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Molecular Biology of Serotonin (5-HT) Receptors

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SHIH, J. C., W. YANG, K. CHEN AND T. GALLAHER. *Molecular biology of serotonin (5-HT) receptors.* PHARMACOL BIOCHEM BEHAV **40**(4) 1053–1058, 1991.—The recent cloning of three types of 5-hydroxytryptamine (5-HT, serotonin) receptors substantiates radioligand-based definitions of 5-HT receptors, and provides a framework in which to understand the function and evolution of the receptors. The primary sequences determined by molecular cloning of the 5-HT_{1c}, 5-HT_{1a} and 5-HT_2 receptors place each of these 5-HT receptor subtypes into the class of G protein-coupled receptors. These receptors all share similar functional and structural features. Each receptor is positioned in the lipid bilayer with seven membrane-spanning domains and corresponding intracellular and extracellular domains. By analogy to the known functional structures of the β-adrenergic receptor, the binding site of 5-HT is proposed to be in the membrane domains and the intracellular domain is important for G protein interaction. The primary sequences and the second messenger systems of the receptors indicate the 5-HT_2 and 5-HT_{1c} receptors are closely related, whereas the 5-HT_{1a} receptor is more distantly related to the 5-HT_2 and 5-HT_{1c} receptors.

Serotonin receptors Molecular biology Primary sequence Second messenger systems G protein

IN the past five years, an explosion of knowledge has occurred in our understanding of the molecular mechanisms of chemically mediated cellular communication. It is now understood that receptors for neurotransmitters belong to gene families which share conserved structural domains and functional mechanisms. Understanding the properties of these receptors has brought the field of neuropharmacology to a new level of understanding, and provides the basis for an encompassing view of the molecular workings of the nervous system. The new knowledge begins to account for the ability of the brain to process myriad environmental messages and the information carried therein. The action of drugs acting on the nervous system is also accounted for at the molecular level. A basis to study the neural relationship to behavior is provided as well by the molecular information that is now available. This review focuses on neural serotonin (5-hydroxytryptamine; 5-HT) receptors which are one of many neurochemical systems of the nervous system. The molecular cloning of serotonin receptors and their related receptors provides a great understanding of the structure and function of these pharmacologically important receptors.

5-HT Receptors

The existence of multiple types of receptors for 5-HT was first demonstrated by Gaddum and Picarelli (10) and were deemed D and M type receptors. The advent of radioligand binding assays made a thorough assessment of 5-HT receptors in isolated membrane fractions possible. Peroutka and Snyder (21) demonstrated the existence of two types of 5-HT receptors which were designated 5-HT_1 and 5-HT_2 receptors. With the synthesis of new organic compounds, the existence of multiple receptors for 5-HT was confirmed and knowledge of their properties was refined. At this point in time, there are three main categories of 5-HT receptors defined by radioligand analysis: 5-HT_1, 5-HT_2 and 5-HT_3 receptors (22). Physiological and biochemical studies support these classifications and delineate the 5-HT_3 receptors into a class of signal transducing proteins that is distinct from the 5-HT_1 and 5-HT_2 receptors. 5-HT_3 receptors are ligand-gated ion channels (4) whereas the others are G protein-coupled receptors (see below). Gaddum and Picarelli's original observations of D and M receptors are confirmed, with the M receptor corresponding to the 5-HT_3 receptor and the D to the 5-HT_2 receptor. The final confirmation of the existence of multiple 5-HT receptors has come by molecular cloning. Cloned 5-HT receptor subtypes are seen to be coded for by individual genes which contain all the information conferring the ligand binding and physiological properties inherent to the receptor subtype.

Molecular Cloning of 5-HT Receptors

The 5-HT_{1c} receptor was first cloned by Lubbert et al. (19) from a choroid plexus papilloma tissue source. They isolated a gene of 5000 nucleotides (five kilobases; 5 kB) which encoded a protein with all the pharmacological characteristics of a 5-HT_{1c} receptor. To isolate this gene, a system was used which is now becoming commonplace for the cloning of proteins. The method involved using a source tissue, in this case the choroid plexus papilloma, which produces the receptor in high quantities, to isolate the mRNA's produced in the cell and to then screen them for functional activity until the receptor gene is identified. To do this, a hybrid-depletion method of screening was used to identify the gene of interest and an expression system utilizing xenopus oocytes was used. The method is described in detail elsewhere (18). Briefly, cDNA created from the mRNA of the papilloma is used to make mRNA transcripts. These transcripts will contain the gene for the 5-HT_{1c} receptor. The functional assay uses the ability of the frog egg (the oocyte) to express foreign mRNA that has been introduced into it by injection. After

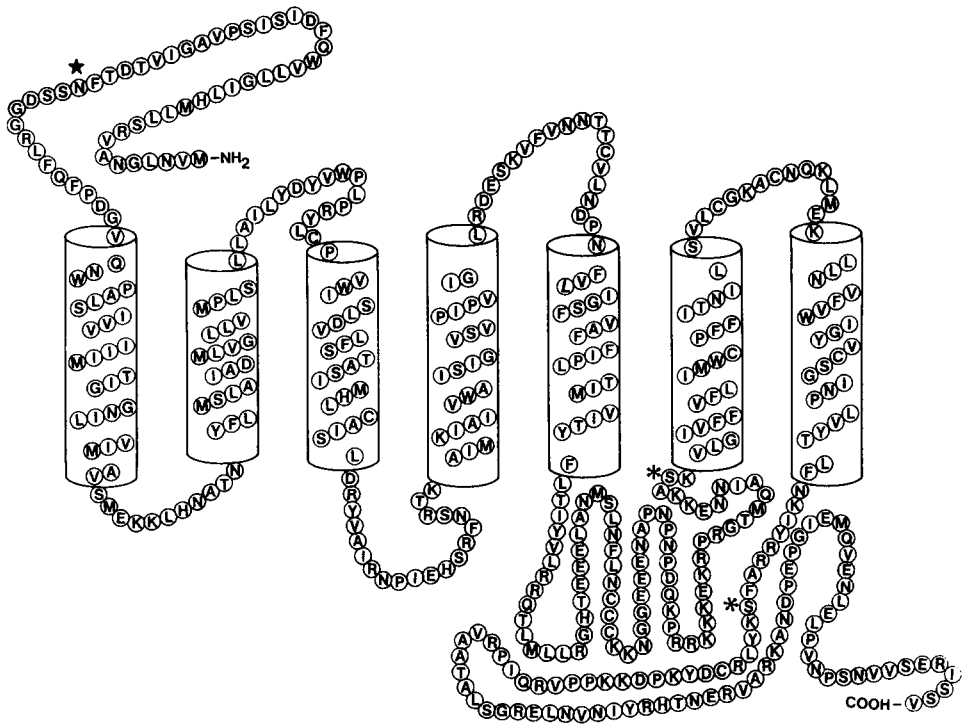

FIG. 1. A depiction of the proposed topology of the 5-HT_{1c} receptor in the lipid bilayer based upon hydrophobicity analysis. The seven transmembrane domains and the extra- and intracellular domains are clearly evident. Asterisks mark potential phosphorylation sites in the cytoplasmic domains and the star indicates a glycosylation site [from (15)].

injection of and subsequent translation of the message, the presence of the desired gene is tested by electrically measuring the oocyte's response to 5-HT. The neurotransmitter elicits a change in membrane potential in eggs injected with the mRNA coding for the receptor. An elimination process is carried out until the individual gene is obtained.

Later Julius et al. (15), using a similar method, also cloned the 5-HT_{1c} receptor and presented the first published amino acid sequence of the receptor. The primary sequence indicated the receptor shared structural similarities with a number of known signal transducing proteins. A method to analyse the structure of membrane proteins is commonly used which is called hydrophobicity analysis. Membrane proteins are embedded in the lipid bilayer of a cell membrane. The hydrophobic interior thermodynamically excludes charged amino acid residues, whereas nonpolar residues are thermodynamically favorable in the nonpolar lipid environment. By examining the polarity of each amino acid in the protein sequence, areas of hydrophobicity can be determined. These hydrophobic regions are postulated to be in the membrane. The hydrophobicity analysis indicates 5-HT_{1c} receptors contain seven regions of hydrophobicity of 20–30 amino acids. These regions are called transmembrane domains (TMD's), and define a two-dimensional topological structure of the protein in the membrane. This seven membrane-spanning domain structure is illustrated for the 5-HT_{1c} receptor in Fig. 1. The same topological structure is seen in many other receptors which form a family of signal transducing receptors which share conserved structural and functional features (2, 8, 20).

The 5-HT_{1a} receptor was cloned by a different approach by Kobilka et al. (16). By using the β-adrenergic receptor gene as a probe, a gene with a high sequence homology was identified. This technique exploited the ability of the adrenergic receptor gene to base pair with a similar but not exact gene from a human genomic library. This cross-hybridization selected a 6 kB intronless gene which was designated G-21. An intronless gene contains only coding DNA, i.e., DNA that codes specifically for the protein. Most mammalian genes contain intervening sequences, called introns, that are not translated but are removed from the gene before processing the mRNA to be translated. The primary sequence and the hydrophobicity analysis indicated 7 TMD's were present which placed this gene in the G protein-coupled family of receptors, but its identity was unknown, as it did not respond to adrenergic drugs. Later, the receptor was shown to be a 5-HT_{1a} receptor (9). The identity of the G-21 gene as a 5-HT_{1a} receptor was determined by introducing the gene into a mammalian cell line derived from monkey tumor cells called cos-7. The gene, thus inside the cell, is translated into its gene product where it can be assayed pharmacologically and functionally. This is called transient transfection. The G-21 product in cos-7 cells had a pharmacological profile that matched the 5-HT_{1a} receptor in that it bound [^{3}H] 8-OH-DPAT, 5-HT and spiperone with high affinity, but not ketanserin. In this manner, the G-21 gene was seen to be a human 5-HT_{1a} receptor. A rat 5-HT_{1a} receptor has also been cloned by this same hybridization approach (1).

The 5-HT_2 receptor was cloned by Pritchett et al. (23) by screening a rat cDNA library with two oligonucleotides based upon the sequence of the 5-HT_{1c} receptor. In the same way that the sequence similarity of the β-adrenergic to the 5-HT_{1a} receptor was used in identifying the 5-HT_{1a} gene, the 5-HT_{1c} sequence was used to identify the similarly sequenced 5-HT_2 receptor. Hydrophobicity analysis of the 5-HT_2 amino acid se-

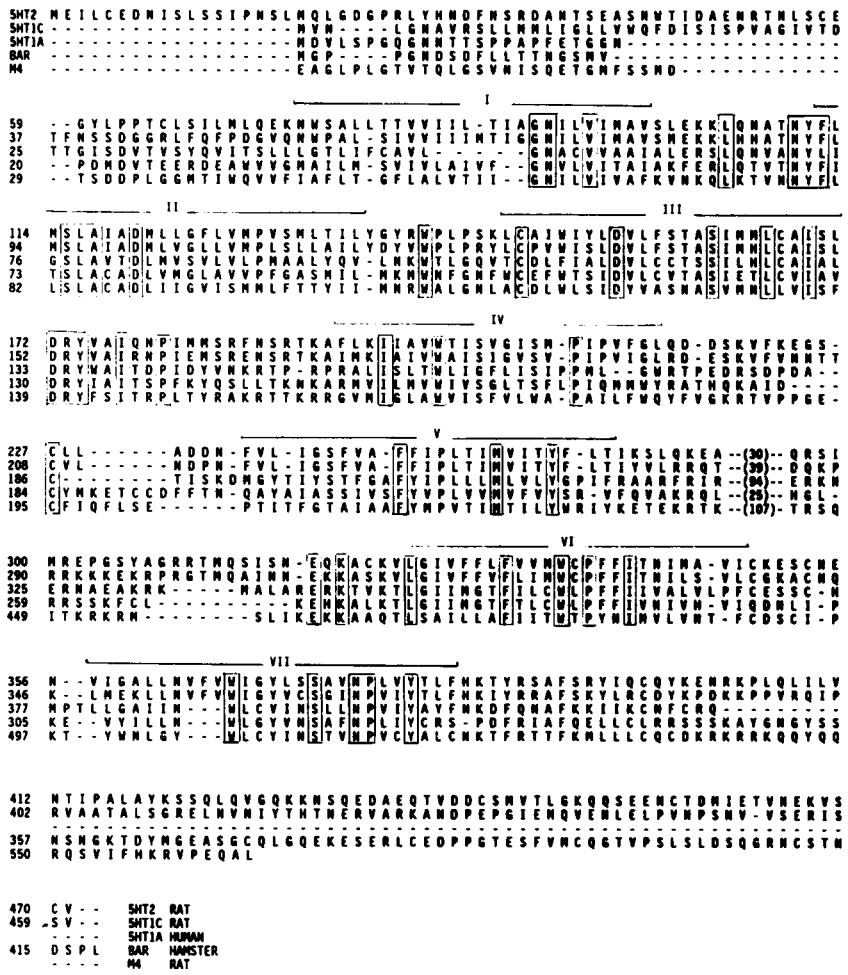

FIG. 2. Primary sequence of five G protein-coupled receptors. Shown are three different 5-HT receptor subtypes, 5-HT_2, 5-HT_{1c} and 5-HT_{1a}, and the primary sequence for the hamster β-adrenergic receptor (5), and rat muscarinic acetylcholine receptor (3). The seven transmembrane domains are designated I–VII. Amino acids conserved in all five receptors are boxed.

quence revealed the seven TMD structure common for all of the receptor family. The receptor was pharmacologically identified by its expression in kidney 293 cells, where the known 5-HT_2 pharmacological profile was obtained. Also, in the transfected cell line, 5-HT caused a rise in intracellular Ca^{++} levels which was consistent with the known properties of these receptor subtypes. Expression of mRNA from the cloned 5-HT_2 receptor injected into xenopus oocytes resulted in a 5-HT elicited change in membrane potential in the egg. These results clearly demonstrated the identity of the gene as the 5-HT_2 receptor. Later Julius et al. (14) confirmed these results. Figure 2 illustrates the primary sequences of the 5-HT receptors and the muscarinic receptor and the adrenergic receptor. The amino acids that are conserved in all five receptors are boxed and the transmembrane domains are identified.

Recently, the mouse and human genomic clones of the 5-HT_2 receptor have been isolated in our laboratory. Unlike the 5-HT_{1a} gene, the 5-HT_2 receptor gene contains introns. The gene consists of three exons and two introns which span a larger than 20 kB region of the chromosome. Genomic information is significant in terms of understanding the regulation of gene expression as well as the evolutionary relationships between the genes and the evolution of the nervous system.

The G Protein-Coupled Receptor Family of Membrane Proteins

The amino acid sequences which have been determined for a number of receptors in the past few years have led to the understanding that receptors can be classified into gene families. These family members are defined such as they share functional mechanisms and structural features. We see that the individuality of the receptor in signaling (i.e., its neurotransmitter and the cellular response it elicits) is due to a small difference in the primary sequence. The G protein-coupled receptor family is one such family. It is so named because the cellular response elicited by the signaling molecule is not directly mediated by the receptor, but is relayed by a class of GTP-hydrolysing enzymes which are allosterically coupled to the receptor. The receptor is stimulated by the agonist which in turn activates the G protein. It is the G protein which acts upon the cellular system, e.g., activation of adenylate cyclase, to produce cyclic AMP or other second messenger responses. The G proteins belong to their own gene family. There are many cloned G proteins which share

conserved structural and functional features but are specific for unique enzymes to elicit the cellular response to the neurotransmitter. The manner in which the receptor activates the G protein is called a receptor-effector system. There is a slight amplification in this process because a receptor bound to its ligand can activate more than one G protein.

Receptors in this family exist in organisms from bacteria to man. The evolutionary precursor for the family is believed to be the light-activated molecule rhodopsin, which is found in the rod and cone cells of the eye. Bacteriorhodopsin is a bacterial counterpart found in *Halobacterium* and much of the structural information now known for the receptors of this family, the 7TMD structure for instance, was first determined for bacteriorhodopsin. The rhodopsins use a retinol molecule analogously to the neurotransmitter. The retinol chromophore is located in the middle of the transmembrane regions and is covalently bound to the protein. Isomerization of the retinol due to a photon is the basis for the allosteric behavior of the rhodopsins. The receptors have evolved so that a transmitter molecule forms a noncovalent interaction with the receptor protein which causes the functional allosterism of the signal transduction mechanism.

Among members of the family are the rhodopsins, yeast-mating factor receptors, cyclic AMP receptors, neurotransmitter and peptide receptors. Table 1 lists the members which have been identified by molecular cloning so far (17).

TABLE 1

A LIST OF THE G PROTEIN RECEPTOR FAMILY MEMBERS WHICH HAVE BEEN CLONED*

Neurotransmitter Receptors
Adrenergic (α and β)
Dopamine (4 types)
Serotonin (3 types)
Muscarinic acetylcholine (many genes)
Histamine
Adenosine
Glutamate
Peptide and Hormone
Substance P
Substance K
Neuromedin K
Thyrotropin
Follicle stimulating hormone
Lutropin/chorionadotropin
Angiotensin
Arginine-vasopressin
Vasoactive intestinal polypeptide
Bombesin/gastrin releasing hormone
Thyrotropin releasing hormone
Thromboxane A_2
Other
Rhodopsins (light receptors)
Odorant receptors
Thrombin
Endothelins
Platelet-activating factor
N-formyl peptide
Yeast mating factors (two types)
cAMP (*Dictyostelium* slime mold)
Cannabinoid (marijuana receptor)

*Each is identified by the presence of seven hydrophobic domains as determined by hydrophobicity analysis of their primary sequences.

Structure and Function of 5-HT Receptors

As mentioned above, the 5-HT receptors share a conserved topological structure within the lipid bilayer shared also with all the GPR's. Specific domains of the receptor have been shown to be determinants for function. The structure of the 5-HT receptors can be defined into three domains: 1) extracellular domains which include the amino terminal and the extracellular domains between transmembrane domains 2 and 3, 4 and 5 and 6 and 7; 2) the membrane domain which consists of the seven hydrophobic membrane spanning regions; and 3) the intracellular domains which include the carboxyl terminus and the intracellular domains between TMD's 1 and 2, 3 and 4 and 5 and 6. These regions are easily identified in Fig. 1. The extracellular domain contains the amino acids called glycosylation sites where complex sugar moieties are bound to the receptor. The sugar groups play a role in processing the receptor to the cytoplasmic membrane, but do not have a role in receptor recognition of the ligand or in functional mechanism. By analogy with the β-adrenergic receptor (6) and the position of the rhodopsin chromophore, the ligand binding site is most probably inside the protein in the lipid domain and is formed by the TMD's. Also, by using the analogous structure of the β-adrenergic receptor, specific amino acids can be identified as the binding sites for 5-HT. The aspartic acid in TMD 3 is predicted to be the counterion for the amine group of 5-HT (25); a serine in TMD 5 (24) is predicted to act as the binding site for the hydroxyl moiety of 5-HT, and a phenylalanine in TMD 6 (7) theoretically serves as the third recognition site for the 5-HT molecule and forms a stacking interaction with the indole moity of 5-HT. These predictions are based upon site-directed mutagenesis of β-adrenergic receptors which allow one amino acid to be substituted for another amino acid and the functional results of the substitution are analyzed. The three moities listed above were seen to be the epitopes for the recognition of the β-adrenergic receptor and its ligand. 5-HT and its receptor share enough similarities with the β-adrenergic receptor and its ligand, adrenaline, to predict the analogous binding mechanism presented above. With the availability of 5-HT receptor clones, site-directed mutagenesis of these receptors will confirm or deny the proposed mechanism.

Also postulated to be necessary for 5-HT receptor activation of its G protein is an aspartic acid in TMD 2. This amino acid is conserved in the 5-HT receptors, adrenergic receptors, muscarinic receptors and the dopamine receptors. Mutagenesis studies of adrenergic receptors (7) have shown this conserved amino acid to be necessary to elicit a second-messenger response, but to have no effect on the binding properties of the ligand. This residue is predicted to be necessary for the allosteric activation of the G protein. This has yet to be confirmed by mutagenesis of the 5-HT receptor, but would be of great surprise if it did not play such a role for the function of 5-HT receptors.

The cytoplasmic domains are proposed to be necessary for G protein coupling and signal transduction. Mutagenesis studies of the third cytoplasmic domain of the β-adrenergic receptor have shown these regions to inhibit signal transduction without impairing agonist binding, indicating a G protein coupling is effected (12), and analogously these regions of the 5-HT receptor should interact with the G protein. The adrenergic receptor is amenable to covalent modification in the cytoplasmic regions as well. Cyclic AMP protein kinase (CAMP-PK), protein kinase C and β-adrenergic receptor protein kinase have been shown to act on the third cytoplasmic domain and at the carboxyl-terminus to phosphorylate the receptor (11). These enzymes work as state specific regulators of receptor function, in a sense "fine tuning" the receptor's response to ligand under different cellular conditions. Serotonin receptors are expected to exhibit these characteristics as well due to the presence of potential phosphorylation sites in the same cytoplasmic domains.

Relationships Between 5-HT Receptors

The cloned 5-HT receptors all belong to the GPR family and share basic structural features, but each has its own unique functional properties exhibited in the second messenger it produces and in its pharmacological profile. Pritchett et al. (23) have shown the 5-HT_2 receptor and 5-HT_{1c} receptor to share a highly homologous primary structure compared to the 5-HT_{1a} receptor. The 5-HT_{1c} and 5-HT_2 receptors share 51% sequence homology, whereas the 5-HT_{1a} receptor shares only 35% with either of these receptors. Thus the 5-HT_2 and 5-HT_{1c} receptors are more closely related to each other than to the 5-HT_{1a} receptor. Further, if only the TMD's are examined, the 5-HT_2 and 5-HT_{1c} receptors share 80–90% sequence homology. These relationships allow us to suggest that the 5-HT_{1c} receptor and the 5-HT_2 receptors should be classified as 5-HT_{2A} and 5-HT_{2B} as they more closely resemble each other in structure and function than they do the 5-HT_{1a} receptor. This relationship is not unexpected, based upon the pharmacological profiles and second messenger systems of the receptors (13).

Future

The cloning of 5-HT receptors and their identification as GPR's have many benefits in the field of neuroscience and behavior. The knowledge of receptor structure will be used in designing and identifying therapeutic agents which can be used to treat mental disorders. The use of the anxioplytic buspirone which is specific for 5-HT_{1a} receptors is an example. The ability to design or select highly selective compounds will produce agents with fewer side-effects in treating the particular disorder. In a similar manner, the effects of drugs can be studied and the receptors which they interact with can be determined which will lead to a greater understanding of the molecular basis of brain function.

The knowledge of receptor structures and their specific interactions with drugs will complement the neural mapping of the nervous system using the receptors as markers. A detailed neurochemical anatomy will be obtained allowing neural networks to be identified which will have a chemical basis to their functioning. The primary sequences will allow a detailed molecular mapping wherein not only will a serotonin receptor be identified at a particular synapse, but a specific catalogued subtype will be identified.

The goal of integrative molecular and anatomical studies to describe the neurochemical anatomy of the brain will be to establish a "hard" basis to the understanding of behavior and mental processes. It is not inconceivable to envision a mathematical analysis of the brain using neuromolecular anatomy and enzyme kinetics. Neural networks actually based on the chemistry and architecture of the brain can be modeled.

The genes for the receptors also will be of use in genetic studies to examine the extent of a genetic component to certain emotional or behavioral states. Molecular genetics have been used in other fields, and neurology will now be able to examine such questions. The fields of molecular biology and neurobehavioral science have converged to begin a new era of understanding the brain and its complexities.

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REFERENCES

1. Albert, P. R.; Zhou, Q-Y.; Van Tol, H. H.; Bunzow, J. R.; Civelli, O. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine 1a receptor gene. J. Biol. Chem. 265:5825–5832; 1990.
2. Bonnes, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. Identification of a family of muscarinic acetylcholine receptor genes. Science 237:527–530; 1987.
3. Braun, T.; Schofield, P. R.; Shivers, B. D.; Seeberg, P. H. A novel subtype of muscarinic receptor identified by homology screening. Biochem. Biophys. Res. Comm. 149:125–132; 1987.
4. Derkach, V.; Suprenant, A.; North, R. A. 5-HT_3 receptors are membrane ion channels. Nature 339:706–709; 1989.
5. Dixon, R. A. F. et al. Cloning of the gene and cDNA for the mammalian β-adrenergic receptor. Nature 321:75–79; 1986.
6. Dixon, R. A. F.; Sigal, I. S.; Rands, E.; Register, R. B.; Candelore, M. R.; Blake, A. D.; Skades, C. D. Ligand binding to the β-adrenergic receptor involves its rhodopsin-like core. Nature 326: 73–77; 1987.
7. Dixon, R. A. F.; Sigal, I. S.; Strader, C. D. Structure-function analysis of the β-adrenergic receptor. Cold Spring Harbor Symp. Quant. Biol. 53:487–497; 1988.
8. Dohlman, H. G.; Caron, M. G.; Lefkowitz, R. J. A family of receptors coupled to guanine nucleotide regulatory proteins. Biochemistry 26:2657–2664; 1987.
9. Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT_{1a} receptor. Nature 335:358–360; 1988.
10. Gaddum, J. H.; Picarelli, Z. P. Two kinds of tryptamine receptors. Br. J. Pharmacol. Chemother. 12:323–328; 1957.
11. Greengard, P.; Huganir, R. L. Regulation of neurotransmitter desensitization by protein phosphorylation. Neuron 5:555–567; 1990.
12. Hausdorf, W. P.; Hnatowich, M.; O'Dowd, B. F.; Caron, M. G.; Lefkowitz, R. J. A mutation of the β-adrenergic receptor impairs agonist activation of adenyl cyclase without affecting high affinity agonist binding. J. Biol. Chem. 265:1358–1393; 1990.
13. Hoyer, D. Molecular pharmacology and biology of 5-HT_{1c} receptors. Trends Pharmacol. Sci. 9:89–94; 1988.
14. Julius, D.; Huang, K. N.; Livelli, T. J.; Axel, R.; Jessell, T. M. The 5-HT_2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors. Proc. Natl. Acad. Sci. USA 87:928–932; 1990.
15. Julius, D.; MacDermot, A. B.; Axel, R.; Jessell, T. M. Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. Science 241:558–564; 1988.
16. Kobilka, B. K.; Frielle, T.; Collins, S.; Yang-Feng, T.; Kobilka, T. S.; Francke, U.; Lefkowitz, R. J.; Caron, M. G. An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. Nature 329:358–360; 1987.
17. Lefkowitz, R. J. Receptor variation on a theme. Nature 351:353–354; 1991.
18. Levitan, E. Molecular cloning of substance-K and 5-HT receptors. Trends Neurol. Sci. 11:41–43; 1988.
19. Lubbert, H.; Hoffman, B. J.; Snutch, T. P.; van Dyke, T.; Levine, A. J.; Hartig, P. R.; Lester, H. A.; Davidson, N. cDNA cloning of a serotonin 5-HT_{1c} receptor by electrophysiological assays of mRNA-injected Xenopus oocytes. Proc. Natl. Acad. Sci. USA 84:4332–36; 1987.
20. Masu, Y.; Nakayama, K.; Tamaki, H.; Motoy, K.; Nakashini, S. cDNA cloning of bovine substance-K receptor through oocyte expression system. Nature 329:836–838; 1987.
21. Peroutka, S. J.; Snyder, S. H. Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Mol. Pharmacol. 16:687–699; 1979.
22. Peroutka, S. J.; Schmidt, A. W.; Sleight, A. J.; Harrington, M. A.

Serotonin receptor "families" in the central nervous system. Ann. NY Acad. Sci. 600:104–113; 1990.
23. Pritchett, D. B.; Bach, A. W. J.; Wozny, M.; Taleb, O.; Dal Toso, R.; Shih, J. C.; Seeberg, P. H. Structure and functional expression of cloned rat serotonin 5-HT_2 receptor. EMBO J. 7:4135–4140; 1988.
24. Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A. F. Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. J. Biol. Chem. 264:13572–13578; 1989.
25. Strader, C. D.; Sigal, I. S.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. F. Conserved aspartic residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. J. Biol. Chem. 263:10267–10271; 1988.