Properties of the $\beta_1$- and $\beta_2$-Adrenergic Receptor Subtypes Revealed by Molecular Cloning

Thomas Frielle, Marc G. Caron, and Robert J. Lefkowitz

The $\beta_1$- and $\beta_2$-adrenergic receptor subtypes are biochemically and functionally similar, because both receptors mediate the catecholamine-dependent activation of adenylate cyclase through the GTP-binding protein, $G_{\alpha}$. Pharmacologically, the two receptors can be distinguished on the basis of their relative affinities for the agonists epinephrine and norepinephrine as well as their affinities for several selective antagonists. The primary structures of the human $\beta_1$- and $\beta_2$-adrenergic receptors have recently been deduced from the cloning of their genes and (or) cDNAs, revealing high sequence homology and a membrane topography of seven putative transmembrane regions similar to that of rhodopsin. Chimeric $\beta_1/\beta_2$-adrenergic receptor cDNAs have been constructed by site-directed mutagenesis and the chimeric RNA transcripts expressed in *Xenopus laevis* oocytes. The pharmacological properties of the expressed chimeric receptor proteins were assessed by radioligand binding utilizing subtype-selective agonists and antagonists. Apparently, several of the putative transmembrane regions contribute significantly to the determination of subtype selectivity, presumably by formation of a ligand-binding pocket, with determinants for agonist and antagonist binding being distinguishable.

The translation of extracellular signals into intracellular metabolic events is often dependent upon a system of three interacting components: (a) a receptor capable of specifically binding selected ligands, (b) an effector enzyme, and (c) a guanine nucleotide-binding regulatory protein that couples the receptor to the effector enzyme. The $\beta$-adrenergic receptor-coupled adenylate cyclase (EC 4.6.1.1) system is one of the most thoroughly characterized transmembrane signaling systems. In this system the agonist-occupied $\beta$-adrenergic receptor is capable of activating adenylate cyclase through its interaction with the guanine nucleotide-binding regulatory protein, $G_\alpha$ (1).

Two pharmacologically distinct subtypes of the mammalian $\beta$-adrenergic receptor, termed $\beta_1$ and $\beta_2$, have been described. The definition of the two subtypes is based upon their relative affinities for the catecholamine agonists epinephrine and norepinephrine (2). The $\beta_1$-adrenergic receptor binds epinephrine and norepinephrine with approximately equal affinities, whereas the $\beta_2$-adrenergic receptor binds epinephrine with about 30-fold greater affinity than it does norepinephrine. Subsequent to this initial pharmacological classification, various $\beta$-adrenergic receptor agonists and antagonists have been developed that distinguish between the two $\beta$-adrenergic receptor subtypes and that have allowed their quantification and characterization (3). The distinction between the $\beta_1$ and $\beta_2$ subtypes is demonstrated most clearly by the competition of a nonselective radiolabeled antagonist by a subtype-selective agonist or antagonist. The same selectivity can also be demonstrated by the ability of selective agonists or antagonists to activate or inhibit stimulation of adenylate cyclase.

The $\beta_1$- and $\beta_2$-adrenergic receptors are functionally homologous, because they are both coupled to the stimulation of adenylate cyclase through activation of $G_{\alpha}$. Biochemically, these two mammalian proteins possess identical molecular masses by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and peptide maps generated from photofinity-labeled receptors are virtually identical (4). Thus, the question has arisen whether the $\beta_1$- and the $\beta_2$-adrenergic receptors represent post-translationally modified products of a common gene or are products of different genes. Recent data, reviewed in this essay, establish that the two receptors are, in fact, products of different genes.

It is becoming increasingly clear that, based on structural and functional similarities, the adrenergic receptors are members of a large family of G-protein-coupled receptors. Recently cloned members of this family include human rhodopsin (5), the human color opsins (6), the $\alpha_1$- (7) and $\alpha_2$-adrenergic receptors (8), the hamster (9) and human $\beta_2$-adrenergic receptors (10), the avian $\beta$-adrenergic receptor (11), and the cerebral (12) and cardiac muscarinic cholinergic receptors (13), as well as the receptor for substance K (14) and two additional recently described muscarinic receptors (15). On the basis of hydrophobicity analyses of the deduced amino acid sequences, it has been proposed (16) that the membrane organization of the receptors mimics the structure of bacterio-rhodopsin as determined by electron diffraction studies. This structure of seven $\alpha$-helical membrane-spanning regions of 20 to 28 amino acid residues, each connected by intra- and extracellular hydrophilic loops, is highly conserved among all of these proteins (17).

Like other members of this family, the human $\beta_1$-adrenergic receptor possesses seven clusters of hydrophobic amino acids, likely representing $\alpha$-helical membrane-spanning regions (18). Figure 1 compares the primary structures of human $\beta_1$-adrenergic receptor with those of the avian $\beta$-adrenergic receptor and the human $\beta_2$-adrenergic receptor. The $\beta_1$-adrenergic receptor consists of 477 amino acid residues, whereas the avian $\beta$-adrenergic receptor and the human $\beta_2$-adrenergic receptor have 483 and 413 residues, respectively. Sequence similarities between the human $\beta_1$-adrenergic receptor and the avian $\beta$-adrenergic receptor (Figure 1A) and the human $\beta_1$- and $\beta_2$-adrenergic receptors (Figure 1B) are striking and highest in the presumed transmembrane regions. The percentage of amino acid homologies within the transmembrane regions and the overall sequence homologies, respectively, between the human $\beta_1$-adrenergic receptor and the other two receptors are: avian $\beta$-adrenergic receptor 84%, 69%, and human $\beta_2$-adrenergic receptor 71%, 54%. Thus the $\beta_1$-adrenergic receptor is remarkably similar to the avian $\beta$-adrenergic receptor but is less closely related to the $\beta_2$-adrenergic receptor.

The extensive homology of the membrane-spanning regions among the three receptors suggests that these regions are subject to a selective pressure that acts to prevent divergence. These regions must therefore embody functions such as ligand binding and G protein coupling common to all

---

Departments of Medicine, Biochemistry, and Cell Biology, Howard Hughes Medical Institute at Duke University Medical Center, Durham, NC 27710.

Received (prereviewed) February 14, 1989; accepted February 15, 1989.
contains 16 additional proline residues. Given the rotational constraints imposed by proline, the third cytoplasmic loop of the \( \beta_1 \)-adrenergic receptor could have a very different tertiary structure than that of the \( \beta_2 \)-adrenergic receptor. Recent mutagenesis studies \((19, 20) \) with the hamster \( \beta_2 \)-adrenergic receptor and the human \( \beta_2 \)-adrenergic receptor \((21) \) have suggested two short segments of this third cytoplasmic loop, situated near transmembrane segments 5 and 6, respectively, as being important in coupling of the \( \beta_2 \) receptor to \( G \). However, the variations in length and composition of this loop among receptors suggest that these domains may confer specificity for \( G \) protein coupling or some other function of the receptors. The data reviewed in this essay indicate that mammalian \( \beta_1 \) and \( \beta_2 \)-adrenergic receptors are products of distinct genes. This conclusively eliminates the possibility discussed previously, that differences in agonist and antagonist affinities for the two subtypes are due to post-translational modifications.

As stated, the only apparent functional differences between the human \( \beta_1 \) and \( \beta_2 \)-adrenergic receptors are the differences in epinephrine and norepinephrine affinities and in subtype-selective antagonist affinities. To determine which sequences within the receptors are responsible for such subtype specificity, chimeric receptor proteins containing sequences derived from both the \( \beta_1 \) and the \( \beta_2 \)-adrenergic receptors have been constructed by using site-directed mutagenesis and expressed in \textit{Xenopus laevis} oocytes \((22) \). Each chimeric receptor was assessed for subtype-specific agonist and antagonist binding characteristics, permitting delineation of those structural domains that determine the pharmacological differences between the \( \beta_1 \) and \( \beta_2 \)-adrenergic receptor subtypes.

Characteristic agonist competition profiles for the \( \beta_1 \) and the \( \beta_2 \)-adrenergic receptors are shown in Figure 2, A and H, respectively. These were generated with oocytes injected with RNA for each of these two receptors. The \( \beta \)-adrenergic agonist isoproterenol is the most potent competing ligand for either receptor subtype. Consistent with the well-established pharmacology of \( \beta_1 \) and \( \beta_2 \)-adrenergic receptors, epinephrine and norepinephrine are approximately equipotent in competing with the antagonist radioisogand for binding to the \( \beta_1 \)-adrenergic receptor, whereas for the \( \beta_2 \)-adrenergic receptor, epinephrine is approximately 20 times more potent than norepinephrine.

Replacement of the N-terminus and the first one, two, or three \( \alpha \)-helices of the \( \beta_1 \)-adrenergic receptor with the corresponding regions of the \( \beta_2 \)-adrenergic receptor chimeras, \( \beta_2(I)/\beta_1(II–VII) \), \( \beta_2(I)/\beta_1(III–VII) \), and \( \beta_2(I–III)/\beta_1(IV–VII) \) resulted in epinephrine and norepinephrine competition profiles resembling the profiles for the \( \beta_1 \)-adrenergic receptor \((22, 23) \). i.e., epinephrine and norepinephrine are approximately equipotent. These results suggest that those amino acid residues that determine \( \beta \)-adrenergic receptor subtype specificity reside within \( \alpha \)-helices IV–VII and that the N-terminus and first three \( \alpha \)-helices do not contribute to agonist subtype specificity.

When helix IV of the \( \beta_2 \)-adrenergic receptor is substituted for the corresponding helix of the \( \beta_1 \)-adrenergic receptor \([\beta_2(I–IV)/\beta_1(V–VII)] \) (Figure 2F), the \( K_D \) for norepinephrine increases by 10-fold when compared with the preceding \( \beta_1 \)-\( \beta_2 \) chimeras \([\beta_2(I–III)/\beta_1(IV–VII)] \) (Figure 2D). If one compares the \( K_D \) norepinephrine/\( K_D \) epinephrine ratio for the native receptors, the value is \( \sim 1 \) for the \( \beta_1 \)-adrenergic receptor, \( \sim 15 \) for the \( \beta_2 \)-adrenergic receptor, and \( \sim 16 \) for \( \beta_2(I–IV)/\beta_1(V–VII) \) (Figure 2, A, H, and E, respectively).
Thus, the relative affinities of norepinephrine and epinephrine binding to \( \beta_2 \) helices (I-IV)/\( \beta_2 \) (V-VII) are essentially identical to those for the \( \beta_2 \) adrenergic receptor. These data suggest that amino acid residues within \( \alpha \) helix IV contribute strongly to determining the agonist subtype specificity of the \( \beta_2 \) adrenergic receptor. The sequential addition of \( \beta_2 \) adrenergic receptor helices V, VI, and VII in chimeras \( \beta_2 \) (I-V)/\( \beta_2 \) (VI-VII), \( \beta_2 \) (I-IV)/\( \beta_2 \) (V-VII), and the \( \beta_2 \) adrenergic receptor, does not result in any further significant changes in the \( K_D \) norepinephrine/\( K_D \) epinephrine ratio. A decrease in the apparent potency of both epinephrine and norepinephrine was observed when the \( \beta_2 \) adrenergic receptor was compared with the chimeras of Figure 2, E, F, and G. This may indicate a potential contribution of helix VII to the affinity of agonists. These data support the conclusion that residues in helices I, II, III, V, and VII do not significantly determine \( \beta_1 \) and \( \beta_2 \) adrenergic receptor agonist subtype specificity.

The subtype specificity of the chimeric receptors was also assessed by using the \( \beta_1 \) adrenergic receptor-selective antagonist betaxolol and the \( \beta_2 \) adrenergic receptor-selective antagonist ICI 118551 as competing ligands. In contrast to the results observed for agonists, it is apparent from Figure 3 that as \( \beta_2 \) adrenergic receptor helices are sequentially substituted for the corresponding \( \beta_1 \) adrenergic receptor helices, the potencies of betaxolol and of ICI 118551 gradually change. However, several of the \( \alpha \) helices appear to contribute more significantly than others to determining antagonist potency. Thus the addition of \( \beta_2 \) adrenergic receptor helices III, VI, and VII each result in six- to 10-fold changes in the \( K_D \) betaxolol/\( K_D \) ICI ratio, when compared with each preceding chimera. Addition of helix I leads to no change in the ratio, whereas addition of helices II, IV, and V lead to more modest (~twofold) changes in the ratio. The addition of helices III, IV, and VII results in significant increases in the \( K_D \) of betaxolol, whereas addition of helices II, V, and VI results in significant decreases in the \( K_D \) of ICI 118551. This suggests that residues within all of these helices are involved in determining \( \beta_1 \) vs \( \beta_2 \) adrenergic receptor agonist specificity.

The high degree of sequence homology in the presumed transmembrane regions of the various adrenergic receptors as well as previous mutagenesis (19) and chimeric receptor work (23) all suggest that several of the transmembrane helices are involved in forming the ligand binding site of these receptors. The extracellular, hydrophilic loops are apparently not essential for ligand binding, because deletion of these residues does not alter ligand affinity (24). Therefore, even though our chimeric receptors contain both hydrophilic and hydrophobic sequences derived from both \( \beta_1 \) and \( \beta_2 \) adrenergic receptors, we have interpreted the data in terms of which receptor subtype contributes a particular membrane-spanning domain.

Interestingly, when \( \beta_1 \) and \( \beta_2 \) adrenergic receptor sequences are compared (Figure 1), \( \alpha \) helices IV, VI, and VII are the most divergent of the putative membrane-spanning domains, suggesting that those residues that determine the pharmacological differences between the subtypes may be located within these \( \alpha \) helices. Results of photoaffinity labeling techniques suggest that residues within several of the \( \alpha \) helices of the adrenergic receptors contribute to the ligand binding site. Labeled by such techniques are residues located within helix II of the \( \beta_2 \) adrenergic receptor (25), and residues within helices II-V and helix VII of the avian \( \beta_1 \) adrenergic receptor (26).
chimeric receptor studies, a model can be proposed for the arrangement of the α-helical membrane-spanning domains within the plasma membrane. In such a configuration, those helices that most significantly contribute to β-adrenergic receptor agonist or antagonist subtype specificity (IV, VI, VII) are grouped together, suggestive of a ligand binding pocket. As a consequence of this arrangement, those additional helices that at least in part determine β-adrenergic receptor antagonist subtype specificity (II, III, V) also could contribute to the formation of such a pocket.

In summary, these results with chimeric β1-β2-adrenergic receptors suggest that subtype specificity is determined by most of the transmembrane spanning regions of the molecules. This further strengthens the emerging idea that binding of ligands by such receptors occurs in a pocket formed by the clustering of these membrane-spanning α-helices. While competitively occluding this binding pocket, antagonists nonetheless interact with structural determinants distinct from those with which agonists interact. These observations are consistent with the older notion of antagonists interacting with "accessory binding sites" on their receptors in addition to the sites with which the natural agonists interact. These studies further underscore the value of chimeric receptors for elucidating the structural basis of receptor functions.

References
18. Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ.


