Identification of a Conserved Switch Residue Responsible for Selective Constitutive Activation of the β2-Adrenergic Receptor*
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A cysteine-to-phenylalanine mutation of residue 116 in the third transmembrane domain of the β2-adrenergic receptor caused selective constitutive activation of Na+/H+ exchange through a pathway not involving cAMP. This selectivity was identified by comparing binding and signaling characteristics of wild-type (WT) versus C116F mutant receptors transiently transfected into COS-1 cells. Indicating constitutive activity, ligand binding to the C116F mutant showed a 78-fold higher than WT affinity for isoproterenol and a 40-fold lower than WT affinity for ICI 118551. Although agonist-independent activation of cAMP production was not exhibited by the C116F mutant, a constitutive stimulation of the Na+/H+ exchanger (NHE1) was observed. This was identified by measuring either basal intracellular pH (pHi) or rate of pHi recovery from cellular acid load. Due to a higher rate of H+ efflux through NHE1, C116F transfectants exhibited a significantly higher pHi (7.42) than did WT transfectants (7.1). Furthermore, the rate of pHi recovery from acid load facilitated by NHE1 was 2.1-fold faster in mutant transfectants than in WT transfectants. The lower rate seen in the WT case was stimulated by epinephrine, and the higher rate seen in the mutant case was inhibited by ICI 118551. These findings, which show that a C116F mutation of the β2-adrenergic receptor evokes selective constitutive coupling to NHE1 over cAMP, form the basis of our prediction that multiple and distinct activation states can exist in G protein-coupled receptors.

The β2AR is one member of a large family of G protein-coupled receptors that mediate the effects of numerous peptidic and nonpeptidic hormones and neurotransmitters. As with all G protein-coupled receptors, the common structural feature of this family is a single polypeptide chain with seven hydrophobic regions that constitute seven transmembrane-spanning domains. Classification of adrenergic receptors (α1, α2, and β), which as a family mediate the physiological effects of the sympathetic nervous system, is based on homology of amino acid sequences and pharmacology of various ligands (1). In the case of the β2-subtype, dogma suggests that the endogenous catecholamines epinephrine and norepinephrine promote receptor coupling to Gαs and downstream cAMP production (2, 3). Since β2ARs are the most abundant in smooth muscle, the best characterized effects of cAMP activation include smooth muscle relaxation in bronchial tubes and in vascular tissue (4).

The process of receptor activation and G protein coupling that evokes cellular effects is described by the widely accepted revised ternary complex model (5, 6). In this allosteric model, the active conformation of a native receptor is the cornerstone of the agonist-receptor-G protein complex that leads to signaling. Without agonist present, the model predicts spontaneous receptor isomerization between the inactive (R) and active (R*) conformations, with equilibrium under native conditions shifted toward R. At any given time, even though most receptors reside in R, a small population will reside in R*, permitting formation of the R*G protein complex that causes effector activation. The model predicts that the addition of agonist does not directly convert the receptor from R to R*. Rather, the agonist will preferentially bind to receptors already in R*, thereby shifting isomerization equilibrium away from R. Receptor mutations that induce an agonist-independent shift in isomerization equilibrium toward the R* conformation are termed constitutively active and so by definition couple to and evoke second messenger responses in the absence of agonist.

The studies contributing to the development of the revised ternary complex model were based primarily on the characterization of β2AR signaling, which was assumed to be through a single effector pathway (adenylate cyclase via coupling to Gαs). However, recent studies indicate that besides coupling to Gαs, β2ARs also couple to an alternate protein in the Gα family (possibly Gα13), which leads to newly discovered cellular effects, including stimulation of NHE1 (7–9), via Cdc42- and RhoA-dependent pathways (10). Therefore, in the present study, β2AR signaling via both cAMP production and Na+/H+ exchange was examined in a receptor that was engineered to have a single amino acid mutation of Cys-116 in the third transmembrane domain. Cys-116 is situated approximately one helical turn below Asp-113, which is the putative counterion that binds the protonated amine of adrenergic ligands (11, 12). Because of the important role of Asp-113 in ligand binding, any perturbation of the third transmembrane helix could affect both the ligand-binding pocket and second messenger coupling. Interestingly, the C116F mutation induces a receptor conformation that constitutively activates Na+/H+ exchange while only maintaining competent coupling to cAMP. This finding is similar to the result observed in an analogous C128F mutation of the α1AR, which exhibits selective constitutive coupling to inositol metabolism over the arachidonic acid pathway (13). Work in our laboratory with C116F β2AR and C128F α1AR has formed the basis of an emerging hypothesis suggesting that receptors are...
capable of forming multiple activation states that are G protein-specific. An understanding of conformational differences between these distinct states may eventually lead to the design of signaling-specific therapeutic agents. At least in the case of receptors that couple to more than one G protein, we suggest that the current model of receptor activation be revised to include isomerization from the R conformation to distinct active conformations that exhibit G protein selectivity.

EXPERIMENTAL PROCEDURES

**Media**—HEM buffer was composed of 20 mM Hepes (pH 7.5), 1.4 mM EGTA, and 12.5 mM MgCl2. Fluorescence medium consisted of 125 mM NaCl, 5 mM KCl, 1.7 mM CaCl2, 0.7 mM NaH2PO4, 0.8 mM Na2SO4, 0.5 mM MgCl2, 15 mM Hepes (pH 7.4), 2 mM t-glutamate, and 10 mM α-glucose. Potassium-based fluorescence medium was identical to fluorescence medium except that it contained 20 mM NaCl and 110 mM KCl.

**Site-directed Mutagenesis**—The cysteine-to-phenylalanine mutation was made at residue 116 of the human β2AR using cassette replacement as described previously (14). cDNAs were sequenced by the dye-deoxy method (Sequenase kit, Amersham Corp.) to confirm the mutation. The synthetic human β2AR was then subcloned into the eukaryotic expression plasmid pMT2 (15) and purified.

**Cell Culture and Transient Transfection—** COS-1 cells (American Type Culture Collection), known to be βAR-negative based on the lack of epinephrine-evoked cAMP responses (16), were grown in Dulbecco’s modified Eagle’s medium (Sigma) + 10% fetal bovine serum (Life Technologies, Inc.). Cells were plated onto 150- or 60-mm tissue culture plates for membrane preparations or second messenger studies, respectively, or onto UV-clear 24-well plates (Biotools) for fluorescence assays. Transient transfection was achieved by the DEAE-dextran method (17). Efficiency of transfection was typically near 20%. Cells were harvested or assayed 60 h after transfection.

**Membrane Preparation and Radioligand Binding—** COS-1 cell membranes were prepared as described previously (15). Membrane protein concentration was determined using the Bradford method (18). Competition binding experiments with the agonist radioligand [125I]CYP were carried out in a final volume of 0.25 ml containing HEM buffer + 0.1% bovine serum albumin, 150 pm [125I]CYP, COS-1 membranes, and varying concentrations of unlabeled ligand. Nonspecific binding was determined in the presence of 10 μM propranolol. Reactions were run at room temperature for 1 h, after which time the mixtures were filtered onto Whatman GF/C glass fiber filters using a Brandel cell harvester. Bound radioactivity was quantitated with a Packard Auto-gamma 500 counter. Binding data were analyzed with the iterative curve-fitting software package GraphPad Prism. Saturation binding studies were performed with increasing concentrations of [125I]CYP (5–600 pm) in the same buffer as used in competition binding experiments. To minimize variation, WT and C116F β2AR binding experiments were always performed simultaneously. Statistical significance in both binding and functional assays was identified by the two-tailed Student’s t test.

**cAMP Determination—** Accumulation of cAMP in WT and C116F β2AR transfectants was measured using a commercially available cAMP assay system (Amersham Corp.) according to the directions supplied by the manufacturer. Cell extracts were derived from cultures in 60-mm dishes that were preincubated for 30 min with 5 mM theophylline and then for 30 min with both theophylline and increasing concentrations of agonist.

**Measurement of Intracellular pH Using BCECF—** Transfected COS-1 cells, cultured in 24-well dishes, were incubated for 15 min at 37 °C with 0.3 mM BCECF/AM (Calbiochem). Unincorporated dye was washed away by perfusion with fluorescence medium until extracellular fluorescence was undetectable. Intracellularly trapped BCECF fluorescence was measured with a Delta Scan dual wavelength spectrofluorometer (Photon Technology International) functionally linked to an Olympus inverted fluorescence microscope. Cells were alternately excited with 440- and 500-nm light while the intensity of the 530 nm emission was measured with a photomultiplier tube. Emission intensities at the two exciting wavelengths were collected every 0.2 s during continuous perfusion of the cells with medium. Using an adjustable shutter, the emission from an area containing only 20–30 cells was collected for analysis. Cellular autofluorescence and contaminating fluorescence from leaked dye were found to be negligible, but background corrections were made to remove contributions from ambient light. Excitation ratios (500/440 nm) were calibrated for pH with 10 μM nigericin in potassium-based fluorescence medium as described previously (19). Fig. 1 shows the averaged results of seven separate calibrations that indi-
shift of agonist affinity seen for the C116F mutant was examined by performing competition studies in the absence and presence of 0.1 mM Gpp(NH)p. $K_i$ values and Hill coefficients for isoproterenol binding to mutant receptors were not affected by the presence of Gpp(NH)p (data not shown).

**Stimulation of cAMP Formation**—Functional coupling of WT and C116F $\beta_2$ARs to the cAMP pathway was examined by measuring accumulation of cAMP. For this experiment, the transfection protocol was modified to assure similar receptor expression levels in WT and C116F transfectants. This was accomplished by titrating down the amount of WT cDNA introduced to the cells until $B_{\text{max}}$ was approximately equal to values seen in the mutant case. Mean receptor expression in titrated WT transfectants was 0.063 pmol/mg compared with 0.056 pmol/mg in mutant transfectants. Fig. 2 compares the isoproterenol concentration dependence of cAMP production in cells expressing similar levels of either WT or C116F receptors. Arguing against constitutive coupling to $G_{\text{so}}$ and cAMP production, the C116F mutant receptor did not evoke a response in the absence of isoproterenol, and it did not affect the potency of isoproterenol to evoke a response compared with the WT receptor. The agonist concentration curves seen in the two transfected cell populations were nearly identical, with mean EC$_{50}$ values of 90.8 and 79.3 nM, respectively. Like isoproterenol, epinephrine concentration-response curves and EC$_{50}$ values seen in WT and C116F transfectants were not different (data not shown).

**Stimulation of Na$^+/H^+$ Exchange**—A possible alternative constitutive coupling of C116F $\beta_2$ARs to $G_{\text{so}}$ and downstream NHE1 was initially examined by studying the effect of this hypothetical constitutive activity on basal pH$_i$. Continuous stimulation of H$^+$ extrusion through the Na$^+/H^+$ exchanger by a constitutively active C116F mutant receptor would likely cause cytosolic alkalosis. Therefore, using basal pH$_i$ as a marker of constitutive activity, resting pH$_i$ levels in COS-1 cells were determined. Fig. 3 illustrates that C116F receptor-transfected cells had a significantly more alkaline pH$_i$ (7.42) than either WT transfectants (7.1) or untransfected cells (7.04). This was despite 6.8-fold more receptors on WT than on mutant cell membranes. As expected, intracellular alkalosis in the C116F transfectants was inhibited by inactivating the mutant receptors with the inverse agonist ICI 188551 (10$^{-8}$ M).

The apparent constitutive coupling of the C116F $\beta_2$AR to Na$^+/H^+$ exchange was further examined by measuring the rate of pH$_i$ recovery from cellular acid load. COS-1 cells transfected with either the WT or C116F $\beta_2$AR cDNA were loaded with H$^+$ by a 6-min exposure to 20 mM NH$_4$Cl. Rate of recovery from this acid load (dpH$_i$/dt) was compared in WT and C116F transfectants under various conditions. In an attempt to factor out effects on H$^+$ extrusion rate caused by the ion's concentration gradient, comparisons were made between groups of cells that loaded to a similar minimum pH$_i$ in the range between 6.35 and 6.4. A time course of the pH$_i$ changes that occur during acid load in C116F receptor-transfected cells is shown in Fig. 4. The initial alkalosis upon addition of NH$_4$Cl was due to the rapid internalization of NH$_3$ and its association with H$^+$ to form NH$_4^+$ (20, 21). Upon abrupt removal of the NH$_4$Cl, intracellular NH$_4^+$ dissociates to form NH$_3$, which rapidly leaves the cells, and H$^+$ ions, which are retained intracellularly. Recovery of the cells from this acid load was detected as a net alkalinization of the cytosol. Also shown in Fig. 4, facilitation of this pH$_i$ recovery was confirmed to be NHE1-specific based on sen-

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**TABLE I**

| Ligand               | WT (S.E.)        | C116F (S.E.)       | WT/C116F     |
|---------------------|------------------|--------------------|--------------|
| Isoproterenol       | $1.2 \times 10^{-7}$ | $2 \times 10^{-7}$ | $7.8^a$      |
| Epinephrine         | $1.7 \times 10^{-7}$ | $1.8 \times 10^{-6}$ | $18^a$       |
| Dichloroisoproterenol| $6.2 \times 10^{-10}$ | $8.2 \times 10^{-10}$ | $6.6^a$      |
| Albuterol           | $1.2 \times 10^{-5}$ | $1.5 \times 10^{-5}$ | $0.02^a$     |
| CGP 12177           | $9.7 \times 10^{-11}$ | $10 \times 10^{-11}$ | $0.04^a$     |
| Metaproterenol      | $3.7 \times 10^{-10}$ | $4.4 \times 10^{-10}$ | $0.27^a$     |
| Propanolol          | $3.8 \times 10^{-10}$ | $4.1 \times 10^{-10}$ | $0.54$       |

* Significant difference in $K_i$ between the WT and mutant receptors ($p < 0.001$).

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**Fig. 2. Stimulation of cAMP accumulation by isoproterenol.**

The efficacy of isoproterenol to evoke a cAMP response was tested in COS-1 cells transfected with either the WT or C116F $\beta_2$AR. Mean receptor expression in WT and C116F receptor-transfected cells was 0.063 and 0.056 pmol/mg, respectively. Based on the concentration-response curves shown, EC$_{50}$ values were determined to be 91 ± 7.8 nM in WT transfectants (□) and 79 ± 7.8 nM in mutant transfectants (○). The basal cAMP level (without agonist; labeled con) was 2.7 ± 0.12 pmol/10$^6$ cells. Values shown represent the mean ± S.E. ($n = 3$).
Furthermore, activation of WT receptors with 10^6 M epinephrine stimulated pH recovery with a similar rate. WT transfectants. WT receptor-transfected cells and untransfected control cells expressed a 2.1-fold higher dpH/dt than WT transfectants. WT receptor-transfected cells and untransfected control cells recovered from the acid load with a similar rate. Furthermore, activation of WT receptors with 10^-6 M epinephrine stimulated dpH/dt to the rate seen in C116F transfectants, whereas treatment with 10^-5 M ICI 118551 had no effect. Comparatively, treatment of the C116F mutant receptor with 10^-5 M ICI 118551 significantly reduced dpH/dt to a value near that seen in WT transfectants, whereas stimulation with 10^-6 M epinephrine had no effect.

**DISCUSSION**

In the current revised ternary complex model describing the activation of G protein-coupled receptors (5, 6), agonists do not directly drive the inactive receptor to assume the active conformation. Rather, the model predicts that agonists will preferentially bind to a receptor's R* conformation, thus stabilizing R* and shifting the isomerization equilibrium away from R. Conversely, inverse agonists, which preferentially bind to R, promote receptor inactivation by shifting the equilibrium away from R* (23, 24). Constitutively active mutant receptors that exhibit an increase in agonist affinity and/or a decrease in inverse agonist affinity do so because a shift in equilibrium toward the active conformation has already occurred. A mutation-induced shift of a receptor toward R* will, by definition, facilitate agonist-independent coupling to G proteins, thus
causing constitutive activation of effector pathways. Based on these tenets of the model, the higher than WT binding affinity of common β2-agonists seen for the C116F β2AR (Table I) is strongly suggestive of constitutive receptor activity. Consistent with receptor theory (6), the degree of high affinity shift was proportional to the intrinsic efficacy of the agonist tested. Also supporting this hypothesis, the β2-inverse agonist ICI 118551 exhibited a higher affinity for the WT receptor than for the C116F mutant (Table I). Again, it appears that the degree of shift to lower affinity was proportional to the inverse efficacy of the antagonist tested. ICI 118551, which is known to be a highly efficacious inverse agonist (24), showed the largest rightward shift in affinity for the mutant receptor (Table I). As expected, alprenolol and propranolol, which are classified either as weak inverse agonists (23) or as neutral antagonists (24), exhibited smaller rightward affinity shifts. Overall, these changes in ligand binding affinity are intrinsic to the mutant receptor and are not due to altered G protein precoupling as evidenced by the inability of Gpp(NH)p to affect Kᵢ values or Hill coefficients (data not shown).

The C116F mutation could evoke these shifts in ligand binding affinity in one of two ways. First, since the 116th residue is located in the third transmembrane domain only one helical turn below Asp-113, the counterion for the protonated amine of catecholamines (11, 12), it seems possible that it could indirectly affect interaction of the receptor with ligand per se. However, since the function of the receptor has also been altered and correlates to an activational paradigm, this scenario seems unlikely. Rather, we suggest that the mutation has affected receptor conformation to mimic the activated state. Since we have postulated that the equivalent mutation in the α₁bAR (C128F) is involved in modulation of an important salt bridge constraint that stabilizes the inactive conformation (25), it is possible to envision that C116F is influencing a similar constraining factor.

Even though the binding data allude to a constitutive activity induced by the C116F mutation, agonist-independent generation of a second messenger signal must be identified to firmly establish this hypothesis. Classically, it is held that the β2AR evokes cellular effects through a coupling to cAMP production via Gₛ (2, 3). Based on this, agonist-dependent and -independent effects on cAMP production were compared in WT and C116F receptor-transfected COS-1 cells. Not only did the C116F mutation fail to potentiate the isoproterenol dose response (EC₅₀) relative to the WT receptor, it also failed to evoke a response in the absence of the agonist (Fig. 2). Similar results were seen with epinephrine (data not shown). These findings indicate that the C116F mutation does not facilitate constitutive coupling to Gₛ. Rather, the mutant and WT receptors show equal competency in the generation of agonist-evoked cAMP responses. If the β2AR indeed only signals through a coupling to Gₛ, these data would suggest that the C116F mutation affects ligand binding without inducing the receptor to assume an activated conformation that is competent to couple to a second messenger signal.

As was mentioned, a coupling of β2ARs to Gₛ has been convincingly established in the literature. Because of this, when studying β2-receptor pharmacology, the inclination has been to measure cAMP as a marker of G protein coupling. Over the past decade, however, a strong case for an alternate pathway has emerged. Early work by Strader et al. (7) showed that β-receptor mutants, uncoupled from Gₛ due to deletion of G protein-interacting domains (residues 222–229 and 258–270), exhibited marked increases in agonist affinity. Similarly, mutagenesis of Asp-130 (in the third transmembrane domain) also led to increases in agonist binding affinity despite receptor uncoupling from Gₛ (26). The Gₛ-uncoupled 222–229 deletion mutant was later shown by Barber and Ganz (8) to activate Na⁺/H⁺ exchange via an unknown G protein. This study established the novel hypothesis that Gₛ-independent cellular responses were evoked by β2ARs. Since then, Barber and co-workers (9) have identified Gₛ₁₂ as responsible for activating a pathway that stimulates Na⁺/H⁺ exchange through the ubiquitous NHE1. At least one other member of the Gₛ subfamily, Gₛ₁₂, has since been implicated in the regulation of various isoforms of NHE1 (27), although the Gₛ₁₂ effects are inhibitory. These studies collectively support a hypothesis implicating Gₛ₁₂ in coupling of the βAR to Na⁺/H⁺ exchange.

With Gₛᵢ-independent coupling of β2ARs to NHE1 having been established, questions are raised about possible effects of the C116F β2AR mutant on Na⁺/H⁺ exchange. NHE1, which is present on the plasma membrane of all mammalian cells, fulfills several distinct physiological functions including control of pHi and maintenance of cellular volume (28). NHE1 function, which is commonly measured by monitoring pHi with the fluorescent dye BCECF, was examined via two approaches. Basal pHi was determined in WT and C116F receptor-transfected COS-1 cells. Due to continuous H⁺ extrusion during the posttransfection period, possible constitutive activation of NHE1 induced by the C116F mutation would be detected via identification of a cytosolic alkalosis relative to pHi values seen in the WT case. However, since pHi is not a direct measure of NHE1 activity, the actual rate of NHE1 function was also determined in WT and C116F transfectants by measuring the rate of pHi recovery following NH₄Cl-induced cellular acid load.

In the first experiment, intracellular alkalosis was evident in C116F receptor-transfected COS-1 cells relative to pHi levels seen in WT transfectants (Fig. 3). This was despite a 6.8-fold lower number of receptors in the C116F case. Locking the mutant receptor in the inactive state with the inverse agonist ICI 118551 blocked the effect, with treated mutants exhibiting a basal pHi similar to the WT case (Fig. 3). These findings not only provide strong evidence of a constitutive activation of Na⁺/H⁺ exchange in C116F receptor-transfected cells, but the effect is shown to be β₂-receptor-specific based on inhibition by the β₂-selective inverse agonist.

In the second experiment, cells transfected with the C116F β2AR showed a significantly faster rate of pHi recovery from acid load than WT transfectants. Confirming facilitation by NHE1, H⁺ extrusion during the posttransfection period, possible constitutive activation of NHE1 by the C116F mutation would be detected via identification of a cytosolic alkalosis relative to pHi values seen in the WT case. However, since pHi is not a direct measure of NHE1 activity, the actual rate of NHE1 function was also determined in WT and C116F transfectants by measuring the rate of pHi recovery following NH₄Cl-induced cellular acid load.

As before, this significant difference was seen despite a 6.8-fold lower receptor number in the C116F receptor-transfected cells. Further implicating the β₂AR as a regulator of NHE1, activation of the WT receptor with epinephrine caused an increase in dPH/dt to values seen in the C116F case, whereas inactivation of the C116F receptor with ICI 118551 reduced dPH/dt to values seen in the WT case. These results (a) corroborate earlier findings demonstrating coupling of β₂ARs to NHE1, (b) indicate selective constitutive coupling of the C116F β₂AR to NHE1, and (c) establish a role for Cys-116 in regulating selective coupling of the β₂AR to either the cAMP pathway or NHE1. It should be noted that coupling of the β₂AR to NHE1 has been demonstrated in cell lines that endogenously express the receptor (29). Therefore, it is unlikely that the results presented in this report are due to a promiscuous coupling of the receptor to Gₛ₁₂ in COS-1 cells.

Interestingly, epinephrine did not affect the rate of H⁺ extrusion seen in C116F receptor-transfected cells (Fig. 5). It is possible that the C116F mutation had itself induced a maximal
by earlier studies of hypothesis that Cys-116 can function as a switch is supported for this amino acid position as a "switch" that determines the receptor to a selectively coupled conformation suggests a role in the organelle pathways."

The findings presented in this report corroborate previously published work (7–9) describing a coupling of β2ARs to NHE1, putatively through Gα13. Additionally, we identify novel selective constitutive activity of NHE1 over cAMP in β2ARs possessing a C116F mutation (Fig. 6). The model predicts that under basal conditions, the C116F mutant will preferentially isomerize to a conformation that couples putatively to Gα13 (R*→Gα13). Fewer receptors will isomerize to the uncoupled state (R), while fewer still will reside in the Gαs-coupled conformation (R*→Gαs). In the presence of agonist, competent coupling to Gαs is finally achieved due to a shift in equilibrium that leads to a significant number of receptors in the Gαs-coupled conformation. Therefore, according to our model, the mutation selectively induces a putative R*→Gα13 conformation that does not restrict agonist induction of the WT receptor activation scenario (i.e., coupling to both second messenger pathways).

The importance of Cys-116 in controlling the transition of the receptor to a selectively coupled conformation suggests a role for this amino acid position as a "switch" that determines the trafficking of the receptor between multiple active states. The hypothesis that Cys-116 can function as a switch is supported by earlier studies of α1b-adrenergic and AT1 receptors. Cys-116 in the β2AR is 14 residues N-terminal to the highly conserved DRY domain of Gαs found on the cytoplasmic border of the third transmembrane domain. DRY, an important functional domain for the binding of G proteins and the catalysis of GDP release (31), provides a convenient reference for comparing the C116F β2 AR mutation with similar point mutations in α1bARs, AT1 receptors (Fig. 7), and possibly other G protein-coupled receptor systems.

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Selective Constitutive Signaling in $\beta_2$-Adrenergic Receptors

Fig. 8. Cubic ternary complex models. A, the cubic ternary complex model of Kenakin (34) in which a single activated state of the receptor partitions into different ternary complexes; B, the proposed revision of the cubic ternary complex model in which a single activated state of the receptor partitions into different ternary complexes; $R_1$, agonist; $R_2$, receptor; $G$, G protein.

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REFERENCES

1. Bylund, D. B. (1992) *FASEB J.* 6, 832–839
2. Strosberg, A. D. (1993) *Protein Sci.* 2, 1198–1209
3. Kubilius, B. (1991) *Trends Cardiovasc. Med.* 1, 189–194
4. Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., and Goodman Gilman, A. (eds) (1996) *The Pharmacological Basis of Therapeutics,* 9 Ed. p. 125, McGraw-Hill Book Co., New York
5. De Lean, A., Stadel, J. M., and Leffkowitz, R. J. (1980) *J. Biol. Chem.* 255, 7108–7117
6. Samama, P., Cotechia, S., Costa, T., and Leffkowitz, R. J. (1993) *J. Biol. Chem.* 268, 4625–4636
7. Strader, C. D., Dixon, R. A. F., Cheung, A. H., Candelore, M. R., Blake, A. D., and Sigal, I. S. (1987) *J. Biol. Chem.* 262, 16439–16443
8. Barber, D. L., and Ganz, M. B. (1992) *J. Biol. Chem.* 267, 20607–20612
9. Voyno-Yasenetskaya, T. A., Conklin, B. R., Gilbert, R. L., Hooley, R., Bourne, H. R., and Barber, D. L. (1994) *J. Biol. Chem.* 269, 4721–47247.
10. Hooley, R., Yu, C.-Y., Symons, M., and Barber, D. L. (1996) *J. Biol. Chem.* 271, 6152–6158
11. Dixon, R. A. F., Sigal, I. S., and Strader, C. D. (1988) *Cold Spring Harbor Symp. Quant. Biol.* 53, 487–497
12. Strader, C. D., Gaffney, T., Sugg, E. E., Candelore, M. R., Keys, R., Patchett, A. A., and Dixon, R. A. F. (1991) *J. Biol. Chem.* 266, 5–8
13. Perez, D. M., Hwa, J., Guavin, R., Mathur, M., Brown, F., and Graham, R. M. (1996) *Mol. Pharmacol.* 49, 112–122
14. Noda, K., Saad, Y., Graham, R. M., and Karnik, S. S. (1994) *J. Biol. Chem.* 269, 6743–6752
15. Perez, D. M., Piascik, M. T., and Graham, R. M. (1991) *Mol. Pharmacol.* 40, 876–883
16. Perez, D. M., DeYoung, M. B., and Graham, R. M. (1993) *Mol. Pharmacol.* 44, 784–795
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
19. Thomas, J. A., Buchsbaum, R. N., Zimniak, A., and Racker, E. (1979) *Biochemistry* 18, 2210–2218
20. Boron, W. F., and De Weer, P. (1976) *J. Gen. Physiol.* 67, 91–112
21. Kohmoto, O., Spitzer, K. W., Movsesian, M. A., and Barry, W. H. (1990) *Circ. Res.* 66, 622–632
22. Benos, D. J. (1982) *Am. J. Physiol.* 242, C131–C145
23. Chidiac, P., Hebert, T. E., Valiquette, M., Dennis, M., and Bouver, M. (1993) *Mol. Pharmacol.* 45, 490–499
24. Samama, P., Pei, G., Costa, T., Cotechia, S., and Leffkowitz, R. J. (1993) *Mol. Pharmacol.* 45, 380–394
25. Porter, J. E., Hwa, J., and Perez, D. M. (1996) *J. Biol. Chem.* 271, 28318–28323
26. Fraser, C. M., Chung, F.-Z., Wang, C.-D., and Venter, J. C. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 5478–5482
27. Lin, X., Voyno-Yasenetskaya, T. A., Hooley, R., Lin, C.-Y., Orlowski, J., and Barber, D. L. (1996) *J. Biol. Chem.* 271, 22604–22610
28. Grinstein, S., Rotin, D., and Mason, M. J. (1989) *Biochim. Biophys. Acta* 988, 73–97
29. Barber, D. L., McGuire, M. E., and Ganz, M. B. (1989) *J. Biol. Chem.* 264, 21038–21042
30. Aronson, P. S. (1985) *Ann. Rev. Physiol.* 47, 545–560
31. Acharya, S., and Karnik, S. S. (1996) *J. Biol. Chem.* 271, 25406–25411
32. Kjelsberg, M. A., Cotecchia, S., Ostrowski, J., Caron, M. G., and Leffkowitz, R. J. (1992) *J. Biol. Chem.* 267, 1430–1433
33. Noda, K., Peng, Y.-H., Liu, X.-P., Saad, Y., Husain, A., and Karnik, S. S. (1996) *Biochemistry* 35, 16435–16442
34. Kenakin, T. (1995) *Trends Pharmacol. Sci.* 16, 232–238
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