonitrile in 0.1 percent trifluoroacetic acid (TFA) over a YM-30 membrane. The final concentrate was lyophilized and then redissolved in 300 μl of 88 percent formic acid. Solid cyanogen bromide was added in three 100-μmol amounts over a period of 3 days, and the entire sample was then fractionated by gel filtration liquid chromatography with a TSK 4000 column (7.5 by 600 mm) and a mobile phase of 10 percent formic acid in water (**A**). Pooled material in the cross-hatched area was then fractionated by reversed-phase liquid chromatography with a Phenomenex (5 μm, W-Porex) C-18 column (4.6 by 250 mm) and a gradient of 8 to 80 percent acetonitrile in 0.1 percent TFA, at a flow of 2 ml/min (**B**). A blank run is shown by the dotted line. Material eluting between 49 and 54.5 minutes was subjected to amino acid sequence analysis on an Applied Biosystems 470A gas-phase sequenator in combination with an Applied Biosystems 120A phenylthiohydantoin analyzer. One peptide (145 pmol) was present that yielded 27 amino acid residues before the signal was lost. Material eluting in earlier and later fractions from the gel filtration step was also fractionated by reversed-phase liquid chromatography, but no other peptides were obtained. In a second purification, the α_2-adrenergic receptor was first treated with 2-mercaptoethanol, and it was then carboxymethylated with iodoacetamide. The receptor preparation was concentrated and diluted as above to remove reactants but with a buffer containing digitonin (10 m*M* tris-HCl, 1 m*M* 2-mercaptoethanol, 0.02 percent digitonin, *p*H 8.5). The receptor preparation was then cleaved by successive treatments with cyanogen bromide, 1 percent (w/w) pepsin, and 1 percent (w/w) thermolysin. Reversed-phase liquid chromatography yielded three additional peptides, which were isolated and sequenced. The amino acid sequences of all the peptides are shown in Fig. 3B.

cardiac M2 (*9*) muscarinic cholinergic receptors are presented in Fig. 4. Clearly the α_2-adrenergic receptor has all the features of these other G protein–coupled receptors. They are all similar in length and range from 413 amino acids (β_2) to 477 amino acids (β_1). Like the other receptors, the α_2-adrenergic receptor has two sites for *N*-linked glycosylation near the amino terminus (at asparagine residues 10 and 14). The greatest homology between the α_2-adrenergic receptor and the other receptors occurs in the putative transmembrane spanning domains. Each of these seven domains has been demarcated on the basis of the hydrophobicity index (*15*), and each consists of 26 predominantly hydrophobic amino acids that are capable of forming an α helix. Separating these hydrophobic domains are variable stretches of polar or charged amino acids. It is proposed that each hydrophobic domain spans the plasma membrane once with the amino terminus being extracellular and the carboxyl terminus being intracellular (*2*). This model predicts that the hydrophilic regions connecting the hydrophobic domains would form loops projecting out of the membrane. Thus, along with the carboxyl terminus, three loops would extend into the cytoplasm of the cell, while the remaining three loops, as well as the amino terminus, would project into extracellular space. Although this structural organization is based primarily on analogy with the apparent structure of rhodopsin (*11*), it provides a useful working model for the study of the family of G protein–coupled receptors.

M I L V Y V R I Y Q I A K R R T R V P P

ATG ATC CTG GTC TAT* GTG CGG* ATC TAC CAG ATT* GCC AAG
(probe 1)

ATC TAC CAG ATT* GCC AAG CGG* CGG* ACC CGG* GTG CCC* CCA*
(probe 2)

Fig. 2. Partial amino acid sequence of peptide 1 and corresponding nucleotide sequences of the probes used for plaque hybridization analysis. Two overlapping 39-nucleotide probes were designed in accordance with most frequent codon usage (*14*). Incorrect choices based upon the actual nucleotide sequence of the gene are indicated by asterisks. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K. Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Table 1. [^{3}H]Yohimbine binding to membranes prepared from RNA-injected *X. laevis* oocytes and competition by adrenergic agonists and antagonists. Experiments were conducted as described in Fig. 6. Apparent equilibrium dissociation constants (K_i) were determined by computer with a nonlinear regression program describing the interaction of ligands with a single class of binding sites (*31*).

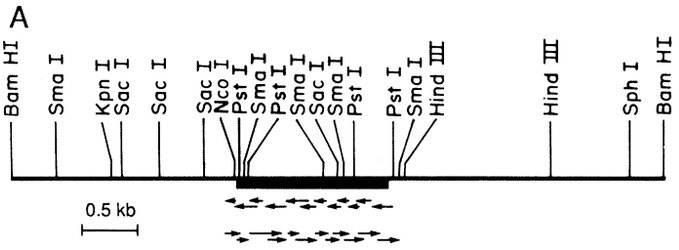
Antagonists	K_i (n*M*)	Agonists	K_i (n*M*)
Rauwolscine	3.5	*p*-Aminoclonidine	140
Yohimbine	4.4	(−)Epinephrine	1,900
Idazoxan	18	(−)Norepinephrine	7,500
Phentolamine	27	(+)Epinephrine	16,000
Prazosin	880	(−)Isoproterenol	480,000
Corynanthine	2,200		

Of the 182 amino acids comprising all of the membrane spanning domains for any of the given receptors (Fig. 4), in the α_2-adrenergic receptor 45 percent are homologous with the human β_1-adrenergic receptor, 42 percent are homologous with G-21, 39 percent are homologous with the human β_2-adrenergic receptor, 31 percent are homologous with the M1 receptor, and 28 percent are homologous with the M2 receptor. The single region of greatest homology occurs between amino acids residues 111 to 136 (third transmembrane and second cytoplasmic loop) where the α_2-adrenergic receptor shares 22 out of 26 amino acid identities (85 percent), with G-21. It is possible, therefore, that the protein coded for by G-21 might be an adrenergic receptor.

There is much less homology between the α_2-adrenergic receptor and the other receptors in the putative intracellular and extracellular domains. Although the carboxyl terminus of the α_2-adrenergic receptor is comparable in length to that of G-21 and the M1 and M2 receptors, it shows significant homology with the initial segments of the longer carboxyl terminal regions of the β_1- and β_2-adrenergic receptors. The greatest homology in the carboxyl terminal regions, however, is found between the α_2-adrenergic receptor and G-21. The third cytoplasmic loop of the α_2-adrenergic receptor (amino acid residues 219 to 374) is 156 amino acids in length. Although comparable in size to the corresponding loops in the M1 receptor (155 residues), the M2 receptor (179 residues), and G-21 (128 residues), significant amino acid homology in this region is practi-

Table 2. Human chromosome content and presence of human platelet α_2-adrenergic receptor sequences in Chinese hamster × human somatic cell hybrids. Data for chromosomes involved in rearrangements or present at a frequency of 0.1 or less were excluded. Somatic cell hybrid analysis was carried out with 13 hybrids of series XII, XIII, XVII, XXI, and 31. The derivation and human chromosome content of these hybrids have recently been summarized (*24*). Southern blot analyses of Pst I– and Pvu II–digested genomic DNA from these hybrids and their parental controls were done with the ^{32}P-labeled 0.95-kb Pst I genomic fragment as a probe.

Enzyme/ fragment size	Hybrid./ chrom.	Human chromosomes																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Pst I / 5.9 kb	+/+	2	1	2	3	0	2	1	2	2	0	0	2	2	3	3	2	0	3	3	0	2	2	1
Pvu II / 3.7 kb	−/−	8	9	4	7	7	5	7	6	8	8	4	7	6	4	5	7	8	4	7	5	4	4	2
	+/−	1	1	1	0	3	1	2	1	1	3	3	1	1	0	0	1	3	0	0	3	1	0	0
	−/+	2	1	5	0	2	5	1	4	1	2	4	3	4	4	5	2	2	4	3	5	5	6	3
Discordant hybrids		3	2	6	0	5	6	3	5	2	5	7	4	5	4	5	3	5	4	3	8	6	6	3
Informative hybrids		13	12	12	10	12	13	11	13	12	13	11	13	13	11	13	12	13	11	13	13	12	12	6
Pst I / 1.8 kb	+/+	1	2	2	2	0	2	1	1	2	0	1	2	2	3	2	1	0	2	2	0	2	1	2
Pvu II / 1.8 kb	−/−	7	10	4	6	7	5	7	5	8	8	5	7	6	4	4	6	8	4	6	6	4	3	2
	+/−	2	0	1	1	3	1	2	2	1	3	2	1	1	0	1	2	3	0	1	3	1	1	0
	−/+	3	0	5	1	2	5	1	5	1	2	3	3	4	4	6	3	2	5	4	4	5	7	2
Discordant hybrids		5	0	6	2	5	6	3	7	2	5	5	4	5	4	7	5	5	5	5	7	6	8	2
Informative hybrids		13	12	12	10	12	13	11	13	12	13	11	13	13	11	13	12	13	11	13	13	12	12	6
Pst I / 0.95 kb	+/+	0	0	2	0	1	2	1	0	0	2	1	1	0	1	1	0	0	0	0	1	1	2	1
Pvu II / 2.2 kb	−/−	7	8	5	7	9	6	8	5	7	11	6	7	5	3	4	7	9	2	5	8	4	4	2
	+/−	2	2	0	0	1	0	1	2	2	0	1	1	2	1	1	2	2	2	2	1	1	0	0
	−/+	4	2	5	3	1	5	1	6	3	0	3	4	6	6	7	3	2	7	6	3	6	6	3
Discordant hybrids		6	4	5	3	2	5	2	8	5	0	4	5	8	7	8	5	4	9	8	4	6	6	3
Informative hybrids		13	12	12	10	12	13	11	13	12	13	11	13	13	11	13	12	13	11	13	13	12	12	6



B

```
    -50.      -40.      -30.      -20.      -10.          1                15
CCCGCGTTCATGTTCCGCCAGGAGGCCAAGGCCGTTGGCCGAGGGCAGCTTTGCGCCC  ATG GGC TCC CTG CAG
                                                             Met Gly Ser Leu Gln

                                                                              75
CCG GAC GCG GGC AAC GCG AGC TGG AAC GGG ACC GAG GCG CCG GGG GGC GGC GCC CGG GCC
Pro Asp Ala Gly Asn Ala Ser Trp Asn Gly Thr Glu Ala Pro Gly Gly Gly Ala Arg Ala
                   *         *

                                                             135
ACC CCT TAC TCC CTG CAG GTG ACG CTG ACG CTG GTG TGC CTG GCC GGC CTG CTC ATG CTG
Thr Pro Tyr Ser Leu Gln Val Thr Leu Thr Leu Val Cys Leu Ala Gly Leu Leu Met Leu

                                                             195
CTC ACC GTG TTC GGC AAC GTG CTC GTC ATC ATC GCC GTG TTC ACG AGC CGC GCG CTC AAG
Leu Thr Val Phe Gly Asn Val Leu Val Ile Ile Ala Val Phe Thr Ser Arg Ala Leu Lys

                                                             255
GCG CCC CAA AAC CTC TTC CTG GTG TCT CTG GCC TCG GCC GAC ATC CTG GTG GCC ACG CTC
Ala Pro Gln Asn Leu Phe Leu Val Ser Leu Ala Ser Ala Asp Ile Leu Val Ala Thr Leu

                                                             315
GTC ATC CCT TTC TCG CTG GCC AAC GAG GTC ATG GGC TAC TGG TAC TTC GGC AAG ACT TGG
Val Ile Pro Phe Ser Leu Ala Asn Glu Val Met Gly Tyr Trp Tyr Phe Gly Lys Thr Trp

                                                             375
TGC GAG ATC TAC CTG GCG CTC GAC GTG CTC TTC TGC ACG TCG TCC ATC GTG CAC CTG TGC
Cys Glu Ile Tyr Leu Ala Leu Asp Val Leu Phe Cys Thr Ser Ser Ile Val His Leu Cys

                                                             435
GCC ATC AGC CTG GAC CGC TAC TGG TCC ATC ACA CAG GCC ATC GAG TAC AAC CTG AAG CGC
Ala Ile Ser Leu Asp Arg Tyr Trp Ser Ile Thr Gln Ala Ile Glu Tyr Asn Leu Lys Arg

                                                             495
ACG CCG CGC CGC ATC AAG GCC ATC ATC ATC ACC TGT TGG GTC ATC TCG GCC GTC ATC TCC
Thr Pro Arg Arg Ile Lys Ala Ile Ile Ile Thr Val Trp Val Ile Ser Ala Val Ile Ser

                                                             555
TTC CCG CCG CTC ATC TCC ATC GAG AAG AAG GGC GGC GGC GGC GGC CCG CAG CCG GCC GAG
Phe Pro Pro Leu Ile Ser Ile Glu Lys Lys Gly Gly Gly Gly Gly Pro Gln Pro Ala Glu
```

```
                                                             615
CCG CGC TGC GAG ATC AAC GAC CAG AAG TGG TAC GTC ATC TCG TCG TGC ATC GGC TCC TTC
Pro Arg Cys Glu Ile Asn Asp Gln Lys Trp Tyr Val Ile Ser Ser Cys Ile Gly Ser Phe

                                                             675
TTC GCT CCC TGC CTC ATC ATG ATC CTG GTC TAC GTG CGC ATC TAC CAG ATC GCC AAG CGT
Phe Ala Pro Cys Leu Ile Met Ile Leu Val Tyr Val Arg Ile Tyr Gln Ile Ala Lys Arg
                         peptide 1

                                                             735
CGC ACC CGC GTG CCA CCC AGC CGC CGG GGT CCG GAC GCC GTC GCC GCG CCG CCG GGG GGC
Arg Thr Arg Val Pro Pro Ser Arg Arg Gly Pro Asp Ala Val Ala Ala Pro Pro Gyl Gly
                                                  peptide 2
                                                             795
ACC GAG CGC AGG CCC AAC GGT CTG GGC CCC GAG CGC AGC GCG GGC CCG GGG GGC GCA GAG
Thr Glu Arg Arg Pro Asn Gly Leu Gly Pro Glu Arg Ser Ala Gly Pro Gly Gly Ala Glu
                                                  peptide 3
                                                             855
GCC GAA CCG CTG CCC ACC CAG CTC AAC GGC GCC CCT GGC GAG CCC GCG CCG GCC GGG CCG
Ala Glu Pro Leu Pro Thr Gln Leu Asn Gly Ala Pro Gly Glu Pro Ala Pro Ala Gly Pro

                                                             915
CGC GAC ACC GAC GCG CTG GAC CTG GAG GAG AGC TCG TCT TCC GAC CAC GCC GAG CGG CCT
Arg Asp Thr Asp Ala Leu Asp Leu Glu Glu Ser Ser Ser Ser Asp His Ala Glu Arg Pro

                                                             975
CCA GGG CCC CGC AGA CCC GAG CGC GGT CCC CGG GGC AAA GGC AAG GCC CGA GCG AGC CAG
Pro Gly Pro Arg Arg Pro Glu Arg Gly Pro Arg Gly Lys Gly Lys Ala Arg Ala Ser Gln

                                                            1035
GTG AAG CCG GGC GAC AGC CTG CGC GGC GCG GGC CGG GGG CGA CGG GGA TCG GGA CGC CGG
Val Lys Pro Gly Asp Ser Leu Arg Gly Ala Gly Arg Gly Arg Arg Gly Ser Gly Arg Arg
peptide 4
                                                            1095
CTG CAG GGC CGG GGG AGG AGC GCG TCG GGG CTG CCA AGG CGT CGC GCT GGC GCG GGC GGG
Leu Gln Gly Arg Gly Arg Ser Ala Ser Gly Leu Pro Arg Arg Arg Ala Gly Ala Gly Gly

                                                            1155
CAG AAC CTC GAG AAG CGC TTC ACG TTC GTG CTG GCC GTG GTC ATC GGA GTG TTC GTG GTG
Gln Asn Arg Glu Lys Arg Phe Thr Phe Val Leu Ala Val Val Ile Gly Val Phe Val Val

                                                            1215
TGC TGG TTC CCC TTC TTC TTC ACC TAC ACG CTC ACG GCC GTC GGG TGC TCC GTG CCA CGC
Cys Trp Phe Pro Phe Phe Phe Thr Tyr Thr Leu Thr Ala Val Gly Cys Ser Val Pro Arg

                                                            1275
ACG CTC TTC AAA TTC TTC TTC TGG TTC GGC TAC TGC AAC AGC TCG TTG AAC CCG GTC ATC
Thr Leu Phe Lys Phe Phe Phe Trp Phe Gly Tyr Cys Asn Ser Ser Leu Asn Pro Val Ile

                                                            1335
TAC ACC ATC TTC AAC CAC GAT TTC CGC CGC GCC TTC AAG AAG ATC CTC TGT CGG GGG GAC
Tyr Thr Ile Phe Asn His Asp Phe Arg Arg Ala Phe Lys Lys Ile Leu Cys Arg Gly Asp

          1350.          .          .          .          .          .
AGG AAG CGG ATC GTG TGA  GGTTTCCGCTGGCGCCCGCGTAGACTCACGCTGACTGCAGGCAGCGGGGGGCAT
Arg Lys Arg Ile Val ---

 .          .          .          .          .          .          .
CGAGGGGTGCTTAGCCCGAGGGCACTCAGAAACCCGGGCGCTGCTGCTCTGCGTTT
```

Fig. 3. Restriction map (**A**) and nucleotide and deduced amino acid sequence (**B**) of the human platelet α_2-adrenergic receptor genomic clone. Three identical clones containing about 15 kb of human genomic DNA were obtained by screening 10^6 recombinants from a λ EMBL 3 human genomic library (Clonetech) with two synthetic oligonucleotide probes (Fig. 2). The probes, synthesized on an Applied Biosystems model 380B DNA synthesizer, were purified on a 16 percent denaturing polyacrylamide gel and were labeled with ^{32}P at the 5′ hydroxyl group by T4 polynucleotide kinase. Duplicate filters were hybridized in 6× SSC, 10× Denhardt's, sodium pyrophosphate (0.1 percent), 0.1 percent SDS, sheared salmon sperm DNA (50 μg/ml), and ^{32}P-labeled oligonucleotide (2×10^6 cpm/ml) at 45°C for 36 hours. Filters were washed at 60°C in 2× SSC. The λ DNA, isolated from positive clones, contained a 5.5-kb Bam HI fragment, which hybridized on Southern blots to both probes at high stringency (0.5× SSC at 65°C). The restriction map of this 5.5-kb Bam-HI fragment is shown above (A). The heavy line indicates the coding region, and the arrows indicate the sequencing strategy. Nucleotide sequencing was done by the Sanger dideoxy method (*35*) with both the Klenow fragment of DNA polymerase I (Pharmacia) and avian reverse transcriptase (Promega) on M13 and PUC templates. The nucleotide sequence and the deduced amino acid sequence are shown in (B). Potential sites of *N*-linked glycosylation are indicated by asterisks. α_2-Adrenergic receptor peptides that were isolated and sequenced (Fig. 1) are underlined.

cally nonexistent. The β_1- and β_2-adrenergic receptors have relatively short third loops of 78 and 52 amino acids, respectively. Three regions of the third loop of the β_1-adrenergic receptor share significant amino acid homology with comparable, although more widely separated, regions of the α_2-adrenergic receptor (Fig. 4).

There are 41 amino acid residues in the α_2-adrenergic receptor that are conserved among all of these receptor proteins (Fig. 4). Thirty of these are located in the 182 amino acid residues assigned to the membrane spanning domains, while 11 are found in the remaining 268 residues. Because the α_2-, β_1-, and β_2-adrenergic receptors can all bind epinephrine, we examined structural features shared by these receptors that are not present, for example, in the muscarinic receptors. There are 29 amino acid residues in the α_2-adrenergic receptor that are also found in the β_1- and β_2-adrenergic receptors but not in the M1 or M2 receptors. All but two of these (93 percent) are in the membrane spanning domains. Thus, the membrane spanning domains of such receptors may collectively determine the ligand binding site (*3, 16*).

The amino acids involved with the effector functions of the α_2-adrenergic receptor would be expected to be more closely related to those of the cardiac muscarinic receptor which can inhibit adenylate cyclase, rather than to the β-adrenergic receptors which stimulate adenylate cyclase. In fact, 44 residues in the α_2-adrenergic receptor are also found in the cardiac M2 receptor but not in the β_1- or β_2-adrenergic receptors. In contrast to the previous comparison of shared amino acids that might be involved in ligand binding, only 14 (32 percent) of the shared residues that might be involved with effector functions are in the membrane spanning domains. However, 25 of the shared amino acids (57 percent) are in the putative intracellular domains where they could be involved in coupling to G proteins.

Phosphorylation of serine or threonine residues by various protein kinases has been implicated as a mechanism for regulating receptor function (*17*). In the third cytoplasmic loop of the α_2-adrenergic receptor, there is a consensus sequence for protein kinase A (residues 244 to 227); however, whether or not the α_2-adrenergic

receptor is actually a substrate for this enzyme has not been tested. In contrast, the α_2-adrenergic receptor can be phosphorylated in an agonist-dependent fashion by the β-adrenergic receptor kinase (*18*). This enzyme and the related enzyme rhodopsin kinase are believed to phosphorylate clusters of serine residues present in the carboxyl terminal regions of the β-adrenergic receptor (*19*) and of rhodopsin (*20*), respectively. In the carboxyl terminus of the α_2-adrenergic receptor, however, there are no serine or threonine residues. This leaves as possibilities the cytoplasmic loops of the α_2-adrenergic receptor, which all contain potential phosphorylation sites. Of particular interest is the third cytoplasmic loop, which contains a total of 19 serine and threonine residues including one stretch of four contiguous serines (residues 296 to 299).

Expression. To prove that the gene we have cloned and sequenced codes for a functional human platelet α_2-adrenergic receptor, RNA was prepared and used for in vivo translation in *Xenopus laevis* oocytes. The Nco I–Hind III fragment of the α_2-adrenergic receptor gene was ligated into the Nco I–Eco RV site of the human β_2-adrenergic receptor RNA expression vector pSPNar (*21*), effectively replacing the coding sequence of the β_2-adrenergic receptor with that of the α_2-adrenergic receptor (Fig. 5). This new construct, pSPhpα_2, retained the 5′ and 3′ untranslated regions of the β_2-adrenergic receptor expression vector to enhance the stability of transcribed RNA. RNA transcribed with SP6 RNA polymerase from the α_2-adrenergic expression vector (pSPhpα_2) was injected into *X. laevis* oocytes, and 2 to 3 days later binding of the α_2-adrenergic antagonist [^{3}H]yohimbine was performed to test for de novo synthesis of α_2-adrenergic receptors. Specific [^{3}H]yohimbine binding was not observed in membranes prepared from uninjected oocytes and from oocytes injected with RNA coding for β_2-adrenergic receptors. In contrast, after the injection of RNA coding for human platelet α_2-adrenergic receptors, [^{3}H]yohimbine binding was saturable (Fig. 6A) and was competed for by various adrenergic antagonists (Fig. 6B) and agonists (Fig. 6C). Computer analysis of the saturation curve data yielded an apparent equilibrium dissociation constant (K_d) for [^{3}H]yohimbine of 2.5 n*M*, which is in good agreement with results obtained from the binding of [^{3}H]yohimbine to intact human platelets and platelet membranes (*22*). Analysis of the antagonist competition curve data (Fig. 6B and Table 1) shows that the α-adrenergic antagonist phentolamine is significantly more potent than the selective α_1-adrenergic antagonist prazosin, which is also consistent with results obtained in intact human platelets (*22*). In addition, the antagonist diastereomers rauwolscine and corynanthine, which are selective for α_2- and α_1-adrenergic receptors, respectively (*23*), also showed the expected order of potency in competition for the binding of [^{3}H]yohimbine to the oocyte membranes. The α_2-selective ligand *p*-aminoclonidine was the most potent agonist followed in order of potency by the endogenous catecholamines (−)epinephrine and (−)norepinephrine and the β-adrenergic selective agonist (−)isoproterenol (Fig. 6C and Table 1). These results, together with the evidence that agonist stereoselectivity was also obtained [(−)epinephrine was eight times more potent than (+)epinephrine], indicate that the gene that we have isolated codes for the human platelet α_2-adrenergic receptor.

Related genes. When human genomic DNA was digested with Pst I and subjected to Southern blot analysis with the 0.95-kb Pst I restriction fragment from the α_2-adrenergic receptor gene as a probe (Fig. 3A), three separate hybridizing bands are observed after washings at low stringency (Fig. 7). The two higher molecular weight bands at 1.8 kb and 5.9 kb were substantially reduced after a high stringency wash (0.2× SSC at 65°C), but the 0.95-kb band, which represents hybridization of the probe to itself, remained unchanged. These results indicate that the 1.8- and 5.9-kb Pst I

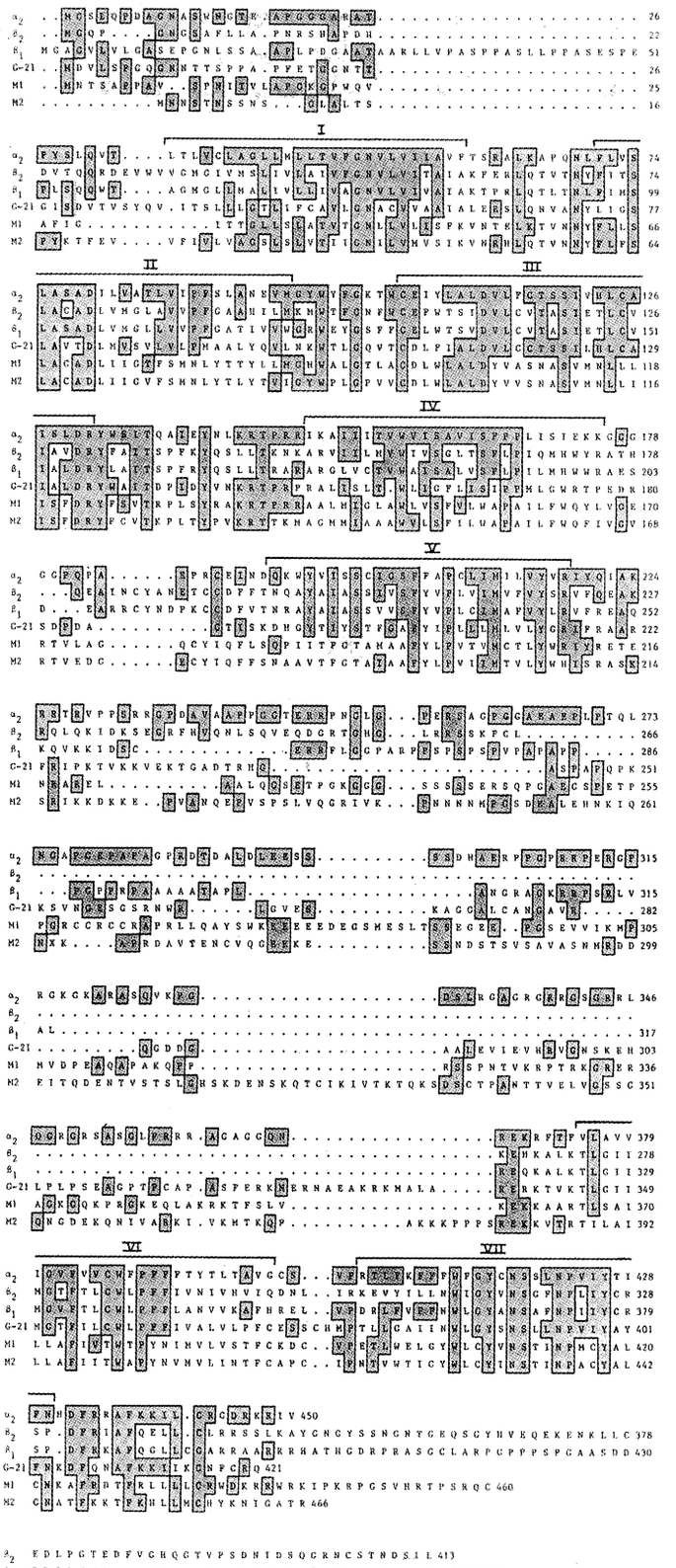


Fig. 4. Homology comparison of the human platelet α_2-adrenergic receptor with other G protein–coupled receptors. Amino acid sequences (single letter code) are aligned to optimize homology with the α_2-adrenergic receptor by the ALIGN computer program (*36*). The optimal alignment is based on a match score of 1, a mismatch score of 0, and a gap penalty of −2. Homology is indicated by shading. The putative transmembrane domains are indicated by brackets identified by roman numerals.

fragments share significant homology with the gene for the platelet α_2-adrenergic receptor. Somatic cell hybridization analysis (*24*) indicates that the 0.95-, 1.8- and 5.9-kb Pst I fragments are located on different chromosomes (Table 2). Thus, the 0.95-kb Pst I fragment, representing the gene for the platelet α_2-adrenergic receptor, was present only in those somatic cell hybrids containing human chromosome 10. Likewise the 1.8- and 5.9-kb Pst I fragments were present only in those hybrids containing human chromosomes 2 and 4, respectively. Further evidence for the location of the human platelet α_2-adrenergic receptor gene on chromosome 10 comes from studies done with a probe made from its 5′ untranslated region. This 2-kb probe (Bam HI to PST I, Fig. 3A) hybridized to only one band on Southern blot analysis and it also segregated with human chromosome 10. In situ hybridization revealed that the regional location of the human platelet α_2-adrenergic receptor gene is the long arm of chromosome 10 (q24-q26). Although confirmation of the chromosomal assignments of the 1.8- and 5.6-kb PST I fragments awaits the cloning of their genes, our results show that they are located on separate chromosomes and do not represent the genes for either the β_2-adrenergic receptor (*5*) or G-21 (*12*), which are both located on chromosome 5.

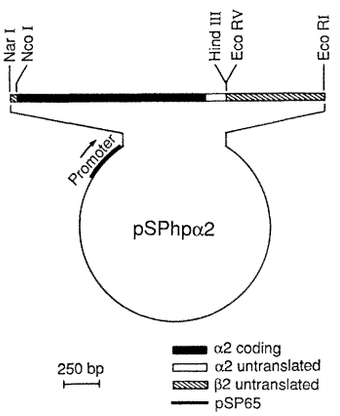


Fig. 5. RNA expression vector (pSPhpα_2) for the human platelet α_2-adrenergic receptor. The Nco I–Hind III fragment (Fig. 3A) containing the coding sequence of the α_2-adrenergic receptor was ligated into the β_2-adrenergic receptor expression vector pSPNar (*21*) after removing the β_2-adrenergic receptor coding sequence contained in the Nco I–Eco RV fragment. The resulting vector contains the 5′ and 3′ untranslated regions of the β_2-adrenergic receptor and the coding sequence of the α_2-adrenergic receptor. This vector was linearized with Hind III, and RNA was prepared with SP6 RNA polymerase according to the method of Melton *et al.* (*37*). RNA prepared from 25 μg of DNA template was dissolved in 50 μl of water to yield a final RNA concentration of about 0.4 μg/μl.

Conclusions. The structural organization of the α_2-adrenergic receptor with its large third cytoplasmic loop and short carboxyl terminus resembles that of the muscarinic receptors to which the effector functions of the α_2-adrenergic receptor may be more closely related. However, sequence homology within the membrane spanning regions is actually greater with the β-adrenergic receptors, which have ligand binding properties closer to those of the α_2-adrenergic receptor than to those of the muscarinic receptors. These findings are consistent with the hypothesis that the membrane spanning regions may form a ligand binding pocket, whereas the cytoplasmic regions may be involved in effector coupling functions.

A striking feature of the α_2-adrenergic receptor gene is that the coding block is contained within a single exon. This is also true of the genes coding for the human and hamster β_2-adrenergic receptors (*3, 4*), the porcine M2 muscarinic receptor (*10*), and G-21 (*12*). Thus, in every case where information is thus far available, G protein–coupled receptors are coded for by single exons. The only exception is the visual pigment rhodopsin, which contains five exons and four introns (*25*). Whether introns were lost at some point in the evolution of this gene family or whether the rhodopsin gene acquired them from a common precursor without introns remains to be discovered.

Speculation about the sites of phosphorylation in the β-adrenergic receptor, by the enzyme β-adrenergic receptor kinase, have focused on the serine-rich carboxyl terminus; however, the exact sites have not yet been determined (*17*). The α_2-adrenergic receptor is an excellent substrate for this enzyme—indeed, it is as good as the β-adrenergic receptor itself (*18*)—yet the α_2-adrenergic receptor completely lacks serines in its short carboxyl terminal tail. This suggests that the cytoplasmic loops, of not only the α_2-adrenergic receptor but of other G protein–coupled receptors as well, might be important in regulatory phosphorylation events. Further support for this idea comes from recent mutagenesis studies that have indicated that agonist-promoted desensitization of β-adrenergic receptors, believed to be a phosphorylation-mediated process, proceeds normally even in the face of deletions of the carboxyl terminus (*21, 26*).

Our results that there are other human genes homologous to the

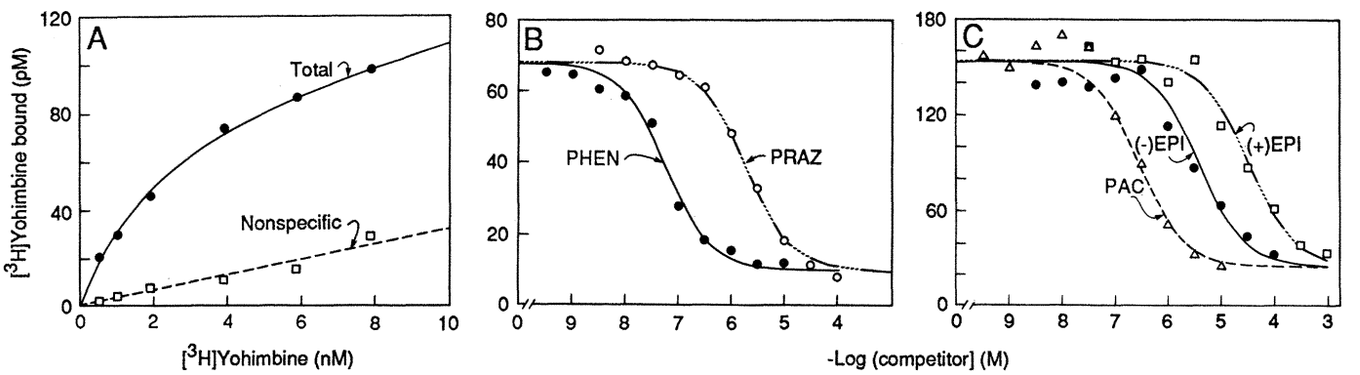


Fig. 6. De novo expression of specific [^{3}H]yohimbine binding to membranes prepared from *Xenopus laevis* oocytes after injection of RNA coding for human platelet α_2-adrenergic receptors. RNA was prepared as described in Fig. 5. *Xenopus laevis* oocytes (stage V to VI) were obtained and were individually injected with 50 to 80 nl of a solution of RNA (*21*). After an incubation period of 48 to 72 hours, membranes were prepared (*21*), and the binding of [^{3}H]yohimbine was determined. (**A**) Saturation isotherm. The binding of [^{3}H]yohimbine was determined in a final volume of 500 μl and with about 100 μg of membrane protein (equivalent to about 30 oocytes). Assays were incubated at 25°C for 30 minutes and were terminated by filtration through Whatman GF/C filters. Nonspecific binding was determined in the presence of 10 μ*M* phentolamine. Data were analyzed by computer with the assumption that binding was to a single class of sites (*38*). The experiment was repeated and the average K_d for the binding of [^{3}H]yohimbine was 2.5 ± 0.5 n*M* (n = 2, ± S.D.). The number of binding sites in the oocyte membranes showed greater variability, but was in the range of 300 to 600 fmol per milligram of protein or 1 to 2 fmol per oocyte. (**B**) Antagonist competition. The binding of [^{3}H]yohimbine (2 n*M*) was assessed in the presence of ten competing concentrations of either phentolamine (PHEN) or prazosin (PRAZ). Assays were conducted as described for the saturation isotherms. (**C**) Agonist competition. The binding of [^{3}H]yohimbine (2 n*M*) was assessed in the presence of ten competing concentrations of either *p*-aminoclonidine (PAC), (−)epinephrine [(−)EPI], or (+)epinephrine [(+)EPI]. Affinity constants calculated from these data are in Table 1.

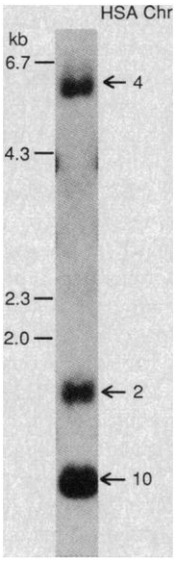


Fig. 7. Southern blot of human genomic DNA. A Pst I digest of human genomic DNA was subjected to agarose gel electrophoresis and was then blotted on to Hybond-N (Amersham) and probed with the 0.95-kb Pst I fragment of the α_2-adrenergic receptor clone in Fig. 3A. The 0.95-kb Pst I fragment was labeled with ^{32}P by the method of random priming (*39*), and the blot was hybridized in 30 percent formamide, 5× SSC, 0.1 percent SDS, 5× Denhardt's and sheared salmon sperm DNA (100 μg/ml) at 42°C and washed in 1× SSC, 0.1 percent SDS, at 42°C. The autoradiogram was exposed for 72 hours. The position of the molecular size standards are indicated on the left, and the chromosomal localization of each band is given on the right. Chromosomal localization was accomplished by somatic cell hybrid analysis (*24*). The data from Southern blot analyses of Pst I–digested genomic DNA from somatic cell hybrids are summarized in Table 2.

platelet α_2-adrenergic receptor gene are significant in light of mounting pharmacologic evidence for α_2-adrenergic receptor heterogeneity. Thus, α_2-adrenergic receptors appear to be heterogeneous on several grounds. These include their different affinities for various adrenergic ligands (*27*), their anatomical locations (*28*), and their coupling to different responses (*29*). Unfortunately, many of these studies have been confounded by questions of species differences in α_2-adrenergic receptors, and to date no biochemical evidence is available to provide a structural basis for this heterogeneity. With regard to the existing nomenclature, the α_2-adrenergic receptor gene that we have cloned is provisionally classified as an α_2A-adrenergic receptor (*30*). This receptor, as characterized by the results of our expression studies, has high and approximately equal affinity for yohimbine and rauwolscine and much lower affinity for prazosin. Other α_2-adrenergic receptor subtypes might be expected to have somewhat lower affinity for yohimbine and significantly higher affinity for prazosin (*27*).

Our findings suggest that we may be entering a new era of pharmacologic discovery. Thus with the isolation of receptor genes, and with the ability to express a single gene product, drug development may proceed in a system containing only one receptor subtype. Such endeavors should lead to a more complete understanding of receptor subtypes as well as to new drugs with more specific therapeutic actions.

REFERENCES AND NOTES

1. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
2. H. G. Dohlman, M. G. Caron, R. J. Lefkowitz, *Biochemistry* **26**, 2657 (1987).
3. R. A. F. Dixon *et al.*, *Nature* (*London*) **321**, 75 (1986).
4. B. K. Kobilka *et al.*, *J. Biol. Chem.* **262**, 7321 (1987).
5. B. K. Kobilka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 46 (1987).
6. T. Frielle *et al.*, *ibid.*, p. 7920.
7. Y. Yarden *et al.*, *ibid.* **83**, 6795 (1986).
8. T. Kubo *et al.*, *Nature* (*London*) **232**, 411 (1986).
9. T. Kubo *et al.*, *FEBS Lett.* **209**, 367 (1986).
10. E. G. Peralta *et al.*, *Science* **236**, 600 (1987).
11. Y. A. Ovchinnikov, *FEBS Lett.* **148**, 179 (1982); P. A. Hargrave, *Prog. Retinal Res.* **1**, 1 (1982).
12. B. K. Kobilka *et al.*, *Nature* (*London*) **329**, 75 (1987).
13. Only one peptide was isolated and sequenced from the initial purified receptor preparation (legend to Fig. 1). Although this receptor preparation had been labeled with a specific photoaffinity ligand, peptide 1 did not contain any labeled amino acid residues. SDS–polyacrylamide gel electrophoresis of the cyanogen bromide digest from the initial purification indicated that the labeled peptides were irreversibly aggregated. The early peak of radioactivity and of ultraviolet absorbance in Fig. 1A represents this aggregated material. Aggregation may have been due to the removal of digitonin in the initial peptide purification scheme or to the formation of intermolecular disulfide bonds that were essentially inaccessible to reduction after aggregation. In a second attempt at peptide purification, measures were taken to address these possibilities. Furthermore, to address the possibilities that the receptor had a low content of methionine or that the cyanogen bromide cleavage was incomplete, additional enzymatic cleavage steps were used (legend to Fig. 1); in retrospect the former was true since there are only four methionine residues (including the initiator methionine) in the platelet α_2-adrenergic receptor (Fig. 3B).
14. R. Lathe, *J. Mol. Biol.* **183**, 1 (1985).
15. J. Kyte and F. F. Doolittle, *ibid.* **157**, 105 (1982).
16. R. A. F. Dixon *et al.*, *Nature* (*London*) **326**, 73 (1987).
17. D. R. Sibley, J. L. Benovic, M. G. Caron, R. J. Lefkowitz, *Cell* **48**, 913 (1987).
18. J. Regan *et al.*, *Clin Res.* **35**, 648A (1987); J. L. Benovic *et al.*, *J. Biol. Chem.*, in press.
19. J. L. Benovic, F. Mayor, Jr., R. L. Sommers, M. G. Caron, R. J. Lefkowitz, *Nature* (*London*) **322**, 869 (1986).
20. P. A. Hargrave *et al.*, *Neurochem. Int.* **1**, 231 (1980); W. Wilden and H. Kühn, *Biochemistry* **21**, 3014 (1982).
21. B. K. Kobilka *et al.*, *J. Biol. Chem.*, in press.
22. H. J. Motulsky, S. J. Shattil, P. A. Insel, *Biochem. Biophys. Res. Commun.* **97**, 1562 (1980).
23. R. Weitzell, T. Tanaka, K. Starke, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **308**, 127 (1979); P. B. M. W. M. Timmermans, A. M. C. Schoop, H. Y. Kwa, P. A. van Zwieten, *Eur. J. Pharmacol.* **70**, 7 (1981).
24. T. Yang-Feng, G. F. Smith, M. Nemer, J. Drouin, U. Francke, *Am. J. Human Genet.* **37**, 1117 (1985).
25. J. Nathans and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4851 (1984).
26. C. D. Strader *et al. Cell* **49**, 855 (1987).
27. Y. D. Cheung, D. B. Barnett, S. R. Nahorski, *Eur. J. Pharmacol.* **84**, 79 (1982); C. B. Neylon and R. J. Summers, *Br. J. Pharmacol.* **85**, 349 (1985); V. A. Alabaster, R. F. Keir, C. J. Peters, *ibid.* **88**, 607 (1986); K. E. J. Dickinson, R. L. M. McKernan, C. M. M. Miles, K. S. Leys, P. S. Sever, *Eur. J. Pharmacol.* **120**, 285 (1986).
28. J. P. Hieble *et al.*, *J. Hypertension* **4**, 5189 (1987). C. L. Boyajian, S. E. Loughlin, F. M. Leslie, *J. Pharmacol. Exp. Ther.* **241**, 1079 (1987).
29. K. A. Clare, M. C. Scrutton, N. T. Thompson, *Br. J. Pharmacol.* **82**, 467 (1984).
30. D. B. Bylund, *Pharmacol. Biochem. Behav.* **22**, 835 (1985).
31. A. DeLean, P. J. Munson, D. Rodbard, *Am. J. Physiol.* **4**, E97 (1978).
32. M. G. Repaske, J. H. Nunnari, L. E. Limbird, *J. Biol. Chem.* **262**, 12381 (1987).
33. J. W. Regan, H. Nakata, R. M. DeMarinis, M. G. Caron, R. J. Lefkowitz, *ibid.* **261**, 3894 (1986).
34. J. W. Regan, R. M. DeMarinis, R. J. Lefkowitz, *Biochem. Pharmacol.* **34**, 3667 (1985).
35. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); J. Vieira and J. Messing, *Gene* **19**, 259 (1982).
36. B. C. Orcutt, N. O. Dayhoff, D. G. George, W. C. Baker, *PIR Report ALI-1284* (National Biomedical Research Foundation, Washington, DC, 1986).
37. D. N. Melton *et al.*, *Nucleic Acid Res.* **12**, 735 (1984).
38. A. DeLean, A. A. Hancock, R. J. Lefkowitz, *Mol. Pharmacol.* **21**, 5 (1982).
39. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).

40. We thank D. Addison for secretarial assistance, R. R. Randall and C. E. Coonley for peptide sequencing, and M. McAdams and K. Theisen for oligonucleotide synthesis.

6 August 1987; accepted 28 September 1987