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Identification of a Single Amino Acid Residue Responsible for the Binding of a Class of β-Adrenergic Receptor Antagonists to 5-Hydroxytryptamine$_{1A}$ Receptors

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SUMMARY

The 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor can bind certain β-adrenergic receptor antagonists, such as pindolol, with high affinity. Such pharmacological cross-reactivity suggests a structural similarity in the ligand binding site between the two receptors. To identify this structural entity, we mutated Asn$_{385}$ in the seventh transmembrane domain of the human 5-HT$_{1A}$ receptor, based on the observation that this residue is conserved in all 5-HT$_{1A}$ and β-adrenergic receptors of different species but is absent in all other cloned guanine nucleotide-binding protein-coupled receptors. This single point mutation (Asn$_{385}$ to valine) causes a highly selective decrease in the affinity of pindolol and other aryloxyalkylamines for the mutant receptor (about 100-fold), while producing only minor changes in the binding of other 5-HT agonists and antagonists. The results provide direct evidence that Asn$_{385}$ is responsible for the high affinity interaction between 5-HT$_{1A}$ receptors and aryloxyalkylamine β-adrenergic antagonists but is not required for the binding of other chemical classes of ligands.

5-HT$_{1A}$ and β_2-adrenergic receptors are both guanine nucleotide-binding protein-coupled receptors with the putative seven-transmembrane topology. The gene for the human 5-HT$_{1A}$ receptor was first identified on low stringency Southern blots and cloned from a size-selected genomic library by using the β_2-adrenergic receptor gene as a probe (1). Certain β-adrenergic receptor antagonists, such as pindolol, propranolol, and alprenolol, which belong to the aryloxyalkylamine family of compounds (Fig. 1A), also bind to the 5-HT$_{1A}$ receptor with relatively high affinity (2). The molecular mechanism for such pharmacological cross-reactivity remains undetermined, although it is assumed that this similar binding behavior results from a common structural domain between the 5-HT$_{1A}$ and β-adrenergic receptors.

Previous studies with chimeric β_2- and α_2-adrenergic receptors suggest that the sixth and seventh transmembrane domains are important determinants of antagonist specificities among the adrenergic receptors (3). Recently, it has been demonstrated that β-adrenergic antagonist binding properties can be acquired by the human α_2-adrenergic receptor with a single amino acid substitution, of phenylalanine to asparagine, in the seventh transmembrane domain (4). An asparagine residue is found in a homologous position in the seventh transmembrane domain in all reported 5-HT$_{1A}$ and β-adrenergic receptors but is absent in other cloned guanine nucleotide-binding protein-coupled receptors, including 5-HT$_{1C}$, 5-HT$_2$, α-adrenergic, muscarinic cholinergic, and dopaminergic receptors, at the equivalent positions (Fig. 2) (28–30). To test whether this common asparagine residue is responsible for the binding of aryloxyalkylamines to the 5-HT$_{1A}$ receptor, Asn$_{385}$ of the human 5-HT$_{1A}$ receptor was mutated to valine, the corresponding amino acid residue present in 5-HT$_{1C}$ and 5-HT$_2$ receptors (31). We found that this single point mutation markedly decreases the affinity of β-adrenergic antagonists pindolol and other aryloxyalkylamines for the mutant receptor, without altering the binding of other classes of ligands.

Materials and Methods

Mutagenesis and expression. The human 5-HT$_{1A}$ receptor gene, cloned in pBC12BI vector (32), was provided by Dr. R. J. Lefkowitz, Duke University. The mutation of Asn$_{385}$ to valine was achieved by altering the DNA codon from AAT to GTG, using a standard polymerase chain reaction technique. The mutation was verified by DNA

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; K_d, equilibrium dissociation constant; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propyl-amino)-1,2,3,4-tetrahydronaphthalene; K_i, equilibrium inhibition constant; 5-CT, 5-carboxamidotryptamine; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate.

sequencing. Both wild-type and mutant receptors were expressed in COS-7 cells by using the DEAE-dextran method (3).

Ligand binding. Cells were lysed in ice-cold 5 mM Tris, 2 mM EDTA buffer (pH 7.4) 72 hr after the transfection and were then homogenized with a Polytron homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min, and then the supernatant was centrifuged again at 38,000 × *g* for 30 min. The pellet was stored at −20° until use. The binding assays were conducted as described before (33). For saturation binding studies to determine K_d values, the nonspecific binding was determined with 10 μM 8-OH-DPAT for [^{3}H]5-HT binding and 10 μM 5-HT for [^{3}H]8-OH-DPAT binding. The competition binding studies to determine the K_i values were performed using 0.5 nM [^{3}H]8-OH-DPAT. Nonspecific binding was determined with 10 μM 5-HT. K_i values were calculated based on the equation $K_i = IC_{50}/(1 + L/K_d)$ (Ref. 34), where L is the final concentration of radioligand and K_d is the equilibrium dissociation constant for the radioligand.

Results and Discussion

Both wild-type and mutant receptor genes were transiently expressed in COS-7 cells. Radioligand binding with [^{3}H]5-HT and [^{3}H]8-OH-DPAT, a selective 5-HT$_{1A}$ agonist, was performed. The affinities (K_d values) of the wild-type and mutant receptors for both 5-HT and 8-OH-DPAT are essentially the same (Table 1). The competition studies show that the wild-type and mutant receptors have similar binding profiles for the natural ligand 5-HT (Fig. 3A). In contrast, the mutant receptor has approximately 100-fold lower affinity for the β-adrenergic receptor antagonist pindolol than does the wild-type receptor (Fig. 3B). A single saturation experiment with ^{125}I-cyanopindolol also shows that, whereas the wild-type receptor binds as expected, with a K_d of about 2 nM, the mutant receptor fails to bind ^{125}I-cyanopindolol (data not shown). A detailed pharmacological characterization of both the wild-type and mutant receptors is provided in Table 1. No major differences (<5-fold) are apparent between the wild-type and mutant receptors, in terms of their affinity for a variety of chemical classes of agents, such as indoles (5-HT and 5-CT), ergots (lisuride, mesulergine, and metergoline), aminotetralins (8-OH-DPAT), and arylpiperazines (buspirone and ipsapirone). In contrast, the affinities of the aryloxyalkylamines (pindolol, propranolol, and alprenolol) for the 5-HT$_{1A}$ receptor are markedly decreased (40–150-fold) in the mutant receptor, compared with the wild-type receptor. However, the binding of other chemical classes of β-adrenergic antagonists, such as labetalol (35) and lisuride (36), to the 5-HT$_{1A}$ receptor is not altered by the mutation (Table 1). The chemical structures of aforementioned ligands are shown in Fig. 1B.

It has been documented that the affinity of agonists for the 5-HT$_{1A}$ receptor is decreased in the presence of GTP or its analogs (33). GTPγS, a GTP analog, causes a similar dose-dependent reduction of [^{3}H]8-OH-DPAT binding in both wild-type and mutant receptors, with K_i values of 1.1 ± 0.17 and 2.7 ± 0.69 μM, respectively (three experiments).

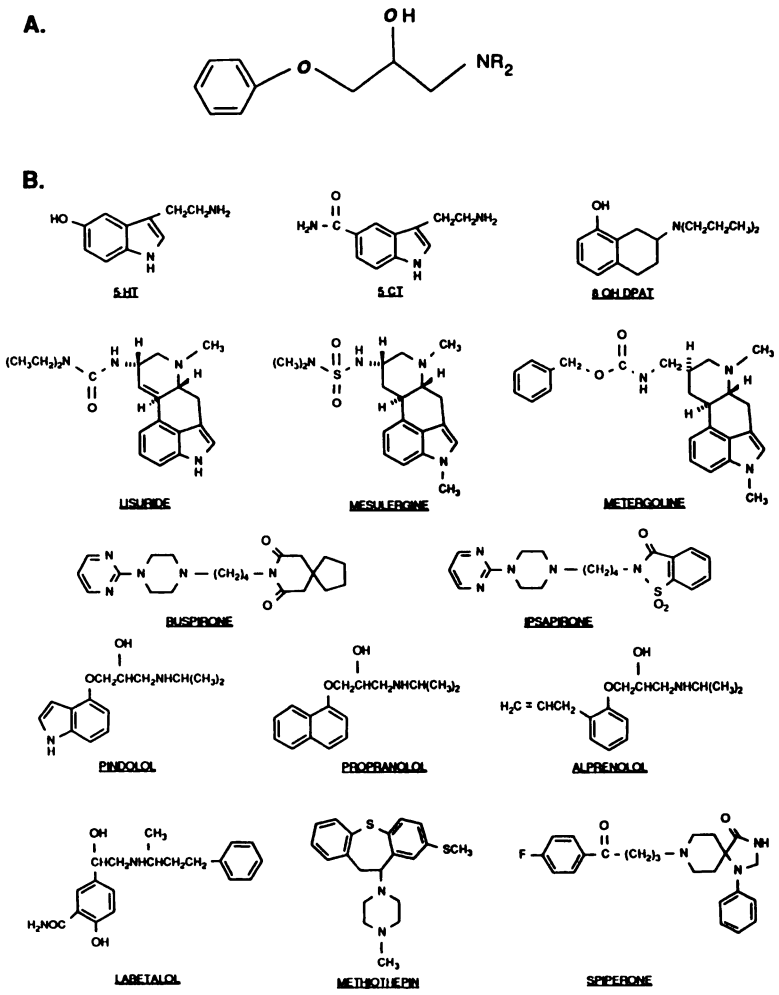

Fig. 1. A, General chemical structure of aryloxyalkylamines; B, chemical structures of various agents used in the study.

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HAMSTER α1B         A V F K V V |F| W L G Y F N S C L N P I I Y P C S S(5)
CANINE α1           A V F K V V |F| W L G Y F N S C L N P I I Y P C S S(6)
BOVINE α1C          T V F K I A |F| W L G Y L N S C I N P I I Y P C S S(7)

HUMAN α2C10         T L F K F F |F| W F G Y C N S S L N P V I Y T I F N(8)
HUMAN α2C4          P L F K F F |F| W I G Y C N S S L N P V I Y T V F N(9)
HUMAN α2C2          G L F Q F F |F| W I G Y C N S S L N P V I Y T I F N(10)
PORCINE α2C10       T L F K F F |F| W F G Y C N S S L N P V I Y T I F N(11)
RAT α2B             G L F Q F F |F| W I G Y C N S S L N P V I Y T V F N(12)
RAT BRAIN α2B       P L F K F F |F| W I G Y C N S S L N P V I Y T V F N(13)

HUMAN β1            R L F V F F |N| W L G Y A N S A F N P I I Y C R S P(14)
RAT β1              R L F V F F |N| W L G Y A N S A F N P I I Y C R S P(15)

HAMSTER β2          E V Y I L L |N| W L G Y V N S A F N P L I Y C R S P(16)
TURKEY β2           W L F V F F |N| W L G Y A N S A F N P I I Y C R S P(17)
HUMAN β2            E V Y I L L |N| W I G Y V N S G F N P L I Y C R S P(18)
RAT β2              E V Y I L L |N| W L G Y V N S A F N P L I Y C R S P(19)
MOUSE β2            E V Y I L L |N| W L G Y V N S A F N P L I Y C R S P(20)

HUMAN β3            P A F L A L |N| W L G Y A N S A F N P L I Y C R S P(21)

HUMAN 5-HT1A        L L G A I I |N| W L G Y S N S L L N P V I Y A Y F N(1)
RAT 5-HT1A          L L G A I I |N| W L G Y S N S L L N P V I Y A Y F N(22)

RAT 5-HT1C          K L L N V F |V| W I G Y V C S G I N P L V Y T L F N(23)
MOUSE 5-HT1C        K L L N V F |V| W I G Y V C S G I N P L V Y T L F N(24)
RAT 5-HT2           A L L N V F |V| W I G Y L S S A V N P L V Y T L F N(25)
HAMSTER 5-HT2       A L L N V F |V| W I G Y L S S A V N P L V Y T L F N(26)
HUMAN 5-HT2         A L L N V F |V| W I G Y L S S A V N P L V Y T L F N(27)
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Fig. 2. Comparison of amino acid sequences of the putative seventh transmembrane domain of cloned α- and β-adrenergic and 5-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors. The *boxed amino acids* are the residues at positions equivalent to that of Asn$_{385}$ of the 5-HT$_{1A}$ receptor. The references are indicated by the numbers in parentheses.

TABLE 1
Affinities of various ligands for [^{3}H]8-OH-DPAT binding to the wild-type and the mutant 5-HT$_{1A}$ receptors expressed in COS-7 cells
Ratio (M/W) is the K_i (or K_d) for the mutant receptor divided by the K_i (or K_d) for the wild-type receptor. The procedures for binding assays are described in Materials and Methods. Data are means ± standard errors of three independent experiments.

	K_i (or K_d)		
	Wild-type	Mutant	Ratio (M/W)
	nM		
Indoles			
5-HT	1.6 ± 0.6[a]	4.4 ± 0.7[a]	2.8
5-CT	0.12 ± 0.01	0.27 ± 0.004	2.3
Aminotetralins			
8-OH-DPAT	1.4 ± 0.3[a]	0.74 ± 0.08[a]	0.5
Ergots			
Lisuride	0.76 ± 0.2	0.42 ± 0.06	0.6
Mesulergine	750 ± 200	1000 ± 300	1.3
Metergoline	2.2 ± 0.2	3.2 ± 0.3	1.5
Arylpiperazines			
Buspirone	23 ± 10	6.5 ± 1	0.3
Ipsapirone	1.9 ± 0.4	2.5 ± 0.3	1.3
Aryloxyalkylamines			
Pindolol	24 ± 4	2700 ± 100	110
Propranolol	150 ± 40	6100 ± 2000	41
Alprenolol	28 ± 5	4100 ± 700	150
Miscellaneous			
Labetalol	240 ± 30	160 ± 4	0.7
Methiothepin	37 ± 5	11 ± 2	0.3
Spiperone	900 ± 100	220 ± 60	0.2

[a] K_d.

It is likely that all ligands capable of competing for [^{3}H]8-OH-DPAT binding sites share at least one common point of interaction with the receptor. However, the results presented here suggest that specific amino acids may selectively interact with a specific class of ligands. The data in the present study support the hypothesis that Asn$_{385}$ in the seventh transmembrane domain of the 5-HT$_{1A}$ receptor plays a critical role in the interaction between aryloxyalkylamines and 5-HT$_{1A}$ receptors. This amino acid does not appear to participate in the binding of other β-adrenergic antagonists or other 5-HT$_{1A}$ receptor ligands to the receptor molecule. These results are in contrast to those from similar experiments with α_2- and β_2-adrenergic receptors (4). When the homologous asparagine in the β_2-adrenergic receptor is changed to phenylalanine (the amino acid residue found at the same position in the α_2-adrenergic receptor), no binding to either α_2 or β_2 antagonists can be detected. Immunohistochemical studies suggest that this mutant is retained in the endoplasmic reticulum, perhaps due to aberrant folding of the protein. When the phenylalanine at position 412 in the seventh transmembrane domain of the α_2 receptor is changed to asparagine, the α_2 receptor acquires affinity for aryloxyalkylamines but loses affinity for α_2-adrenergic antagonists and agonists and the common adrenergic receptor agonist epinephrine. Thus, changing the amino acid at this position in adrenergic receptors has more general effects on receptor binding properties than those observed for the mutation in the 5-HT$_{1A}$ receptor.

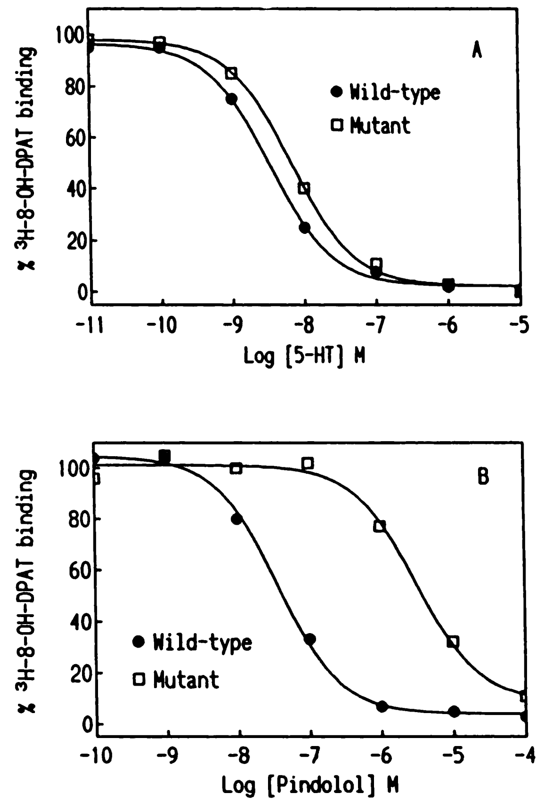

Fig. 3. Competition studies of 5-HT (A) and pindolol (B) versus [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ and mutant (Asp$_{385}$ to valine) 5-HT$_{1A}$ receptors expressed in COS-7 cells. [^{3}H]8-OH-DPAT was used at 0.5 nM for all assays. Nonspecific binding was defined using 10 μM 5-HT. Data are plotted as means of triplicates of a representative experiment. A total of three independent experiments were performed for each ligand.

The mechanism underlying the Asn$_{385}$-pindolol interaction remains unknown. Although Asn$_{385}$ may influence the binding of aryloxyalkylamines by an indirect allosteric effect, the selective nature of changes in the binding properties of the mutant receptor suggests a direct interaction between this amino acid and these ligands. The difference in the free energy ($\Delta\Delta G$) of pindolol binding between the wild-type and mutant receptors is calculated to be 2.8 kcal/mol, according to the equation $\Delta G = -RT\ln(1/K_d)$ (37). This value would be consistent with the free energy of a weak bond interaction such as hydrogen bonding (38). A recent study of the structure-affinity relationships between propranolol analogs and the 5-HT$_{1A}$ receptor indicated

that the oxygen atom directly linked to the aromatic ring (the ether oxygen) of propranolol analogs, but not the side chain hydroxy group (see Fig. 1), is important for 5-HT_{1A} binding (39). It is interesting to note that, among the β-adrenergic antagonists tested in the present study, the binding of ligands with an ether oxygen is markedly affected by the asparagine to valine mutation in the 5-HT_{1A} receptor. Labetalol, on the other hand, does not have this ether oxygen, and its affinity for the 5-HT_{1A} receptor is not reduced by the mutation. These observations demonstrate that site-specific mutagenesis studies may be used to identify the molecular basis of previously documented chemical structure-activity relationships.

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