Phenylobenzamine Binding Reveals the Helical Orientation of the Third Transmembrane Domain of Adrenergic Receptors*

Phenylobenzamine (PB), a classical α-adrenergic antagonist, binds irreversibly to the α-adrenergic receptors (ARs). Amino acid sequence alignments and the predicted helical arrangement of the seven transmembrane (TM) domains suggested an accessible cysteine residue in transmembrane 3 of the α₂-ARs, in position C₃₃₅₆ (in subtypes A, B, and C corresponding to amino acid residue numbers 117/96/135, respectively), as a possible site for the PB interaction. Irreversible binding of PB to recombinant human α₂-ARs (90 μM, 30 min) reduced the ligand binding capacity of α₂A-, α₂B-, and α₂C-AR by 81, 96, and 77%. When the TM3 cysteine, Cys₁₁₇, of α₂A-AR was mutated to valine (α₂A-C₁₁₇V), the receptor became resistant to PB (inactivation, 10%). The β₂-AR contains a valine in this position (V₃₃₅₆; position number 117) and a cysteine in the preceding position (Cys₁₁₆) and was not inactivated by PB (10 μM, 30 min) (inactivation 26%). The helical orientation of TM3 was tested by exchanging the amino acids at positions 116 and 117 of the α₂A-AR and β₂-AR. The α₂A-F₁₁₆C/C₁₁₇V mutant was resistant to PB (inactivation, 7%), whereas β₂-V₁₁₇C was irreversibly inactivated (inactivation, 93%), confirming that position 3.36 is exposed to receptor ligands, and position 3.35 is not exposed in the binding pocket.

In the adrenergic receptor subfamily, the seven α-helical transmembrane (TM) domains form a crevice for the recognition and binding of ligands (1). The amino acid sequences are highly conserved within the seven hydrophobic TM domains of the three human α₂-adrenoreceptor (α₂-AR) subtypes (≈ 75% amino acid identity) (2–5). The α₂-AR subtypes also share significant structural identity within the TM domains with other members of the adrenoreceptor family (2, 6). For example, of the 182 amino acids comprising the TM domains of the human α₂A-AR, about 40% are identical with the human β₂-AR. The conserved regions are known to contain structural determinants responsible for recognizing and binding the endogenous hormones/neurotransmitters adrenaline and noradrenaline and other receptor ligands (2, 7–9).

* This work was financially supported by the Academy of Finland and the Technology Development Center of Finland. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: TM, transmembrane domain; PB, phencylobenzamine; AR, adrenergic receptor; CHO, Chinese hamster ovary.

Phenylobenzamine (PB) is an irreversible, subtype-nonselective α-AR antagonist. PB has been used as a pharmacological tool to study α-AR subpopulations in tissue preparations. PB was also the first α-AR antagonist to be therapeutically evaluated in humans. It produces long-lasting α-AR blockade and reduces blood pressure, but its clinical use was limited by severe side effects (10–12). Although the pharmacology of PB has been studied quite extensively, the molecular basis of its interaction with α-ARs has not been examined in detail. It is known that β-haloalkylamines, such as PB, cyclize in aqueous solution to form an unstable aziridinium ion, which can bind to target proteins with a strong ionic bond. The aziridinium ion then opens to create a reactive intermediate, with the consequence that a covalent bond between the drug molecule and the binding site can be formed. Side chains of amino acid residues that can be alkylated by haloalkylamines include –SH, –OH, –NH, and –COOH (13). Of the susceptible amino acid residues, cysteine (–SH) is the most reactive (14).

The helical arrangement of the TM domains indicated by receptor modeling (15, 16), in conjunction with analysis of sequence alignments (see Fig. 1), suggested to us an interaction between PB and the TM3 region of the α-ARs. The amino acid sequence alignment of TM3 of adrenergic receptors presented in Fig. 1 is validated by the position of the conserved aspartate residue (D₃₃₅₆ according to the nomenclature of Ballesteros and Weinstein (17) or position 113 in the α₂A-AR), known to be crucial for binding the charged nitrogen present in adrenergic phenethylamine ligands (7, 9). According to our hypothesis, the reactive aziridinium derivative of PB forms a covalent bond with Cys₁₁₇ in TM3 of the α₂A-, α₂B- and α₂C-ARs (Fig. 2) (corresponding to amino acid residues 117, 96 and 135, respectively). Also the three α₁-AR subtypes have a cysteine in this position, but the three β-AR subtypes have a valine residue in its place (Fig. 1). To test our hypothesis, we determined the irreversible binding of PB to the three human α₂-AR subtypes and constructed and tested an α₂A-F₁₁₆C/C₁₁₇V mutant lacking the Cys₁₁₇ (Cys₁₁₇ was substituted with valine; α₂A-C₁₁₇V). We also tested the effect of PB on the human β₂-AR, which has a valine (V₃₃₅₆) in the position corresponding to Cys₁₁₇ in α₂A-AR and a cysteine (C₃₃₅₅) in the preceding position (Fig. 1). The wild-type recombinant β₂-AR was resistant to the alkylating effect of PB, which indicated that this position is not exposed in the cavity. To confirm the structural orientation of TM3, α₂A-AR was mutated to resemble β₂-AR (α₂A-F₁₁₆C/C₁₁₇V) and vice versa (β₂-V₁₁₇C). The α₂A-F₁₁₆C/C₁₁₇V mutant was resistant to the alkylating effect of PB, whereas β₂-V₁₁₇C was irreversibly inactivated by PB treatment, confirming that a cysteine in position 3.36 is required for alkylation of adrenergic receptors by PB and that position 3.35 is unreachable by ligands in the binding crevice and probably points toward another TM helix or the lipid bilayer.
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**EXPERIMENTAL PROCEDURES**

Materials—Ethyl-[^3]H]RS79948—197 (8aR, 12aS, 13aS)-5,6,8a,9,10,11,12a,13,13a-decahydro-2-methoxy-12-(ethylsulphonyl)-6H-isouquinolin-2(1-1)-amine (specific activity 86.0 Ci/mmol) and [^3]HCGP-12177 (1,12-dihydro-3-(3-tert butylamino-2-hydroxypropoxy)-5,7,9 3-H]benzimidazol-2-one (specific activity 48.0 Ci/mmol) were from Amersham Pharmacia Biotech. Phenoxybenzamine and phentolamine was from Sigma. Cell culture reagents were supplied by Life Technologies, Inc. Other chemicals were of analytical grade and were purchased from commercial suppliers.

**Mutagenesis and Receptor Production**—The cDNA encoding the human a_2A-AR (2) was inserted into the Smal site, and the cDNA of the human a_2B-AR (18) was inserted into the HindIII/XbaI sites of the vector pALTER-1 (Promega, Madison, WI). Site-directed mutagenesis was performed utilizing the Altered Sites II site-directed mutagenesis system (Promega). Mutated a_2A-AR cDNAs were subcloned into the KpnI/BamHI sites of the expression vector pREP4 (Invitrogen). The wild-type a_2C- and mutated a_2B-AR cDNAs were subcloned into the PvuII site of the vector pREP4.

Chinese hamster ovary (CHO) cells expressing wild-type a_2A-, a_2C-, and a_2B-AR were established as described earlier (19). Adherent CHO cells (American Type Culture Collection, Manassas, VA) were cultured as reported previously (16). The pREP4-based expression constructs were transfected into cells using the Lipofectin reagent kit (Life Technologies, Inc.). Hygromycin B (Roche Molecular Biochemicals)-resistant (450 µg/ml) cell cultures expressing wild-type and mutated a_2A- and a_2B-ARs were tested for their ability to bind the radioligands ethyl-[^3]H]RS79948—197 or [^3]HCGP-12177. The transfected cells chosen for further experiments were subsequently maintained in 150 µg/ml hygromycin B. Saturation isotherms of ethyl-[^3]H]RS79948—197 and [^3]HCGP-12177 binding and LIGAND-derived K_d (receptor affinity) and B_max (receptor density) values were determined for each cell line. The binding affinities of the receptor mutants a_2A-C117V, a_2A-F116C/C117V, and a_2B-V117C were assessed by incubation (0.2–0.4 mg protein/assay tube) with 2 nM ethyl-[^3]H]RS79948—197 or [^3]HCGP-12177. Non-specific radioligand binding was again determined by including 10 µM phentolamine or propranolol in parallel assays.

**RESULTS**

Site-directed Mutagenesis and Transfections—To investigate the orientation of a cysteine residue in position 117 in TM3 of the a_2A-AR (C3.36) and a valine in this position in the a_2B-AR (V3.36) and to study the a-helical orientation of this region, the following three receptor variants were generated: a_2A-C117V, a_2A-F116C/C117V, and a_2B-V117C. The introduced mutations and the absence of secondary mutations were confirmed with an ABI3737 automated sequencer.

Mutated and wild-type receptors were expressed in CHO cells. Hygromycin B-resistant cell cultures were tested for their ability to bind the a_2A-AR antagonist ethyl-[^3]H]RS79948—197 or the a_2B-AR antagonist [^3]HCGP-12177. The transfected cells chosen for further experiments (Table I) were maintained in 150 µg/ml hygromycin B. Saturation isotherms of ethyl-[^3]H]RS79948—197 and [^3]HCGP-12177 binding and LIGAND-derived K_d (receptor affinity) and B_max (receptor density) values were determined for each cell line. The binding affinities of the receptor mutants a_2A-C117V, a_2A-F116C/C117V, and a_2B-V117C for the employed radioligands were comparable with the wild-type receptors (Table I).

**Receptor Inactivation Studies**—To validate the experimental conditions in the PB inactivation assay, we first compared the effect of PB treatment (10 µM–100 µM) with two incubation times (30 or 60 min) on the capacity of ethyl-[^3]H]RS79948—197 and [^3]HCGP-12177 binding in CHO cell homogenates expressing wild-type a_2A- and a_2B-ARs (data not shown). In subsequent experiments, PB was used at concentrations of 30, 60, and 90 nM for a_2A-ARs and 1 and 10 µM for a_2B-ARs. The incubation of cell homogenates for 30 min at 37 °C in the absence (control) and presence of PB was chosen as optimal for inactivation assays.

We first tested the alkylating effect of PB on the three human a_2 AR subtypes (a_2A, a_2B, and a_2C) expressed in CHO cells. PB treatment (90 nM, 30 min) reduced the binding capacity of a_2A-, a_2B-, and a_2C-AR by 81, 96, and 77%, respectively (Fig. 3). This was in agreement with the involvement of an exposed cysteine in TM3 of the a_2-ARs in the alkylating effect.
Results from saturation and competition binding assays are shown. The concentration of PB and phentolamine that inhibited specific \( {^3}H \)CGP-12177 binding in competition assays by 50% (\( IC_{50} \)) was used to calculate apparent \( K_i \) values (inhibition constant) according to the Cheng-Prusoff equation. Results are means ± S.E. from three to six separate experiments performed in duplicate. ND, not determined; wt, wild-type.

| Receptor | Saturation assay with [ethyl\(^3\)H]RS79948–197 or \(^3\)HCGP-12177 (fmol/mg of protein) | Competition assay |
|----------|---------------------------------------------------------------|------------------|
| \( \alpha_{2B} \)-wt | 1740 ± 133 | 0.01 ± 0 | ND |
| \( \alpha_{2A} \)-wt | 3610 ± 193 | 0.61 ± 0.11 | 0.06 ± 0.09 | ND |
| \( \alpha_{2A} \)-C117V | 4340 ± 61 | 0.21 ± 0.01 | 0.06 ± 0.0 | 0.01 ± 0 |
| \( \alpha_{2A} \)-F116C/C117V | 410 ± 7 | 0.25 ± 0.01 | 1.05 ± 0.07 | 0.45 ± 0.01 |
| \( \beta_2 \)-wt (Val\(^{111}\)) | 441 ± 2 | 0.30 ± 0.02 | 1.27 ± 0.04 | 0.28 ± 0.0 |
| \( \beta_2 \)-V117C | 896 ± 4 | 0.11 ± 0.01 | 36.3 ± 2.65 | 1940 ± 1250 |
| | 187 ± 5 | 0.16 ± 0.0 | 1.54 ± 0.06 | 740 ± 270 |

FIG. 3. Effect of preincubation with PB on ligand binding capacity of human \( \alpha_2 \)-AR subtypes. Membranes from CHO cells expressing \( \alpha_{2A} \), \( \alpha_{2B} \), and \( \alpha_{2C} \)-AR were incubated in the absence (control) and presence of PB (30, 60, and 90 nM) for 30 min at 37 °C, followed by two washes. Residual \( \alpha_2 \)-AR binding was determined by incubation with 2 nM [ethyl\(^3\)H]RS79948–197. Nonspecific binding was defined with 10 μM phentolamine. Results are expressed as percent of specific [ethyl\(^3\)H]RS79948–197 binding remaining after preincubation with PB compared with control and represent the means ± S.E. of three separate experiments performed in duplicate. wt, wild-type.

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Phenoxylbenzamine (PB) is a long-acting \( \alpha_2 \)-adrenoceptor antagonist. To further characterize the interaction of PB with TM3 cysteines, we compared the effects of PB treatment on \( \alpha_{2A} \)- and \( \beta_2 \)-ARs. Instead of a cysteine, the human \( \beta_2 \)-AR contains a valine in the corresponding position (V\(^{3,36}\)). Incubation with PB (90 nM, 30 min) inactivated 81% of \( \alpha_{2A} \)-AR, but \( \beta_2 \)-AR binding was not affected (inactivation 0%). When C\(^{3,36}\) of \( \alpha_{2A} \)-AR was mutated to valine, the mutated receptor \( \alpha_{2A} \)-C117V became relatively resistant to the alkylation effect of 10 μM PB (inactivation 26%) (Fig. 5). This confirmed our hypothesis of a structure-activity relationship between the alkylation effect of PB and an exposed cysteine residue in position 3.36 (117 in \( \alpha_{2A} \)-AR and \( \beta_2 \)-V117C) in TM3 of adrenergic receptors. This also indicated that the preceding position (F\(^{3,35}\) in \( \alpha_{2A} \)-AR and C\(^{3,35}\) in \( \beta_2 \)-AR) is not exposed in the binding pocket and thus not accessible for PB and other receptor ligands.

Covalent Binding of PB to Cysteine-containing Receptor Peptide—To confirm the covalent bonding of PB to Cys\(^{117}\) in TM3 of \( \alpha_{2A} \)-AR, PB was reacted in aqueous solution with a synthetic 9-mer peptide corresponding to residues 114–122 of TM3 of the receptor. After 2 h of incubation, mass spectrometric analysis revealed that all PB had hydrolyzed (M+H\(^+\) m/z 286.4), acylated in the employed acetate buffer (M+H\(^+\) m/z 328.5), or reacted with the thiol group of the peptide (M+2H\(^+\) m/z 619.0, M+H\(^+\) m/z 1236.1). The reference peptide with a valine substituted for the cysteine in position 117 did not react with PB. The identified reaction products are illustrated in Fig. 6.

 Competition Binding Assays—All wild-type and mutated receptors were tested with PB also under competition binding assay conditions (Table I). At all receptor variants, PB inhibited specific binding of 2 nM [ethyl\(^3\)H]RS79948–197 or \(^3\)HCGP-12177 in the competitive assays with steep monophasic competition curves, indicating that the lack of alkylation by PB was not because of lack of binding affinity but rather because of the absence of an accessible cysteine in the binding cavity. The three PB-resistant receptors, \( \alpha_{2A} \)-C117V, \( \alpha_{2A} \)-F116C/C117V and \( \beta_2 \)-AR, were also capable of binding PB, although with lower apparent affinity than the PB-sensitive receptors (Table I). Competition binding assays were also per-
formed with phentolamine, another subtype-nonselective \(\alpha_{2A}\)-AR antagonist. The substitution of Cys 117 of \(\alpha_{2A}\)-AR with valine resulted in 28–45-fold decreases in the affinity of the receptor for phentolamine (Table I). Phentolamine was bound with low affinity to both wild-type \(\alpha_{2A}\)-AR and the \(\alpha_{2A}\)-V117C mutant receptor.

**DISCUSSION**

We have here demonstrated that an exposed cysteine residue, C3.36, in position 117/96/135 in the binding cavity of \(\alpha_{2A}\)/\(\alpha_{2B}\)/\(\alpha_{2C}\)-AR is required for the alkylating effect of the irreversible \(\alpha\)-AR antagonist, PB. The present work also shows that the depth of the ligand binding cavity extends to at least one helical turn below the conserved D3.32 in the adrenergic receptors. Introduction of a valine into position 3.36 makes the \(\alpha_{2A}\)-AR resistant to PB (\(\alpha_{2A}\)-C117V). The \(\beta_2\)-AR has a valine in this position and a cysteine in the preceding position and is resistant to the alkylating effect of PB. However, when V3.36 was substituted by cysteine, the \(\beta_2\)-V117C receptor mutant became susceptible to irreversible inactivation by PB. It has been reported previously that position 116 in \(\beta_2\)-AR, i.e. C3.35, is not involved in recognition and binding of \(\beta\)-AR antagonists (21). Site-directed mutagenesis and modeling of the \(\alpha_{1B}\)-AR has also clearly indicated that the residue in position 3.35 points toward...
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TM2 and is not exposed in the cavity (22, 23). Our second PB-resistant α2A receptor, α2A-2F116C/C117V, also supports a hypothetical structural orientation of TM3, where position 116 (3.35) is not exposed in the binding cavity and is unreachable by PB. Position 3.36 in the middle of the TM3 segment has also been shown to be exposed in the receptor binding-site crevice of other non-adrenergic monoamine receptors, such as the dopamine D2 receptor (Cys118) (25–27) and the 5-HT₂A receptor (Ser159) (28).

Inactivation of the PB-resistant α2A-AR mutants with high PB concentrations indicated that there must be other sites in the receptor protein in addition to the investigated TM3 cysteine that are susceptible to alklylation by PB. Mass spectrometric analysis of the reaction products obtained from incubation of PB with a synthetic peptide corresponding to this region of TM3 verified that PB indeed forms a covalent bond with the cysteine residue. No reaction product was generated when PB was incubated with a control peptide with a valine in place of the cysteine.

In the inactivation assay with 10 μM PB, [³H]CGP-12177 binding to the wild-type β²-AR was inhibited by 26%, indicating that PB might have interactions with other sites in the β²-AR binding cavity, lacking a cysteine in position 3.36. However, Gether et al. (21) have shown previously that none of the five cysteines located in the TM domains of the β²-AR (Cys77, Cys116, Cys125, Cys285, and Cys327) are essential for β²-antagonist binding. Indeed, studies with the cysteine-reactive reagent 2-aminoethyl methane thiosulfate indicated that none of these cysteines is exposed in the water-accessible binding site crevice (29). The observed effect of the employed high PB concentration may thus represent interactions with other reactive amino acid side chains than –SH of an exposed cysteine.

The mutant receptors bound the employed radioligands with similar affinities compared with the wild-type receptors, which verified the proper expression and folding of the modified proteins. Competition binding assays showed that the PB-resistant receptors α2A-C117V, α2A-F116C/C117V, and β²-AR were also capable of binding PB, although with reduced apparent affinity, indicating that the lack of alklylation by PB was not because of lack of binding affinity. It should be noted that the true affinity of an irreversible ligand cannot be determined reliably in competition binding assays, and the apparent affinity of PB determined in this way actually represents both reversible and irreversible binding and grossly overestimates the affinity of the PB-susceptible receptors (16, 24).

Cysteine substitutions in the binding cavity can, however, have structure-dependent selective effects on ligand binding affinities. That this indeed is the case for α2A-AR was indicated by our preliminary experiments with another α2-AR radioligand, [³H]RX821002. No specific binding was detected for this radioligand at α2-AR or α2-F116C/C117V (results not shown), despite adequate receptor expression levels as determined in assays with [³H]RS79948–197, RX821002 is an imidazoline derivative that interacts with TM3 of α₂-ARs, and we therefore wanted to test another widely used imidazoline derivative, phentolamine, as a reference compound. Phentolamine does not bind irreversibly to adrenoceptors, and its true affinity can be determined in competition binding assays. The affinity of phentolamine at mutated α₂-ARs lacking C₃.₃₆ was clearly lower than at the wild-type receptor. This indicates that C₃.₃₆ is important for binding of imidazoline derivatives and further supports the location of this residue in the binding cavity. PB is not an imidazoline, and its binding mode in the α₂-AR binding cavity is different from phentolamine and RX821002. Our subsequent competition binding assays with structurally diverse α₂-AR ligands indicate that the effects of the C₃.₃₆ substitution are specific for imidazoline and imidazole derivatives.²

Using irreversible binding of the α-AR antagonist PB as a criterion for identifying a sulphydryl side chain of an endogenous or introduced cysteine as being exposed in the ligand-accessible binding cavity of the receptor, we have demonstrated that C₃.₃₆ (Cys117) in the TM3 domain of the α₂-AR is responsible for binding PB. This cysteine also appears to be important in interactions with other receptor antagonists, as indicated by the observed decrease in the affinity for the imidazoline derivative phentolamine when C₃.₃₆ was substituted with valine. The structural orientation of the TM3 domain of α₂-AR, where amino acid residues D₃.₃₂ and C₃.₃₆ point into the binding cavity, and F₃.₃₅ is not exposed in the cavity, may, however, represent only one receptor conformation. All α₂-protein-coupled receptors are thought to exist in an equilibrium between two or more conformations or allosteric states, R and R* (29–31). Being antagonists, the ligands tested in this study mainly interact with the receptor in its predominant, inactive conformation (R). The orientation of the TM domains may change upon receptor activation and agonist binding (1, 21, 30). Nevertheless, results obtained with catecholamine agonists (9, 22, 32) indicate that D₃.₃₂ indeed is accessible also in the active conformation (R*), and if TM3 has typical α-helical periodicity, then C₃.₃₆ would be expected to be exposed also in the active conformation (R*).

Acknowledgments—Ulla Uoti and Raisa Vuorinen are gratefully acknowledged for skillful technical assistance. We thank Dr. Jonathan Javitch and Dr. Jukka Hellman for valuable comments on the manuscript.

REFERENCES

1. Scheer, A., Fanelli, F., Costa, T., De Benedetti, P. G., and Coteccia, S. (1996) EMBO J. 15, 3566–3578
2. Kobilka, B. K., Matsu, H., Kobilka, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J., and Regan, J. W. (1987) Science 238, 650–656
3. Lomasney, J. W., Lorenz, W., Allen, L. F., King, K., Regan, J. W., Yang-Feng, T. L., Caron, M. G., and Lefkowitz, R. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5094–5098
4. Kobilka, B. K. (1990) Pharmacol. Rev. 41, 119–124
5. Lomasney, J. W., Coteccia, S., Lefkowitz, R. J., and Caron, M. G. (1991) Biochim. Biophys. Acta 1095, 127–139
6. Regan, J. W., Kobilka, T. S., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R. J., and Kobilka, B. K. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6301–6305
7. Pepperl, J., and Regan, J. W. (1994) in Handbook of Receptors and Channels (Peroutka, S. ed.) pp. 45–78, CRC Press, Inc., Boca Raton, FL
8. Kobilka, B. K. (1995) Pharmacol. Rev. 47, 119–124
9. Salmienia, T., Varis, M., Nyrönien, T., Pihlilaito, M., Hoffren, A. M., Lönnberg, T., Marjamaki, A., Frang, H., Savola, J. M., Scheinin, M., and Johnson, M. S. (1999) J. Biol. Chem. 274, 23405–23413
10. Wang, C. D., Buck, M. A., and Fraser, C. M. (1991) Mol. Pharmacol. 40, 168–179
11. Hoffman, B., and Lefkowitz, R. J. (1996) in The Pharmacological Basis of Therapeutics (Hardman, J. and Limbird, L., eds) pp. 199–248, McGraw-Hill Book Co., New York
12. Phillips, D. (1980) in Handbook of Experimental Pharmacology. Adrenergic Activators and Inhibitors (Starkman, L. ed.) pp. 5–61, Springer, Berlin
13. Verducci, R. J., Bondell, W., and Hieble, J. P. (1995) J. Med. Chem. 38, 3681–3716
14. Johnson, D. (1996) in Textbook of Receptor Pharmacology (Foreman, J. and Johnson, T., eds) pp. 3–62, CRC Press, Inc., Boca Raton, FL
15. Shulman-Roess, E. M., Noe, D. A., Gamcsik, M. P., Marlow, A. L., Hilton, J., Haashe, N. H., Culvin, O. M., and Luedeman, S. M. (1988) J. Med. Chem. 31, 513–529
16. Cockerell, V., Frang, H., Pihlilaito, M., Marjamaki, A., and Scheinin, M. (2000) J. Neurochem. 74, 1705–1710
17. Marjamaki, A., Pihlilaitto, M., Vakkuri, M., Heinonen, P., Savola, J. M., and Scheinin, M. (1998) Mol. Pharmacol. 53, 570–576
18. Ballesteros, J. A., and Weinstein, H. (1995) in Receptor Molecular Biology (Sealfon, S. C., ed) pp. 366–427, Academic Press, San Diego, CA
19. Dixon, R. A., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohman, H. G., Frielie, T., Bolanowski, M. A., Bennett, C. D., Rand, E., and Diehl, R. E. (1988) Nature 321, 75–79
20. Pohjanpalo, J., Janssen, C. C., Luomala, K., Marjamaki, A., Savola, J. M., and Scheinin, M. (1997) Eur. J. Pharmacol. 335, 53–63
21. Halme, M., Sjoholm, B., Savola, J. M., and Scheinin, M. (1995) Biochim. Biophys. Acta 124, 2–12

² H. Frang, A.-M. Hoffren, A. Marjamaki, and M. Scheinin, manuscript in preparation.
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21. Gether, U., Lin, S., Ghanouni, P., Ballesteros, J. A., Weinstein, H., and Kobilka, B. K. (1997) EMBO J. 16, 6737–6747
22. Perez, D. M., Hwa, J., Galvin, R., Mathur, M., Brown, F., and Graham, R. M. (1996) Mol. Pharmacol. 49, 112–122
23. Porter, J. E., and Perez, D. M. (1999) J. Biol. Chem. 274, 34535–34538
24. Michel, M. C., Kerker, J., Branchek, T. A., and Forray, C. (1993) Mol. Pharmacol. 44, 1165–1170
25. Javitch, J. A., Li, X., Kaback, J., and Karlin, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10355–10359
26. Javitch, J. A., Fu, D., Chen, J., and Karlin, A. (1995) Neuron 14, 825–831
27. Javitch, J. A., Fu, D., and Chen, J. (1996) Mol. Pharmacol. 49, 692–698
28. Almaula, N., Ebersole, B. J., Zhang, D., Weinstein, H., and Sealfon, S. C. (1996) J. Biol. Chem. 271, 14672–14675
29. Javitch, J. A., Fu, D., Liapakis, G., and Chen, J. (1997) J. Biol. Chem. 272, 18546–18549
30. Marjamaki, A., Frang, H., Pihlavisto, M., Hoffren, A.-M., Salminen, T., Johnson, M. S., Kallio, J., Javitch, J. A., and Scheinin, M. (1999) J. Biol. Chem. 274, 21867–21872
31. Samama, P., Cotechia, S., Costa, T., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 4625–4636
32. Nyronen, T., Pihlavisto, M., Peltonen, J. M., Hoffren, A.-M., Varis, M., Salminen, T., Wurster, S., Marjamaki, A., Kanerva, L., Katainen, E., Laaksonen, L., Savola, J.-M., Scheinin, M., and Johnson, M. S. (2001) Mol. Pharmacol. 59, 1343–1354
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J. Biol. Chem. 2001, 276:31279-31284.
doi: 10.1074/jbc.M104167200 originally published online June 6, 2001

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