Chimeric alpha-2-, beta-2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity.

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Chimeric [alpha].sub.2.-, [beta].sub.2.-Adrenergic Receptors: Delineation of Domains Involved in Effector Coupling and Ligand Binding Specificity

THE ADRENERGIC RECEPTORS ([ALPHA].sub.1.-, [alpha].sub.2.-, [beta].sub.1.-, AND [beta].sub.2.-), which mediate the physiological effects of catecholamines, belong to the family of plasma membrane receptors that are coupled to guanine nucleotide regulatory proteins (G proteins) (1). This receptor family also includes rhodopsin and the visual color opsins, the muscarinic cholinergic receptors, and many other neuro-transmitter receptors and receptors for peptide hormones. A common feature of G protein-coupled receptors is that agonist occupancy of the receptor leads to receptor activation of a G protein, which in turn modulates the activity of an effector enzyme or ion channel. Several of the G protein-coupled receptors (including the major subtypes of adrenergic receptors) have been cloned and found to share structural features with rhodopsin (2). The most consistently conserved of these features is the existence of seven clusters of hydrophobic amino acids. In addition, there is significant amino acid sequence similarity among these receptors, which is most striking in the hydrophobic domains. For bovine rhodopsin, physical and biochemical studies have revealed that these hydrophobic domains may form seven alpha helices that span the lipid bilayer (3). It has been suggested that these alpha helices form a pocket for the chromophore 11-cis-retinal (3). Thus, in an analogous fashion, the hydrophobic domains of the adrenergic receptors may form a pocket in the plasma membrane for binding ligands.

Because so many different hormones, neurotransmitters, and drug receptors are likely to have structures homologous with the adrenergic receptors, it is necessary to achieve an understanding of the structural basis for the various functional properties of these receptors, in particular the specificity of ligand binding and effector coupling. This has been done heretofore (i) by mutagenesis, especially the deletion of specific peptide sequences (4-6), and (ii) biochemically, where proteases have been used to cleave defined peptide segments from the digitonin solubilized receptor (7). These methods, although useful in delineating regions of the receptor that do not influence its function, suffer from difficulties in that it is difficult to draw compelling inferences about the role of specific domains based on loss of functions.

In order to circumvent such problems, and to establish a potentially general approach to the study of G protein-coupled receptors so that positive inferences can be drawn about functions associated with specific receptor domains, we have constructed and expressed a series of chimeric [alpha].sub.2.,[beta].sub.2.-adrenergic receptor genes. All of the subtypes of adrenergic receptors are activated by epinephrine, but they differ in their affinity for various subtype selective agonists and antagonists. Furthermore, the [beta].sub.2.-adrenergic receptors ([beta].sub.2.-AR)'s couple to Gs (the stimulatory G protein for adenylyl cyclase) while the [alpha].sub.2.-adrenergic receptors ([alpha].sub.2.-AR)'s couple to G.sub.i (the inhibitory G protein for adenylyl cyclase). These two receptors therefore, respectively, stimulate and inhibit the enzyme. By studying the ligand binding and adenylyl cyclase activating properties of these chimeric receptors, in which various regions of the [alpha].sub.2.- and [beta].sub.2.-adrenergic receptors have been interchanged, we have deduced structural domains that determine the specificity of ligand binding and effector coupling.

The [alpha].sub.2.- and [beta].sub.2.-adrenergic receptors. We have described the cloning of the genes for both the human [alpha].sub.2.-AR (8) and the human [beta].sub.2.-AR (9). Both genes have been expressed in Xenopus laevis oocytes by injecting the oocytes with receptor-specific mRNA (8, 10). Receptors expressed in this way can be detected by binding to specific radioactively labeled ligands. [.sup.125.I]Cyanopindolol can be used to detect expressed [beta].sub.2.-AR (10). The [beta].sub.2.-AR expressed in Xenopus oocyte membranes has an affinity for [.sup.125.I]Cyanopindolol of 63 pM and has a typical [beta].sub.2.-AR agonist order of potency, with isoproterenol ([beta]-AR agonist) being more potent than epinephrine ([alpha].sub.2.- and [beta]-AR agonist), which in turn is much more potent than p-aminoclonidine ([alpha].sub.2.-AR agonist) (Table 1). These agonists, with the exception of p-aminoclonidine, stimulate [beta].sub.2.-AR's, expressed in Xenopus oocyte membranes, to activate endogeneous adenylyl cyclase (Table 2).

In contrast to the [beta].sub.2.-AR, [alpha].sub.2.-AR expressed in Xenopus oocytes cannot be detected with
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[.sup.125.I]cyanopindolol, but instead binds [.sup.3.H]yohimbine ([alpha].sub.2.-AR antagonist) with high affinity (2.5 nM). Competition binding studies with [.sup.3.H]yohimbine for [alpha].sub.2.-AR expressed in Xenopus oocytes show a typical [alpha].sub.2.-AR agonist order of potency, with p-aminoclonidine ([alpha].sub.2.-AR agonist) being more potent than epinephrine ([alpha].sub.2.- and [beta]-AR agonist), which is much more potent than isoproterenol ([beta]-AR agonist). These binding studies on [alpha].sub.2.-AR expressed in Xenopus oocytes (8) are in agreement with studies on [alpha].sub.2.-AR expressed in simian COS-7 cells (Table 3). Thus, the [beta].sub.2.-AR, expression of the [alpha].sub.2.-AR in Xenopus oocyte membranes can be documented and characterized by ligand binding. However, unlike the [beta].sub.2.-AR, a functional interaction of adenylyl cyclase with the [alpha].sub.2.-AR expressed in Xenopus oocyte membranes has not been observed. Thus, stimulation of [alpha].sub.2.-AR in Xenopus oocyte membranes does not lead to inhibition of adenylyl cyclase activity.

Chimeric receptors. To determine which structural domains of these two receptors confer specificity for agonist and antagonist binding as well as G protein coupling, we constructed ten chimeric receptor genes from the human [beta].sub.2.-AR and human platelet [alpha].sub.2.-AR genes. These chimeric receptor genes were expressed in Xenopus oocytes and COS-7 cells, and the ability of the chimeric receptors to bind [beta].sub.2.-AR- and [alpha].sub.2.-AR-specific ligands and to activate adenylyl cyclase was determined. When the ligand binding properties and G protein-coupling specificities of the various chimeric receptors are correlated with the [alpha].sub.2.-AR and [beta].sub.2.-AR amino acid sequences of these chimeric receptors, it is possible to assign functional properties to specific structural domains.

The structures of each of the ten chimeric receptors and the ability of each chimeric receptor to bind to [.sup.125.I]cyanopindolol or [.sup.3.H]yohimbine and to activate adenylyl cyclase after stimulation with epinephrine are compared in Fig. 1B. Chimeric receptors (CR) 1, 2, 3, and 4, expressed in Xenopus oocytes, were able to bind [.sup.125.I]cyanopindolol. Since CR 3 and CR 4 are structurally similar with respect to the composition of their putative membrane spanning domains, detailed pharmacologic studies were done on CR 3 as well as CR 1 and CR 2. Saturation binding isotherms and competition binding studies were done on the [beta].sub.2.-AR and on CR 1, CR 2, and CR 3 to determine the affinity constants for the [beta]-AR antagonists [.sup.125.I]cyanopindolol and alprenolol and the agonists isoproterenol, epinephrine, and p-aminoclonidine (Fig. 2 and Table 1). The [alpha].sub.2.-AR antagonist yohimbine at a concentration of 0.1 mM did not compete with [.sup.125.I]cyanopindolol for binding sites on these chimeric receptors.

[.sup.3.H]Yohimbine ([alpha].sub.2.-AR antagonist) binding was assayed in COS-7 cell membranes after transient transfection of these cells with the chimeric receptor genes. While CR 6 bound [.sup.3.H]yohimbine weakly, only CR 8 (Fig. 3) and CR 9 bound [.sup.3.H]yohimbine with an affinity high enough to permit determination of affinity constants for agonists and antagonists (Table 3).

The ability of each chimeric receptor to couple to Gs and thus activate adenylyl cyclase was determined by studying epinephrine-stimulated adenylyl cyclase activity in oocyte membranes expressing the chimeric receptor. Control oocyte membranes exhibited little or no epinephrine-stimulated adenylyl cyclase activity. Epinephrine was used because it is an agonist for both [alpha].sub.2.-AR and [beta].sub.2.-AR, and thus would be expected to act as an agonist for an [alpha].sub.2.-[beta]-chimeric receptor. Chimeric receptors 1, 2, 6, 8, and 9 were capable of activating adenylyl cyclase while CR’s 3, 4, 5, 7, and 10 were not. An epinephrine dose response study was done to determine the efficiency of agonist stimulated receptor activation of adenylyl cyclase for CR’s 1, 2, 6, 8, and 9 relative to the [beta].sub.2.-AR (Fig. 4 and Table 2). The agonist potency for adenylyl cyclase stimulation of each chimeric receptor was determined from results of isoproterenol, epinephrine, and p-aminoclonidine dose response studies on each chimeric receptor capable of mediating epinephrine-stimulatable adenylyl cyclase activity (Fig. 5 and Table 2).

G protein coupling. One of our goals was to locate the region of the [beta].sub.2.-AR that is responsible for coupling to G.sub.s and to determine whether this domain is also involved in ligand binding. Of the chimeric receptors that can activate adenylyl cyclase, CR 8 and CR 9 contain the shortest stretches of [beta].sub.2.-AR. Furthermore, the agonist order of potency for both cyclase activation and ligand binding for both of these chimeric receptors resembles [alpha].sub.2.-AR in that p-aminoclonidine > epinephrine > isoproterenol (Figs. 3 and 5 and Tables 2 and 3). The [beta].sub.2.-AR sequence in CR 8 extends from amino acid 174 at the NH.sub.2.-terminal portion of the second putative extracytoplasmic loop, fifth hydrophobic domain and the third cytoplasmic loop, and ending at amino acid 295 at the COOH-terminal portion of the sixth hydrophobic domain (Fig. 6). CR 10, which contains [beta].sub.2.-AR amino acid sequence 174 to 261 does not activate adenylyl cyclase. Chimeric receptor 3, which

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contains [beta].sub.2.-AR amino acid sequence 262 to 413 also does not activate adenylyl cyclase even though it binds [.sup.125.I]cyanopindolol. Chimeric receptor 9 contains [beta].sub.2.-AR amino acid residues 215 to 295 and activates adenylyl cyclase, but the efficiency of this activation is weak compared to activation by CR 8, as can be seen by comparing the median effective concentration (EC.sub.50.) for agonists and the maximal stimulation of adenylyl cyclase for these chimeric receptors (Fig. 4 and Table 2). These results suggest that, at least, portions of the fifth and sixth hydrophobic domains may be required for determining the specificity of [beta]sub.2.-AR coupling to G.sub.s.. Conversely, hydrophobic domains 1, 2, 3, 4, and 7 as well as the first and second cytoplasmic loops and the COOH-terminus appear to have little influence in determining the specificity for G protein coupling.

Studies on site-directed mutagenesis of the hamster [beta]sub.2.-AR (5, 6) and proteolysis of digitonin-solubilized turkey [beta]-AR (7) have addressed the issue of which structural domains may be involved in coupling of the [beta]-AR to G.sub.s. Deletion of several small segments of the third cytoplasmic loop of the hamster [beta].sub.2.-AR does not affect G protein coupling (5, 6). The region of the human [beta].sub.2.-AR analogous to the hamster [beta].sub.2.-AR in the region of these deletions extends from amino acid residues 229 through 262 (Fig. 6). Also, deletion of sequences at the NH.sub.2- and COOH-terminal portions of the third cytoplasmic loop in the hamster [beta].sub.2.-AR leads to loss of G.sub.s. activation (6). In the human [beta].sub.2.-AR (Fig. 6), these deletions would correspond to amino acid 222 to 229 and amino acid 258 to 270, respectively. These studies therefore provide clues to the potential sites of interaction between the [beta].sub.2.-AR and G.sub.s.; however, it is also possible that the negative effect of these deletion mutations might be due to an allosteric rather than a direct effect on the actual G protein coupling domain.

Proteolysis studies on the turkey [beta]-AR suggest that deletion of even larger regions of the third cytoplasmic loop, and possibly of the fifth hydrophobic domain, do not affect the ability of the receptor to couple to G.sub.s. (7). However, with this approach it was difficult to define the precise position of some of the proteolytic cleavage sites.

Our results define a limited region of the human [beta].sub.2.-AR which, when placed in the analogous position of the human [alpha].sub.2.-AR, confers the ability to couple to and activate G.sub.s. with an [alpha].sub.2.-AR agonist order of potency. More detailed resolution of the precise sequences necessary for receptor-G.sub.s coupling will be achieved by insertion of small segments of the [beta].sub.2.-AR into the [alpha].sub.2.-AR and by single amino acid substitutions.

Ligand binding. A number of studies have suggested that the hydrophobic domains of the [beta].sub.2.-AR are involved in the formation of the ligand binding pocket. In constructing and studying the series of [alpha].sub.2.- and [beta].sub.2.-AR chimeric receptors an attempt was made to determine which domains conferred ligand binding specificity for agonists and antagonists. Since each chimeric receptor is an artificial combination of [alpha].sub.2.- and [beta].sub.2.-AR, it might not be expected to function as well as either of the native receptors (see below). Attention was therefore focused on the relative order of potencies for agonists and antagonists rather than the absolute affinities for the different agents. Thus, each chimeric receptor can be classified as having an [alpha].sub.2.-AR or [beta].sub.2.-AR agonist or antagonist potency series. These determinations were made on the basis of both ligand binding (for those chimeric receptors capable of binding either [.sup.125.I]cyanopindolol or [.sup.3.H.]yohimbine) and adenylyl cyclase assays.

A comparison of [.sup.125.I]cyanopindolol binding studies (Fig. 2 and Table 1) and adenylyl cyclase studies (Table 2) on the native [beta].sub.2.-AR with those on CR 1, 2, and 3 suggests that hydrophobic domains 1 to 5 are not involved in a major way in determining [beta].sub.2.-AR antagonist specificity. All of these chimeric receptors bind [.sup.125.I]cyanopindolol with an affinity equivalent to or higher than the native [beta].sub.2.-AR (Table 1).

A somewhat different picture emerges for the agonists. The affinity of all agonists for CR 1, 2 and 3 was significantly lower than for the [beta].sub.2.-AR. Moreover, a progressively changing specificity for agonists can be appreciated by considering the ratio of K.sub.i. for the [alpha].sub.2.-AR agonist p-aminoclonidine and the [beta].sub.2.-AR agonist isoproterenol [K.sub.i.([PAC])/K.sub.i.([ISO])]. At the two extremes, the ratio of K.sub.i.([PAC]) to K.sub.i.([ISO]) was 1800 for the [beta].sub.2.-AR and 0.00032 for the [alpha].sub.2.-AR. The values for the chimeric receptors were: CR 1 94; CR 2, 9.4; and CR 3, 2.2. Thus, as the extent of [alpha].sub.2.-AR sequence increases the receptor becomes progressively less "[beta].sub.2.-AR-like" in its agonist binding properties.

Our data on the chimeric receptors, in particular the ligand binding properties of CR 9, suggest that the sixth hydrophobic domain does not exert a major influence on either agonist or antagonist order of potency. However, the seventh hydrophobic domain appears to be a major determinant of both agonist and antagonist ligand binding...
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specificity. A study of CR 6 shows that replacement of the seventh hydrophobic domain in the [beta].sub.2.-AR with the seventh hydrophobic domain from the [alpha].sub.2.-AR leads to a loss of [.sup.125.I]cyanopindolol binding and the acquisition of the ability to bind [.sup.3.H.]yohimbine, albeit with low affinity. Furthermore, isoproterenol, epinephrine, and norepinephrine (Fig. 7). This "split receptor" also activate cyclase, although it is only [isapproximately] 25 percent as efficient as the wild-type [beta].sub.2.-AR in doing so. However, injection of mRNA for SR(6-7) alone does not lead to the expression of a sup.125.I-labeled cyanopindolol binding protein in the oocyte membranes. This suggests that, even though hydrophobic domain 7 (or 6 and 7) appear to be the major determinants of [beta].sub.2.-AR ligand binding specificity, this region of the molecule by itself is insufficient to bind [beta].sub.2.-AR ligands or activate adenyl cyclase.

The importance of the seventh hydrophobic domain in conferring [alpha].sub.2.-agonist and antagonist specificity can be further illustrated by comparing CR 2 and 8. In CR 2, hydrophobic domains 1 to 4 are derived from the [alpha].sub.2.-AR and hydrophobic domains 5 to 7 are derived from the [beta].sub.2.-AR. This chimeric receptor exhibits predominantly [beta].sub.2.-AR ligand binding properties (Fig. 2C). Chimeric receptor 8 is made by changing the seventh hydrophobic domain in CR 2 from [beta].sub.2.-AR to [alpha].sub.2.-AR (see Fig. 1B). In contrast to chimeric CR 2, CR 8 exhibits [alpha].sub.2.-AR agonist and antagonist ligand binding properties (Fig. 3 and Table 3), and activates adenyl cyclase with an [alpha].sub.2.-AR agonist order of potency (Fig. 5). Thus, changing the seventh hydrophobic domain in CR 2 from [beta].sub.2.-AR to [alpha].sub.2.-AR results in a change in the ratio of K.sub.i.(Pac) to K.sub.i.(ISO) from 9.4 to 0.0022.

These data indicate that most of the hydrophobic domains influence agonist ligand binding specificity, while antagonist ligand binding specificity (at least for [.sup.3.H.]yohimbine, [.sup.125.I]cyanopindolol, and alprenolol) in influenced primarily by the seventh hydrophobic domain or the combination of the sixth and the seventh. Thus, CR 3, which contains only hydrophobic domains 6 and 7 from the [beta].sub.2.-AR, has affinities for [.sup.125.I]cyanopindolol and alprenolol that are close to the affinities of these ligands for the wild-type [beta].sub.2.-AR.

Split receptor. The role of hydrophobic domains 6 and 7 in binding to [.sup.125.I]cyanopindolol was then explored. The [beta].sub.2.-AR was expressed as two separate peptides (see Fig. 1C), one encoding amino acid 1 to 262, containing hydrophobic domains 1 to 5, SR(1-5), and the other containing hydrophobic domains 6 to 7, SR(6-7). We constructed SR(1-5) by inserting a termination codon after amino acid 262. This mutant does not bind ligands or activate adenyl cyclase (10). We made SR(6-7) by deleting the region between the second amino acid of the [beta].sub.2.-AR and amino acid 262. It is possible to express SR(1-5) and SR(6-7) together in Xenopus oocytes and obtain a functional receptor with a K.sub.d for [.sup.125.I]cyanopindolol of 44 pM and normal [beta].sub.2.-AR affinities for isoproterenol, epinephrine, and norepinephrine (Fig. 7). This "split receptor" also activate cyclase, although it is only [isapproximately] 25 percent as efficient as the wild-type [beta].sub.2.-AR in doing so. However, injection of mRNA for SR(6-7) alone does not lead to the expression of a sup.125.I-labeled cyanopindolol binding protein in the oocyte membranes. This suggests that, even though hydrophobic domain 7 (or 6 and 7) appear to be the major determinants of [beta].sub.2.-AR ligand binding specificity, this region of the molecule by itself is insufficient to bind [beta].sub.2.-AR ligands or activate adenyl cyclase.

While these results suggests that the seventh hydrophobic domain is involved in dictating ligand binding specificity, it cannot be concluded that this hydrophobic domain forms the ligand binding pocket. This domain may confer ligand binding specificity by interaction with the domains directly involved in the formation of the binding site. The [beta]-AR-specific photoaffinity antagonist pBABC covalently binds to a peptide in the second hydrophobic domain (11) suggesting that this domain may form or lie adjacent to the ligand binding pocket. Site-directed mutagenesis of various residues in different domains of [beta].sub.2.-AR leads to alteration of ligand binding properties (4, 12). While these findings may indicate that the mutated regions are involved in the formation of the ligand binding site, they may also be due to allosteric effects.

Possible arrangement of hydrophobic domains. The study of this set of chimeric receptors has provided insight into the function of various structural domains. However, these molecules may also provide clues about the arrangement of the various hydrophobic domains within the parent molecules. These hydrophobic domains may form a [alpha]-helices that span the plasma membrane, as has been suggested by electron diffraction studies on bacteriorhodopsin (13). For the following discussion we therefore refer to the hydrophobic domains as membrane spanning [alpha]-helices. The arrangement of these [alpha]-helices with respect to each other might be dictated by the interactions of various charged, noncharged polar, and nonpolar amino acids as well as the possible formation of covalent bonds (that is, disulfide bridges). The less hydrophobic amino acids of these [alpha] helices are likely to project toward the interior of the molecule, while the more hydrophobic residues may form a boundary with the plasma membrane. The [alpha] helices that lie adjacent to each other have presumably evolved in such a way as to minimize steric and electrostatic repulsive forces between each other.
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In the process of making chimeric receptors these favorable molecular interactions may be lost. For example, in making CR 1, [alpha]-helix 2 from the [alpha].sub.2.-AR and [alpha]-helix 3 from the [beta].sub.2.-AR are forced to lie adjacent to each other. Furthermore, other [alpha] helices that may normally cluster around [alpha]-helices 1 and 2 from the [beta].sub.2.-AR may be less compatible with [alpha]-helices 1 and 2 from the [alpha].sub.2.-AR. These molecular incompatibilities might be expected to destabilize the molecule, and thus make it less efficient or even nonfunctional. This is consistent with the finding that all chimeric receptors in this series are less efficient than either parent molecule in binding agonists, and less efficient than the [beta].sub.2.-AR in activating G.sub.2.

Furthermore, chimeric receptors containing two molecular splice junctions such as CR 7, 8, 9, and 10 (Fig. 1), might be expected to function even less well than chimeric receptors with only one splice junction. For example, while CR 1 and CR 6 are both to couple to and activate G.sub.s, CR 7, which contains both molecular splice junctions found in CR 1 and CR 6, is nonfunctional even though it contains the essential elements of [beta].sub.2.-AR necessary to activate G.sub.s.

Chimeric receptor 8 is of particular interest, therefore, since it contains two molecular splice junctions, yet has a higher affinity for epinephrine (Tables 1 and 3) and is more efficient at activating adenylyl cyclase (Table 2) than either CR 2 or CR 6, each of which contains only one of the two molecular splice junctions found in CR 8 (Fig. 1). This observation might be explained by considering the possible arrangement of hydrophobic domains in the [alpha].sub.2.-AR and various chimeric receptors (Fig. 8). The model proposes that [alpha]-helix 7 of the [alpha].sub.2.-AR and CR 1, 6, 7, and 8 lie adjacent to [alpha]-helices 3 and 4. In CR 6 (Fig. 8B) [alpha]-helix from the [alpha].sub.2.-AR is paired with a [alpha]-helix 3 and 4 from the [beta].sub.2.-AR and is therefore less stable. Similarly in CR 2 (Fig. 8C), [alpha]-helix 7 from the [beta].sub.2.-AR is paired with [alpha]-helices 3 and 4 from the [alpha].sub.2.-AR. However, in CR 8 (Fig. 8D) [alpha]-helices 3, 4, and 7 are all from the [alpha].sub.2.-AR. Thus, the potential molecular incompatibilities between [alpha]-helix 4 and [alpha]-helix 5 and between [alpha]-helix 6 and [alpha]-helix 7 in CR 8 are compensated for by the opportunity for [alpha]-helix 7 to interact normally with [alpha]-helices 3 and 4 from the same receptor. In the nonfunctional CR 7 (Fig. 8E), potential incompatibilities between [alpha]-helix 7 and [alpha]-helices 3 and 4 as well as between [alpha]-helix 2 and [alpha]-helix 3 and between [alpha]-helix 6 and [alpha]-helix 7 may contribute to the lack of activity of this receptor. Thus, on the basis of the functional capacity of chimeric receptors, it may be possible to predict the arrangement of [alpha]-helices within the parent molecules.

Only CR 5, 7, and 10 are nonfunctional; that is, they do not bind [.sup.3.H]yohimbine or [.sup.125.I]cyanopindolol, nor do they activate adenylyl cyclase. Sequence analysis of the splice junctions of these chimeric receptor genes confirmed that these chimeric receptors were properly constructed. Furthermore, in vitro translation of mRNA made from these chimeric receptor genes produced a protein of the predicted molecular size. While the lack of function of these chimeric receptors might be explained by molecular incompatibilities between [alpha]-helices ad discussed above, it is also possible that these chimeric receptors failed to insert properly in the plasma membrane as a result of specific amino acid sequences created at the splice junctions. This may be particularly important for CR 5 and CR 10 which have a splice junction in the putative third cytoplasmic loop in a region where the [alpha]-AR and [beta].sub.2.-AR share little amino acid sequence similarity.

The results from our study of chimeric receptors made from the [beta].sub.2.-AR and [alpha].sub.2.-AR have provided new insights into the functional role of several structural domains. The fifth and sixth hydrophobic domains and the third cytoplasmic loop are capable of conferring specificity for G.sub.s coupling to the [beta].sub.2.-AR. The seventh hydrophobic domain of the [alpha].sub.2.- and [beta].sub.2.-adrenergic receptors is a major determinant of both agonist and antagonist ligand binding specificity. Finally, several of the first five hydrophobic domains may contribute to agonist binding specificity. The strength of this approach for the study of these functionally complex molecules is that conclusions can be drawn from qualitative changes in receptor function, or from the acquisition of new functions that can be correlated with specific protein sequences. This is in contrast with more standard mutagenesis approaches where the end point is the loss of function resulting from amino acid deletions or substitutions. Our results provide an initial structural map for understanding the various functions of two model G protein-coupled receptors. The map requires further refinement and testing of its generally for understanding receptors of this class.