

NOVEL MUTANTS OF THE HUMAN β_1 -ADRENERGIC RECEPTOR REVEAL AMINO ACIDS RELEVANT FOR RECEPTOR ACTIVATION*

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Activation of G protein-coupled receptors like the β_1 -adrenergic receptor results in conformational changes which ultimately lead to signal propagation through a G protein to an effector like adenylyl cyclase. In this study we identify amino acids which seem to be critical for activation of the human β_1 -adrenergic receptor. Activation patterns of mutant receptors were analyzed using two structurally different ligands for β -adrenergic receptors which both are mixed agonist/antagonists. Broxaterol and terbutaline are agonists at β_2 - and β_3 -receptors, however, they act as antagonists at the β_1 -subtype. We reasoned that this functional selectivity may be reflected by a corresponding sequence pattern in the receptor subtypes. Therefore, we exchanged single amino acids of the β_1 -adrenergic receptor for residues that were identical in the β_2 - and β_3 - subtypes but different in the β_1 -receptor. Pharmacological characterization of such receptor mutants revealed that binding of a panel of agonists and antagonists including broxaterol and terbutaline was unaltered. However, two of the mutants (I185V and D212N) were activated by broxaterol and terbutaline which acted as antagonists at the wild-type receptor. Two additional mutants (V120L and K253R) could be activated by terbutaline

alone which is structurally more closely related to endogenous catecholamines like epinephrine than to broxaterol. A model of the human β_1 -adrenergic receptor showed that the four gain-of-function mutations are outside of the putative ligand-binding domain substantiating the lack of an effect of the mutations on binding characteristics. These results support the notion that V120, I185, D212 and K253 are critically involved in conformational changes occurring during receptor activation.

Introduction

G protein-coupled receptors (GPCR)² comprise a large number of structurally related membrane receptors many of which are important drug targets. Among the receptors targeted in established therapies β -adrenergic receptors comprise one of the most important subgroups. The use of β -blockers is indicated in virtually all major cardiovascular diseases whereas β_2 -selective agonists are a mainstay in the treatment of asthma. Activation of GPCR is a complex process which results in a state that propagates a signal to the corresponding G protein. It is thought that more than one activated state may exist (1, 2) providing a basis for transduction of different ligand-specific signals via a given receptor subtype

(3). The notion that receptor activation is a multistep process whereby the receptor assumes a series of conformational intermediates provides an explanation for such functionally distinct states (4). Such functionally defined states could serve as distinct mediators of more specific drug actions.

The investigation of conformational intermediates should benefit from ligands which are able to distinguish their functional identity. Alternatively, it would be interesting to study distinct activation patterns at closely related receptor subtypes which might be different for a given ligand. We have recently discovered that some ligands exhibit distinct functional properties at the three subtypes of β -adrenergic receptors. In the course of a previous project for the development of subtype-selective ligands for human β -adrenergic receptors we noticed that the experimental compound broxaterol which is thought to be a β_2 -selective agonist, binds in fact with the same affinity to all three β -receptor subtypes (5, 6). Interestingly, this compound turned out to be an agonist at β_2 - and β_3 -receptors, but an antagonist at the β_1 -receptor (5, 6) making it a β_2 -/ β_3 -selective agonist in functional terms although it does not bind selectively to these subtypes. In a recent study we found similar functional β_2 -selectivity for clinically used compounds including terbutaline and salbutamol (6). This discovery prompted us to undertake the present investigation where we generated several gain-of-function mutants of the β_1 -adrenergic receptors which are activated by compounds like broxaterol and terbutaline. Based on the close sequence similarity between β -adrenergic receptor subtypes we reasoned that the functional selectivity of such compounds may have a corresponding sequence pattern in amino acids that are identical in β_2 - and β_3 - but different in the β_1 -subtype. Sequence comparison of the entire receptor proteins revealed a total of 17 amino acids following this pattern with eight of these positions being located in transmembrane domains. We generated six mutants where a β_1 -amino

acid was substituted for the corresponding β_2 -/ β_3 -amino acid and the functional characteristics of these mutants were analyzed. For mutation of such positions functionally different regions were selected. As the putative ligand binding domain is embedded in the transmembrane domains (7, 8) several mutations were introduced in transmembrane regions. The two mutants L154V and K253R are at the interface between transmembrane domains and intracellular loops two and three, respectively, which are thought to be involved in receptor-G protein coupling (9). Mutant D212N is located in the second extracellular (E2) loop in a position that has been shown to be relevant for ligand-receptor interaction in other GPCR (10-13). Four gain-of-function mutants were found which showed distinct ligand-dependent changes of activation patterns. Our data including a receptor model suggest that we have identified amino acid residues outside the ligand-binding domain that are critical for the activation of human β_1 -adrenergic receptors.

Experimental Procedures

Materials - Oligonucleotides were synthesized by MWG-Biotech. Cell culture media and fetal calf serum were from PanSystems; penicillin (100 U/ml), streptomycin (100 μ g/ml), L-glutamine and G-418 were purchased from Gibco-Life Technologies. Ligands were purchased from the following sources: (-)-epinephrine, (-)-norepinephrine, CGP-20712 ((\pm)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide), (-)-isoproterenol, terbutaline, from Sigma; and ICI-118551 ((\pm)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol), from RBI. Salmeterol was kindly provided by Dr. H. Krohn (GlaxoSmithKline). Broxaterol was kindly synthesized by Prof. M. De Amici (Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Milano, Italy). (-)-3-¹²⁵I-Iodocyanopindolol

(^{125}I -CYP, specific radioactivity, 2200 Ci/mmol) was from Amersham Biosciences. [α - ^{32}P]ATP was from PerkinElmer LifeScience. All other materials were from sources as described earlier (6, 14).

Mutagenesis and cell transfection - The cDNA encoding for human β_1 -adrenergic receptor (15); GenBank entry J03019) was mutated by PCR-mediated mutagenesis technique using *VENT* DNA polymerase (New England Biolabs). After confirmation of the mutation, the PCR products were digested with the appropriate enzymes and cloned into the expression vector pcDNA3 containing the wild type β_1 -adrenergic receptor cDNA to obtain full length mutated β_1 -adrenergic receptors. The sequences of all resultant cDNAs were verified by automated sequencing. The cDNA of the β_2 -adrenergic receptor (16); GenBank entry Y00106) was cloned into the pcDNA3 expression vector as described earlier (6).

Cell culture and membrane preparation - CHO cells stably transfected with human β -adrenergic receptor subtypes and different mutants of the β_1 -adrenergic receptor respectively, were grown and splitted in Dulbecco's Modified Eagle's Medium with nutrient mixture F12 (DMEM/F12), as described before (6). Before cells were harvested the culture medium was removed and cells were washed twice with PBS. Then membranes were prepared or cells were frozen on the dishes for later preparation of membranes. Crude membrane fractions were prepared from fresh (measurement of adenylyl cyclase) or frozen cells (radioligand binding) according to two different protocols, which have been described recently (14). The resulting membrane pellets were resuspended in 50 mM Tris/HCl pH 7.4 at a final protein concentration of 1 to 2 mg/ml. Protein concentration was determined by the method of Bradford (17) with bovine serum albumin (Sigma) as a standard.

Adenylyl cyclase activity and radioligand binding studies - Determination of adenylyl cyclase activity in cell

membranes was based on the method originally described by Jakobs et al. (18), for details see (6). Accumulation of [α - ^{32}P]-cAMP was linear over at least 20 min under all conditions. The basal and isoproterenol-stimulated adenylyl cyclase activity, respectively, in the different cell clones characterized in Table 3 was (all values in pmol/mg membrane protein/min): 13.5 ± 1.26 and 19.1 ± 1.57 (β_1 -Wildtype); 16.9 ± 1.22 and 25.6 ± 1.91 (β_2 -Wildtype); 12.4 ± 0.73 and 15.3 ± 0.91 (β_1 -V120L); 16.3 ± 1.24 and 23.0 ± 2.35 (β_1 -L154V); 11.3 ± 1.21 and 13.9 ± 1.38 (β_1 -I185V); 13.2 ± 0.39 and 16.2 ± 1.45 (β_1 -D212N); 12.3 ± 0.83 and 18.9 ± 1.65 (β_1 -K253R); 12.5 ± 2.58 and 15.1 ± 3.14 (β_1 -F362L).

The radioligand binding experiments were performed with membranes prepared as described above and followed the procedure as outlined previously (6). For competition binding approximately 50 pM ^{125}I -CYP were used and assays were done in the presence of 100 μM GTP to ensure monophasic competition curves for agonists. Membranes with β_1 -wildtype and mutant receptors used in competition binding experiments showed comparable receptor expression (Table 1). K_D -values for ^{125}I -CYP and K_i -values from competition experiments were calculated from saturation experiments by nonlinear curve fitting with the program SCTFIT (19).

Receptor homology modeling - The alignment of the primary sequences (Swiss-Prot entries P02699: rhodopsin, P08588: β_1 -adrenergic receptor) was generated with the program ClustalW. To ensure that residues in corresponding secondary-structure elements were properly classified, the aligned structures were examined using the program Jpred (20). For every receptor 15 regions were identified according to the topology of rhodopsin (21, 22): 7 transmembrane helices, 3 cytoplasmic loops, 3 extracellular loops, the extracellular N-terminus and the cytoplasmic C-terminus. The amino acid sequences of these areas flanked by 4 additional amino acid were submitted to the WU-Blast server to search

in the RCSB Protein Databank (23) for additional fragments with a known 3D structure. The structure-based multiple sequence alignments of all the fragments plus the rhodopsin data were merged as input for the homology modeling to obtain three-dimensional models of the β_1 -adrenergic receptor (24-26). The program MODELLER (24-26) was used to generate the three-dimensional structures of β_1 -receptor and during this process the models were refined by molecular dynamics routines with incremental increases in simulation temperature from 150°K to 1000°K, followed by incremental temperature decreases from 1000°K to 300°K. Several slightly different models have been calculated by varying the initial parameters, and the variability and energy among these models has been used to estimate the lower limit of the error in the corresponding regions of the fold. The models obtained were submitted to the PROCHECK package for the structure evaluation (27).

Ligand docking - The AutoDock 3.0 program was used to perform an automatic docking exploration for different conformations of the ligand in the β_1 models (28). This automated docking program uses a grid based method for energy calculation of the flexible ligand in complex with a rigid protein. Points on the 3D grid are placed such that they cover the entire inner cavity of the β_1 -adrenergic receptor, and are probed with the atoms that constitute the ligand. The docking experiments explored the interaction of terbutaline with the binding region of β_1 -subtype.

The simulation was carried out within a 22Å cube using a 0.375Å grid spacing. AutoDock uses an adaptive global-local search method based on Lamarckian genetics (LGA) in conjunction with an empirical force field that allows the prediction of binding free energies for docked ligand. In our setup we used a starting population of 50 ligand conformations with a stopping criterion of a maximum of ten million energy evaluations. The number of dockings was set to 250 to get good statistics of the docked complex and the resulting bound states were

clustered in groups on the basis of an RMSD value of 0.5 Å relative to the initial starting position of the ligand. These numbers together with AutoDock's default parameters has been shown to be a useful setting for blind docking (29). From the 250 simulations performed the binding modes with the most populated clusters was selected.

Results

Figure 1 shows a sequence comparison documenting the high sequence identity of the human β -adrenergic receptor subtypes in particular in the transmembrane domains. Broxaterol and terbutaline activate only β_2 and β_3 receptors as opposed to agonists like isoproterenol or fenoterol which activate all three subtypes of β -receptors. Therefore, amino acids which are shared between the β_2 - and β_3 -adrenergic receptor but are different in the β_1 -subtype are investigated as to their importance for the specific activation pattern of broxaterol and terbutaline. In Figure 1 respective amino acids are emphasized with a box. Mutation of such amino acids in the β_1 receptor to the corresponding amino acid of the β_2/β_3 subtypes was accomplished by a PCR-approach (see Experimental Procedures). A selection of the sites following this pattern was chosen for mutation (arrows in Figure 1, Table 1).

The functional characteristics of the mutated receptors were investigated in CHO-cells stably transfected with mutant and wildtype receptors. Figure 2A shows that broxaterol does not stimulate adenylyl cyclase activity via β_1 -receptors but has partial agonistic activity at the β_2 -subtype as has been shown before (5, 6). The β_1 -receptor mutants V120L, L154V, K253R and F362L behave like the wildtype receptor as broxaterol does not mediate a stimulation of adenylyl cyclase. In contrast, broxaterol does stimulate adenylyl cyclase via mutants I185V located in TM4 and D212N in the second extracellular loop (Figure 2A). The stimulation of adenylyl cyclase activity mediated by these gain-of-function mutants

is comparable to the stimulation achieved by the full agonist isoproterenol.

In addition to broxaterol we tested the efficacy of terbutaline which is another compound that had shown a mixed β_1 -antagonist- β_2 -/ β_3 -agonist profile (6). Figure 2B confirms the minimal efficacy of terbutaline at the β_1 -adrenergic receptor (19% compared to isoproterenol) and near full efficacy at the β_2 -receptor. Terbutaline behaves similar to broxaterol at the β_1 -receptor mutants L154V and F362L as it shows an identical minimal functional response at these mutants as well as at the wildtype receptor (Figure 2B). The mutants I185V and D212N mediate a stimulation of adenylyl cyclase by terbutaline which is in correspondence to the agonistic activity shown for broxaterol at these mutants. In contrast to the structurally different compound broxaterol, terbutaline activates adenylyl cyclase also via the mutants V120L at the extracellular end of TM2 and K253R which is located at the cytoplasmic face of TM5 (Figure 2B). Again, the efficacy of terbutaline at these mutants is similar to isoproterenol. All gain-of-function mutants showed the same or a slightly lower basal activity compared to the β_1 -wildtype (see Experimental Procedures).

The mutant β_1 -adrenergic receptors were tested for their binding characteristics and expression levels. Table 1 shows that wild-type β_1 - and β_2 -receptors and all mutant β_1 -receptors bind the nonselective antagonist ^{125}I -CYP with similar K_D values. Also, the expression levels of the cell lines studied are in the same order of magnitude excluding that the observed gain-of-function may be a result of massive overexpression of the respective mutant receptor.

As a next step it was confirmed that the mutations leading to activation by broxaterol or terbutaline did not cause a pharmacological conversion of β_1 - into β_2 - (or β_3 -) adrenergic receptors. One of the main characteristics of β_2 -adrenergic receptors is the low affinity for norepinephrine compared to epinephrine whereas the β_1 -subtype does

not distinguish between these two endogenous catecholamines. In contrast, the β_3 -subtype shows a marked preference for norepinephrine over epinephrine. Table 2 shows that all mutants clearly maintain the pharmacological identity of a wildtype β_1 -adrenergic receptor.

In addition, more detailed competition studies were performed with a panel of agonists that confirmed that the mutants share all their pharmacological characteristics with the wildtype β_1 -receptor (Table 3). The affinity of some prototypical subtype-selective antagonists was also not affected by any of the mutations introduced into the β_1 -sequence (Table 3).

The 3D model of the β_1 -adrenergic receptor in Figure 3A shows the location of the mutations which changed the functional responses to broxaterol and terbutaline. The model is based on the crystal structure of rhodopsin (21) and was generated as described in the Experimental Procedures. In Figure 3B some amino acids thought to be involved in agonist recognition are shown. These amino acids were previously identified as critical for agonist binding in models for both β_1 - (30) and β_2 -receptors (7, 8). In addition, terbutaline is docked into the putative binding site of the receptor. The docking of terbutaline was simulated with various starting positions but independent of the starting conditions about a quarter of the obtained structures fitted to the same position (RMSD 0.5Å). The model suggests that all mutated amino acids are too far away from the binding pocket to be directly implicated in ligand binding. The closest distance between the ligand and a mutated amino acid in the transmembrane region of 7.7 Å is found between terbutaline and I185.

Discussion

Recently it was found that not only the experimental drug broxaterol but, surprisingly, also clinically used compounds like terbutaline bind nonselectively to human β -receptors. However, they exhibit functional selectivity as they act as agonists

only at β_2 - and β_3 -adrenergic receptors whereas they are antagonists at the β_1 -subtype (5, 6). The lack of binding selectivity of such compounds suggests that their interaction with the ligand-binding domain does not provide a sufficient explanation for the observed functional differences at β -adrenergic receptor subtypes. There seem to exist common features of the ligand-binding domains that dictate the binding affinity. However, it may be reasonable to assume that receptor activation is additionally controlled by subtype-specific sequence patterns of amino acids outside the ligand-binding domain. Therefore, nonselective ligands that are mixed agonist/antagonists like broxaterol or terbutaline should be interesting tools to study receptor activation. Based on the high sequence identity between the subtypes of β -adrenergic receptors it seemed possible to trace down amino acids that are responsible for the subtype-specific activation patterns of broxaterol and terbutaline. Such positions should play a prominent role in receptor activation in general. We reasoned that the observed functional pattern for mixed agonists/antagonists might be reflected by a corresponding sequence pattern in the three β -adrenergic receptor subtypes. The gain-of-function mutants of the β_1 -receptor that were found in the course of this study confirm this initial hypothesis.

Several of the generated mutants showed altered functional characteristics compared to the wildtype β_1 -adrenergic receptor. It can be excluded that these changes are caused by a decrease in structural constraints as all gain-of-function mutants showed the same or a slightly lower basal activity compared to the β_1 -wildtype. Interestingly, we found two β_1 -mutants at which broxaterol turned into an agonist whereas for terbutaline two additional gain-of-function mutations were identified. Broxaterol is an unusual β -agonist as it does not share a catechol or related structural motif with the endogenous ligands epinephrine and norepinephrine or most typical β -adrenergic agonists. It seems reasonable, therefore, that more gain-of-

function mutants were found for terbutaline than for broxaterol as the catechol and related structures are important for receptor activation (31).

One obvious explanation for the functional effect of exchanging amino acids in the β_1 -receptor for β_2 - or β_3 -residues would be a change of the pharmacological characteristics and a concomitantly altered activation pattern. Our data clearly show that the mutated β_1 -receptors are pharmacologically absolutely identical with the wildtype receptor. Closer investigation with a number of subtype-selective agonists and antagonists confirmed that all mutants independent of their activation by broxaterol or terbutaline are pharmacologically indistinguishable from β_1 -wildtype receptors. The receptor model shown in Figure 3 also suggests that the mutations did not alter the ligand binding pocket. Therefore, our data provide evidence that single amino acids in various positions which do not seem to be involved in ligand recognition are decisive for the agonistic properties of a receptor ligand.

Overall, the number of mutations of the β_1 -adrenergic receptor reported in the literature is rather limited compared to numerous mutations of the β_2 -receptor. A number of mutations turning β_1 -receptors into constitutively active receptors has been described (32). A polymorphism of the human β_1 -subtype with functional consequences is known in position 389 (33). It turned out that an Arg in position 389 is more common than the Gly originally identified as the amino acid in the wildtype receptor. The variant with an Arg-389 is functionally different from the wildtype as it shows both higher basal and isoproterenol-stimulated adenylyl cyclase activity based on enhanced G_s coupling (33). To our knowledge, so far no β_1 -receptor mutants were known that turn an antagonist into an agonist.

Activation of a GPCR is thought to be associated with conformational changes in the receptor protein. Current concepts suggest that after initial contact between an

agonist and the ligand binding domain a multistep process guides the receptor through a series of conformational intermediates (4, 34). Different conformational states may be responsible for the selective activation of signaling cascades mediated through different G proteins like in the case of the β_2 -adrenergic receptor (35). They may also be the basis for functionally distinct responses to different concentrations of a ligand as has been shown for CGP 12177 and other compounds (3). It is clear that any motion in the receptor protein resulting in conformational changes requires the existence of hinges or pivots to allow for structural changes to occur. In addition to amino acids responsible for ligand-receptor interaction other critical amino acids should exist that act as key points for such motion. It is not surprising, therefore, that mutations of amino acids that are not involved in ligand-recognition may have effects on receptor function. Although we do not know the exact mechanism which underlies the functional changes caused by mutation it is obvious though that residues V120, I185, D212 and K253 play a critical role in the activation of the human β_1 -adrenergic receptor.

Isogaya et al. (8) describe a number of mutations including mutation of V120 which is one of the positions leading to a gain-of-function mutation in our study. The mutation of V120 to A shows an indirect contribution to subtype-selectivity of selected agonists (8). Although ligand binding is not affected in our study we also find that activation by terbutaline, but not by broxaterol is changed by mutation of V120 to L in our case, confirming a significant role of this position.

A number of studies suggest that the E2 loop plays an important role in ligand binding and receptor activation at least in some GPCR (10-13). Our data support this notion as the D212N mutant shows a functional change compared to the wildtype receptor as it can be activated by both broxaterol and terbutaline. A number of mutations in the E2 loop of the C5a receptor resulted in constitutive activity, therefore, a

role as a negative regulator of receptor activation was proposed for the E2 loop (13). Position D212 in the human β_1 -adrenergic receptor does not seem to function in such a way as the mutation to N does not result in a change in basal receptor activity. In the case of the dopamine D₂ receptor a contribution of the E2 loop to the ligand binding site was suggested (12). In particular our binding data led us to conclude that D212 is not directly involved in ligand binding as the D212N mutation like all other mutations presented in this study did not affect agonist or antagonist binding. All functionally changed mutants including the D212N mutation support the concept that amino acids outside the ligand-binding domain contribute relevant structural elements for receptor activation and thus for ligand efficacy.

The model of the human β_1 -adrenergic receptor shows that the gain-of-function mutants described here are very unlikely close enough to the docked ligand terbutaline to directly interfere with ligand-receptor interaction. Further confirmation of this notion comes from the absolutely unaffected pharmacological characteristics of all functionally significant mutants investigated in our study. The mutations affect residues outside the ligand-binding domain and reveal, therefore, that the respective amino acids contribute to the control of the activation process independent of ligand-receptor recognition. It turned out that the ligands broxaterol and terbutaline which are nonselective in binding but functionally selective are ideal pharmacological tools to analyze the activation process in conjunction with point mutation and receptor modeling.

In summary, we present data revealing an important role of V120, I185, D212 and K253 for the activation of the human β_1 -adrenergic receptor. The mutation of these positions to the corresponding amino acids of the β_2 -/ β_3 -subtype results in a gain-of-function as the β_1 -antagonists broxaterol and terbutaline turn into agonists at these mutants. We conclude that the mutated positions represent critical residues for

conformational changes that occur during receptor activation. This notion is supported by a receptor model suggesting that the

amino acids mutated in this study are not involved in direct ligand-receptor recognition.

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Footnotes

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¹¹These authors contributed equally to this work

²The abbreviations used are: GPCR, G protein-coupled receptor(s); ¹²⁵I-CYP, ¹²⁵I-iodocyanopindolol; TM, transmembrane domain; E2 loop, second extracellular loop

Legends to Figures

Figure 1

Sequence comparison of β -adrenergic receptor subtypes. Amino acids identical in two or three subtypes are shown in bold, homologous residues are given in black. All other amino acids are in gray. Transmembrane domains are marked by a line below the β_3 -sequence. The boxed positions represent amino acids that are identical in the β_2 - and β_3 -receptor but different in the β_1 -subtype. Arrows mark the mutations characterized in this study.

Figure 2

Gain-of-function of β_1 -adrenergic receptor mutants. Open and black columns show the broxaterol- or terbutaline-stimulated activity of adenylyl cyclase in the β_1 - and β_2 -wildtype receptors, respectively, relative to the signal of the full agonist isoproterenol. **A** In four of the β_1 -mutants broxaterol does not show agonistic activity similar to the wildtype receptor. However, mutants I185V and D212N are activated by broxaterol almost to the level of activation observed in the β_2 -receptor. **B** No activation of adenylyl cyclase was observed in

mutants L154V and F362L whereas mutants V120L, I185V, D212N and K253R are activated by terbutaline.

Figure 3

Model of the human β_1 -adrenergic receptor. **A** Amino acids shown correspond to the mutations that caused functional changes for broxaterol and/or terbutaline. **B** Amino acids thought to be directly involved in ligand recognition are shown in detail. Two of the mutated amino acids (I185 and D212) are also shown. The closest distance (7.7 Å) between terbutaline (bonds in light gray) and a mutated amino acid is marked with a white line to I185.

Table 1

Characteristics of wild-type human β_1 - and β_2 -receptors, and mutants of the β_1 -adrenergic receptor. Values are means from 3-6 experiments.

	K_D (pM)	95% confidence limits	B_{max} ± SEM (fmol/mg)
β_1	38.5	26.6 – 55.9	398 ± 84
β_2	16.7	9.1 – 30.6	124 ± 7
V120L	13.9	7.4 – 26.5	411 ± 21
L154V	33.6	25.4 – 44.5	273 ± 16
I185V	12.7	7.5 – 21.6	576 ± 125
D212N	23.5	12.7 – 43.4	370 ± 54
K253R	45.5	35.2 – 58.9	205 ± 19
F362L	27.6	22.0 – 34.7	287 ± 31

Table 2

Affinity of epinephrine and norepinephrine at wild-type human β_1 - and β_2 -receptors, and functionally altered mutants of the β_1 -adrenergic receptor.

	epinephrine		norepinephrine	
β_1 *	4000	(2,800 – 5,500)	3,600	(2,400 – 5,200)
β_2 *	700	(500 – 1,000)	26,000	(23,000 – 30,000)
β_3 *	130,000	(120,000 – 140,000)	4,000	(2,800 – 5,500)
V120L	5,400	(2,700 – 10,700)	2,100	(1,100 – 3,800)
I185V	5,600	(3,600 – 8,700)	3,800	(1,400 – 10,400)
D212N	4,500	(3,400 – 5,900)	2,600	(1,900 – 3,600)
K253R	6,900	(2,800 – 17,000)	3,100	(1,000 – 9,300)

** values are from (6)

Table 3 Affinity of agonists and antagonists at wild-type and mutant β_1 -adrenergic receptors. K_i values are from competition experiments with ^{125}I -CYP as a radioligand. K_i values are in nM with 95% confidence intervals in parentheses.

compound	β_1 WT*	V120L	I185V	D212N	K253R
<i>isoproterenol</i>	220 (150 - 340)	280 (210 - 360)	260 (170 - 400)	300 (200 - 400)	450 (170 - 1,190)
<i>broxaterol</i>	1,300 (930 - 1,900)	1,100 (1,000 - 1,100)	1,400 (980 - 2,000)	910 (560 - 1,500)	870 (560 - 1,400)
<i>terbutaline</i>	31,000 (19,000 - 52,000)	34,000 (30,000 - 39,000)	40,000 (22,000 - 73,000)	48,000 (24,000 - 98,000)	50,000 (39,000 - 63,000)
<i>salmeterol</i>	1,600 (1,100 - 2,300)	1,400 (1,100 - 1,700)	900 (700 - 1,200)	1,300 (560 - 3,000)	1,200 (780 - 2,000)
<i>ICI 118551</i>	50 (40 - 361)	53 (32 - 89)	56 (32 - 98)	100 (44 - 240)	85 (56 - 130)
<i>CGP 20712A</i>	4.7 (4.0 - 5.5)	2.9 (1.8 - 4.7)	2.8 (1.8 - 4.2)	3.9 (1.6 - 9.9)	4.7 (2.5 - 8.7)

* values are from (6)

FIGURE 1

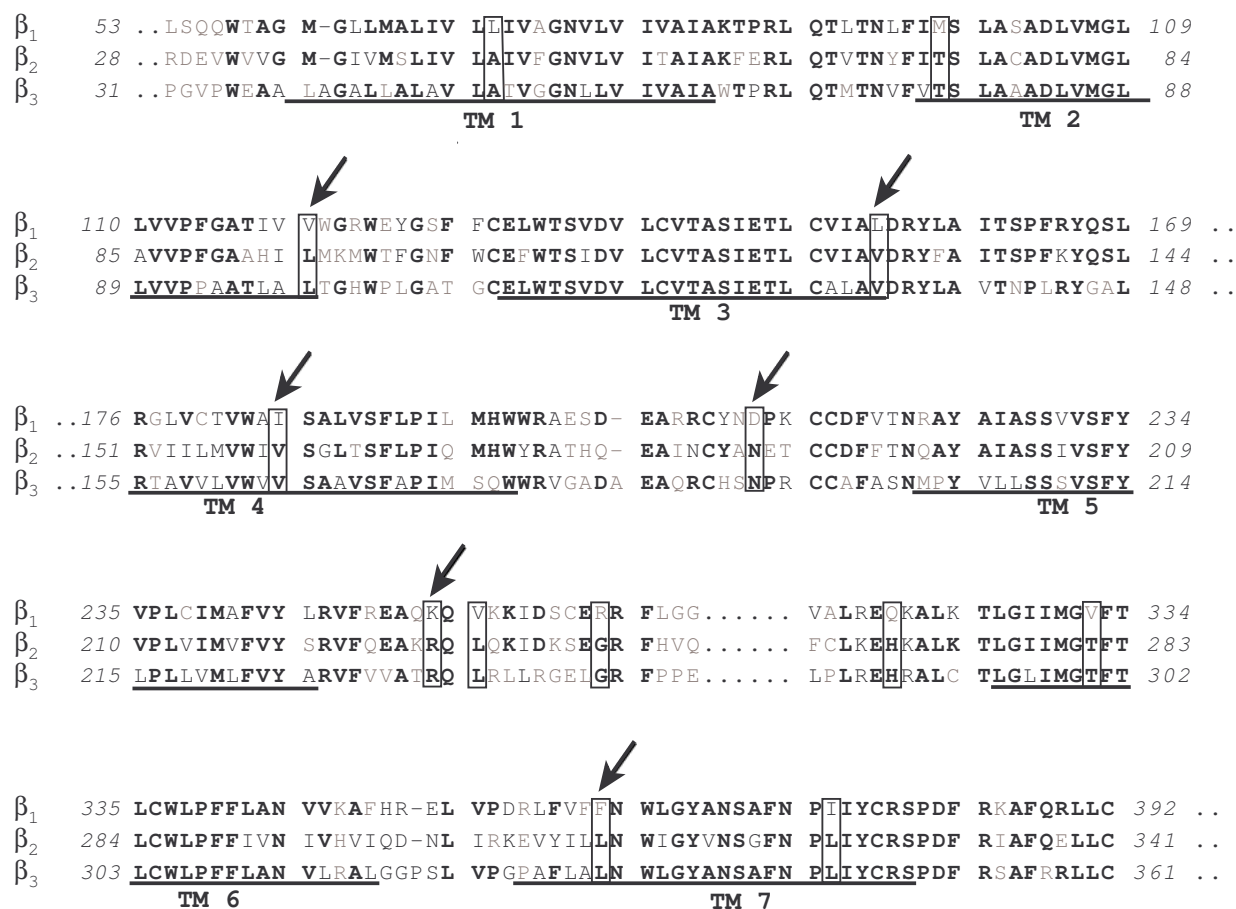


FIGURE 2A

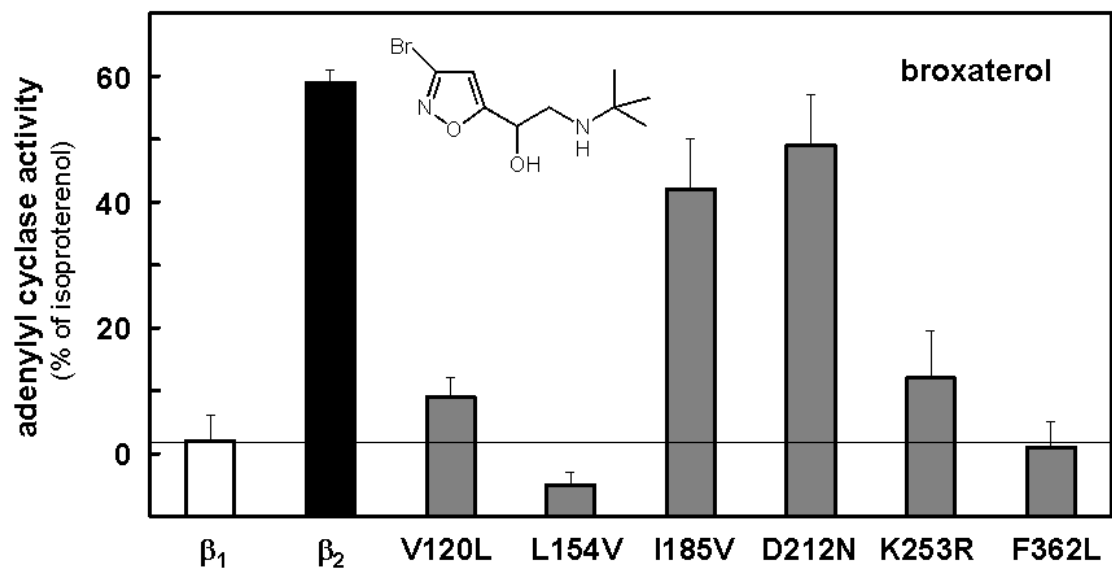


FIGURE 2B

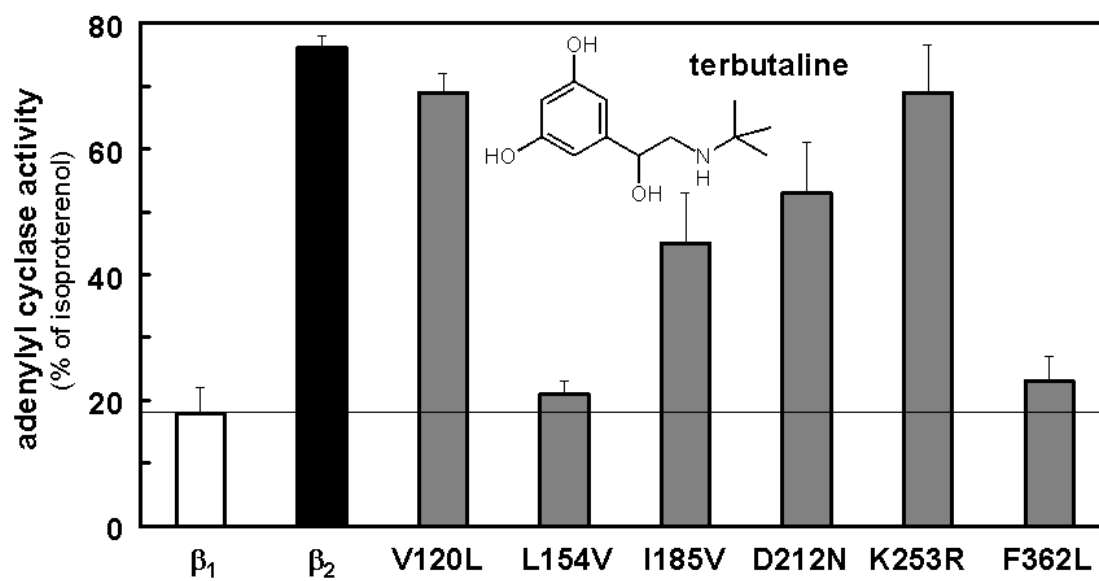


FIGURE 3A

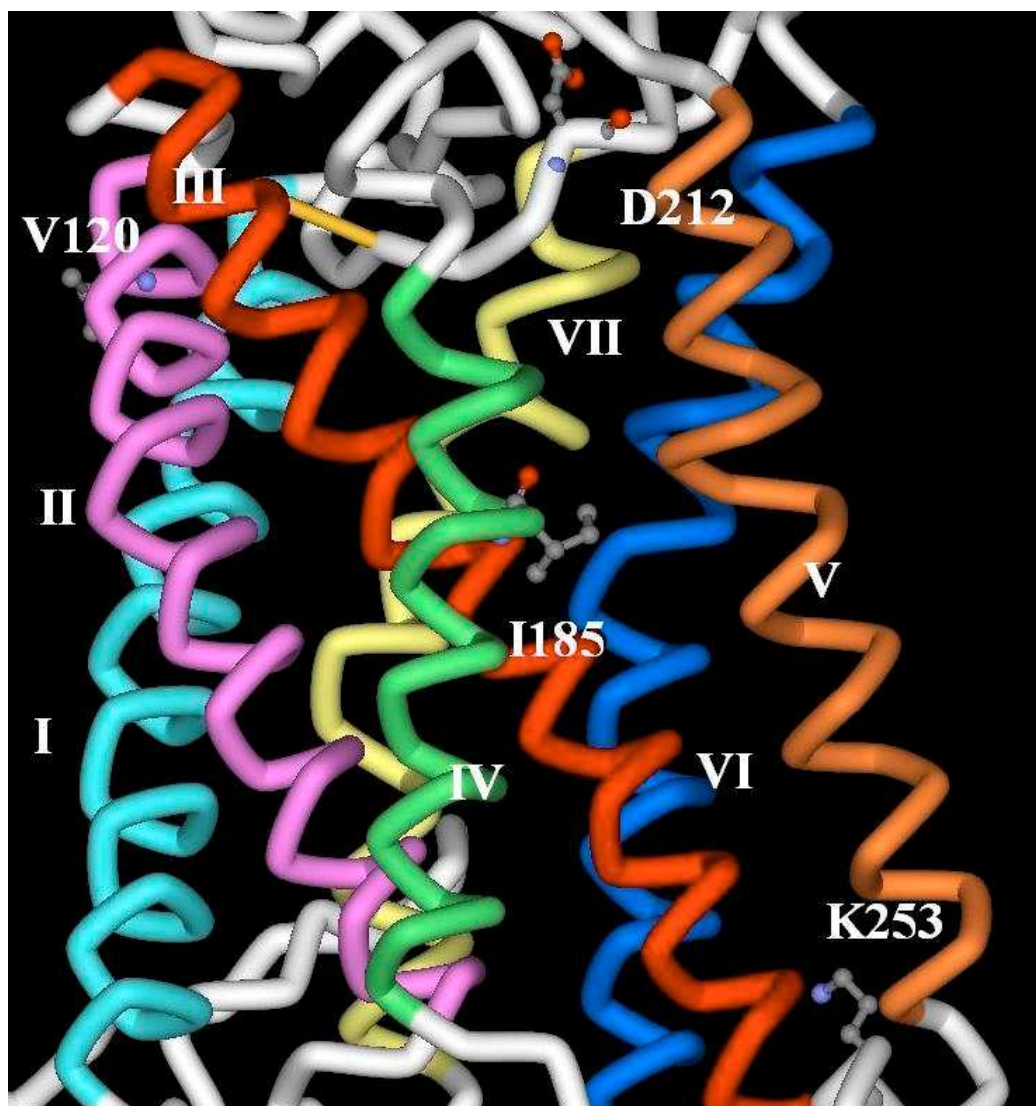
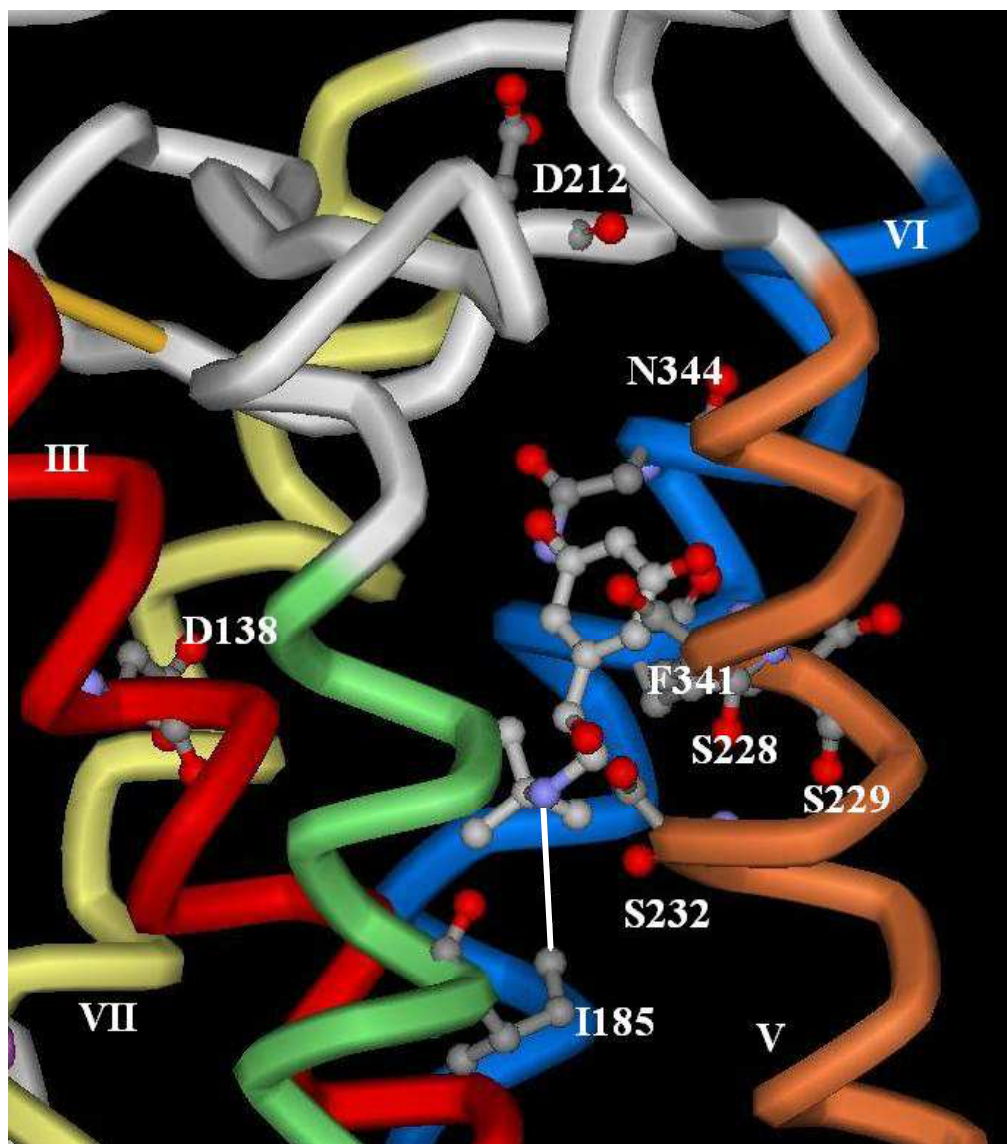


FIGURE 3B



Novel mutants of the human β 1-adrenergic receptor reveal amino acids relevant for receptor activation

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