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Cloning of the cDNA for the human β_1-adrenergic receptor

(catecholamines/transmembrane signaling/receptor subtypes)

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ABSTRACT **Screening of a human placenta λgt11 library has led to the isolation of the cDNA for the human β_1-adrenergic receptor (β_1AR). Used as the probe was the human genomic clone termed G-21. This clone, which contains an intronless gene for a putative receptor, was previously isolated by virtue of its cross hybridization with the human β_2-adrenergic receptor (β_2AR). The 2.4-kilobase cDNA for the human β_1AR encodes a protein of 477 amino acid residues that is 69% homologous with the avian βAR but only 54% homologous with the human β_2AR. This suggests that the avian gene encoding βAR and the human gene encoding β_1AR evolved from a common ancestral gene. RNA blot analysis indicates a message of 2.5 kilobases in rat tissues, with a pattern of tissue distribution consistent with β_1AR binding. This pattern is quite distinct from the pattern obtained when the β_2AR cDNA is used as a probe. Expression of receptor protein in *Xenopus laevis* oocytes conveys adenylate cyclase responsiveness to catecholamines with a typical β_1AR specificity. This contrasts with the typical β_2 subtype specificity obser.ed when the human β_2AR cDNA is expressed in this system. Mammalian β_1AR and β_2AR are thus products of distinct genes, both of which are apparently related to the putative G-21 receptor.**

Several recently cloned receptors, which are coupled to guanine nucleotide regulatory proteins (G proteins), show significant sequence homology, suggesting the existence of an extensive gene family. Homologous members of this group that have been sequenced thus far include: adenylate cyclase stimulatory β-adrenergic receptors (βAR) from hamster (1), human (2), and turkey (3); two subtypes of muscarinic acetylcholine receptor coupled respectively to stimulation of phosphatidylinositol turnover (M_1) (4) and inhibition of adenylate cyclase (M_2) (5, 6); the family of visual pigments (opsins) (7); and G-21, an as yet unidentified human receptor isolated by cross-hybridization with the human β_2-adrenergic receptor (β_2AR) (8). The conserved structural features of these receptor proteins include hydropathicity profiles consistent with seven transmembrane spanning domains, sites of N-linked glycosylation near the amino termini, consensus sequences for phosphorylation by regulatory kinases on presumed cytoplasmic domains, and striking amino acid homology within the membrane spanning segments (9).

At least four pharmacologically well-characterized subtypes of adrenergic receptors are known and are referred to as α_1, α_2, β_1, and β_2. Of these, both β_1AR and β_2AR stimulate adenylate cyclase, although they subserve distinct physiological functions. Moreover, a variety of drugs, both agonists and antagonists, selective for either β_1 or β_2 receptors have important applications in clinical medicine (10). The sequences of the hamster and human β_2AR are known and are almost identical. However, the situation for the β_1AR is much less clear. Although the avian βAR, which is ≈50% homologous with the mammalian β_2AR, is often referred to as "β_1," its pharmacological specificity is in fact quite discrepant from that of either the mammalian β_1 or β_2 receptor (11). Moreover, repeated attempts in our laboratory to obtain a mammalian β_1AR cDNA or gene by cross-hybridization with mammalian β_2AR probes have failed. Here we report the unexpected cloning of the human β_1AR cDNA from a human placenta cDNA library screened with human genomic clone G-21. This clone, containing an intronless gene for an as yet unidentified putative receptor, was itself obtained by its cross-hybridization with the human gene encoding β_2AR. Our results serve to clarify the nature and evolutionary relationships of the β_1 and β_2 subtypes of adrenergic receptors as well as the structural basis for pharmacological specificity of two closely related receptors that couple to the same effector. A cDNA clone encoding the human β_1AR was isolated and its sequence determined.§

METHODS

cDNA Library Screening. A human placenta cDNA λgt11 library was generously provided by Evan Sadler (Washington University School of Medicine). The cDNA was prepared from term placenta poly(A)$^+$ RNA. Recombinants (2.5×10^6) were screened (12) at high stringency [0.2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7) at 65°C] using the ^{32}P-labeled 700-base-pair (bp) *Sph* I–*Bam*HI fragment of clone G-21 (8).

DNA Sequencing. Sequencing of both strands of DNA was performed by the dideoxy chain-termination method (13, 14) using overlapping restriction fragments in M13mp10 and pUC19.

Blot-Hybridization Analysis. Routine procedures were performed as described (15, 16). Total cellular RNA was prepared by the method of Chirgwin *et al.* (17), and RNA blots were performed as described (18, 19). RNA blots were probed with the 1.3-kilobase (kb) *Sma* I fragment of the clone 11 insert or the 1.3-kb *Hin*dIII fragment of hamster β_2AR, both labeled by nick-translation (20).

Expression of βAR Transcripts. The 2.4-kb insert was ligated into pSP65 (21). RNA transcribed with SP6 RNA polymerase (22) was injected into *Xenopus laevis* oocytes (23). Crude membranes were prepared after 48 hr and assayed for catecholamine-sensitive adenylate cyclase activity as described (24, 25). Basal activity was ≈0.2 pmol of cAMP/min/mg of membrane protein for both membranes prepared from uninjected or injected oocytes. A second pSP65 expression vector was constructed by utilizing the cDNA insert lacking the first 80 bp of the 5′ untranslated region. Transcripts from this construct were expressed more

Abbreviations: βAR, β-adrenergic receptor; β_1AR and β_2AR, human β_1 and β_2-adrenergic receptors; G proteins, guanine nucleotide regulatory proteins.

§This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03019).

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efficiently and, therefore, were used when binding of ^{125}I-labeled cyanopindolol, a βAR-specific ligand, was determined (26). A pSP65 vector containing the human β_2AR cDNA insert also was transcribed, expressed, and assayed as described above. Protein was assayed as described by Bradford (27).

RESULTS

Screening of the human placenta cDNA library with the G-21 probe resulted in the isolation of nine positive clones, seven of which were found to have restriction maps identical to the previously isolated β_2AR cDNA. Two clones hybridized only weakly with the β_2AR cDNA and had distinct restriction maps. The restriction map and sequence of one of these, clone 11, is presented in Fig. 1 *Upper*.

The nucleotide sequence and deduced protein sequence of clone 11 are shown in Fig. 1 *Lower*. The 2.4-kb clone contains a short 5′ untranslated sequence of 86 bp. An initiator methionine codon with sequence matching Kozak's consensus sequence (28) begins an open reading frame that encodes

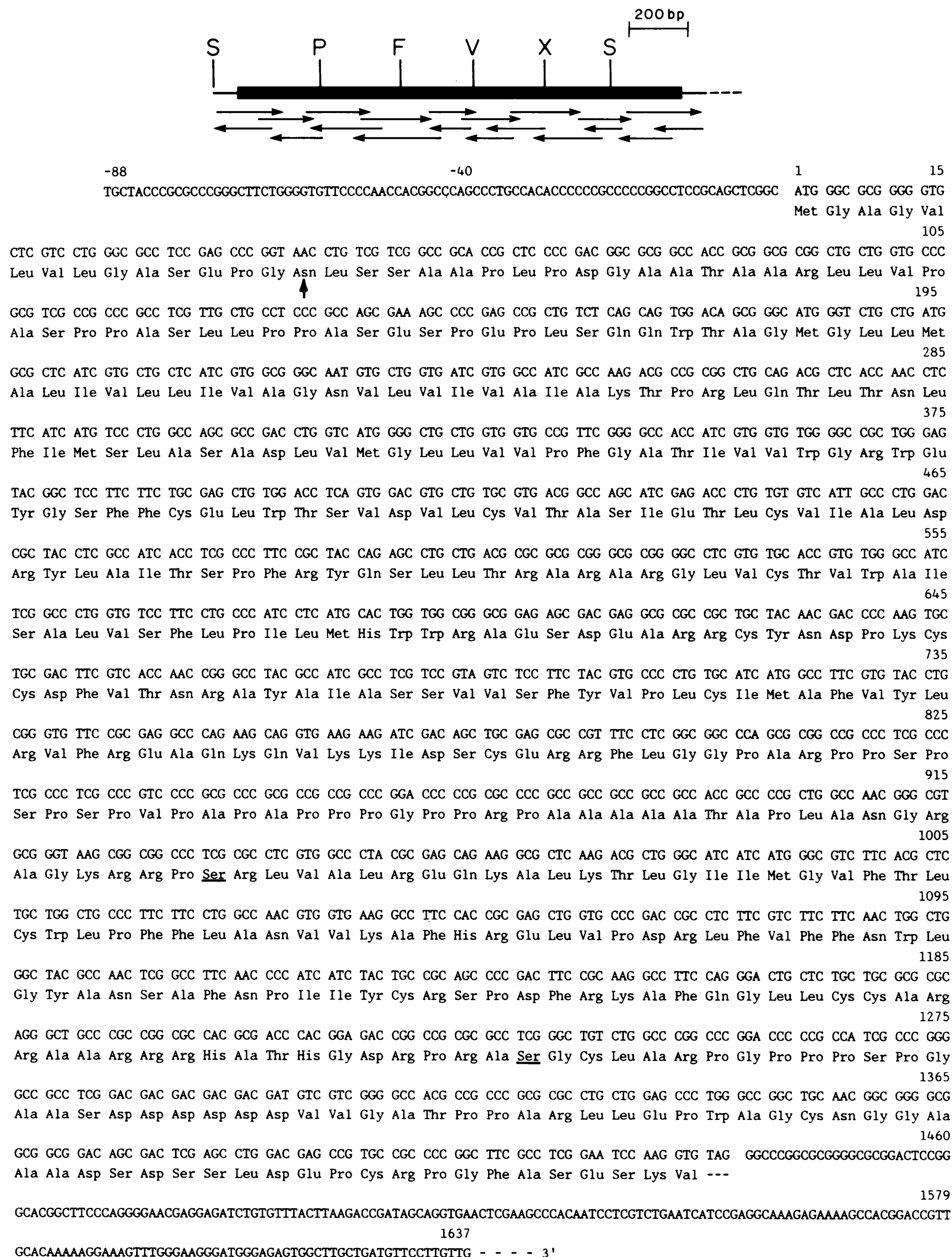

FIG. 1. (*Upper*) Restriction map of the 2.4-kb cDNA clone 11. S, *Sma* I; P, *Pst* I; V, *Pvu* II; F, *Sfi* I; X, *Xma* I. The shaded region represents the human β_1AR coding sequence. Arrows indicate overlapping sequencing reactions. As indicated by the dashed line, the 3′ noncoding sequence has not been sequenced in its entirety. (*Lower*) Nucleotide and deduced amino acid sequence of the human β_1AR cDNA clone 11. The N-linked glycosylation site is indicated by ↑. The consensus cAMP-dependent protein kinase A phosphorylation sites are underlined.

a protein of 477 amino acid residues (51,220 daltons), followed by a 3′ untranslated sequence of ≈900 bp.

The deduced protein sequence of clone 11 is compared with those of the avian βAR, the human β_2AR, and G-21 in Fig. 2. Like other members of this gene family, the encoded protein has seven clusters of hydrophobic amino acid residues likely representing membrane-spanning regions. There is one consensus site for N-linked glycosylation at Asn-15 and two consensus cAMP-dependent kinase phosphorylation sites at Ser-312 and Ser-412; Ser-312 could possibly serve as a site for protein kinase C phosphorylation as well. While these sites are found in similar locations in the human β_2AR, the avian βAR lacks a phosphorylation site comparable to Ser-412. The carboxyl terminus possesses seven serine residues, five of which are conserved in the avian βAR. Thus, the carboxyl terminus represents a possible locus for regulatory phosphorylation by the βAR kinase (31) by analogy to the phosphorylation of rhodopsin by rhodopsin kinase (32).

Sequence similarity with other βARs and with G-21 is striking and, as observed previously, is highest in the membrane-spanning regions (9). The identities in the presumed transmembrane regions and overall homologies with each of the receptors, respectively, are: avian βAR, 84% and 69%; human β_2AR, 71% and 54%; G-21, 50% and 39%. Thus, the encoded protein is remarkably similar to the avian βAR and is less closely related to both the human β_2AR and G-21. In contrast to the extensive similarity within the membrane-spanning regions, the amino and carboxyl termini of all of the βARs are quite divergent. The first two cytoplasmic loops (residues 85–96 and 157–172, respectively) are generally well conserved, whereas the extracellular loops are more divergent. The third cytoplasmic loop (residues 247–321) of the putative human β_1AR diverges significantly from that of the

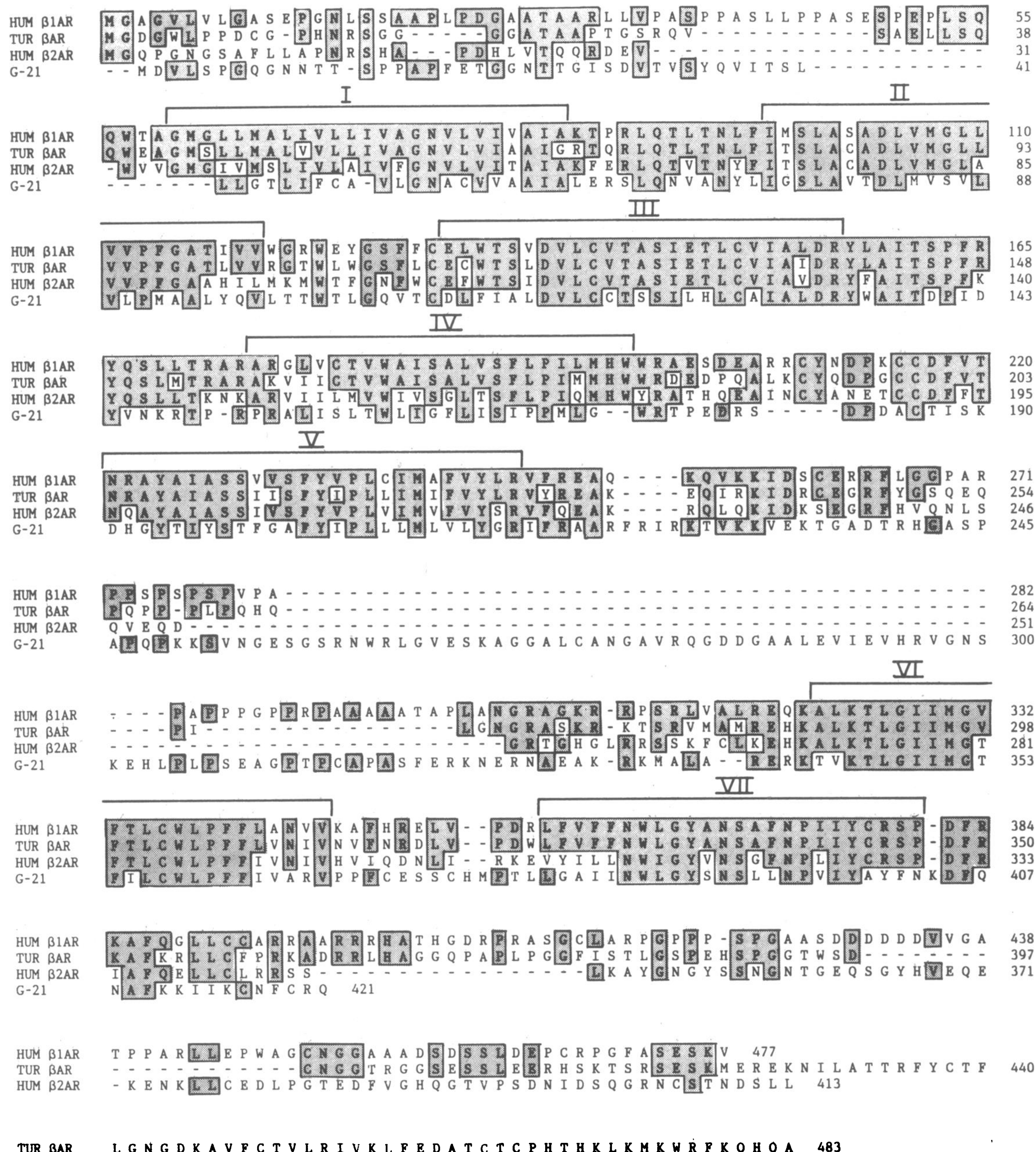

FIG. 2. Comparisons of receptor primary structures. Deduced protein sequences of human β_1AR (HUM β_1AR), turkey βAR (TUR βAR), human β_2AR (HUM β_2AR), and G-21 were aligned to optimize homology with the human β_1AR by the ALIGN computer program (29). Homologies between the β_1 and other receptors are noted by shading. Putative membrane-spanning domains of 24–26 amino acid residues were assigned using the hydropathicity index of Kyte and Doolittle (30).

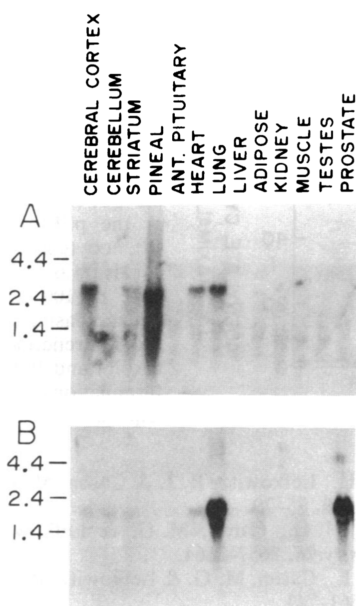

FIG. 3. Blot-hybridization analysis of rat tissue RNA. Forty micrograms of total cellular RNA isolated from various rat tissues (except pineal, 26 μg) were subjected to RNA blot analysis using the human β_1AR cDNA probe (*A*) or the hamster β_2AR cDNA probe (*B*). Hybridizations were performed in 5× SSC at 42°C, and the filters were washed in 0.1× SSC at 55°C. Autoradiograms were developed after a 48-hr exposure. Positions of RNA markers are indicated.

avian receptor and includes an additional 17 amino acid residues. Thus, it is the largest such loop of any βAR cloned to date. The third cytoplasmic loop of all of the βARs is rich in proline residues. However, the human β_1AR contains an additional 10 proline residues in this loop when compared to the avian βAR.

Since the tissue distribution of β_1AR and β_2AR is quite distinct, blot-hybridization analysis was performed on total RNA isolated from 13 rat tissues with both the putative human β_1AR and the hamster β_2AR cDNAs as probes. The results (Fig. 3) are in excellent agreement with the levels of β_1AR and β_2AR expressed in each tissue as previously determined by radioligand binding techniques (10). Thus, the highest levels of mRNA ($\approx$2.5 kb) homologous with the putative β_1AR cDNA were found in pineal gland and cerebral cortex, whereas the highest levels of β_2AR mRNA ($\approx$2.1 kb) were found in lung and prostate. These data indicate that the human β_1AR and β_2AR are clearly products of distinct genes.

To document the functional activity of the expressed protein product of the cDNA, RNA was prepared from a pSP65 expression construct containing the clone 11 cDNA. After injection of this RNA into *Xenopus laevis* oocytes, membranes from such oocytes, but not from control oocytes, bound the β-adrenergic ligand ^{125}I-labeled cyanopindolol with high affinity, $K_D = 6.6 \times 10^{-11}$ M (data not shown). The number of cyanopindolol binding sites in membranes derived from oocytes injected with either the putative β_1AR or the human β_2AR transcripts ranged from 400 to 600 fmol/mg of membrane protein. Moreover, injection of the putative β_1AR RNA also conveyed catecholamine sensitivity to the endogenous oocyte adenylate cyclase. Thus, whereas the adenylate cyclase of control membranes responded to NaF and forskolin but not to isoproterenol, isoproterenol stimulated the adenylate cyclase of membranes from injected eggs $\approx$3- to 4-fold. The order of potency of agonists for this stimulation was isoproterenol > norepinephrine $\simeq$ epinephrine (Fig. 4*A*)—entirely typical of a β_1AR. In contrast, when the previously cloned human β_2AR cDNA was expressed in this system, the typical β_2AR-potency series of isoproterenol > epinephrine >> norepinephrine was found (Fig. 4*B*).

The agonist stimulation of the oocyte adenylate cyclase was competitively inhibited by β-adrenergic antagonists. The β_1AR-selective antagonist betaxolol was $\approx$10 times more potent than the β_2AR-selective antagonist ICI 118551 (Fig. 5*A*). This specificity is again the converse of the typical β_2AR specificity displayed when the human β_2AR cDNA is expressed in this system (Fig. 5*B*). These data confirm that clone 11, in fact, encodes the human β_1AR.

DISCUSSION

When the human β_2AR cDNA was used to probe human genomic DNA, a minor cross-hybridizing species was observed at low stringency. This DNA, termed G-21, was determined to encode a protein with striking sequence and structural similarities with the family of G protein-coupled receptors (8). In screening a human placenta cDNA library for a cDNA clone for this G-21 DNA, we fortuitously isolated a cDNA clone encoding the human β_1AR. Both human β_1AR and β_2AR cDNAs have thus been isolated from the same placenta library (2). This is not surprising in view of the $\approx$50% mixture of these receptor subtypes in this tissue (33).

The remarkable amino acid similarities of the isolated clone with the previously reported turkey βAR strongly suggests that, despite the peculiarities of its pharmacology, the avian receptor is apparently related to the human β_1AR. Additionally, the mammalian β_1AR is considerably more homologous with the avian βAR than with the human β_2AR. Currently unknown is the evolutionary relationship of the G-21 DNA to those encoding these βARs. As noted above, G-21 was originally obtained by using the human β_2AR cDNA as a probe at reduced stringency. G-21 in turn was able to select the β_1AR cDNA, although it is actually less homologous to it than is the β_2AR cDNA. These considerations tie the G-21 DNA even more closely to the family of adrenergic receptors, even though expression studies have indicated that it is not one of the presently known adrenergic receptor subtypes (8).

Comparison of the deduced structures of the human β_1AR and β_2AR highlights informative similarities and differences.

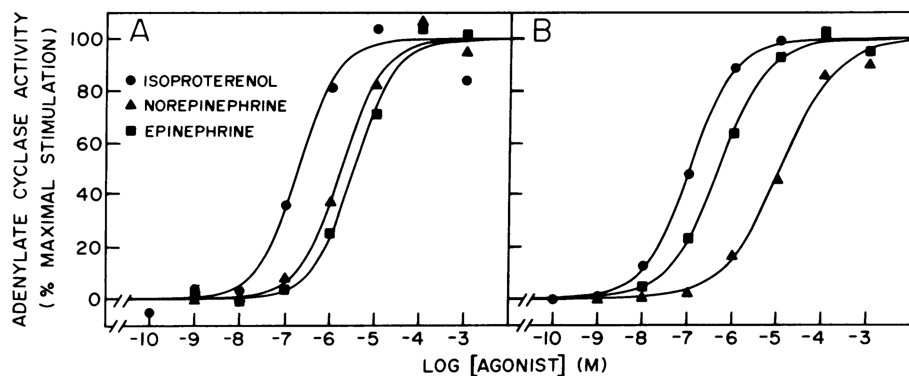

FIG. 4. Activation of *Xenopus laevis* oocyte membrane adenylate cyclase by β-adrenergic agonists. Membranes of oocytes previously injected with 40 ng of either pSP65/β_1AR transcript (*A*) or pSP65/β_2AR transcript (*B*) were assayed for adenylate cyclase activity. Maximal isoproterenol-stimulated activities for adenylate cyclase coupled to β_1AR and β_2AR were 30 and 100 pmol of cAMP per min/mg, respectively. All determinations were performed in triplicate.

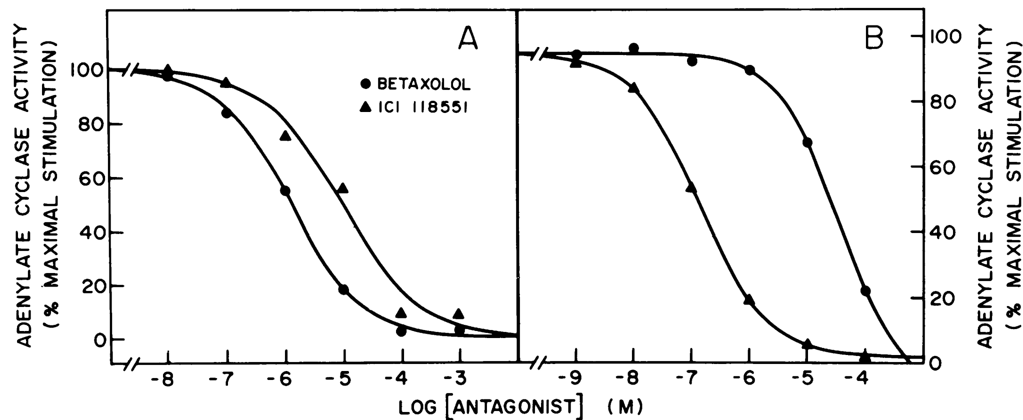

FIG. 5. Inhibition of isoproterenol-stimulated oocyte adenylate cyclase by β-adrenergic antagonists. After injection of the pSP65/β_1AR transcript (*A*) or the pSP65/β_2AR transcript (*B*), oocyte membrane adenylate cyclase was assayed in the presence of 100 μM isoproterenol and increasing concentrations of the β-adrenergic antagonists betaxolol and ICI 118551. All determinations were performed in triplicate.

Similarity between the two proteins is typically greatest in the membrane-spanning domains, which are currently thought to be involved in forming a membrane pocket to bind ligands. Thus, the differences in sequence in these regions may explain the pharmacological differences between these subtypes.

In contrast to their unique pharmacological properties, β_1AR and β_2AR appear to couple to precisely the same effector protein, termed G_s (34). In analogy with rhodopsin (35, 36), it is possible that the cytoplasmic domains of the receptor are involved in these interactions. Whereas there is significant similarity in the first two cytoplasmic loops, among the human β_1AR and β_2AR and the avian βAR, the large third loop and carboxyl-terminal cytoplasmic tail are quite divergent in all of these proteins. Coherent models to explain receptor–G_s coupling will have to accommodate these findings. The availability of well-characterized clones for both human β_1AR and β_2AR should facilitate further analysis of the structural basis for receptor functions and regulation.

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