Accelerated Publications

Identification and Sequence of a Binding Site Peptide of the β_2-Adrenergic Receptor

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ABSTRACT: *p*-(Bromoacetamido)benzyl-1-[^{125}I]iodocarazolol (^{125}I-pBABC) is a potent derivative of the β-adrenergic receptor antagonist *p*-aminobenzylcarazolol. Treatment of the receptor with ^{125}I-pBABC results in efficient covalent incorporation of the ligand into the receptor binding site. Extensive degradation of ^{125}I-pBABC-labeled β_2-adrenergic receptor with either cyanogen bromide or *Staphylococcus aureus* V8 protease results in specifically labeled fragments having M_r's of about 1600 and 3500, respectively. Because the primary structure of the β_2-adrenergic receptor is known, and these proteolytic reagents are highly sequence specific, the site of ^{125}I-pBABC incorporation may be deduced from the sizes of the specifically labeled fragments. Thus the fragment generated by cyanogen bromide cleavage corresponds to residues 83–96, a region of 14 amino acids included in the second membrane spanning domain (helix II) of the β_2-adrenergic receptor. This assignment was confirmed by direct amino acid sequencing of this labeled fragment, though the actual amino acid modified could not be determined. These data permit the assignment of a part of the hormone binding region of the β_2-adrenergic receptor.

The β_2-adrenergic receptor (β_2-AR) is one of a class of integral membrane glycoproteins involved in guanine nucleotide regulatory protein (G-protein) mediated transmembrane signaling (Dohlman et al., 1987a). When occupied by a hormone or agonist drug, the β_2-AR is coupled to the stimulation of adenylate cyclase through the stimulatory G-protein, G_s (Lefkowitz et al., 1983). Molecular cloning of the β_2-AR gene provided a deduced amino acid sequence and revealed significant homology with the visual pigment rhodopsin (Dixon et al., 1986; Kobilka et al., 1987a; Chung et al., 1987). Like rhodopsin, the β_2-AR is also known to have a multiple membrane spanning domain topography [Figure 1 and Dohlman et al. (1987b)], and it now appears that these two homologous proteins are members of a large family of structurally related G-protein coupled receptors. So far, these are known to also include four subtypes of muscarinic cholinergic receptors (Kubo et al., 1986a,b; Peralta et al., 1987; Bonner et al., 1987), as well as the human α_2 (Kobilka et al., 1987b), human β_1 (Frielle et al., 1987), and avian β_1-like (Yarden et al., 1986) adrenergic receptors.

What we know about the hormone binding site of adrenergic receptors comes largely from in vitro mutagenesis and expression of the β_2-AR gene. An array of short deletions in the extracellular hydrophilic regions of the protein failed to affect binding of agonists or antagonists (Dixon et al., 1987), while single amino acid substitutions in the hydrophobic transmembrane regions (helices II, III, and VII) resulted in striking changes in the binding properties of β_2-AR (Strader et al., 1987). Truncation or deletion of the sixth and/or seventh membrane spanning domain(s) abolishes ligand binding and G-protein coupling functions (Dixon et al., 1987; Kobilka et al., 1987c). Thus, the binding of hormones to β_2-AR may occur in a manner analogous to that of retinal in the light-sensitive proteins such as rhodopsin, where the chromophore binding site is thought to be within a bundle of seven membrane spanning α-helices (Thomas & Stryer, 1982). In order to further elucidate the structural determinants of the hormone binding domain of the β_2-AR, we sought to identify the site of incorporation of the specific β-adrenergic alkylating agent ^{125}I-pBABC (Figure 1, inset). This probe binds and incorporates covalently into the β_2-AR binding site with pharmacological specificity characteristic of a high-affinity β-adrenergic ligand (Dickinson et al., 1985). This particular ligand was chosen because it could be radioiodinated to a high specific activity and because it incorporates into β_2-AR with unusually high (30–40%) efficiency. Thus, the ^{125}I-pBABC-modified peptide could be readily identified and isolated in sufficient quantity for protein sequencing. In this paper, we present the sequence of a 14 amino acid fragment of the β_2-AR containing the site of incorporation of ^{125}I-pBABC. These data are the first to make a direct assignment of a portion of the hormone binding region of an adenylate cyclase coupled receptor, and support the hypothesis (Dixon et al., 1986; Dohlman et al., 1987a) that the binding site for adrenergic ligands is within a hydrophobic region of the receptor formed by a bundle of transmembrane segments.

Experimental Procedures

Materials. All reagents for electrophoresis were obtained from Bethesda Research Laboratories and Bio-Rad, except for molecular weight standards (LKB). Phosphate-buffered saline was from Gibco. XAR-5 film and developing solutions for autoradiography were from Kodak. Organic solvents for chromatography were purchased from Mallinckrodt and were

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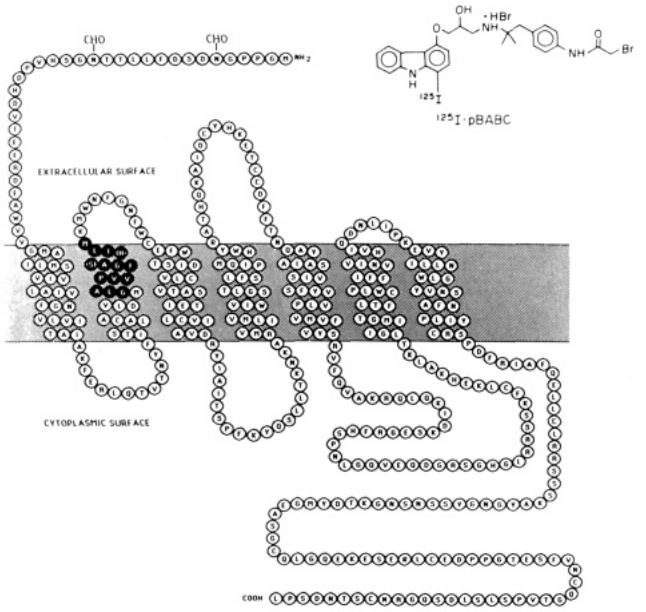

FIGURE 1: Sequence and topography of the hamster β_2-AR. The β_2-AR is shown spanning the membrane 7 times as presented in Dohlman et al. (1987b and references cited therein). The highlighted amino acid residues (83–96) in helix II contain the site(s) of ^{125}I-pBABC incorporation, likely at Ser-92 and/or His-93, as presented in the text. Inset: the chemical structure of the β-adrenergic alkylating probe ^{125}I-pBABC (Dickinson et al., 1985).

of the highest grade available. (−)-Alprenolol, cysteine, and cyanogen bromide were from Sigma, and digitonin was from Gallard-Schlessinger. V8 protease from *Staphylococcus aureus* was purchased from ICN, and dithiothreitol was from Bachem, Inc. (−)-[^{3}H]Dihydroalprenolol and carrier-free Na^{125}I were from New England Nuclear.

Purification of β_2-AR. The β_2-AR was purified to homogeneity according to the method Benovic et al. (1984). Briefly, β_2-AR was purified from digitonin-solubilized hamster lung membranes by alprenolol–Sepharose affinity chromatography and steric exclusion high-performance liquid chromatography (HPLC). Binding activity was assessed with [^{3}H]dihydroalprenolol according to Caron and Lefkowitz (1976). Purity was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and autoradiography of ^{125}I-labeled protein (Benovic et al., 1984; Laemmli, 1970).

Synthesis of ^{125}I-pBABC and Labeling of β_2-AR. ^{125}I-pBABC and I-pBABC were synthesized as described previously (Dickinson et al., 1985), except that in some cases NaI was used instead of Na^{125}I. Ten nanomoles of I-pBABC in 2 mL of ethanol was added to 200 mL of 100 mM NaCl, 20 mM NaH_2PO_4, pH 7.2, and 0.05% digitonin, and the mixture was incubated for 1 h at 25 °C with 1 nmol of pure β_2-AR. Separately, 10 pmol of β_2-AR was incubated under identical conditions with 100 pmol of 2200 Ci/mmol ^{125}I-pBABC in either the presence or absence of 10 μM alprenolol. The labeling reaction was stopped by addition of cysteine (1 mM final) and alprenolol (10^{-5} M final). I-pBABC-labeled β_2-AR was concentrated on a YM20 (Amicon) membrane and then further purified and resolved from unbound ligand by a second steric exclusion HPLC step. Peak fractions were pooled, concentrated, and dialyzed with several additions of 50 mM tris(hydroxymethyl)aminomethane (Tris)–SO_4, pH 7.5, to reduce the digitonin and then lyophilized.

CNBr Digestions. Receptor preparations were resuspended in 1 mL of 70% formic acid containing 0.4 mM cyanogen bromide, digested for 20 h at 23 °C, and then lyophilized (Gross, 1967).

V8 Protease Digestion. Pure hamster lung β_2-AR labeled with either ^{125}I-pBABC alone or ^{125}I-pBABC in the presence of alprenolol was incubated at 37 °C in 75 μL of phosphate-buffered saline plus 0.1% digitonin, 0.07% sodium dodecyl sulfate, and 25 mM dithiothreitol. After 1 h, 135 μL of *S. aureus* V8 protease in water was added to a final concentration of 500 μg/mL and incubated for 24 h at 37 °C. Digestions were stopped by freezing and lyophilization. Sodium dodecyl sulfate–urea–polyacrylamide gel electrophoresis (SDS–urea–PAGE) using 8.5% acrylamide and 8 M urea was performed as described (Hanoaka et al., 1979).

Reverse-Phase HPLC Purification of Receptor Fragments. The cyanogen bromide treated samples were resuspended in 0.2 mL of 20 mM trifluoroacetic acid (TFA) and loaded on a Synchropak C-4 reverse-phase HPLC column (250 mm × 4 mm × 0.3 mm pore size) equilibrated with 10% acetonitrile in 20 mM TFA. Peptides were eluted over 60 min with a 10–70% linear acetonitrile gradient in 20 mM TFA at 1.5 mL/min flow. The elution profile was monitored by absorbance at 214 nm; γ-radioactivity was measured for each eluted fraction. Digitonin alone was used to determine background A_{214}. ^{125}I-pBABC in 100 mM NaCl, 20 mM NaH_2PO_4, pH 7.2, 0.05% digitonin, 1 mM cysteine and 10 μM alprenolol was used to determine background radioactivity. One nanomole of unlabeled, cyanogen bromide digested β_2-AR was used to establish the normal elution profile under these conditions. Relevant peak fractions were frozen and lyophilized and then subjected to SDS–urea–PAGE or prepared for amino acid sequencing.

Amino Acid Sequencing. N-Terminal amino acid sequencing was kindly performed by Dr. Carl Bennett (Merck Sharp and Dohme Laboratories, West Point, PA) and by Dr. Richard Randall (Howard Hughes Medical Institute, Duke University) on an Applied Biosystems 470A sequenator (Hewik et al., 1981; Speiss et al., 1979). γ-Radioactivity of anilinothiazolinone or phenylthiohydantoin-amino acids was measured after each cycle.

Results and Discussion

The purpose of this study was to determine the location and sequence of the site of covalent incorporation of the β-adrenergic antagonist ^{125}I-pBABC into the β_2-AR. Previous studies in our laboratory have demonstrated this ligand to be an extremely potent affinity label for the hormone binding site of β_2-AR, and the specificity of ^{125}I-pBABC incorporation into the β_2-AR hormone binding site has been established by standard pharmacological criteria (Dickinson et al., 1985). Furthermore, while most commonly used β-adrenergic probes incorporate into less than 10% of the available binding sites, incorporation of ^{125}I-pBABC is unusually efficient, approximately 35%, as determined by measurement of the specific activity of ^{125}I-pBABC incorporated into the receptor band migrating at M_r 64 000 on SDS–PAGE (data not shown).

In order to localize the site of incorporation of this hormone analogue into the β_2-AR, we chose to proteolytically map the labeled β_2-AR using two agents with narrow and well-defined substrate specificities. Cyanogen bromide cleaves the peptide bond on the carboxyl side of methionine residues (Gross, 1967), while V8 protease from *S. aureus* will cleave (in phosphate buffer at pH 7.2) at the carboxyl side of both glutamic and aspartic acid residues (Drapeau, 1976). Since the location of methionine, aspartic acid, and glutamic acid residues is known from the deduced amino acid sequence of the β_2-AR (Figure 1), determination of the sizes of the radiolabeled peptide fragments resulting from cyanogen bromide and V8 protease treatment of ^{125}I-pBABC-labeled β_2-AR should provide the

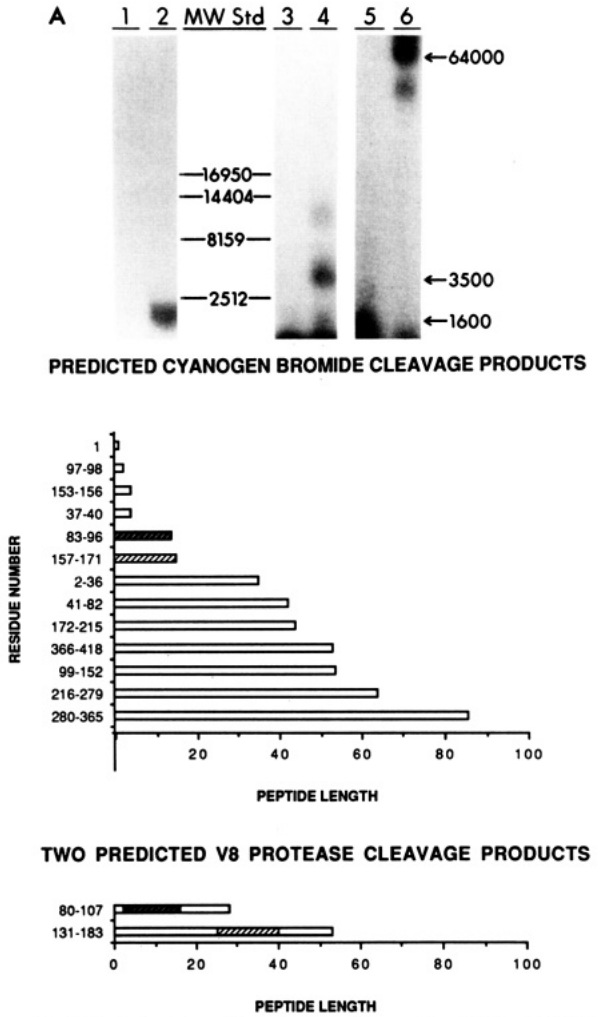

FIGURE 2: Gel electrophoresis and autoradiography of ^{125}I-pBABC-labeled β_2-AR after proteolysis. (A) β_2-AR was labeled with ^{125}I-pBABC in the presence (lanes 1, 3, and 5) or absence (lanes 2, 4, and 6) of competing concentrations of alprenolol to define specificity. Samples were then treated with cyanogen bromide (lanes 1 and 2) or V8 protease (lanes 3 and 4) or were left untreated (lanes 5 and 6) and subjected to SDS–urea–PAGE (8.5% acrylamide, 8 M urea) and autoradiography, as detailed under Experimental Procedures. The faint M_r ~10000 band in lane 4 is an intermediate digestion product. The faint M_r ~40000 band in lane 6 is the result of proteolysis during purification and storage of the β_2-AR. The mobilities of the resultant specifically labeled fragments as well as of the molecular weight standards (MW Std) are shown. Standards are horse heart myoglobin fragments of 16950 (residues 1–153), 14404 (1–131), 8159 (56–131), and 2512 (132–153). (B) The predicted sizes of the cyanogen bromide and relevant V8 protease digestion products are presented. Highlighted are two cyanogen bromide cleavage products with predicted molecular weights near 1600. These fragments are included in two predicted V8 protease digestion products, peptides 80–107 and 131–183, as shown and as discussed in the text.

data for a unique and unambiguous assignment of the site of covalent attachment of ^{125}I-pBABC within the polypeptide.

As shown in Figure 2A, pure hamster lung β_2-AR was labeled with ^{125}I-pBABC in the presence or absence of the reversible β-adrenergic antagonist alprenolol. After complete digestion with cyanogen bromide, the samples were subjected to SDS–urea–PAGE and autoradiography (lanes 1 and 2). A single specifically labeled peptide band migrating at M_r ~1600 was observed, and only with the addition of cyanogen bromide. Steric exclusion HPLC of a similar sample yielded a specific peak of radioactivity eluting in the molecular weight range of 1900 (data not shown), thus confirming the results obtained with our gel electrophoresis system. Of the 12 proteolytic fragments that would result from cleavage at each of the 11 methionine residues within the β_2-AR, only 2 are predicted to have molecular weights in the 500–4000 range. These are the peptides representing residues 83–96 and 157–171 (Figure 2B).

In order to distinguish between the two candidate sites of labeling identified by cyanogen bromide cleavage, another sample of ^{125}I-pBABC-labeled β_2-AR was treated with V8 protease. Under the conditions of this experiment, V8 protease cleavage at the carboxyl side of all glutamic and aspartic acid residues would result in as many as 37 fragments of the β_2-AR. Two of these V8 protease generated fragments, peptides 80–107 and 131–183, would encompass the cyanogen bromide generated peptides 83–96 and 157–171, respectively (Figure 2B). Peptide 80–107 has a predicted molecular weight of 3200, while peptide 131–183 would be significantly larger, 6300. The affinity label has a molecular weight of 685. As shown in Figure 2 (lanes 3 and 4), cleavage of ^{125}I-pBABC-labeled β_2-AR with V8 protease yields a predominant specifically labeled peptide migrating at M_r ~3500. Again, no such band is observed when alprenolol is present during labeling or when no protease is added (lanes 5 and 6). The specifically labeled fragment of M_r ~3500 is very close to the predicted mobility of peptide 80–107 (plus covalently bound probe) and is inconsistent with labeling of the much larger peptide 131–183. We can therefore identify the cyanogen bromide generated β_2-AR peptide 83–96 as containing the unique binding site for ^{125}I-pBABC.

Having identified peptide 83–96 of β_2-AR as uniquely containing the site of ^{125}I-pBABC incorporation, we sought to determine by amino-terminal sequencing the precise amino acid substrate for alkylation by ^{125}I-pBABC. We utilized an approach similar to that of our initial report on the partial amino acid sequence of the hamster β_2-AR (Dixon et al., 1986). As previously observed, reverse-phase HPLC of cyanogen bromide digested β_2-AR produces at least nine distinct peaks of absorbance (Dixon et al., 1986). A similar elution pattern was observed with a cyanogen bromide digest of 1 nmol of β_2-AR labeled with ^{125}I-pBABC (Figure 3A). When fractions were measured for γ-radioactivity, a single peak was observed eluting at 29 min that was absent either when no receptor was present or when receptor was incubated with ^{125}I-pBABC in the presence of alprenolol (Figure 3B). This radioactive peak coelutes at 29 min with a peak of absorbance present in both the ^{125}I-pBABC-labeled and control (unlabeled) preparations of β_2-AR [Figure 3 and Dixon et al. (1986)]. This fraction was subjected to amino-terminal protein sequence analysis, and two different but coeluting receptor peptides were identified (residues determined indirectly from the gene are indicated in parentheses):

peptide 83–96: NH_2-Gly-Leu-Ala-Val-Val-Pro-Phe-(Gly)-Ala-(Ser)-(His)-(Ile)-(Leu)-(Met)-COOH

peptide 157–171: NH_2-Val-(Trp)-Ile-Val-Ser-Gly-Leu-Thr-Ser-Phe-Leu-Pro-Ile-(Gln)-(Met)-COOH

However, in the control preparation of β_2-AR not labeled with ^{125}I-pBABC, only the one sequence corresponding to peptide 157–171 was found to be eluting at 29 min. In the control preparation of β_2-AR, peptide 83–96 elutes exclusively at 22 min, suggesting that modification by ^{125}I-pBABC resulted in an altered mobility of the peptide on reverse-phase HPLC. Consistent with this possibility is that, of the two peptides eluting at 29 min, peptide 83–96 constituted about one-third as much protein as peptide 157–171 (54 pmol vs 148 pmol recovered in the first cycle). That is, given an efficiency of incorporation of ^{125}I-pBABC into the β_2-AR of about 35%,

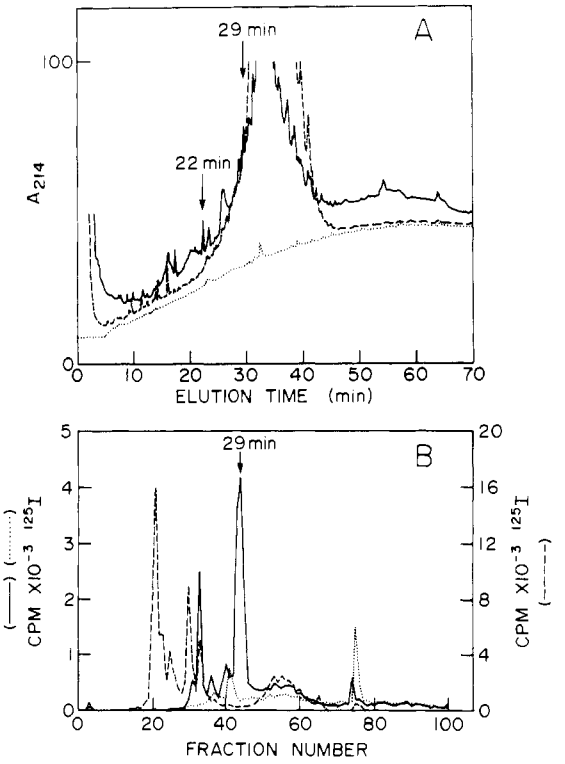

FIGURE 3: Reverse-phase HPLC of cyanogen bromide digested, ^{125}I-pBABC-labeled β_2-AR. (A) One nanomole of ^{125}I-pBABC-labeled β_2-AR (—) or digitonin buffer alone (- - -) was treated with cyanogen bromide, and the resultant digest was subjected to reverse-phase HPLC as detailed under Experimental Procedures. Also shown is the elution profile of a blank sample containing TFA alone (···). The elution was monitored by absorbance at 214 nm, as detailed under Experimental Procedures. (B) The same chromatogram as in (A), except that γ-radioactivity was measured for each 1-min fraction. Elution profiles of cyanogen bromide digested β_2-AR labeled with ^{125}I-pBABC in the absence (—) or presence (···) of competing concentrations of alprenolol and of a sample containing no β_2-AR (- - -) are shown. A single specifically labeled peak of radioactivity is shown to elute at 29 min, corresponding to a peak of absorbance (arrows) as described in the text.

this lower value for recovery of peptide 83–96 vs peptide 157–171 was to be expected.

While Edman degradation of the radiolabeled peptide yielded a sequence of part of the cyanogen bromide fragment predicted by proteolytic mapping studies, we could not identify the precise amino acid residue radiolabeled in the alkylation reaction. Further attempts to identify a radiolabeled residue by measuring γ-radioactivity in the cleavage products after sequential Edman degradation of ^{125}I-pBABC-labeled peptides, generated by either cyanogen bromide or V8 protease, were unsuccessful. In every case, radioactivity was recovered quantitatively in the wash. We postulate that nonspecific cleavage of ^{125}I-pBABC may have occurred during the initial cycles of protein sequencing. Indeed, treatment of ^{125}I-pBABC-labeled β_2-AR with trifluoroacetic acid but not trimethylamine (12.5%) or water (in the gas phase for 90 min at 45 °C) results in complete loss of radioactivity from the labeled receptor, as assessed by SDS–PAGE and autoradiography (data not shown). Thus, the labeling method used for these experiments, although efficient and specific, is unstable to the acidic conditions of Edman degradation and unsuitable for the direct determination of the precise modified residue.

Although the exact amino acid residue of peptide 83–96 modified by ^{125}I-pBABC could not be determined, alkylating agents such as ^{125}I-pBABC will generally react with nucleophilic groups such as the thiol moiety of cysteine, the amino group of lysine, the serine hydroxyl, or the imidazole of histidine. Indeed, nucleophilic residues are present in peptide 83–96 at positions Ser-92 and His-93 (highlighted in Figure 1), and we propose that one or both of these residues represent the substrate amino acid(s) for the alkylation reaction.

Considerable progress has been made in elucidating structure–function relationships of the β_2-AR in vitro mutagenesis methods. Biochemical approaches such as those utilized here complement the very powerful genetic methods in providing information necessary for a more complete understanding of hormone–receptor interactions. Thus, while all or some of the transmembrane segments may contribute to forming a ligand binding pocket, the probe used in this study recognizes a single site in the second membrane spanning domain. This observation is especially interesting in light of mutagenesis experiments demonstrating a complete loss of binding when Asp-113 is changed to asparagine and an alteration of agonist but not antagonist binding when Asp-79 or Asn-318 is changed to an alanine or lysine, respectively (Strader et al., 1987). Asp-79 is located within the second transmembrane segment just four amino acids amino terminal to the cyanogen bromide fragment labeled by ^{125}I-pBABC. By analogy with the organization of chromophores in light-sensitive proteins such as rhodopsin and bacteriorhodopsin (Henderson & Unwin, 1975; Thomas & Stryer, 1982; Ovchinnikov, 1982) where retinal is covalently attached to a lysine in the seventh and sixth transmembrane segments, respectively, it is reasonable to suggest that a seven-helix bundle within the plasma membrane might in some way accommodate the binding of specific adrenergic ligands as well. Further supporting this notion is a remarkably high degree of sequence similarity in these helices among all of the G-protein coupled receptors, including in each a conserved aspartic acid in the second membrane spanning domain (Dohlman et al., 1987a). This residue, determined by mutation analysis to play a critical role in agonist binding to β_2-AR, has been suggested to act as a counterion for the positively charged amine of adrenergic agents (Applebury & Hargrave, 1986).

The present study was undertaken to test the hypothesis that hormones bind to and activate the β_2-AR in a manner analogous to that of retinal and opsin; more specifically, that the binding site for the adrenergic ligand ^{125}I-pBABC is in a hydrophobic region of the receptor formed by a bundle of transmembrane segments. Certainly, labeling by ^{125}I-pBABC of helix II does not preclude a role for the remaining six membrane spanning regions in forming the ligand binding site. In fact, the high degree of sequence conservation in the transmembrane segments among the G-protein coupled receptors (Dohlman et al., 1987a) and data from mutagenesis studies (Dixon et al., 1987; Strader et al., 1987; Kobilka et al., 1987c) strongly support a role for multiple membrane spanning regions in determining binding specificity. Determining the exact role of each of the individual residues that constitute the binding site of β_2-AR, however, will require further analysis involving a combination of biochemical and genetic approaches. This work provides a framework for a more detailed characterization of the structural determinants of hormone–receptor interaction of the family of G-protein coupled receptors.

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REFERENCES

Applebury, M. L., & Hargrave, P. A. (1986) *Vision Res. 26*, 1881–1895.

Benovic, J. L., Shorr, R. G. L., Caron, M. G., & Lefkowitz, R. J. (1984) *Biochemistry 23*, 4510–4518.

Bonner, T. I., Buckley, N. J., Young, A. C., & Brann, M. R. (1987) *Science (Washington, D.C.) 237*, 527–532.

Caron, M. G., & Lefkowitz, R. J. (1976) *J. Biol. Chem. 251*, 2374–2384.

Chung, F.-Z., Lentes, K.-U., Gocayne, J., Fitzgerald, M., Robinson, D., Kerlavage, A. R., Fraser, C. M., & Venter, J. C. (1987) *FEBS Lett. 211*, 200–206.

Dickinson, K. E. J., Heald, S. L., Jeffs, P. W., Lefkowitz, R. J., & Caron, M. G. (1985) *Mol. Pharmacol. 27*, 499–506.

Dixon, R. A. F., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., Bolanowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., Mumford, R. A., Slater, E. E., Sigal, I. S., Caron, M. G., Lefkowitz, R. J., & Strader, C. D. (1986) *Nature (London) 321*, 75–79.

Dixon, R. A. F., Sigal, I. S., Rands, E., Register, R. B., Candelore, M. R., Blake, A. D., & Strader, C. D. (1987) *Nature (London) 326*, 73–77.

Dohlman, H. G., Caron, M. G., & Lefkowitz, R. J. (1987a) *Biochemistry 26*, 2657–2664.

Dohlman, H. G., Bouvier, M., Benovic, J. L., Caron, M. G., & Lefkowitz, R. J. (1987b) *J. Biol. Chem. 262*, 14282–14288.

Drapeau, G. R. (1976) *Methods Enzymol. 45*, 469–475.

Frielle, T., Collins, S., Daniel, K. W., Caron, M. G., Lefkowitz, R. J., & Kobilka, B. K. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7920–7924.

Gross, E. (1967) *Methods Enzymol. 11*, 238–255.

Hanoaka, F., Shaw, J. D., & Muelter, G. C. (1979) *Anal. Biochem. 99*, 170.

Henderson, R., & Unwin, P. N. T. (1975) *Nature (London) 157*, 28–32.

Hewik, R. M., Hunkapiller, M. E., Hood, L. E., & Dreyer, W. J. (1981) *J. Biol. Chem. 256*, 7790–7797.

Kobilka, B. K., Dixon, R. A. F., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Sigal, I. S., Yang-Feng, T. L., Francke, U., Caron, M. G., & Lefkowitz, R. J. (1987a) *Proc. Natl. Acad. Sci. U.S.A. 84*, 46–50.

Kobilka, B. K., Matsui, H., Kobilka, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J., & Regan, J. W. (1987b) *Science (Washington, D.C.) 238*, 650–656.

Kobilka, B. K., MacGregor, C., Daniel, K., Kobilka, T. S., Caron, M. G., & Lefkowitz, R. J. (1987c) *J. Biol. Chem. 262*, 7321–7327.

Kubo, T., Fukuda, K., Mikami, A., Maeda, A., Takahashi, H., Mishina, M., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., & Numa, S. (1986a) *Nature (London) 232*, 411–416.

Kubo, T., Maeda, A., Sugimoto, K., Akiba, I., Mikami, A., Takahashi, H., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Matsuo, H., Hirose, T., & Numa, S. (1986b) *FEBS Lett. 209*, 367–372.

Laemmli, U. K. (1970) *Nature (London) 117*, 680–685.

Lefkowitz, R. J., Stadel, J. M., & Caron, M. G. (1983) *Annu. Rev. Biochem. 52*, 159–186.

Ovchinnikov, Yu. A. (1982) *FEBS Lett. 148*, 179–191.

Peralta, E. G., Winslow, J. W., Peterson, G. L., Smith, D. H., Ashkenazi, A., Ramachandran, J., Schimerlik, M., & Capon, D. J. (1987) *Science (Washington, D.C.) 236*, 600–605.

Speiss, J., Rivier, J. E., Rodkey, S. A., Bennett, C. D., & Vale, W. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2974–2978.

Strader, C. D., Sigal, I. S., Register, R. B., Candelore, M. R., Rands, E., & Dixon, R. A. F. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4384–4388.

Thomas, D. D., & Stryer, L. (1982) *J. Mol. Biol. 154*, 145–157.

Yarden, Y., Rodriguez, H., Wong, S. K.-F., Brandt, D. R., May, D. C., Burnier, J., Harkins, R. N., Chen, E. Y., Ramachandran, J., Ullrich, A., & Ross, E. M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6795–6799.